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Design, synthesis and characterization of new molecules which counteract drug resistance mechanisms

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ABSTRACT

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"Design, synthesis and characterization of new molecules which counteract drug resistance mechanisms"

Multidrug Resistance (MDR) is a type of acquired resistance that cancer cells develop against structurally and mechanistically unrelated drugs to which they are initially sensitive. MDR is mainly due to the overexpression of proteins, as P-gp, MRP1 and BCRP, that work as efflux pumps, reducing the intracellular concentration of drugs below their active dose. P-gp is the most studied transporter, overexpressed in many blood and solid tumors. BCRP is often overexpressed in solid tumors and leukemia, together with P-gp. A possible approach to overcome MDR is to co-administer efflux pump inhibitors with anticancer drugs, to increase drugs' intracellular concentration and restore their therapeutic effects. Interestingly, on the membrane of several resistant cancer cells, P-gp is co-localized and physically associated to the isoform XII of human carbonic anhydrase (hCA XII). Moreover, the pharmacological inhibition of hCA XII reduced the ATPase efflux activity of P-gp. So, compounds with dual P-gp/hCA XII inhibitory effects could be useful to target resistant cancer cells that overexpress both proteins. The aim of my PhD project was to design and synthesize new compounds able to reverse MDR in cancer cells. This PhD thesis consists in two main projects:

1. The first part focused on compounds with dual P-gp/hCA XII inhibitory effects. To maintain a high potency on P-gp and introduce a selective activity towards hCA XII, we designed hybrid inhibitors characterized by both P-gp and hCA XII binding moieties (Figure 1). In these three series of compounds (Figure 1, **A**, **B** and **C**), we introduced on the structure of our potent P-gp inhibitors, as the *N*,*N*-bis(alkanol)amine aryl diesters (series **A** and **B**) and the methoxy-substituted arylpiperazine (series **C**) scaffolds, two specific residues, the benzene sulfonamide (only series **A**) or the coumarin moieties, to target hCA XII.

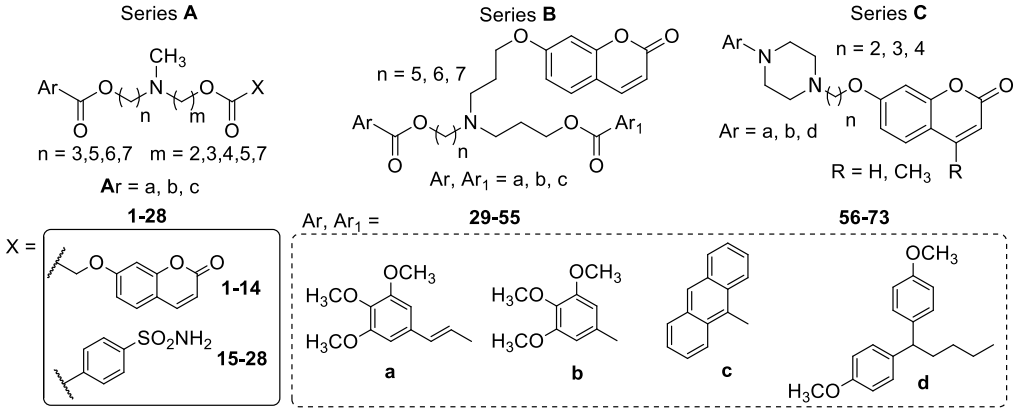

Figure 1. *Structure of dual P-gp/hCA XII inhibitors* ***1-73****.*

As regards the activity on P-gp, all these molecules were able to enhance the intracellular accumulation of two P-gp substrates, Rhodamine-123 and Doxorubicin, in K562/DOX cells that overexpress only P-gp. Moreover, coumarin derivatives were selective inhibitors of the tumor-associated hCA IX and hCA XII isoforms. Interestingly, most of our compounds displayed the highest MDR reverser effects on the tested resistant cell lines (LoVo/DOX, HT29/DOX and A549/DOX), that overexpress both proteins, showing an interesting synergistic effect.

2. The second main project of this thesis is based on the design and synthesis of MDR reversers active as ABC modulators: Tariquidar analogues (Figure 2, **D**) and quinazoline derivatives (Figure 2, **E**). The Tariquidar analogues derive structurally from two potent P-gp inhibitors, Tariquidar and Elacridar. First,

we designed and synthesized a series of compounds, bearing the 6,7-dimethoxy-2-phenethyl-1,2,3,4-tetrahydroisoquinoline nucleus present in the lead compounds, linked to an aryl-substituted amide or ester group (Figure 2, **D**). Then, we modified the amide function, by introducing two bioisosteric heterocycles, the tetrazole and the oxadiazole ones, linked to methoxy-substituted aryl groups. Notably, we designed and synthesized both the 1,5- and the 2,5-disubstituted tetrazoles, and the 2,5-disubstituted-1,3,4-oxadiazoles (Figure 2, **D**). Otherwise, quinazoline derivatives (Figure 2, **E**) maintain the quinazoline-4-amine scaffold of two tyrosine kinase inhibitors (TKIs), Gefitinib and Erlotinib, that have been identified as ABC transporters modulators. In this series of quinazoline derivatives, we introduced secondary or tertiary protonable amines in position 4 of the quinazoline scaffold, while in position 2 we inserted aromatic groups, such as anthracene or methoxy-substituted aryl moieties (Figure 2, **E**).

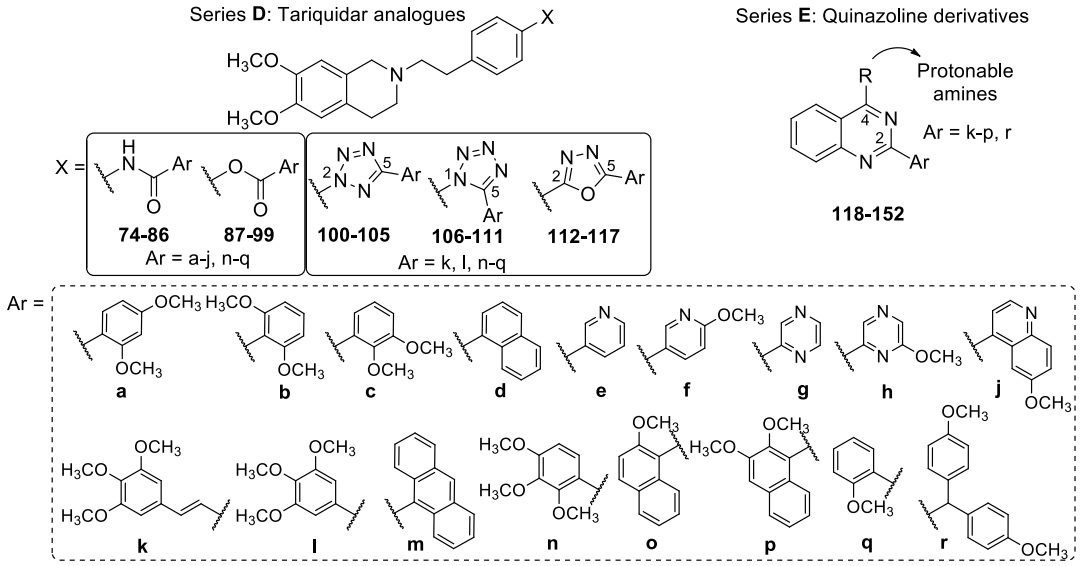

Figure 2. *MDR modulators **74-152**.*

All these MDR modulators **74-152** were studied on three different transfected cell lines (MDCK-MDR1, MDCK-MRP1 and MDCK-BCRP that overexpress P-gp, MRP1 and BCRP, respectively) to evaluate their activity on these ABC proteins, by measuring the inhibition of the transport of two fluorescent probes. In general, all these derivatives showed high inhibitory effects on P-gp: the most potent P-gp modulators were further studied in association with Doxorubicin on resistant cancer cells (MDCK-MDR1, HT29/DOX) that overexpressed P-gp. Moreover, some compounds also displayed a good or moderate activity on the other two transporters MRP1 and BCRP.

Moreover, during my PhD thesis, I also performed a series of chemical stability tests on derivatives bearing liable ester groups: these experiments were carried out to evaluate the susceptibility of our ester molecules towards spontaneous and enzymatic hydrolysis. Stability analyses were performed by liquid chromatography coupled with mass spectrometry (LC-MS/MS) methods. First, I studied the chemical stability of the previously synthesized *N*,*N*-bis(alkanol)amine aryl diesters active as P-gp inhibitors (Figure 3, linear and chiral derivatives); then, I performed these stability tests on all the other ester derivatives synthesized in this PhD project (Figure 1: series **A** and **B**; Figure 2: Tariquidar analogues **87-99**).

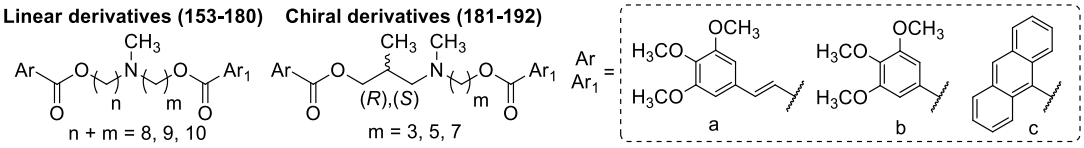

Figure 3. *Structures of the previously synthesized linear and chiral derivatives*.

Concerning the chiral P-gp inhibitors (Figure 3), we also developed a valid method to evaluate the enantiomeric excess of (*R*) and (*S*) enantiomers by enantioselective liquid chromatography coupled with diode array detector (LC-DAD) analysis.

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List of abbreviations and acronyms

AAZ	Acetazolamide
ABC	ATP-Binding Cassette
AML	Acute Myeloid Leukemia
BBB	Blood-Brain Barrier
BCRP	Breast Cancer Resistance Protein
CA	Carbonic Anhydrase
CARPs	CA-Related Proteins
CHX	Cyclohexane
CSC	Cell Surface Capturing
CSF	Cerebrospinal Fluid
DBP	Drug Binding Pocket
DIPEA	*N*,*N*-Diisopropylethylamine
DMF	*N*,*N*-Dimethylformamide
DMAP	4-Dimethylaminopyridine
Doxo	Doxorubicin
EC_{50}	Half Maximal Effective Concentration
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ee	Enantiomeric Excess
ESI	Electrospray Ionization Source
FWHM	Full Width At Half Maximum
FR	Fluorescence Ratio
HATU	1-[Bis(Dimethylamino)Methylene]-1*H*-1,2,3-Triazolo[4,5-*B*]Pyridinium 3-Oxide Hexafluorophosphate
hCAs	Human Carbonic Anhydrases
HOBt	1-Hydroxybenzotriazole Hydrate
HRMS	High-Resolution Mass Spectrometry
IC_{50}	Half Maximal Inhibitory Concentration
K_i	Inhibition Constant
K_M	Michaelis-Menten Constant
LC-MS/MS	Liquid Chromatography Coupled with Mass Spectrometry
LC-DAD	Liquid Chromatography Coupled with Diode Array Detector
MDR	Multidrug Resistance
MRM	Multiple Reaction Monitoring
MRP1	Multidrug-Resistance-Associated Protein-1
MRP7	Multidrug-Resistance-Associated Protein-7
NBD	Nucleotide-Binding Domain
Papp	Apparent Permeability
P-gp	P-Glycoprotein
PBS	Phosphate Buffer Solution
R	Resolution
RF	Reversal Fold
Rhd 123	Rhodamine-123
RT	Retention Time
$t_{1/2}$	Half-Life
THF	Tetrahydrofuran
TKIs	Tyrosine Kinase Inhibitors
TMD	Transmembrane Domain
SG	Sticky Group
ZBG	Zinc-Binding Group

1. Introduction

1.1. Multidrug Resistance (MDR) and ABC transporters

Drug Resistance is nowadays a serious limitation to successful anticancer therapy. Multidrug Resistance (MDR) is a type of acquired cross-resistance that cancer cells develop against a great variety of structurally and mechanistically unrelated drugs to which they are initially sensitive[1,2].

Different types of biochemical MDR mechanisms have been described[3], but the so-called classical MDR is mainly due to the overexpression of transmembrane proteins that work as efflux pumps and prevent drugs' intracellular accumulation needed to exert their therapeutic activity. In human MDR cells, these proteins are part of the ATP-binding cassette (ABC) transporter family[4], and they use the energy derived from ATP hydrolysis to actively transport substrates through the membrane, against the concentration gradient[5]. In addition to be overexpressed in cancer cells, these proteins are also present in many healthy tissues where they arouse different physiological and pharmacological actions[6], by regulating the permeability of biological membranes. Indeed, they often protected these tissues from xenobiotics and can influence ADME properties and bioavailability of many drugs. The human genome encodes 49 ABC transmembrane proteins, divided into seven subfamilies (ABC-A to ABC-G), based on the similarity of their amino acid sequences[7]. The main ABC transporters overexpressed by human MDR cancer cells are P-glycoprotein (P-gp, ABCB1)[8], Multidrug-Resistance-associated Protein-1 (MRP1, ABCC1)[9] and Breast Cancer Resistance Protein (BCRP, ABCG2)[10].

1.2. P-glycoprotein (P-gp)

P-gp is the most studied ABC transporter, and it was the first efflux pump discovered to play a role in human cancer MDR. Many compounds are substrates of this protein, as neutral or positively charged molecules. P-gp is a transmembrane glycoprotein present, beside cancer cells, in several important tissues, as kidneys and liver, and blood-tissue barriers (BBB and gastrointestinal barrier), where it regulates some important physiological processes such as the secretion of lipophilic molecules and the extrusion of exogenous agents[11]. Physiologically, P-gp protects tissues and organs, as the brain, from xenobiotics; it is also the main responsible of the reduced uptake of oral drugs[12]. Unfortunately, this efflux protein is overexpressed in cancer cells as a result of the upregulation of the human MDR1 gene expression. P-gp confers the strongest resistance to the widest variety of compounds, and it extrudes the chemotherapeutic drug from the cells, lowering its concentration below that necessary for anticancer action. Several antitumoral drugs are P-gp substrates, as Vincristine, Daunorubicin, Irinotecan, Imatinib and Gemtuzumab[13].

In 1973, Dano and coll. found that a membrane glycoprotein, belonging to the ABC transporter family, was involved in the development of resistance to Daunorubicin in Ehrlich ascites carcinoma cells[14]. Notably, Dano observed that in the cell line that developed resistance, an ABC transporter was overexpressed and that this cell line was also resistant to other anticancer drugs (vinca alkaloids and anthracyclines)[14]. Three years later, Juliano and Ling discovered that

the ABC transporter, identified by Dano, was responsible for the development of drug resistance in other cell lines[15].

1.2.1. P-gp structure and mechanism of action

The MDR1 gene encoding P-gp was cloned by three research group between 1984 and 1986: this discovery allowed to determine the primary structure of P-gp. P-gp isoforms have greater than 70% sequence identity.
P-gp is a 170 kDa integral membrane protein[15], and it consists in a single polypeptide of 1280 amino acids arranged in two homologous units (Figure 1.1): each unit contains 610 amino acid residues, connected by a segment, called the linker region, of 60-70 phosphorylated amino acid residues[16]. Each unit contains a transmembrane domain (TMD), consisting of six α-helices separated from each other by hydrophilic loops, which seems to be the main drugs binding site. These transmembrane domains form the pathway through which solute crosses the membrane, and they play a major role in determining substrate specificity[17]. The hydrophobic domain is linked to an hydrophilic one that is located on the cytoplasmatic side of membrane, and contains the nucleotide-binding domain (NBD)[18]. This domain is conserved in all the ABC transporters and couples the hydrolysis of ATP with the transport of the substrate: the two NBD domains dimerize in order to bind and hydrolyze ATP[17]. P-gp is also *N*-glycosylated on the extracellular side.

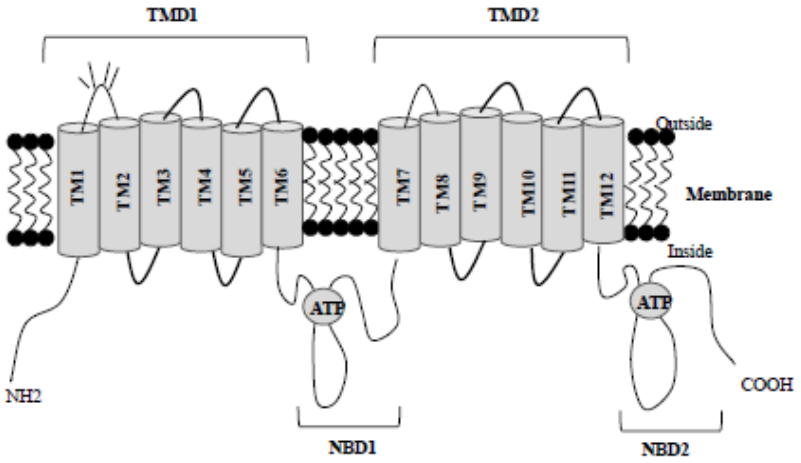


Figure 1.1: schematic structure of P-gp.

The first information about the human P-gp structure was obtained starting from the bacterial transporter proteins (Sav 1866, Figure 1.2), on which homology models were formulated, due to the difficulties to crystallize membrane proteins. Today, the homology model to define the structure of human P-gp is also based on the 3D images of the murine P-gp, which shares a sequence identity of 87% with the human one.

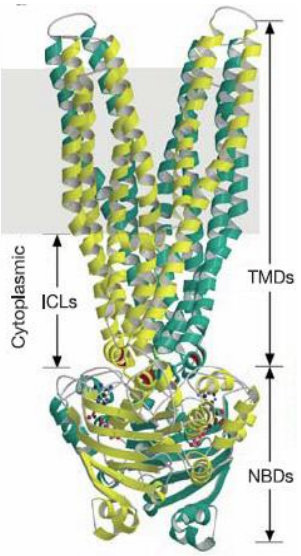


Figure 1.2: Sav1866 structure. ICLs: intracellular loop.

In 2009 Aller et al.[19] obtained an X-ray crystallography of mouse P-gp, with a resolution of 3.8 Å, and in 2014, Jaimes and Aller[20] obtained the same crystallography but with an higher resolution (9.4 Å). Murine P-gp was studied both in its Apo form, i.e. without any ligand in its binding site, and co-crystallized with two stereoisomers of cyclic hexapeptide inhibitors (QZ59-RRR e QZ59-SSS) (Figure 1.3).

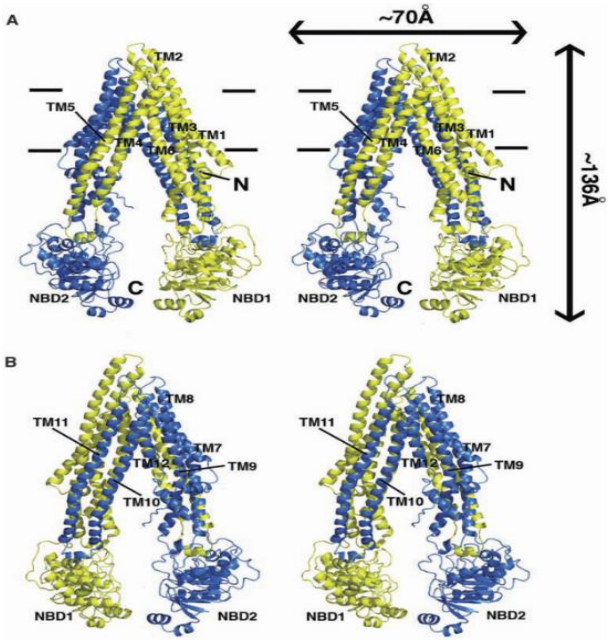


Figure 1.3: structure of P-gp. (A) Front and (B) back views of P-gp.

Each of these two crystalline forms is made up of two molecules of P-gp (P-gp1 and P-gp2) and have structurally similar TMDs, while they have small differences on NBDs. TMD4-TMD6 and TMD10-TMD12 transmembrane domains form two "portals" that allow hydrophobic molecules to enter the cavities, directly through the membrane phospholipid bilayer, up to the binding pocket, called Drug Binding Pocket (DBP). The DBP upper half is rich in hydrophobic and aromatic residues, while the lower half has a higher number of polar and charged amino acids. Docking studies on P-gp revealed how aromatic hydrophobic interactions (such as π-π interactions) are important for the affinity between modulator and P-gp binding pocket.

Furthermore, hydrophobic bonds between modulator and amino acid residues, such as the Van Der Waals interactions, compared to electrostatic bonds, guarantee greater stability of the ligand-protein complex. Also according to docking studies, to design good P-gp modulators, we focused on both hydrophobicity and lipophilicity. Therefore, a good P-gp modulator should have a logP of at least 2.92, to form hydrophobic interactions with the DBP[18].
When substrates interact within the binding side, two ATP molecules bind at the level of the NBDs: this phenomenon determines a deep conformational rearrangement of the protein, which passes from an open conformation towards the intracellular side, with high affinity for the substrate ("*inward facing"*) to an open conformation towards the extracellular side ("*outward facing"*) with low affinity for the substrate, which is thus expelled from the cell (Figure 1.4).

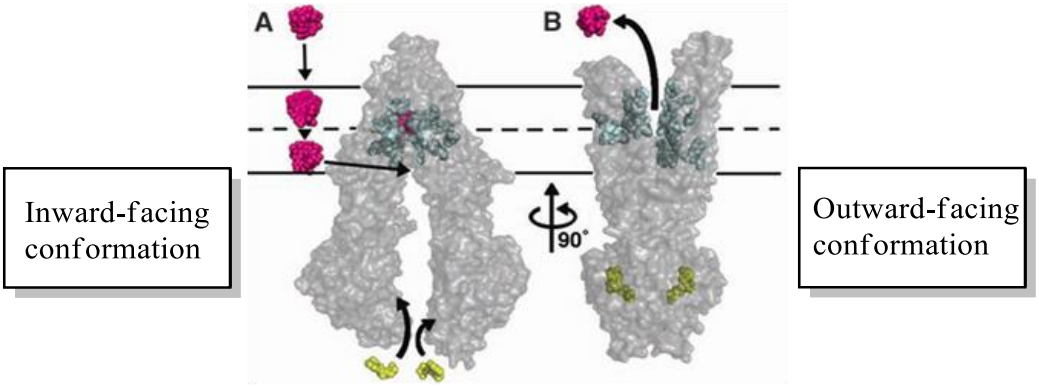


Figure 1.4: model of substrate transport by P-gp. (A) substrate (magenta) crosses the membrane bilayer from outside of the cell to the inner leaflet and enters the internal drug-binding pocket through an open portal. (B) two ATP molecules (yellow) bind to the NBDs, causing a large conformational change which present the substrate and drug-binding site to the extracellular space[19].

1.3. Breast Cancer Resistance Protein (BCRP)

Breast Cancer Resistance Protein (BCRP, ABCG2) is another transporter belonging to the ABC family that is involved in MDR. BCRP was first identified in 1998 when human breast cancer cells (MCF-7 cells), in presence of the P-gp inhibitor Verapamil, developed resistance to Doxorubicin[21].
BCRP is the last discovered ABC transporter that is involved in MDR[21]: it is physiologically expressed in many tissues, and together with P-gp, is located at the blood-brain barrier (BBB) where it is responsible of the limited BBB penetration of several drugs[22]. BCRP is also overexpressed in several hematological and solid tumors, compromising the therapeutic efficacy of many antitumoral agents[10]: it is able to transport a wide variety of anticancer drugs such as Mitoxantrone, Methotrexate, Topotecan, Irinotecan and Doxorubicin[23–25]. Moreover, also the tyrosine kinase inhibitors (TKIs), Gefitinib, Imatinib and Erlotinib, were BCRP substrates[26,27].
BCRP expression is associated with negative outcomes in acute myeloid leukemia (AML) and other cancers, including acute lymphoblastic leukemia, breast and lung cancer[25,28]. BCRP expression is correlated in tumor cells with an increase in self-renewal capacity and tumorigenic potential, suggesting that BCRP inhibition may be an approach to target cancer development[28]. Furthermore, BCRP is implicated in the acquired drug resistance to Topotecan in breast cancer. Further studies show that BCRP ablation in tumor cells increases the survival of animals treated with Topotecan[29].

1.3.1. BCRP structure and mechanism of action

BCRP is a 72 kDa protein made up of 655 amino acids (Figure 1.5); it is considered a half-transporter, since it has a single nucleotide binding domain (NBD) and one transmembrane domain (TMD). The TMD domain is formed by 6 transmembrane helices: it binds the substrates through a binding site located on the cytosolic side and transports them out of the cell[30]. The structure of the NBD domain is strongly conserved, as in all the ABC transporters[30]. This domain binds ATP and hydrolyzes it. However, BCRP requires at least two NBDs to function as a drug efflux pump: hence, functional BCRP exists as either homodimer[28]. To fully bind an ATP molecule, two NBDs dimerize: two ATP molecules bind NBD domains simultaneously, using the structural elements of the opposite NBD in a complementary way. This interaction induces conformational changes and provides the energy to allow TMDs to transport the substrate out of the cell[30].

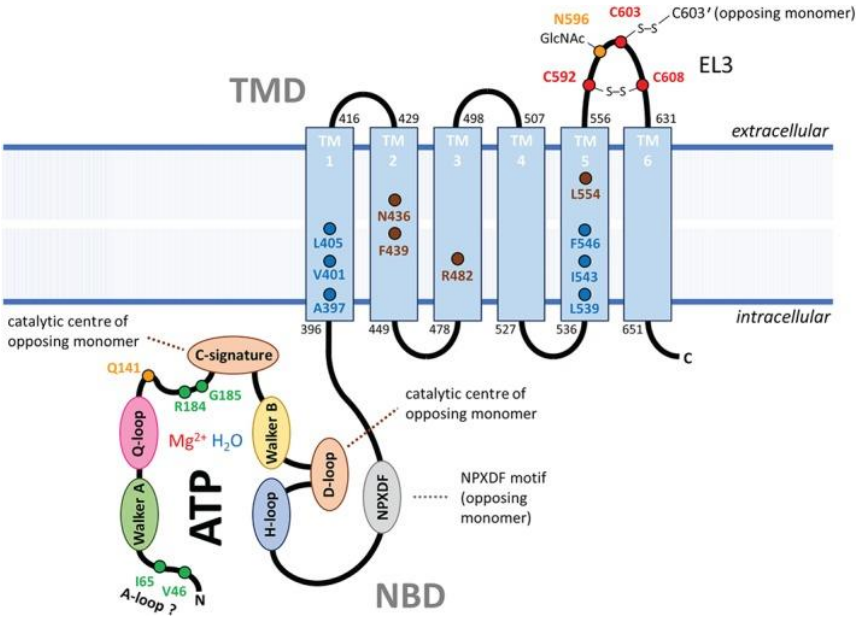


Figure 1.5: schematic overview of the BCRP transporter.

Also for BCRP, two different conformations are discovered: the "*inward-facing*" conformation and the "*outward-facing*" conformation (Figure 1.6)[31]. The "*inward-facing*" conformation is characterized by the presence of a cavity, *Cavity 1*, formed by TMDs and accessible from the cytosolic side. *Cavity 1* is characterized by the presence of hydrophobic residues, and it seems to be the binding site for BCRP substrates. To bind within *Cavity 1*, compounds must to be flat, with hydrophobic features and rich in polycyclic rings[31]. On the extracellular side, in the "*inward-facing*" conformation we observe the presence of a second cleft, *Cavity 2*. This cavity is much smaller than *Cavity 1* and is not accessible from the extracellular side in the "*inward-facing*" conformation. Furthermore, *Cavity 2* has fewer hydrophobic residues than *Cavity 1* and this suggests that it is the site of expulsion of the BCRP hydrophobic substrates. The two cavities are separated by a cap consisting of the amino acid leucine[31]. In the "*outward-facing*" conformation, BCRP binds two molecules of ATP, and then dimerizes. In this conformation, the substrate could move from *Cavity 1* to *Cavity 2* which is open to allow its efflux[32].

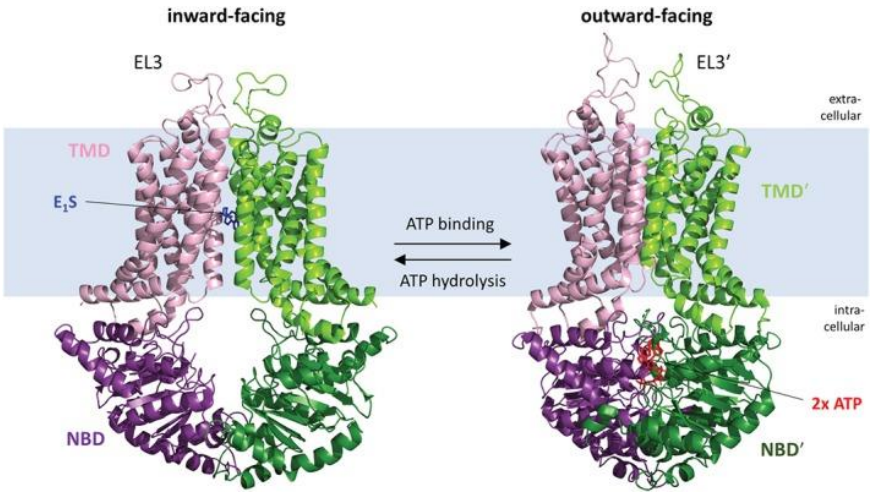


Figure 1.6: *3D structure of the BCRP homodimer that binds to the substrate E_1S (left) or to two ATP molecules (right).*

The transport cycle proposed by Manolaridis[32] is illustrated in Figure 1.7. In the Apo state, the BCRP transporter is in the "*inward-facing*" conformation, where the NBD domains are far from each other. The substrates enter from the cytosolic side and bind to *Cavity 1*. Binding with ATP induces dimerization of the NBD domains, leading to conformational change. In the "*outward-facing*" conformation, the proximity of the NBD domains and the collapse of the *Cavity 1* are responsible for the displacement of the substrate in *Cavity 2*. The lower presence of hydrophobic amino acids in *Cavity 2* helps release the substrate in the extracellular side. Finally, the hydrolysis of ATP provides the energy that allows BCRP to return in its Apo state[32].

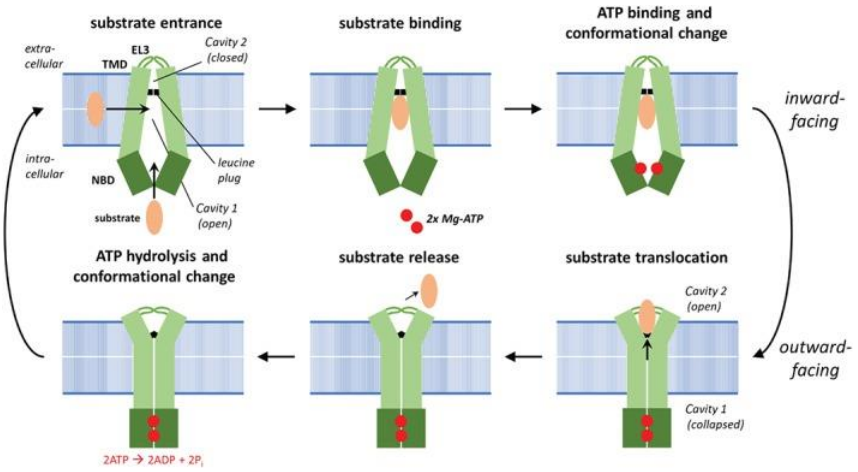


Figure 1.7: BCRP transport cycle proposed by Manolaridis[32].

1.4. Multidrug Resistance Protein (MRP1)

Multidrug Resistance Protein-1 (MRP1, ABCC1) was discovered in cancer cells, which did not express P-gp, but that were resistant to anticancer drugs[33,34].

The MRP1 transporter shares similar structural features with P-gp, except for the presence in MRP1 of a transmembrane domain, TMD0, at the *N*-terminal end of the transporter. Although the sequence identity is very low (19%), P-gp and MRP1 show many common substrates. Indeed, both proteins carry unmodified hydrophobic molecules. MRP1 confers resistance to several chemotherapeutic agents, such as Cisplatin, Etoposide, Doxorubicin, Vincristine,

Methotrexate, Irinotecan and Mitoxantrone[9,35]. Thanks to the presence of TMD0, MRP1 can transport neutral or negatively charged substrates, also conjugated with the sulfate group, glucuronic acid or glutathione[35].
MRP1 is widely expressed in healthy humans and mouse tissues and plays important roles in the protection of various tissues from xenobiotics[36,37]. Mice lacking the gene encoding MRP1 are normal, but show hypersensitivity to the MRP1 substrate Etoposide, resulting in loss of body weight and mortality[38,39]. After the treatment with Etoposide, mice lacking the gene encoding MRP1 showed damages of the mucosal layer of the tongue and cheek, and to the inhibition of the spermatogenesis. These studies indicate the importance of MRP1 in physiological barriers (including the testis-blood barrier, the oropharyngeal mucosa, the urinary collecting tubules, and the blood-CSF barrier) and also highlight the tissues for which side effects of MRP1 substrate drugs may be increased by concomitant administration of MRP1 inhibitors[40,41].
High levels of MRP1 were associated with the poor outcome of chemotherapy in breast cancer[42], non-small cell lung cancer patients, and also pediatric solid neuroblastoma. The ABCC1 gene, which encodes MRP1, is transcriptionally regulated by the oncogene MYCN[43], a tumor-genesis driver in neuroblastoma[44]. Furthermore, among the first-line agents for the treatment of neuroblastoma, therapy includes several MRP1 substrates, as Etoposide, Doxorubicin, Vincristine and Irinotecan. Thus, MRP1 inhibitors appear useful to overcome drug resistance, but unfortunately no specific inhibitors for MRP1 have been tested in clinical trials. Promising lead compounds with a high degree of selectivity for MRP1 have been developed, including tricyclic isoxazoles linked to cyclohexyl[45] and flavonoid derivatives[46]. However, these substances have not been comprehensively evaluated in preclinical studies.

1.5. Strategies to overcome the human cancer MDR

Strategies to reverse MDR have been extensively studied and the inhibition of the functions of ABC transporter proteins has been considered a suitable approach; for this reason, many modulators of these proteins have been synthesized over the past few decades. These compounds are chemosensitizers that, when administered in combination with antineoplastic drugs that are substrates of ABC efflux pumps, could restore their efficacy in resistant cancer cells[47,48].

1.5.1. P-gp inhibitors

ABC transporter-targeting drugs present a variety of chemical structures, however some general features for the interaction with these proteins have been identified:

- high lipophilicity;
- the presence of one or more protonable nitrogen atoms and aromatic moieties;
- the ability of establishing hydrogen bond interactions[49].

These structural features are in agreement with the information collected on the structure of ABC transporters suggesting that their recognition sites, in particular for P-gp, result characterized as large, polymorphous drug binding domains where a variety of molecules can be accommodated in a plurality of binding modes establishing π–π, π-ion, hydrogen bonds and hydrophobic interactions[3].

Verapamil[50] (Figure 1.8) was the first compound showing P-gp modulating activity and, together with many other molecules as Cyclosporine A[51] (Figure 1.8) and quinidine[52], belongs to the first generation of P-gp modulators. However, the toxicity of this first series of compounds prevented their clinical use and, at present, verapamil is only used as gold standard in biological assays.

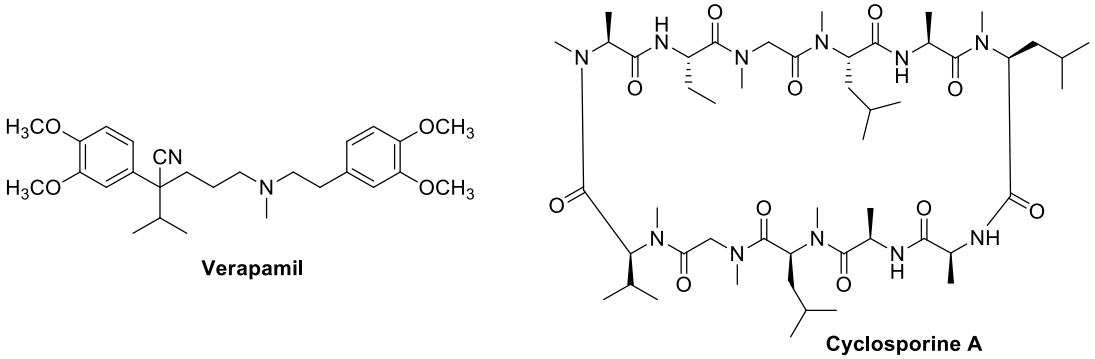


Figure 1.8: structures of Verapamil and Cyclosporine A, two first-generation P-gp inhibitors.

Since then, many P-gp modulators, belonging to three generations of compounds have been identified[53]. Two of the most interesting third-generation chemo-sensitizers are the tetrahydroisoquinoline derivatives Elacridar (GF-120918 or GW120918)[54] and Tariquidar (XR9576)[55] (Figure 1.9).

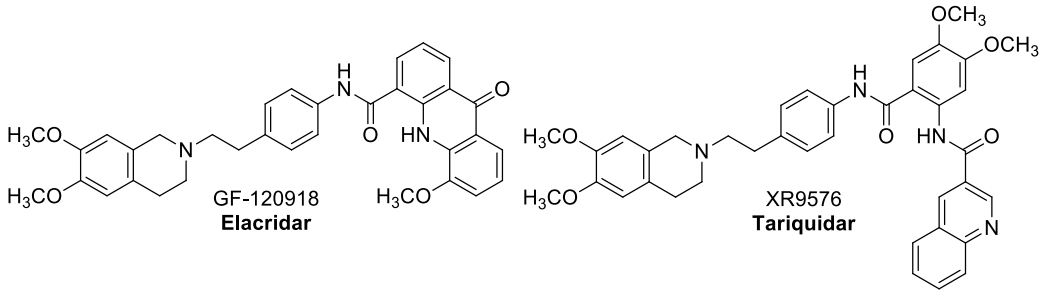


Figure 1.9: Elacridar e Tariquidar, two of the most interesting third-generation P-gp inhibitors.

These compounds displayed a high affinity towards the ABC proteins, a reduced effect on cytochromes, and few pharmacokinetic interactions with cytotoxic drugs[56]. Disappointingly, they have not been approved for therapy since they did not show an improvement of the efficacy of the co-administered antitumoral drugs[57]. Early studies indicated that both derivatives are not specific for P-gp because they are also able to bind the BCRP transporter[53], and although several P-gp modulators have been studied, till now only a few compounds displayed activity on both these two proteins. In the case of Tariquidar, recent evidences indicated that this molecule is able to bind also the MRP1 transporter[58]; the same compound was shown to potentiate the sensitivity to Paclitaxel in resistant cells transfected by another member of the ABCC family, the MRP7 protein[59]. Therefore, despite the failure in clinical trials, Tariquidar and Elacridar have been considered lead compounds to discover new MDR modulators able to target the three ABC proteins involved in MDR.

Several of third-generation P-gp inhibitors have reached pre-clinical or clinical trials[56], but none of these compounds has been approved for therapy, because of their low potency, toxicity and inhibitory effect on isoforms of cytochrome[60].

The failure of pre-clinical and clinical investigational studies led to considerable pessimism regarding the validity of such therapeutic approach to overcome MDR[61]. Nevertheless, the

search for new, safer, more potent and efficacious multidrug transporter modulators is still of interest.

1.6. Carbonic anhydrases (CAs)

Carbonic anhydrases (CAs, EC4.2.1.1) are a superfamily of ubiquitous metalloenzymes, widely express in prokaryotic and eukaryotic organisms. They are encoded by eight unrelated gene families:

- α-CAs, of which 15 isoenzymes are known, are mainly present in vertebrates, fungi, protozoa, algae, some bacteria and in the cytoplasm of green plants[62]. They represent the most studied class.
- β-CAs have been found in bacteria, algae, fungi, Archaea and in mono- and dicotyledonous chloroplasts[62].
- γ-CAs have been described in bacteria, Archaea as well as some plants[62].
- δ-CAs, present in phytoplankton, dinoflagellates and diatoms, play an important role in the fixation of CO_2 by these marine organisms[62].
- ζ-CAs present only in marine diatoms
- η-CAs have been identified in the Plasmodium Falciparum genome[63]
- θ-CAs were localized in the chloroplasts of diatoms where they play a fundamental role in the photosynthesis process[64].
- The first ι-CA was identified in marine diatom and more recently also found in bacteria[65].

CAs catalyze several hydrolytic reactions, as the conversion of carbon dioxide to bicarbonate and a proton. They play a crucial role for the maintenance of pH homeostasis in the body and also have a metabolic function in several biosynthetic processes[66].

CAs are metalloenzymes that are catalytically effective when a metal ion is bound within the active site. Zn(II) is the metal ion spread in all CA genetic families, but it can be exchanged with Cd(II) in ζ-CAs, while Fe(II) and Co(II) are presumably present in γ-CAs in anaerobic conditions[62,67].

The metal ion is usually coordinated by a water molecule or hydroxide ion and three amino acid residues: three His in the α, γ and δ-CA (Figure 1.10 A) and by one His and two Cys in the β and ζ-CA (Figure 1.10 B). Moreover, some β-CAs possess a Zn(II) coordinated by four amino acid residues: one His, two Cys and one Asp (Figure 1.10 C)[62,67,68].

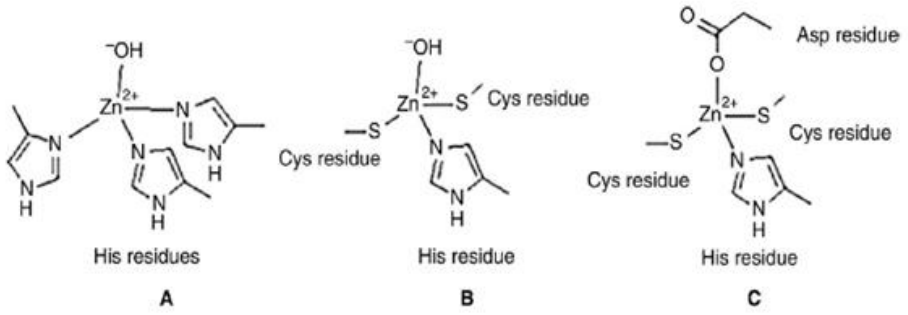


Figure 1.10: different metal ion coordination in CAs families[68].

All the CAs families showed a similar two-stage catalytic mechanism, reported in Figure 1.11. In the first phase, the hydroxide ion, coordinated by the metal ($E\text{-}M^{2+}\text{-}OH^-$, Figure 1.11 **B**), acts

as a strong nucleophile against the CO_2 molecule bound in a hydrophobic pocket of the active site, with the consequent formation of HCO_3^- (Figure 1.11 **C**). Therefore, the bicarbonate ion is displaced by a second water molecule present in the active site, generating the acid form of the enzyme, catalytically inactive (Figure 1.11 **D**). Subsequently, the hydroxide ion is regenerated through a proton transfer reaction from the metal-bound water molecule to a secondary acceptor with the restoration of enzymatic activity[62] (Figure 1.11 **A**). Classic CAs inhibitors are the primary sulfonamides which coordinate with the Zn ion (II) to give the tetrahedral complex (Figure 1.11 **E**) and displace the hydroxide ion from its position.

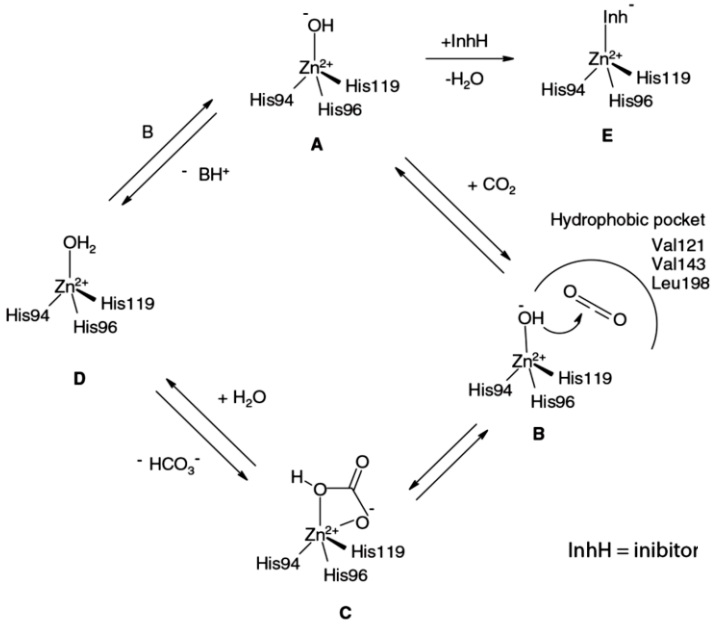


Figure 1.11: mechanism of enzymatic catalytic activity of α-CA[69].

1.6.1. Human carbonic anhydrases (hCAs)

Human CAs (hCAs) comprise 15 different α-CA isoforms varying for their catalytic activity, tissue distribution and subcellular localization (membrane, cytosol, mitochondria)[62]. Only twelve are catalytically active isoforms (hCA I, II, III, IV, VA, VB, VI, VII, IX, XII, XIII and XIV), while the remaining three (VIII, X and XI) are catalytically inactive and are called CA-related proteins (CARPs)[62].

The twelve catalytically active hCAs can be divided in four group based on their subcellular localization[62]:

- hCA I, II, III, VII, X, XI and XIII are cytosolic isoforms;
- hCA VA and VB are present in mitochondria;
- hCA VI is secreted in milk and saliva;
- hCA IV is a glycosylphosphatidylinositol (GPI)-anchored protein;
- hCA IX, XII, XIV are transmembrane proteins.

These enzymes are widely distributed in many tissues and organs where they are involved in essential physiological processes. Among these isoforms, hCA IX and XII are extracellular, membrane-bound CAs associated with tumor progression and metastases formation[66].

1.6.2 Structure and mechanism of α-CA inhibition.

To date, the 3D structures of all hCA isoforms have been determined. The analyzes conducted on the primary sequence of hCAs showed a high structural homology between the different isoforms: notably, the primary sequence of the active site is remarkably conserved. From the secondary structure, also this highly conserved, derives a tertiary globular structure[70] (Figure 1.12), characterized by a conical cavity, the active site, approximately 12Å wide and 13Å deep, which extends from the center of the protein to the surface. The Zn(II) ion is located at the bottom of this cavity, and it coordinates three residues of His (His94, His96 and His119), conserved in the various isoforms, and a water molecule or hydroxide ion[62,71–73]. Crystallographic studies of some α-AC isoforms have shown that the water molecule/hydroxide ion forms hydrogen bonds with a well-preserved residue of threonine (Thr199) and with two further water molecules, located on opposite sides of the cavity.

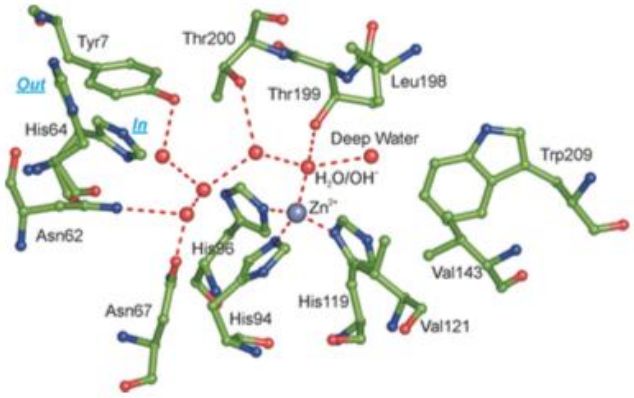


Figure 1.12: X-ray of the hCA II active site[67].

CA inhibitors could be divided in four classes, based on their different inhibition mechanism:

- Metal ion chelating compounds, such as inorganic ions (cyanate and thiocyanate), sulfonamides, sulfamates, *N*-hydroxy-sulfonamides, dithiocarbamates, xanthates, hydroxamates[74];
- Compounds that anchor the zinc-bound water molecule/hydroxide ion, such as phenols, thiophenols, carboxylates, 2-thiocoumarins, sulfocoumarins[74];
- Compounds that occlude the entrance of the active site, such as coumarins and their bioisosters[74];
- Compounds binding outside the active site, recently discovered thanks to crystallographic studies on the complex between the enzyme and 2-(benzylsulfonyl)benzoic acid[74].

1.6.2.1 Metal ion chelating compounds: sulfonamides

Sulfonamides were the only compounds used in clinical practice as CA inhibitors for years.
It is commonly accepted that the sulfonamide group is the ideal zinc-binding group (ZBG), able to coordinate the metal ion, with a tetrahedral or trigonal bipyramidal geometry, displacing the water molecule/hydroxide ion (zinc-bound nucleophile) essential for the enzymatic activity[62,74] (Figure 1.13 A). The sulfonamide group, thanks to the acidic environment of the active site, interacts within the catalytic site in its deprotonated form (SO_2NH^-), forming a dative bond with

the metal ion. Crystallographic studies have shown that this bond is further stabilized by two hydrogen bonds between the sulfonamide group and two amino acid residues (Thr199 and Glu106), highly conserved between the α-AC isoforms, which control the access of water molecules to the site[62,74,75] (Figure 1.13B).

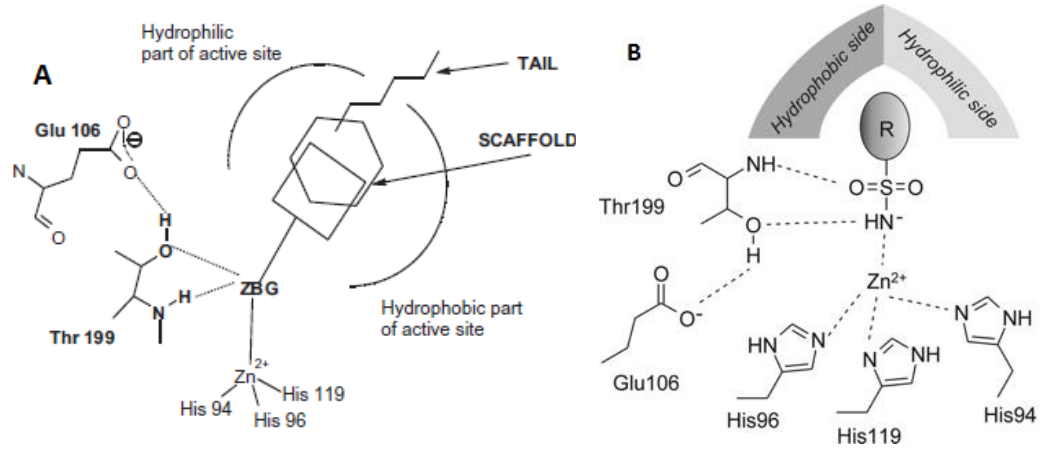


Figure 1.13: general structure of zinc-binding group inhibitors[62,74].

The sulfonamide derivatives act as non-selective CAs inhibitors, causing several side effects due to the inhibition of the physiological CAs not involved in the targeted pathology. To overcome this problem, several Drug Design studies were performed to obtain isoform-selective CA inhibitors for the various isoforms involved specifically in different pathologies. This is however not an easy task, considering that the 12 catalytically active hCA isoforms, have an active site architecture quite similar with each other[74].

1.6.2.2 CA inhibition by occlusion of the active site entrance: coumarins

Coumarins belong to the class of carbonic anhydrase inhibitors that bind the entrance of the cavity of the catalytic site, where there is the most variability of amino acid residues between various isoforms: these inhibitors bind in a region further away from the metal ion compared to sulfonamides that directly coordinate it. Coumarins occlude the CA active site and prevents the entrance of substrates into the internal enzymatic cavity or the exit of products. The binding of these inhibitors with the enzyme therefore occurs in a region far.
This class of inhibitors possess[74] (Figure 1.14):

- a Sticky Group (SG), as a phenolic -OH, -COOH or $CONH_2$, which interacts with the amino acids present at the entrance of the active site in the internal part of the cavity;
- a central aromatic, aliphatic or heterocyclic scaffold, connected to the SG, which binds to the entrance of the cavity, occluding the entrance to the active site;
- a tail, which may not be present, that interacts with the amino acid residues present on the external surface of the enzyme.

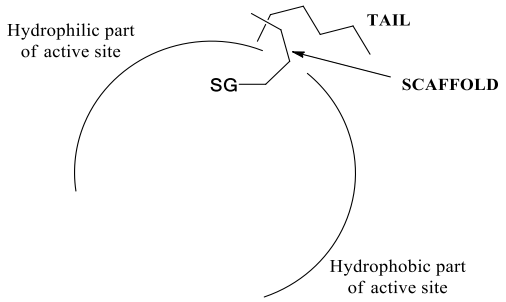


Figure 1.14: general structure of compounds which inhibit the CAs by occluding the entrance to the active site[74]. (SG = Sticky Group).

This mechanism of action was first discovered for coumarins and subsequently for other compounds as lactones, thiolactones or quinolones, that showed a significant hCA inhibitory effects[74].

CA hydrolyzes the lactone ring of coumarins (Figure 1.15), obtaining the corresponding *cis* (Figure 1.15 **4a**) or *trans* (Figure 1.15 **4b**) 2-hydroxycinnamic acid that inhibit the enzyme by placing itself at the entrance of the enzymatic pocket, physically occluding it. The carboxylic function of *trans* 2-hydroxycinnamic acid forms a hydrogen bond interaction with NH groups of residues Asn62 and His64, while the phenolic OH interacts with a water molecule (Wat257) and with the amide function of the Gln92[76] (Figure 1.15).

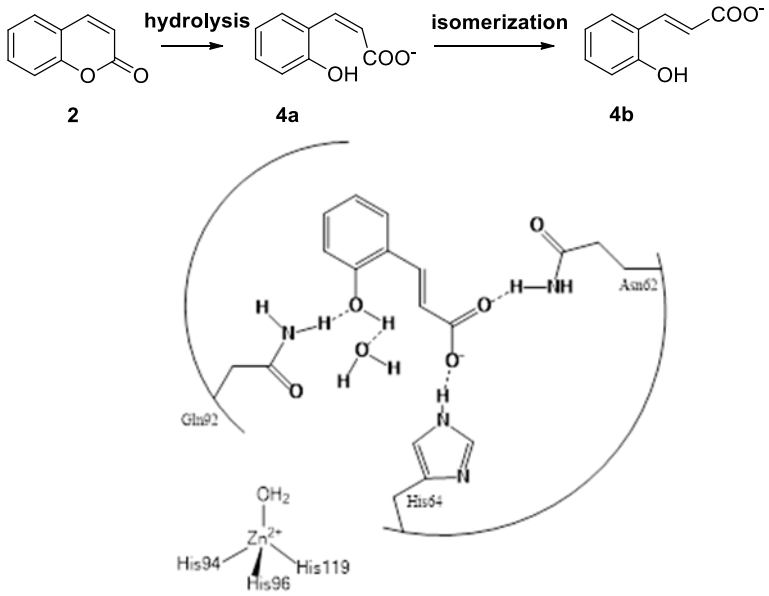


Figure 1.15: enzymatic hydrolysis of coumarin and interactions between *trans* 2-hydroxycinnamic acid and CA cavity[76].

1.7. P-gp and hCA XII synergism

In a recent work, Kopecka and coll. reported that the activity of P-gp can be modulated by hCA XII[77]. hCA XII is extracellular, membrane-bound hCA associated with tumor progression and metastases formation. hCA XII appears as a bitopic dimeric glycoprotein since it crosses the double phospholipid layer only once, like hCA IX (Figure 1.16). Both hCA XII and hCA IX expose the N-terminal portion to the extracellular matrix, while the C-terminal portion turns inward. The secondary structure is similar to that observed in the other isoenzymes. Comparing

hCA XII with the more studied isoform II, we can see how it contains wider loops in three distinct portions. One of the two glycosylation sites of hCA XII is located on Asn52, in the first of these loops; while the second site, on Asn136, is located in a loop region almost superimposable to that of the other isoenzymes. There is a single disulfide bridge between Cys23 and Cys203[78]. In the catalytic site, oriented in the extracellular portion, the Zn(II) ion is positioned at the bottom of the cavity, where it is linked through coordination bonds to three Histidine residues (His94, His96, His119), to a water molecule and an acetate anion (Figure 1.16)[78]. In cancer cells, hCA XII contributes to the acidification of the extracellular compartment, damaging healthy cells, and maintains an optimal intracellular pH, since the bicarbonate ion is retained by the tumor cell to buffer the pH[77].

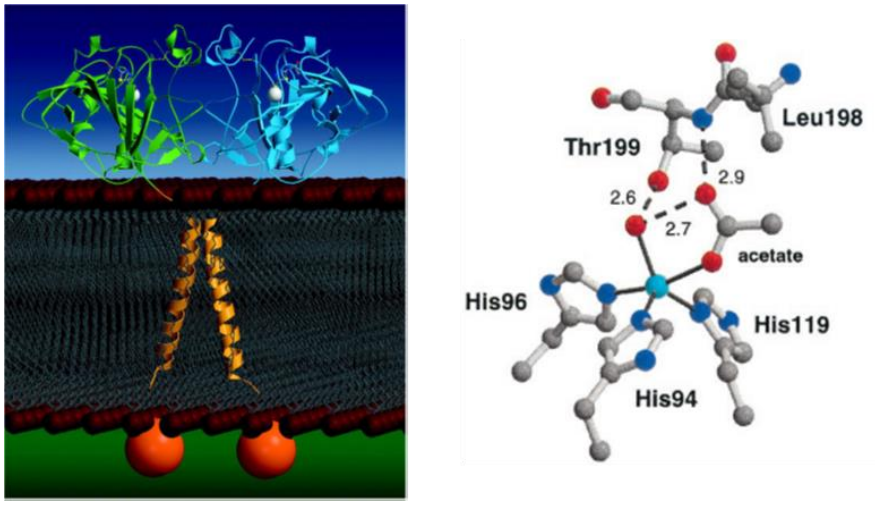


Figure 1.16: structure of the hCA XII dimer in the membrane (left), and of hCA XII active site (right)[78].

In 2015, Kopecka and coll.[77] applied the Cell Surface Capturing (CSC) technology to investigate the cell membrane proteome of a human chemosensitive adenocarcinoma colon cancer HT29 and human chemoresistant adenocarcinoma colon cancer HT29/DOX cells. Results enable the identification of 380 glycoproteins and, among these, MRP1, P-gp and hCA XII were highly expressed in the resistant HT29/DOX cell line[77]. This phenomenon was also found in chemoresistant non-small cell lung cancer A549/DOX cells compared to the chemosensitive A549 cell line[77] (Figure 1.17).

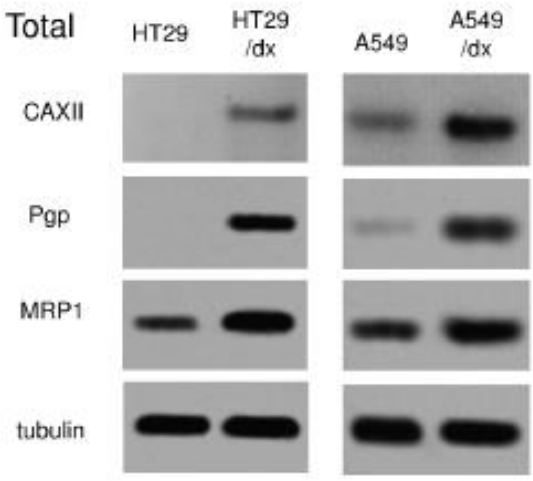


Figure 1.17: expression of hCA XII and P-gp in chemosensitive and chemoresistant cancer cells.

Confocal microscope analysis showed that on the membrane of HT29/DOX cells, hCAXII is co-localized and physically associated with P-gp[77]. Moreover, Kopecka and coll. found that on HT29/DOX cells, the hCA XII silencing led to a highly reduction of P-gp ATPase activity, and a consequently increased intracellular accumulation of the antineoplastic drug Doxorubicin, reaching the same amount measured in the chemosensitive HT29 cell line[77] (Figure 1.18 **A**). Furthermore, Doxorubicin and Irinotecan, that are P-gp substrates, displayed cytotoxic effects on HT29/DOX cells silenced for hCA XII, with highly reduced cells' viability (Figure 1.18 **B**).

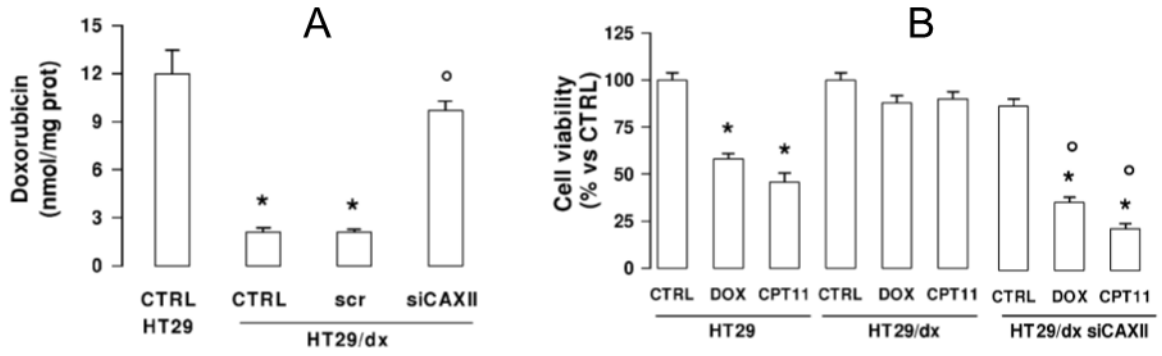


Figure 1.18: comparison between chemosensitive, chemoresistant and hCA XII-silenced HT29/DOX cells: Doxorubicin accumulation (A) and Doxorubicin cytotoxicity effect (B).

hCA XII contributes to extracellular acidification and maintains a slightly alkaline intracellular pH that is optimal for P-gp activity. The pharmacological inhibition of the hCA XII reduces the intracellular pH[79] (between 6.2 and 7.6), impairing the ATPase P-gp efflux effect, suggesting that hCA XII influences the catalytic activity of the transporter: indeed, in the presence of Acetazolamide, a significant increase of Doxorubicin uptake was measured in HT29/DOX cells[77].

In 2016, Kopecka and coll.[79] found that hCA XII inhibitors increased the antineoplastic effect of the P-gp substrate Doxorubicin. They selected a series of potent hCA XII inhibitors, that displayed Ki values < 10 nM and evaluate their activity in reversing MDR. Tested compounds are able to increase the intracellular concentration of Doxorubicin in several resistant cancer cells that overexpressed both P-gp and hCA XII, such as HT29/DOX and A549/DOX cell lines[77], showing the same potency as the P-gp inhibitor, Tariquidar. In contrast, they did not have any effects on cells' viability in cells with low or undetectable levels of hCA XII, as the HT29 cell line. These compounds also increased the intracellular accumulation of Vinblastine and Paclitaxel, two other P-gp substrates. To confirm that the ability of these molecules to restore Doxorubicin cytotoxicity was dependent on hCA XII activity, Kopecka and coll. knocked-out the *ca12* gene in several resistant cell lines that overexpress both proteins: HT29/DOX, A549/DOX, MDA-MB-231 and U2OS/DOX cells[79]. In these conditions, compounds were not able to further increase the Doxorubicin intracellular accumulation, and to inhibit P-gp activity. These results suggest that the MDR reversal effect of these compounds was related to hCA XII inhibition[79].

These observations suggest that hCA XII inhibitors are potent chemosensitizing agents in cancer cells that overexpressed both hCA XII and P-gp. Moreover, a dual P-gp/hCA XII inhibition could be a valid strategy to overcome the P-gp-mediated MDR.

2. P-gp/hCA XII inhibitors

Based on the study of Kopecka and coll.[77] who reported the role of hCA XII in maintaining an intracellular alkalinization that is optimal for P-gp activity, compounds with a dual P-gp/hCA XII inhibitory effect could be useful as synergistic MDR inhibitors to target resistant cancer cells that overexpress both proteins. Therefore, in this project, to maintain a high potency on P-gp and introduce a selectivity towards hCA XII, we designed hybrid inhibitors characterized by the presence of both P-gp and hCA XII binding moieties. For this purpose, we incorporated in a typical scaffold of potent P-gp modulators[80,81] a residue to target hCA XII.

2.1. Coumarin and sulfamoyl benzoate diester compounds

For several years, the research group where I carried out my PhD project has been involved in the design and synthesis of P-gp modulators able to counteract MDR. All these molecules maintained, in general, the structural features considered important for the interaction with P-gp, as high lipophilicity, the presence of hydrogen bond acceptor groups, basic nitrogen atoms and aromatic rings. Among these MDR reversers, the *N*,*N*-bis(alkanol)amine aryl diester compounds were potent P-gp modulators[80], thus we selected their chemical scaffold to design some series of dual P-gp/hCA XII inhibitors[82] (Figure 2.1). Indeed, the first series of dual P-gp/hCA XII inhibitors **1-28**[82] carry a nitrogen atom linked by two polymethylene chains of variable length, to two different aryl ester groups:

1. the (*E*)-3-(3,4,5-trimethoxyphenyl)vinyl, 3,4,5-trimethoxyphenyl or the anthracene residues (Figure 2.1 **a**, **b** or **c**) able to confer a good activity and selectivity towards P-gp[80,83];
2. the benzene sulfonamide or coumarin moieties, to target hCA XII.

Benzene sulfonamide group is a non-selective CAs inhibitor, which chelates Zn^{2+} ions present in the CAs binding site[74]. Coumarin, instead, occludes the entrance of the CAs active site and displayed a high selectivity towards hCA IX and XII isoforms[74].

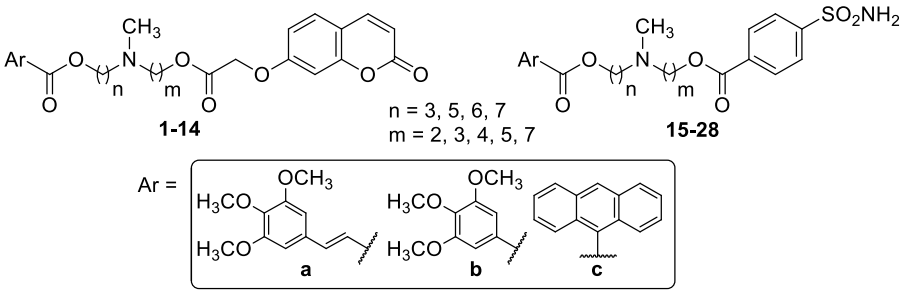


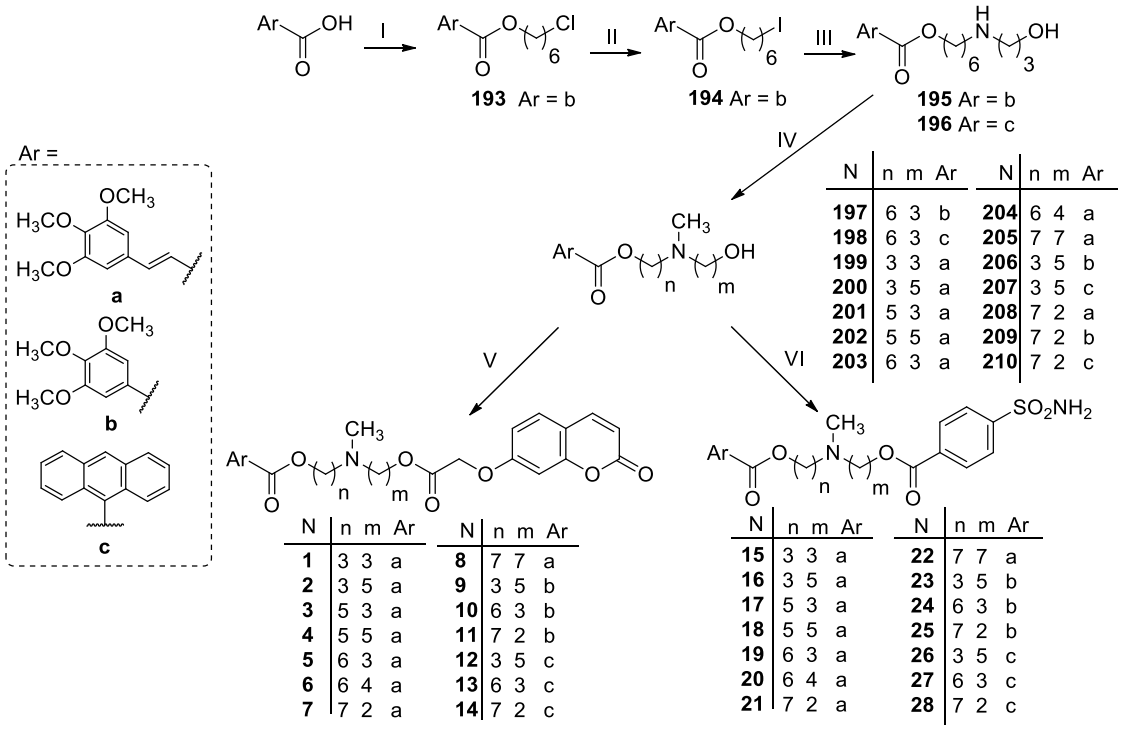
Figure 2.1: structures of coumarin and sulfamoyl benzoate diester compounds **1-28**[82], synthesized in this PhD thesis.

All these derivatives were evaluated, by Prof. Coronnello from the University of Florence, for their P-gp modulating activity by measuring the increased uptake of the specific P-gp substrate Rhodamine-123 on Doxorubicin-resistant erythroleukemia K562 cells (K562/DOX) that overexpress only P-gp. Moreover, their hCAs inhibition efficacy was evaluated, by the research group of Prof. Supuran from the University of Florence, on the tumor associated hCA IX and hCA XII and on the cytosolic hCA I and II isoforms. Selected compounds were also studied by the co-administration assay with the antineoplastic drug Doxorubicin on K562/DOX cells, that overexpress only P-gp, and on Doxorubicin-resistant human colorectal carcinoma LoVo/DOX

cells, that overexpress both P-gp and hCA XII. Finally, their susceptibility to hydrolysis was evaluated in phosphate buffer solution (PBS) and human plasma samples.

2.1.1. Chemistry

Compounds **1-28**[82] were prepared using the reaction pathway reported in Scheme 2.1. Some new intermediates were synthesized to afford final compounds **1-28**[82], while most of them were previously obtained, following the procedure reported in literature[80,84–86]. Thus, chloroester **193** was obtained by esterification of 6-chlorohexan-1-ol with the commercially available 3,4,5-trimethoxybenzoic acid using EDC hydrochloride and DMAP in dry CH_2Cl_2. The chloroalkyl ester was transformed in the corresponding iodo derivative **194** using NaI in acetone, to achieve higher yield in the following reaction. Treatment of **194** with 3-aminopropan-1-ol in dry CH_3CN gave the desired secondary amine **195**. The same reaction performed on the already described 6-iodohexyl anthracene-9-carboxylate[80] gave **196**. These compounds were alkylated by reductive methylation with HCOOH/HCHO to give the corresponding tertiary amines **197** and **198**. Final compounds **1-14**[82] were obtained by esterification of **197**, **198** and the previously reported analogues **199-210**[80,84–86] with 2-((2-oxo-2*H*-chromen-7-yl)oxy)acetic acid **212** (described in Scheme 2.2), using EDC hydrochloride and HOBt in dry CH_3CN. In turn, compounds **15-28**[82] were prepared by esterification of **197-210** with 4-sulfamoylbenzoyl chloride, obtained from 4-sulfamoylbenzoic acid by reaction with $SOCl_2$.



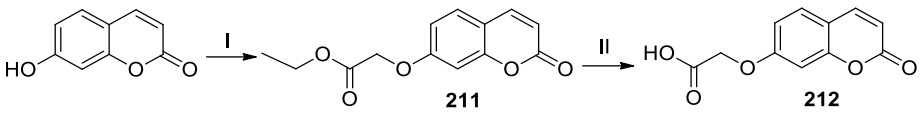
N	n	m	Ar	N	n	m	Ar
197	6	3	b	**204**	6	4	a
198	6	3	c	**205**	7	7	a
199	3	3	a	**206**	3	5	b
200	3	5	a	**207**	3	5	c
201	5	3	a	**208**	7	2	a
202	5	5	a	**209**	7	2	b
203	6	3	a	**210**	7	2	c

N	n	m	Ar	N	n	m	Ar
1	3	3	a	**8**	7	7	a
2	3	5	a	**9**	3	5	b
3	5	3	a	**10**	6	3	b
4	5	5	a	**11**	7	2	b
5	6	3	a	**12**	3	5	c
6	6	4	a	**13**	6	3	c
7	7	2	a	**14**	7	2	c

N	n	m	Ar	N	n	m	Ar
15	3	3	a	**22**	7	7	a
16	3	5	a	**23**	3	5	b
17	5	3	a	**24**	6	3	b
18	5	5	a	**25**	7	2	b
19	6	3	a	**26**	3	5	c
20	6	4	a	**27**	6	3	c
21	7	2	a	**28**	7	2	c

Scheme 2.1: *Reagents and conditions*: I) 6-chlorohexan-1-ol, EDC hydrochloride, DMAP, dry CH_2Cl_2, rt, 48 h; II) NaI, acetone, reflux, overnight; III) $H_2N(CH_2)_3OH$, dry CH_3CN, reflux, 18 h; IV) HCOOH/HCHO, EtOH, 80 °C, 5 h; V) **212**, EDC hydrochloride, HOBt, dry CH_3CN, rt, 5 h; (VI) 4-sulfamoylbenzoyl chloride, $CHCl_3$ (free of ethanol), rt, 17 h.

2-((2-Oxo-2*H*-chromen-7-yl)oxy)acetic acid **212** was synthesized as reported in Scheme 2.2: alkylation of the commercially available 7-hydroxy-2*H*-chromen-2-one with ethyl bromoacetate gave ester **211**, which was hydrolyzed under alkaline conditions to obtain **212**.



Scheme 2.2: *Reagents and conditions*: I) $BrCH_2COOC_2H_5$, K_2CO_3, acetone; II) NaOH 10%.

2.1.2. Results and discussions

2.1.2.1. Pharmacological assays on the single target proteins, hCA XII and P-gp: hCA inhibitory activity and Rhodamine-123 (Rhd 123) uptake test on K562/DOX cells

The hCA inhibitory efficacy of compounds **1-28** was evaluated, by the research group of Prof. Supuran from the University of Florence, on four human hCA isoforms, the two cytosolic hCA I and II and the transmembrane tumor-associate hCA IX and XII isoforms, by the Stopped-Flow CO_2 hydrase assay[87]. Results are reported in Table 2.1 together with those of Acetazolamide (AAZ), used as reference inhibitor.

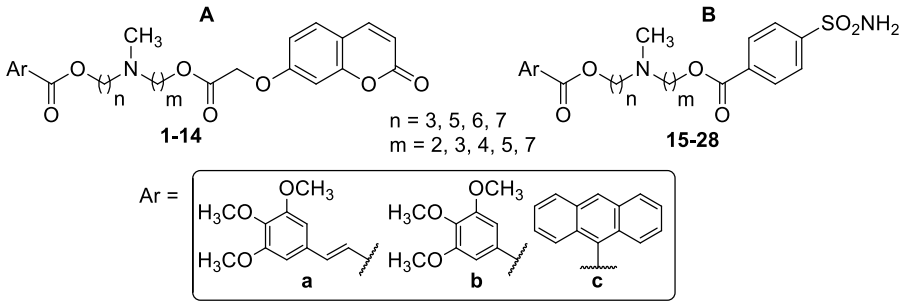
As expected, derivatives **1-14**, carrying the coumarin moiety, inhibited only hCA IX and XII, while they were inactive against the off target hCA I and II isoforms. Interestingly, most of the coumarin derivatives displayed a high selectivity towards hCA XII with Ki values < 10 nM, as the reference inhibitor AAZ. On hCA XII, the most potent compound was **5** (Ki = 6.4 nM) which carries the *(E)*-3-(3,4,5-trimethoxyphenyl)vinyl moiety (**a**), while the anthracene derivatives were, in general, the less potent and selective of the series. The linkers' length seemed not to be crucial for the interaction with hCA XII: for instance, compounds **1** having the shortest linkers (n, m = 3) was equipotent with **8** which showed the longest ones (n, m = 7). On the contrary, compounds **15-28**, incorporating a benzene sulfonamide moiety, inhibited all the four hCA isoforms, showing Ki values in the range of 20.6-241.2 nM and 8.0-96.7 nM for hCA IX and XII, respectively. In this set of molecules, **21**, **26** and **27** showed a preference for hCA XII *vs* hCA I (15-20 times), while **28** displayed a 20-times greater hCA XII *vs* hCA II selectivity.

The ability of compounds **1-28** to inhibit the P-gp transport activity was evaluated, by Prof. Coronnello from the University of Florence, by measuring the uptake of the specific P-gp substrate Rhd 123 on Doxorubicin-resistant erythroleukemia K562/DOX cells, which overexpress only P-gp[88]. Results were expressed as FR (Fluorescence Ratio) values that are the ratios between the average fluorescence intensity of Rhd 123 in the presence and absence of modulators. Results are reported in Table 2.1 together with those of Verapamil, used as standard inhibitor.

At 3 μM concentration, only few compounds (**4**, **7**, **8**, **22** and **26**) inhibited the P-gp transport activity by at least twice o even more, and compound 5 was active as verapamil (FR = 3.67 and 3.30, respectively). At 30 μM concentration, a significant increase in the intracellular Rhd 123 fluorescence intensity was observed for all the compounds (FR values = 4.15-5.85). Notably, **2**, **4**, **5** and **18** showed FR values higher than that of Verapamil, tested at the same concentration (FR = 5.81, 5.45, 5.85 and 4.99, respectively, *vs* FR = 4.71 of Verapamil).

Thus, the introduction of a coumarin or a benzene sulfonamide group maintains good effects on both the target proteins taken individually. Notably, coumarin derivatives were in general more potent than the benzene sulfonamide series, and considering the aryl groups, compounds bearing the (*E)*-3-(3,4,5-trimethoxyphenyl)vinyl moiety were generally the most active ones.

Table 2.1: Rhd 123 uptake enhancement on K562/DOX cells and inhibitory activity on hCA I, II, IX and XII isoforms of compounds **1-28** and of the two reference compounds Verapamil (Ver) and Acetazolamide (AAZ).



Cmpd	n	m	Ar	Struct	K_I (nM)[a]				FR[b]	
					hCA I	hCA II	hCA IX	hCA XII	3 μM	30 μM
1	3	3	a	A	>10000	>10000	142.5	9.3	1.44	4.76
2	3	5	a	A	>10000	>10000	47.4	8.9	1.57	5.81
3	5	3	a	A	>10000	>10000	54.6	48.8	1.00	2.58
4	5	5	a	A	>10000	>10000	26.6	18.9	1.99	5.45
5	6	3	a	A	>10000	>10000	26.7	6.4	3.67	5.85
6	6	4	a	A	>10000	>10000	40.2	36.4	1.39	1.98
7	7	2	a	A	>10000	>10000	29.7	9.1	2.08	4.48
8	7	7	a	A	>10000	>10000	24.2	7.1	2.33	4.15
9	3	5	b	A	>10000	>10000	133.3	9.2	1.00	3.95
10	6	3	b	A	>10000	>10000	103.1	8.9	1.16	2.18
11	7	2	b	A	>10000	>10000	97.1	8.3	1.11	1.58
12	3	5	c	A	>10000	>10000	150.8	45.7	1.00	2.53
13	6	3	c	A	>10000	>10000	82.7	9.1	1.43	2.45
14	7	2	c	A	>10000	>10000	125.8	42.9	1.25	4.22
15	3	3	a	B	55.1	151.7	178.4	91.0	1.00	2.30
16	3	5	a	B	447.2	554.8	210.6	94.3	1.08	1.83
17	5	3	a	B	56.6	203.7	215.3	33.1	1.13	3.90
18	5	5	a	B	62.6	424.8	226.5	62.6	1.00	4.99
19	6	3	a	B	509.6	8.4	39.7	58.6	1.06	3.23
20	6	4	a	B	84.5	14.0	33.9	41.7	1.01	2.58
21	7	2	a	B	533.2	48.9	68.4	35.2	1.30	3.70
22	7	7	a	B	73.7	445.0	171.9	96.7	2.85	3.20
23	3	5	b	B	284.4	127.5	26.6	63.7	1.00	1.66
24	6	3	b	B	83.5	8.9	25.3	57.6	1.42	2.88
25	7	2	b	B	42.7	90.2	117.0	8.0	1.44	3.50
26	3	5	c	B	338.7	81.2	241.2	23.4	2.10	4.45
27	6	3	c	B	616.7	422.4	28.2	31.3	1.08	2.03
28	7	2	c	B	237.9	475.5	20.6	23.5	1.45	4.25
Ver									3.30	4.71
AAZ					250.0	12.0	25.0	5.7	1.00	1.00

[a] Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10 % of the reported values). [b] Inhibition of the P-gp transport activity on K562/DOX cells expressed as FR that is the ratio between the average fluorescence intensity of Rhd 123 in the presence and in absence of modulators (FR = Rhd uptake + modulator/Rhd uptake – modulator).

2.1.2.2. Rhodamine-123 uptake test on LoVo/DOX cells

Compounds with the best profile in term of potency on P-gp and selectivity towards hCA XII (coumarin **2**, **5**, **7** and **8**, and benzene sulfonamide **21**, **25** and **26** derivatives) were further studied, by Prof. Coronnello from the University of Florence, in the Rhd 123 uptake test on Doxorubicin-resistant human colorectal carcinoma LoVo/DOX cells, which overexpress both P-gp and hCA XII[82].

At 3 μM, only coumarins derivatives enhanced the uptake of Rhd (FR values = 1.32-6.70), while the benzene sulfonamide ones were inactive. At 10 μM, instead, all molecules showed significant P-gp inhibitory effects: compounds **2**, **7** and **8** showed FR values of 10.50, 12.60 and 9.90, respectively, that resulted higher than that of Verapamil (6.40) tested at the same concentration (Figure 2.2).

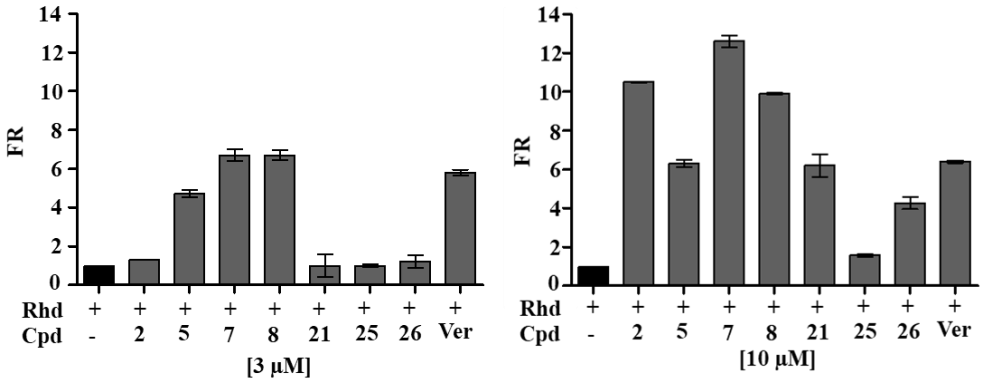


Figure 2.2: FR values in LoVo/DOX cells incubated with Rhd 123 in the presence and absence of modulators and Verapamil, tested at 3 μM (left) and 10 μM (right) concentrations. Value 1 was attributed to the average fluorescence intensity of the samples exposed only to Rhd.

2.1.2.3. Enhancement of Doxorubicin cytotoxicity assay

The most promising compounds (**2**, **5**, **7**, **8**, **21**, **25** and **26**) were further evaluated, at 3 and 10 μM concentrations, on the Doxorubicin co-administration test on K562/DOX and LoVo/DOX cell lines[82]: results are reported in Table 2.2. The antineoplastic drug Doxorubicin is a P-gp substrate and is usually inactive in tumors overexpressing the pump. In this test, Doxorubicin was used at the low toxic concentrations that caused a 20% cell growth inhibition (IC_{20}) on the two resistant lines (IC_{20} = 0.5 μM for K562/DOX cells and 0.3 μM for LoVo/DOX cells).

On K562/DOX cells, at 3 μM only **5** reduced the cell growth from 80 % (with Doxorubicin alone) to 51.7 %, while at 10 μM, all tested compounds increased drug's cytotoxicity: interestingly, **5** reduced the cells' growth to 25.1 %[82]. On LoVo/DOX cells, at 3 μM only compounds **5**, **7** and **8** displayed a moderate activity, while at the higher dose (10 μM) almost all compounds were able to increase the Doxorubicin cytotoxicity. Notably, compounds **7** and **8** caused a reduction of the cells' growth from 80 % to 34.9 and 32.2%, respectively[82].

Interestingly, in this assay, these compounds displayed a higher effect on LoVo/DOX cells, that overexpress both P-gp and hCA XII, than on K562/DOX cells, overexpressing only P-gp.

Table 2.2: Cells' growth experiments performed on K562/DOX and LoVo/DOX cells in the presence of 0.5 μM (K562/DOX) or 0.3 μM (LoVo/DOX) concentrations of Doxorubicin (Doxo) alone or in association with compounds **2**, **5**, **7**, **8**, **21**, **25** and **26** at 3 and 10 μM concentrations.

	Cell growth (%)[a]	
Compounds	**K562/DOX cells**	**LoVo/DOX cells**
Doxo	80.0 ± 3.6	80.0 ± 2.5
Doxo (IC_{20}) + **2** (3 μM)	85.6 ± 4.7	82.7 ± 10.2
Doxo (IC_{20}) + **2** (10 μM)	65.0 ± 5.2	41.5 ± 7.3
Doxo (IC_{20}) + **5** (3 μM)	51.7 ± 3.0	58.0 ± 2.4
Doxo (IC_{20}) + **5** (10 μM)	25.1 ± 12.0	51.0 ± 1.9
Doxo (IC_{20}) + **7** (3 μM)	78.1 ± 4.9	61.0 ± 3.3
Doxo (IC_{20}) + **7 (**10 μM)	48.0 ± 5.0	34.9 ± 8.1
Doxo (IC_{20}) + **8** (3 μM)	82.0 ± 5.9	68.0 ± 2.9
Doxo (IC_{20}) + **8** (10 μM)	49.0 ± 2.3	32.2 ± 10.7
Doxo (IC_{20}) + **21** (3 μM)	78.8 ± 10.2	82.0 ± 2.7
Doxo (IC_{20}) + **21** (10 μM)	68.7 ± 3.6	59.0 ± 3.3
Doxo (IC_{20}) + **25** (3 μM)	80.3 ± 5.9	81.7 ± 2.0
Doxo (IC_{20}) + **25** (10 μM)	76.0 ± 3.7	73.7 ± 0.015
Doxo (IC_{20}) + **26** (3 μM)	77.9 ± 6.1	82.0 ± 2.0
Doxo (IC_{20}) + **26 (**10 μM)	74.0 ± 2.6	58.2 ± 5.9
Doxo (IC_{20}) + **Ver** (3 μM)	69.0 ± 0.03	54.0 ± 0.06
Doxo (IC_{20}) + **Ver (**10 μM)	62.0 ± 0.1	51.0 ± 0.09

[a] Data are the mean ± SE of at least three determinations performed with quadruplicate cultures.

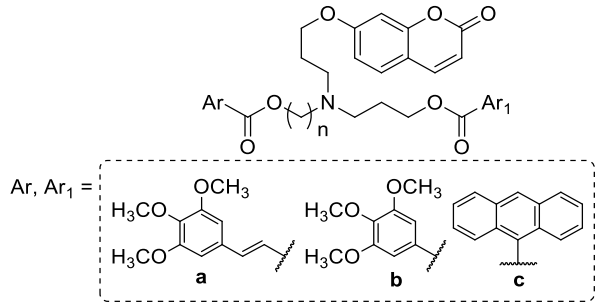
2.1.2.4. Chemical stability tests

Finally, in the laboratory of Prof. Bartolucci from the University of Florence, I evaluated the chemical stability of all these diester derivatives: the analyses were performed by liquid chromatography coupled with mass spectrometry (LC-MS/MS) methods, operating in Multiple Reaction Monitoring (MRM) mode. The LC-MS/MS system and parameters used were reported in Par. 7.2.1. The obtained results demonstrated that all the compounds were stable in PBS, while most of them were susceptible to enzymatic hydrolysis: notably, the hydrolysis occurs only to the ester group linked to the coumarin or benzene sulfonamide moieties (data not shown).

2.2. (*N*-Alkylcoumarin)aminoaryl diester compounds

To continue this project on dual P-gp/hCA XII inhibitors, we synthesized a new series of compounds maintaining the *N*,*N*-bis(alkanol)amine aryl diester scaffold. As regards residues targeting hCAs, we introduced only the coumarin group, since coumarin derivatives of the first series were selective toward hCA IX and XII, while the benzene sulfonamide compounds inhibited all the tested hCA isoforms[82]. In this second series of dual P-gp/hCA XII inhibitors, the nitrogen atom is linked by a propyl chain and a 5, 6 or 7 methylene chain to two ester groups, carrying a combination of the same aryl residues of the first series, as the (*E*)-3-(3,4,5-trimethoxyphenyl)vinyl, 3,4,5-trimethoxyphenyl or the anthracene ones (Figure 2.3 **a**, **b** or **c**). Moreover, the coumarin moiety is connected through a propyl chain to the nitrogen atom by an

ethereal bond, since the corresponding ester group of the first series resulted susceptible to the enzymatic hydrolysis (Figure 2.3).



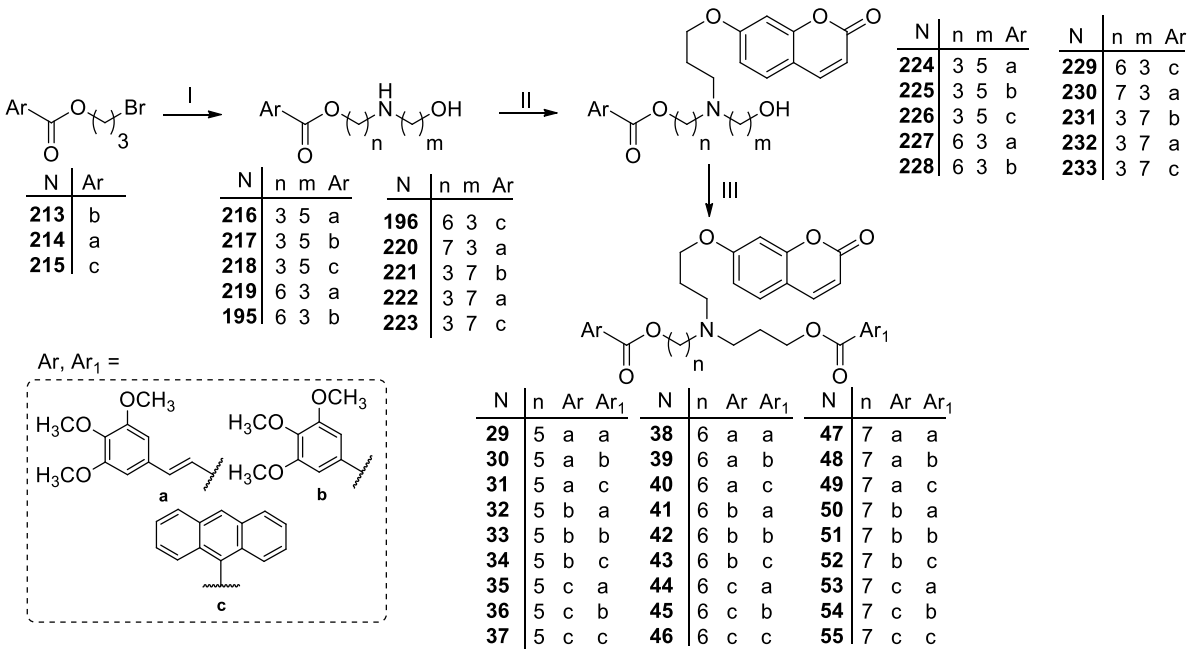
N	n	Ar	Ar_1	N	n	Ar	Ar_1	N	n	Ar	Ar_1
29	5	a	a	**38**	6	a	a	**47**	7	a	a
30	5	a	b	**39**	6	a	b	**48**	7	a	b
31	5	a	c	**40**	6	a	c	**49**	7	a	c
32	5	b	a	**41**	6	b	a	**50**	7	b	a
33	5	b	b	**42**	6	b	b	**51**	7	b	b
34	5	b	c	**43**	6	b	c	**52**	7	b	c
35	5	c	a	**44**	6	c	a	**53**	7	c	a
36	5	c	b	**45**	6	c	b	**54**	7	c	b
37	5	c	c	**46**	6	c	c	**55**	7	c	c

Figure 2.3: structures of the (*N*-alkylcoumarin)aminoaryl diester compounds **29-55**, synthesized in this PhD thesis.

These dual P-gp/hCA XII inhibitors were first studied for their inhibitory activity on the single proteins taken individually. As regards the P-gp inhibition, all these diester were tested in the co-administration assay with Doxorubicin on K562/DOX cells, then they were evaluated for their hCA inhibitory efficacy. All these new compounds were also tested on Doxorubicin-resistant human adenocarcinoma colon cells (HT29/DOX) and on Doxorubicin-resistant non-small cell lung cancer cells (A549/DOX), that overexpress both P-gp and hCA XII[77]: thus, the dual effect of these compounds was analyzed in a specific environment where these two proteins coexisted. Finally, the chemical stability of all these diester derivatives was investigated both in PBS and human plasma samples.

2.2.1. Chemistry

The reaction pathway used to synthesize the designed derivatives **29-55** are reported in Scheme 2.3. The bromoesters **213-215**[84,86] and most of the (hydroxyalkyl)aminoesters **216-220**, needed to achieve final compounds **29-55**, were previously synthesized following the procedures reported in literature[80,84,86]. **195** and **196** were obtained as reported in Par 2.1.1, while **221-223** were synthesized by reaction of the proper bromoester **213-215**[84,86] with 7-aminoheptan-1-ol[89] in dry CH_3CN (Scheme 2.3). Then, the (hydroxyalkyl)aminoesters **195**,**196** and **216-223** were alkylated with 7-(3-bromopropoxy)-2*H*-chromen-2-one **234** (described in Scheme 2.4) in dry CH_3CN, affording the intermediates **224-233** (Scheme 2.3). Finally, compounds **29-55** were obtained by esterification of **224-233** with the proper carboxylic acid ((*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid, 3,4,5-trimethoxybenzoic acid or anthracene-9-carboxylic acid) using EDC hydrochloride and DMAP in dry CH_2Cl_2 or with the acyl chloride obtained by treatment of the suitable acid with $SOCl_2$ in $CHCl_3$ (free of ethanol), as reported in Scheme 2.3 (for details, see the Experimental Section).



N	Ar
213	b
214	a
215	c

N	n	m	Ar
216	3	5	a
217	3	5	b
218	3	5	c
219	6	3	a
195	6	3	b

N	n	m	Ar
196	6	3	c
220	7	3	a
221	3	7	b
222	3	7	a
223	3	7	c

N	n	m	Ar
224	3	5	a
225	3	5	b
226	3	5	c
227	6	3	a
228	6	3	b

N	n	m	Ar
229	6	3	c
230	7	3	a
231	3	7	b
232	3	7	a
233	3	7	c

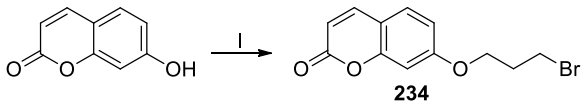
N	n	Ar	Ar1
29	5	a	a
30	5	a	b
31	5	a	c
32	5	b	a
33	5	b	b
34	5	b	c
35	5	c	a
36	5	c	b
37	5	c	c

N	n	Ar	Ar1
38	6	a	a
39	6	a	b
40	6	a	c
41	6	b	a
42	6	b	b
43	6	b	c
44	6	c	a
45	6	c	b
46	6	c	c

N	n	Ar	Ar1
47	7	a	a
48	7	a	b
49	7	a	c
50	7	b	a
51	7	b	b
52	7	b	c
53	7	c	a
54	7	c	b
55	7	c	c

Scheme 2.3: *Reagents and conditions:* I) 7-aminoheptan-1-ol[89], K_2CO_3, dry CH_3CN, 80 °C, overnight; II) **234**, K_2CO_3, dry CH_3CN, 60 °C, 20 h; III) Ar_1COOH, EDC hydrochloride, DMAP, dry CH_2Cl_2, rt, 48 h or Ar_1COCl, $CHCl_3$ (free of ethanol), rt, 18 h.

7-(3-bromopropoxy)-2*H*-chromen-2-one **234** was obtained by reaction of the commercially available 7-hydroxy-2*H*-chromen-2-one with 1,3-dibromopropane in acetone with very good yields, as reported in Scheme 2.4.



Scheme 2.4: *Reagents and conditions:* I) 1,3-dibromopropane, K_2CO_3, acetone, reflux, overnight.

2.2.2. Results and discussions

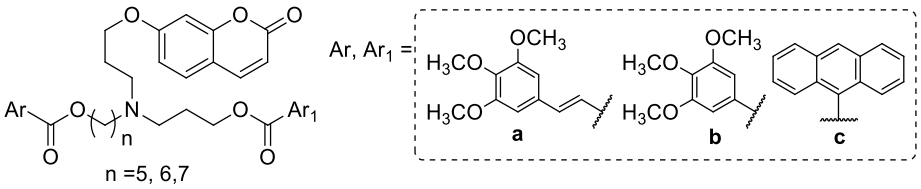
2.2.2.1. Pharmacological assays on the single target proteins, hCA XII and P-gp: hCA inhibitory activity and Doxorubicin cytotoxicity enhancement assay on K562/DOX cells

As for the first series of compounds, these new dual P-gp/hCA XII inhibitors were first studied on the single proteins taken individually: the obtained results are reported in Table 2.3.
Concerning the hCA inhibitory assay, all these coumarin derivatives inhibited only the tumor-associate hCA IX and XII with Ki values in the nanomolar range, and they were inactive on the off-target hCA I and II isoforms. Interestingly, in this series of compounds, the linkers' length seemed to influence the interaction with hCA XII: indeed, derivatives **29-46**, carrying a total spacer of 8 or 9 methylenes, displayed a high selectivity towards hCA XII, except for **31**, **38** and **40**. Instead, compounds characterized by n = 7 were, in general, more active on hCA IX, except for **50**, **54** and **55**. Notably, compounds **33**, **42** and **50** showed the highest selectivity towards hCA XII, with K_i values < 10 nM (K_i = 8.9 nM, 6.8 nM and 4.6 nM, respectively), as the reference compound AAZ. **33** and **42** present in both cases the 3,4,5-trimethoxyphenyl ester moieties (**b**), but they have a 5 and 6 methylene chain, respectively; **50**, instead, show a combination of the *(E)*-3-(3,4,5-trimethoxyphenyl)vinyl (**a**) and the 3,4,5-trimethoxyphenyl (**b**)

groups, and a 7 methylene chain. Moreover, compounds **31**, **38** and **48** were more active on the hCA IX isoform than AAZ, showing Ki values < 10 nM.

The ability of compounds **29-55** to inhibit the P-gp transport activity was evaluated with the Doxorubicin co-administration assay on K562/DOX cells, which overexpress only P-gp[88]. Results were expressed as RF (Reversal Fold) values that are the ratio between the IC_{50} value of Doxorubicin alone and in presence of our P-gp inhibitors, tested at 1 and 3 μM: the higher the RF values, the higher the MDR reversal activity. Results are reported in Table 2.3: all our compounds enhanced the cytotoxicity of Doxorubicin with different extent. The best results were obtained for derivatives carrying the aryl residues **a** and **b**: among these, the most potent compounds were **29**, **32** and **33** (n = 5), **38**, **39** and **42** (n = 6) and **48**, **50** and **51** (n = 7) with RF values upper than 5.0 and 12.0, when used at 1 and 3 μM, respectively. Otherwise, the anthracene derivatives showed, in general, the lowest effects. Notably, the potent P-gp inhibitors **33**, **42** and **50** showed the highest inhibitory effect on hCA XII.

Table 2.3: Inhibitory activity on hCA I, II, IX and XII isoforms and Doxorubicin cytotoxicity enhancement assay on K562/DOX cells of compounds **29-55**.



Cmpd	n	Ar	Ar_1	K_I (nM)[a]				RF[b]	
				hCA I	hCA II	hCA IX	hCA XII	1μM	3μM
29	5	a	a	>10000	>10000	39.5	34.6	9.5	25.7
30	5	a	b	>10000	>10000	24.2	16.2	3.3	7.7
31	5	a	c	>10000	>10000	7.9	44.9	3.1	8.4
32	5	b	a	>10000	>10000	58.8	22.3	6.9	25.7
33	5	b	b	>10000	>10000	40.7	8.9	5.2	12.3
34	5	b	c	>10000	>10000	36.2	30.8	3.1	7.7
35	5	c	a	>10000	>10000	104.5	56.4	3.6	8.9
36	5	c	b	>10000	>10000	93.7	55.2	2.4	7.1
37	5	c	c	>10000	>10000	136.8	73.4	1.0	1.0
38	6	a	a	>10000	>10000	8.1	32.4	22.5	30.0
39	6	a	b	>10000	>10000	50.2	21.6	8.2	45.0
40	6	a	c	>10000	>10000	26.8	66.3	2.2	9.0
41	6	b	a	>10000	>10000	71.7	10.1	1.1	11.1
42	6	b	b	>10000	>10000	82.7	6.8	6.4	16.0
43	6	b	c	>10000	>10000	54.1	43.3	3.9	8.9
44	6	c	a	>10000	>10000	148.3	74.0	4.3	13.6
45	6	c	b	>10000	>10000	123.2	23.9	4.4	9.3
46	6	c	c	>10000	>10000	166.5	90.2	1.2	1.3
47	7	a	a	>10000	>10000	27.8	50.9	1.8	26.7
48	7	a	b	>10000	>10000	5.2	17.2	8.0	26.7
49	7	a	c	>10000	>10000	14.2	37.6	2.0	3.0

50	7	b	a	>10000	>10000	43.8	4.6	16.0	22.8
51	7	b	b	>10000	>10000	18.3	31.7	8.0	20.0
52	7	b	c	>10000	>10000	38.5	62.5	2.3	6.1
53	7	c	a	>10000	>10000	71.1	113.1	3.0	6.4
54	7	c	b	>10000	>10000	41.3	10.1	2.0	6.1
55	7	c	c	>10000	>10000	102.2	83.8	1.0	2.4
AAZ				250.0	12.0	25.0	5.7		

[a] Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10 % of the reported values). [b] Inhibition of the P-gp transport activity on K562/DOX cells expressed as RF that is the ratio between the IC_{50} of Doxorubicin alone and in presence of modulators (RF = IC_{50} of Doxo – modulator/IC_{50} of Doxo + modulator).

2.2.2.2. Enhancement of Doxorubicin cytotoxicity assay on HT29/DOX and A549/DOX

All these compounds were also tested in the Doxorubicin cytotoxicity enhancement assay on human adenocarcinoma colon cells (HT29) and on non-small cell lung cancer cells (A549). These specific cell lines were selected since the Doxorubicin-resistant counterparts (HT29/DOX and A549/DOX) overexpress both P-gp and hCA XII[77]: thus, the effect of these dual P-gp/hCA XII inhibitors was analyzed in a specific environment where the target proteins coexisted. Also in these assays, performed by Prof. Riganti from the University of Turin, our compounds were tested, at 1 and 3 μM, in combination with Doxorubicin and the RF values were measured. Results demonstrated that all the compounds were able to restore the antineoplastic effect of the drug, with a highly reduced cells' viability. Table 2.4 reported, as an example, the results obtained for **33** and **42** (Ar, Ar_1 = **b**, n = 5 and 6, respectively), and for **50** (Ar = **b**, Ar_1 = **a**, n = 7) that displayed the highest inhibitory activity on the single proteins taken individually (see Par. 2.2.2.1): these compounds displayed a synergistic effect on the two resistant cell lines (HT29/DOX and A549/DOX), that overexpress both proteins, with RF values higher than those obtained on K562/DOX cells, that overexpress only P-gp (Table 2.4). Notably, when **33**, **42** and **50** were tested at 3 μM on A549/DOX cell lines, we measured RF values of 155.0, 103.0 and 82.4, respectively; on K562/DOX cells, instead, they displayed RF values of 12.3, 16.0 and 22.8, respectively.

Table 2.4: RF values of compounds **33**, **42** and **50** on the three tested resistant cell lines (K562/DOX, HT29/DOX and A549/DOX).

Cmpd	**RF**[a]					
	K562/DOX		**HT29/DOX**		**A549/DOX**	
	1μM	**3μM**	**1μM**	**3μM**	**1μM**	**3μM**
33	5.2	12.3	44.4	85.7	70.4	155.0
42	6.4	16.0	46.1	63.1	67.4	103.0
50	16.0	22.8	37.6	61.9	61.9	82.4

[a] Inhibition of the P-gp transport activity on three tested resistant cell lines expressed as RF that is the ratio between the IC_{50} of Doxorubicin alone and in presence of modulators (RF = IC_{50} of Doxo – modulator/IC_{50} of Doxo + modulator).

2.2.2.3. Chemical stability tests

Finally, in the laboratory of Prof. Bartolucci from the University of Florence, I evaluated the chemical stability all these diester derivatives: the analyses were performed by liquid chromatography coupled with mass spectrometry (LC-MS/MS) methods, operating in Multiple Reaction Monitoring (MRM) mode. The LC-MS/MS system and parameters used were reported in Par. 7.2.1.

In these assays, we monitored the variation of our diester molecules' concentration at four different incubation times both in PBS and human plasma samples, to evaluate their susceptibility towards spontaneous and enzymatic hydrolysis, respectively. By plotting these data (analyte concentrations *vs* the incubation time), their corresponding degradation profiles were obtained. The analyte concentration (1 μM) used during the stability tests is generally smaller than its Michaelis-Menten constant (K_M), and the enzymatic degradation rate is described by a first-order kinetic. Therefore, by plotting the natural logarithm of the quantitative data versus the incubation time, a linear function can be used, and its slope represents the degradation rate constant (*k*). Accordingly with the linear function, the half-life ($t_{1/2}$) of each tested compound can be calculated as follows:

$$t_{1/2} = \ln(0.50\,\mu M) / k$$

The $t_{1/2}$ value < 2 h of ketoprofene ethylester, used as reference compound, demonstrated that the employed human batch was enzymatically active. At the contrary, when the k values of our compounds were close to 0, extremely high $t_{1/2}$ values can be calculated. Since under the proposed experimental conditions a half-life over 240 min is not measurable, it is reasonable to consider that their $t_{1/2}$ values could be equal or greater than 240 min.

Results demonstrated that all these compounds were stable both in PBS and in human plasma samples with a $t_{1/2}$ equal or greater than 240 min. These observations are very interesting since in this *N*,*N*-bis(alkanol)amine aryl diester series, the introduction of the coumarin group through a propyl chain on the nitrogen atom seems to protect the two ester groups from the enzymatic hydrolysis. Indeed, also compounds carrying the *(E)*-3-(3,4,5-trimethoxyphenyl)vinyl ester group linked to a three methylene chain, and combined with the 3,4,5-trimethoxyphenyl moiety, were stable in human plasma samples (see as an example, compound **41** in Figure 2.4), while the corresponding linear and chiral compounds with a *N*-methylated group were not (see compounds **153** and **(*R*)-183** in Figure 5.3, Par 5.1).

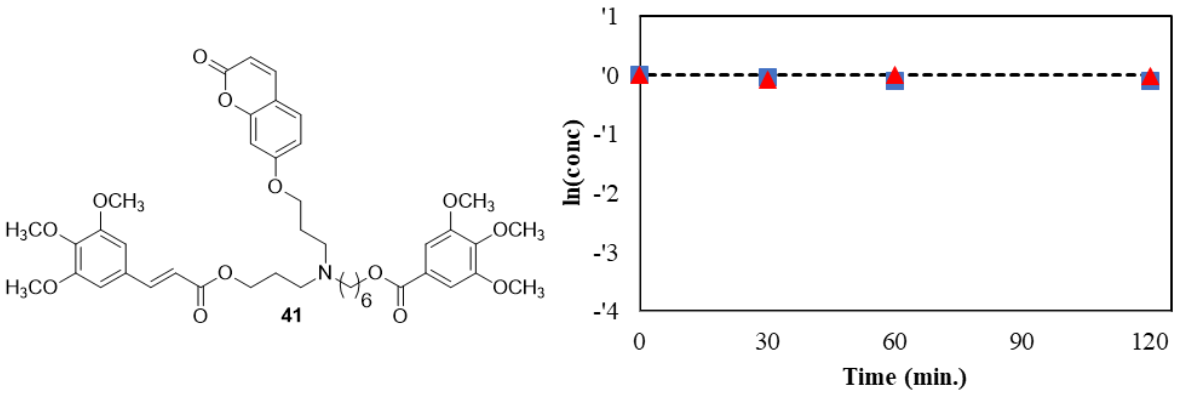


Figure 2.4: Degradation profiles in PBS (blue) and human plasma (red) of the stable compound **41**.

2.3. Piperazine derivatives

To avoid any possible problem associated with the metabolic lability of the ester function, in 2018 our group designed and synthesized a series of P-gp inhibitors characterized by the piperazine ring[81], since this scaffold is present in several MDR reversers, as Zosuquidar, a third-generation P-gp inhibitor (Figure 2.5). The previously synthesized piperazine derivatives carried different arylalkyl groups on the two nitrogen atoms (Figure 2.5), and displayed in general good inhibitory activity on the target protein[81].

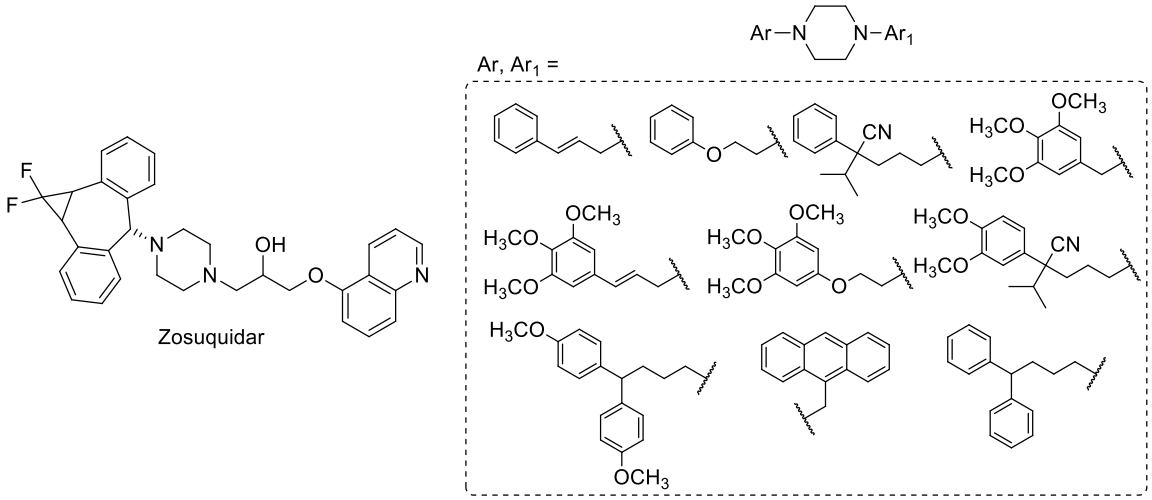
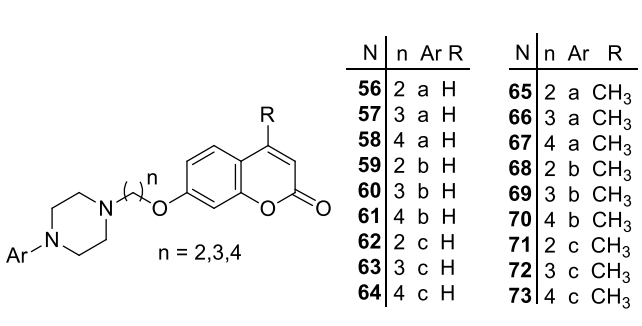


Figure 2.5: structures of Zosuquidar, a third-generation P-gp inhibitor, and of the previously synthesized piperazine derivatives[81].

Based on these results, in this PhD project, we chose to functionalize the piperazine ring obtaining a new series of dual P-gp/hCAXII inhibitors: we introduced on one nitrogen atom the methoxy-substituted aryl moieties that confer good P-gp inhibitory effects, and on the other, a coumarin group to target hCA XII (Figure 2.6). In this series, the selected methoxy-substituted aryl groups are the (*E*)-3-(3,4,5-trimethoxyphenyl)vinyl (**a**), 3,4,5-trimethoxyphenyl (**b**) or the 4,4-bis(4-methoxyphenyl)butyl (**c**) ones (Figure 2.6). To evaluate the selectivity towards hCA XII, two different coumarins were chosen (7-hydroxycoumarin and the 4-methylated one) that are linked to the piperazine ring by a 2, 3 or 4 methylene chain. The 7-hydroxy-4-methyl-coumarin was chosen since it showed high inhibitory activity on hCA XII[90].



N	n	Ar	R
56	2	a	H
57	3	a	H
58	4	a	H
59	2	b	H
60	3	b	H
61	4	b	H
62	2	c	H
63	3	c	H
64	4	c	H

N	n	Ar	R
65	2	a	CH_3
66	3	a	CH_3
67	4	a	CH_3
68	2	b	CH_3
69	3	b	CH_3
70	4	b	CH_3
71	2	c	CH_3
72	3	c	CH_3
73	4	c	CH_3

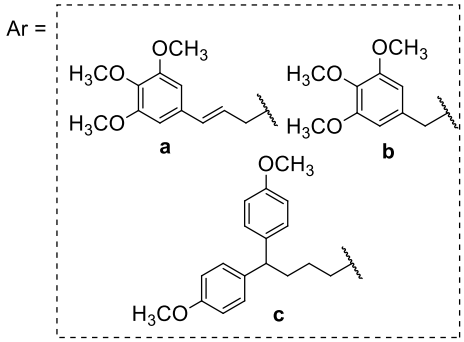


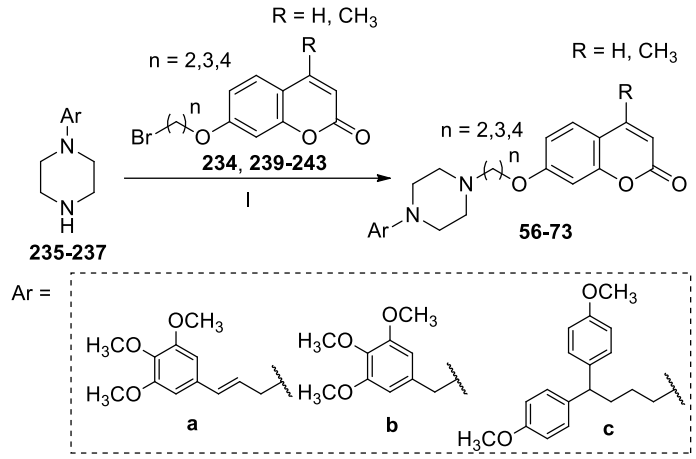
Figure 2.6: structures of the piperazine derivatives **56-73**, synthesized in this PhD thesis.

All these new piperazine compounds were evaluated for their P-gp and hCA XII inhibitory activity, following the same procedures described previously (see Par. 2.2.2.1). Then, the most

promising compounds will be studied on HT29/DOX and A549/DOX cells, that overexpress both the target proteins[77].

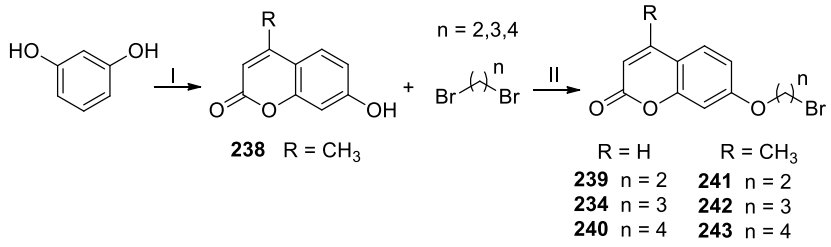
2.3.1. Chemistry

The reaction pathway used to obtain the piperazine derivatives **56-73** is described in Scheme 2.5. The proper methoxy-substituted aryl piperazines **235-237**[81], needed to achieve final compounds **56-73**, were previously synthesized following the procedures reported in ref. [81]. These intermediates **235-237**[81] were *N*-alkylated with the suitable 7-(bromoalkoxy)-2*H*-chromen-2-one (**234**, described in Scheme 2.4, and **239-243**, described in Scheme 2.6) in dry CH_3CN to yield final compounds **56-73** (Scheme 2.5).



Scheme 2.5: *Reagents and conditions*: I) K_2CO_3, dry CH_3CN, 60 °C, overnight.

The 7-(bromoalkoxy)-2*H*-chromen-2-ones **234** and **239-243** were obtained by alkylation of the commercially available 7-hydroxy-2*H*-chromen-2-one or the synthesized 7-hydroxy-4-methyl-2*H*-chromen-2-one **238** with the proper dibromoalkane (1,2-dibromoethane, 1,3-dibromopropane, 1,4-dibromobutane) in acetone, as reported in Scheme 2.4 and 2.6. 7-hydroxy-4-methyl-2*H*-chromen-2-one **238** was obtained by condensation of resorcinol with ethyl acetoacetate under acidic conditions (Scheme 2.6).



Scheme 2.6: *Reagents and conditions*: I) H_2SO_4, ethyl acetoacetate, rt, 15 min; II) K_2CO_3, acetone, refluxed, overnight.

2.3.2. Results and discussions

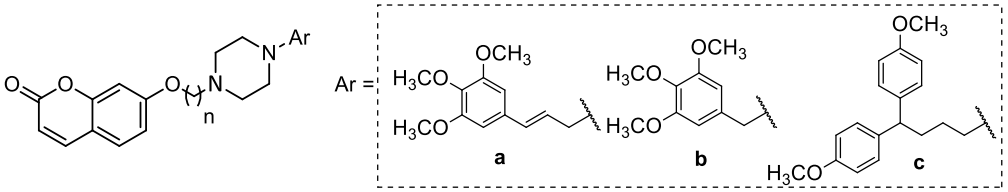
2.3.2.1. Pharmacological assays on the single target proteins, hCA XII and P-gp: hCA inhibitory activity and Doxorubicin cytotoxicity enhancement assay on K562/DOX cells

Regarding this series of compounds, at the moment, we have some preliminary results on P-gp and hCA XII inhibition assays, only for piperazines **56-64**, which carry the non-methylated coumarin group (Table 2.5).

As regard the Doxorubicin co-administration assay on K562/DOX cells (Table 2.5), the highest P-gp inhibitory effect was measured for the 4,4-bis(4-methoxyphenyl)butyl derivatives **62-64**: interestingly, when used at 3 μM, **64** showed an RF value of 90.5.

Otherwise, in the hCA inhibition assay, all the tested piperazine compounds resulted active only on the tumor-associated hCA IX and XII isoforms, showing a high selectivity towards hCA XII. The lowest Ki values were measured for derivatives bearing the aryl residues **a** and **b**: among these compounds, the linker's length seemed to influence the interaction with hCA XII, since Ki values decrease as the number of methylenes of the chain increases.

Table 2.5: Inhibitory activity of compounds **56-64** on the P-gp transport activity on K562/DOX cells expressed as the RF values, and on hCA I, II, IX and XII isoforms.



Cmpd	n	Ar_1	RF[a]		K_I (nM)[b]			
			1 μM	3 μM	hCA I	hCA II	hCA IX	hCA XII
56	2	a	1.6	2.3	>10000	>10000	51.3	13.0
57	3	a	3.8	4.5	>10000	>10000	62.6	29.4
58	4	a	3.1	3.7	>10000	>10000	82.0	34.6
59	2	b	1.0	1.9	>10000	>10000	23.7	6.2
60	3	b	1.0	3.4	>10000	>10000	40.1	13.6
61	4	b	1.1	2.3	>10000	>10000	29.0	42.9
62	2	c	5.9	21.1	>10000	>10000	112.8	84.7
63	3	c	5.5	17.3	>10000	>10000	152.5	115.0
64	4	c	8.1	90.5	>10000	>10000	145.3	92.1

[a] Inhibition of the P-gp transport activity on K562/DOX cells expressed as RF that is the ratio between the IC_{50} of Doxorubicin alone and in presence of modulators (RF = IC_{50} of Doxo – modulator/IC_{50} of Doxo + modulator). [b] Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10 % of the reported values).

3. Tariquidar analogues

These series of compounds are part of a wide project based on the design and synthesis of molecules that display potent inhibitory effects on both P-gp and BCRP transporters. Indeed, these two proteins are mainly involved in MDR, and they are co-overexpressed in several resistant cancer cells: thus, compounds able to inhibit both P-gp and BCRP could be very useful to overcome MDR.
Elacridar (GF120918 or GW120918)[54] and Tariquidar (XR9576)[55] (Figure 3.1) are two of the most interesting third-generation chemo-sensitizers: they both carry a 6,7-dimethoxy-2-phenethyl-1,2,3,4-tetrahydroisoquinoline moiety linked to an aryl-substituted amide function (Figure 3.1).

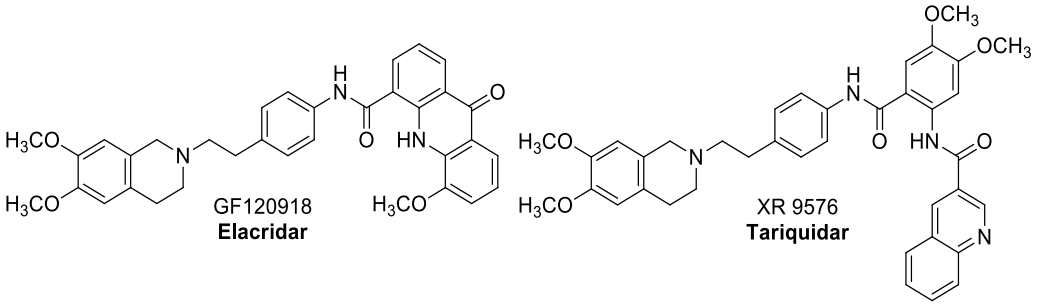


Figure 3.1: Elacridar e Tariquidar, two of the most interesting third-generation P-gp inhibitors.

These compounds displayed a high affinity towards the ABC proteins, a reduced effect on cytochromes, and few pharmacokinetic interactions with cytotoxic drugs[56]. Disappointingly, they have not been approved for therapy since they did not show an improvement of the efficacy of the co-administered antitumoral drugs[57]. Early studies indicated that both derivatives are not specific for P-gp because they are also able to bind the BCRP transporter[53], and although several P-gp modulators have been studied, till now only a few compounds displayed activity on both these two proteins. Notably, recent evidences indicated that Tariquidar is able to bind also the MRP1 transporter[58]; the same compound was shown to potentiate the sensitivity to Paclitaxel in resistant cells transfected by another member of the ABCC family, the MRP7 protein[59].
Therefore, despite the failure in clinical trials, Tariquidar and Elacridar have been considered lead compounds to discover new MDR modulators able to target the three ABC proteins involved in MDR.

3.1. Amide and ester compounds

In a previous study[91], performed in our laboratory, a series of derivatives bearing the 6,7-dimethoxy-2-phenethyl-1,2,3,4-tetrahydroisoquinoline moiety linked, as Elacridar and Tariquidar, to an aryl-substituted amide function, was designed and synthesized. Moreover, also the corresponding isosteric ester derivatives were obtained[91] (Figure 3.2). The aryl residues were chosen based on their presence in potent MDR reversers[86,92,93].

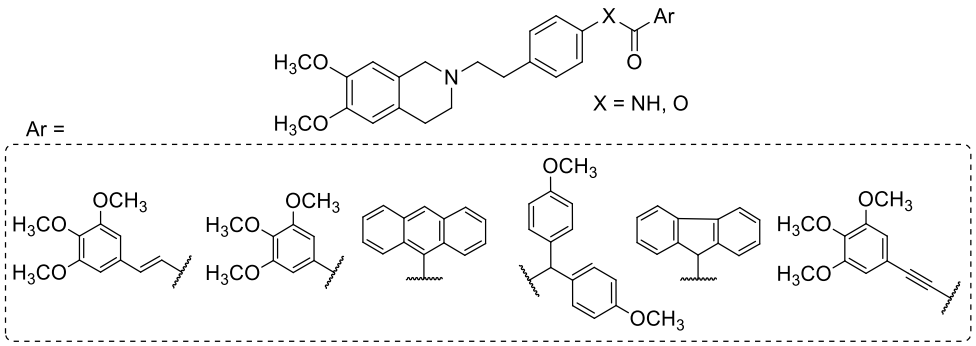
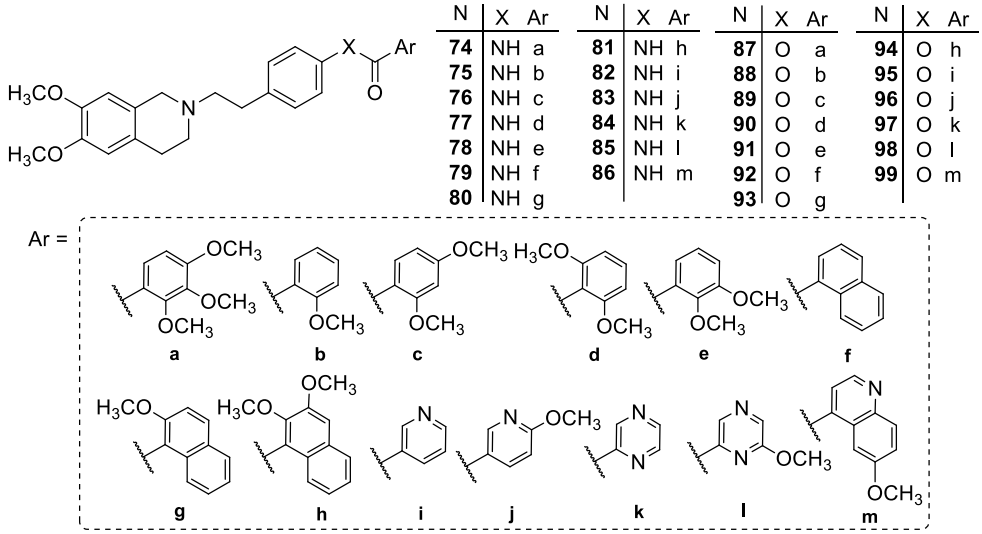


Figure 3.2: structures of the previously synthesized amide and ester derivatives[91].

All the amide derivatives were active, although less potent than Elacridar and Tariquidar, and selective on P-gp. Interestingly, also ester compounds maintained good P-gp inhibitory effects and three of them were also active on BCRP, highlighting that the amide function was not essential for modulating the transporter proteins. On the contrary, none of these compounds display an inhibitory effect on MRP1. The obtained results are reported in ref [91].

Therefore, as a continuation of this study, to deepen the structure-activity relationships of Tariquidar analogues, in this PhD project, we designed and synthesized a new series of amide and ester derivatives characterized by the presence of the 6,7-dimethoxy-2-phenethyl-1,2,3,4-tetrahydroisoquinoline scaffold linked to additional different aryl moieties[94] (Figure 3.3). The new aryl moieties are methoxy-substituted phenyl or naphthyl nuclei, or nitrogen-containing hetero-aromatic residues. In this series, we decided to modulate both the position and the number of methoxy groups. In this way, we investigated if the steric hindrance of an *ortho*-methoxy substituent could influence the interaction with the ABC transporters (Figure 3.3 **a-e** residues). Moreover, to increase the steric hindrance and the lipophilicity of these molecules, we selected the naphthalene ring (**f**) and its 2-methoxy (**g**) and 2,3-dimethoxy-substituted (**h**) analogues (Figure 3.3).



N	X	Ar	N	X	Ar	N	X	Ar	N	X	Ar
74	NH	a	**81**	NH	h	**87**	O	a	**94**	O	h
75	NH	b	**82**	NH	i	**88**	O	b	**95**	O	i
76	NH	c	**83**	NH	j	**89**	O	c	**96**	O	j
77	NH	d	**84**	NH	k	**90**	O	d	**97**	O	k
78	NH	e	**85**	NH	l	**91**	O	e	**98**	O	l
79	NH	f	**86**	NH	m	**92**	O	f	**99**	O	m
80	NH	g				**93**	O	g			

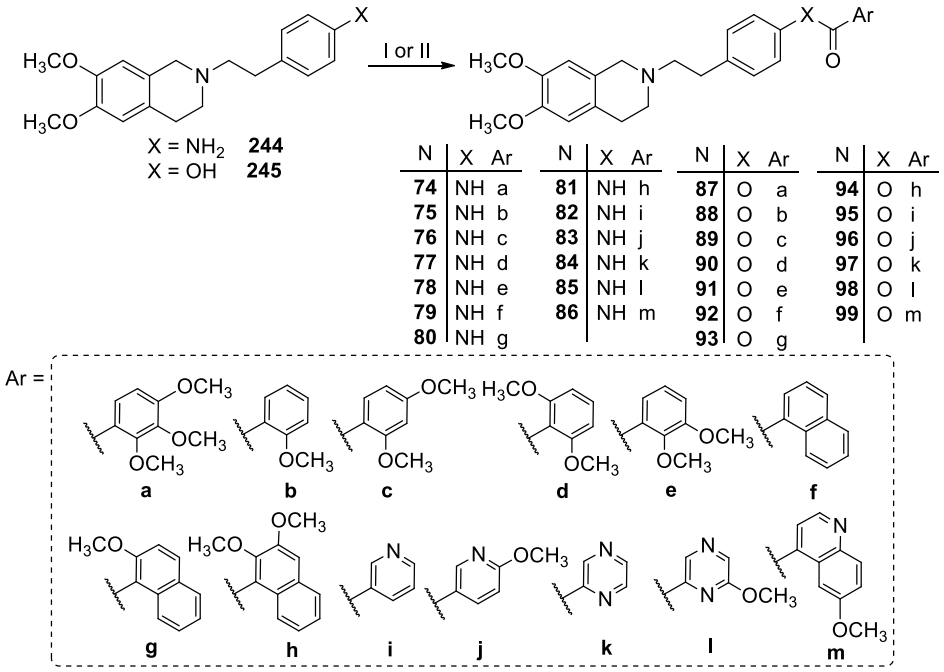
Figure 3.3: new amide and ester Tariquidar analogues **74-99**[94], synthesized in this PhD thesis.

All these compounds were evaluated for their P-gp interaction profile, combining three assays: 1) apparent permeability (Papp) determination (BA/AB) in Caco-2 cell monolayer; 2) ATP cell depletion in cells overexpressing the transporter (MDCK-MDR1); 3) inhibition of the Calcein-AM transport in MDCK-MDR1 cells. Then, the activity on MRP1 and BCRP was evaluated on

cancer cell lines overexpressing each transporter (MDCK-MRP1 and MDCK-BCRP cells, respectively), by measuring the inhibition of the efflux of the pro-fluorescent probe Calcein-AM in MDCK-MRP1 cells or the fluorescent probe Hoechst 33342 in MDCK-BCRP cells[94]. Furthermore, two selected compounds were further tested alone and in co-administration with the antineoplastic drug Doxorubicin in different cancer cell lines (MDCK-MDR1, HT29/DOX and A549/DOX cell lines) with various levels of P-gp. Finally, the stability of amide and ester derivatives was investigated in PBS and human plasma samples[94].

3.1.1. Chemistry

The reaction pathways used to synthesize derivatives **74-99** are reported in Schemes 3.1-3.2. The key intermediates needed to achieve amides and esters were the aniline **244** and the phenol **245**, respectively, which were prepared as reported in literature[91]. Amides **74-86**[94] and esters **87-99**[94] were obtained by reaction of **244**[91] and **245**[91], respectively, with the proper carboxylic acid using EDC hydrochloride and DMAP in dry CH_2Cl_2 or CH_3CN (*Method A*), or with the acyl chloride obtained by treatment of the suitable acid with $SOCl_2$ in $CHCl_3$ (free of ethanol) or dry CH_3CN (*Method B*) (Scheme 3.1; for details see the Experimental Section).

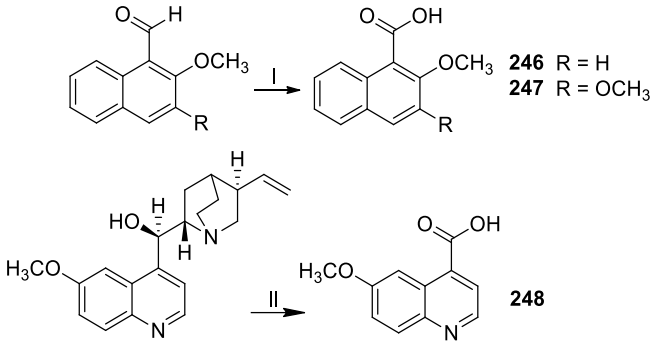


N	X	Ar	N	X	Ar	N	X	Ar	N	X	Ar
74	NH	a	**81**	NH	h	**87**	O	a	**94**	O	h
75	NH	b	**82**	NH	i	**88**	O	b	**95**	O	i
76	NH	c	**83**	NH	j	**89**	O	c	**96**	O	j
77	NH	d	**84**	NH	k	**90**	O	d	**97**	O	k
78	NH	e	**85**	NH	l	**91**	O	e	**98**	O	l
79	NH	f	**86**	NH	m	**92**	O	f	**99**	O	m
80	NH	g				**93**	O	g			

Scheme 3.1: *Reagents and conditions:* I) *Method A*: ArCOOH, EDC hydrochloride, DMAP, dry CH_2Cl_2 or CH_3CN, rt, 48 h; II) *Method B*: ArCOCl, ethanol-free $CHCl_3$ or dry CH_3CN, rt, 18 h.

Most of the carboxylic acids were commercially available. 2-Methoxy-1-naphthoic acid **246**, instead, was synthetized following two different procedures: the commercially available 2-methoxy-1-naphthaldehyde was treated with $CuBr_2$ and *t*-BuOOH (70% solution in water)[95] or with Na_2CO_3 and $KMnO_4$[96], yielding **246** with good yields (Scheme 3.2). To synthesize 2,3-dimethoxy-1-naphthoic acid **247**, the commercially available 2,3-dimethoxy-1-naphthaldehyde was treated with $CuBr_2$ and *t*-BuOOH (70% solution in water)[95], but this procedure did not allow to obtain the desired compound. Thus, 2,3-dimethoxy-1-naphthaldehyde was oxidated in presence of Na_2CO_3 and $KMnO_4$[96] (Scheme 3.2), obtaining 2,3-dimethoxy-1-naphthoic acid

247 with high yields. 6-Methoxyquinoline-4-carboxylic acid **248** was synthesized following the procedure described by Kowanko[97] with 10 % H_2SO_4, MnO_2 and CrO_3 (Scheme 3.2).



Scheme 3.2: *Reagents and conditions:* R = H: I) $CuBr_2$, *t*-BuOOH (70% solution in water), dry CH_3CN, rt, 4 days, or Na_2CO_3, $KMnO_4$, acetone, rt, 5 h. R = CH_3: I) Na_2CO_3, $KMnO_4$, acetone, rt, 5 h. II) H_2SO_4 (10 % solution in water), MnO_2, CrO_3, NH_4OH (15N).

3.1.2. Results and discussions

3.1.2.1. Biological activity: characterization of P-gp interacting profile and ABC transporters selectivity

These compounds were studied, by the research group of Prof. Colabufo from the University of Bari, to evaluate their interaction profile towards P-gp, MPR1 and BCRP, using Madin-Darby Canine Kidney (MDCK) transfected cells[94]. In these assays, we measured the transport inhibition of a pro-fluorescent probe, Calcein-AM, that is a P-gp and MRP1 substrate, in a cell line overexpressing P-gp (MDCK-MDR1) or MRP1 (MDCK-MRP1), and of a fluorescent probe Hoechst 33342, that is a BCRP substrate, in cells overexpressing BCRP (MDCK-BCRP cells). The P-gp interacting profile was further investigated with two other assays, to evaluate if our compounds can be classified as P-gp substrates or inhibitors: the Apparent Permeability (P_{app}) determination (BA/AB) in Caco-2 cell monolayer, and the ATP cell depletion in the MDCK-MDR1 cell line. P_{app} determination measures the ratio between two fluxes: from the basolateral to apical compartments (BA, representative of passive diffusion) and from the apical to basolateral compartments (AB, representative of active transport)[98]. A (BA/AB) value < 2 suggests that the compound can be considered an inhibitor, since it is able to enter the cell membrane avoiding the P-gp-mediated efflux at the apical level. In the same manner, if (BA/AB) > 2, the compound should probably be classified as a substrate, since it is able to enter the cell membrane only by passive diffusion, while it is effluxed by P-gp at the apical level[99]. The second assay detects the consumption of ATP elicited by the transport mediated by the pump; generally, a substrate induces ATP cell depletion being transported by the pump (unambiguous substrate, category I), while a P-gp inhibitor does not induce ATP consumption. There is also a third substrate category (known as category IIB3) displaying a P_{app} value > 2 but not inducing an ATP cell depletion[98].

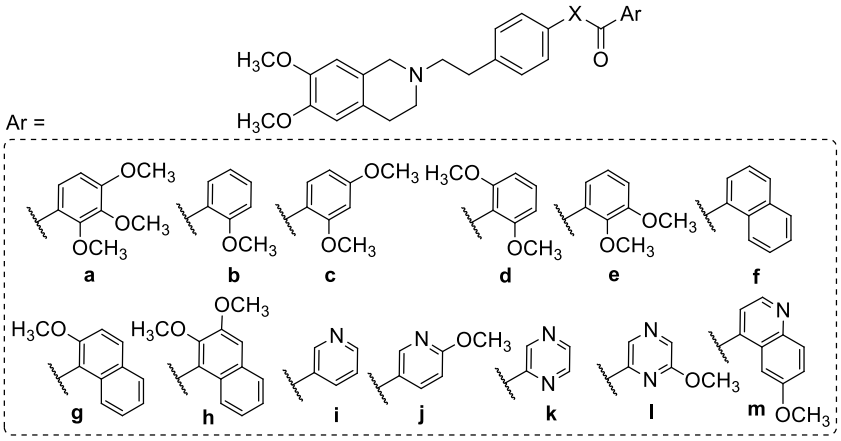
Results on the three inhibition assays of compounds **74-99** are reported in Table 3.1 together with those of Tariquidar and Elacridar, used as reference compounds.

As shown in Table 3.1, all the compounds were active on P-gp with different extent. As a general trend, substituted benzene and naphthalene derivatives displayed activities in the submicromolar or nanomolar range (EC_{50} ranging from 40.5 nM to 0.67 μM for amides and from 0.10 μM to 0.84 μM for esters), except for amide **77**. The most interesting results were

obtained for the 2,4-dimethoxy phenyl derivative **76** (EC_{50} = 40.5 nM) and the 2,3-dimethoxy naphthalene derivative **81** (EC_{50} = 47.8 nM), that showed an outstanding potency in the nanomolar range as the reference compounds Tariquidar and Elacridar. In this set of derivatives, it was not easy to define a relationship between potency values and structural characteristics: some compounds with interesting EC_{50} values could be found both among the amides and the esters, but the nature of the aryl moieties differently influenced the two groups of isosteres, without a definite trend. However, biological data confirmed that the presence of an *ortho*-methoxy substituent is favorable for the activity. Moreover, compounds **82-86** and **95-99**, carrying a nitrogen-containing heterocycle displayed lower potency: only amides **85** and **86**, and ester **98** showed good inhibitory activity on P-gp (EC_{50} = 0.87 μM, 0.66 μM and 0.22 μM, respectively). Interestingly, the presence of the methoxy substituent improved the inhibitory activity on P-gp, particularly for compounds **85** and **98**, bearing a methoxy-substituted pyrazine. As regards BCRP, only six of these derivatives showed a moderate activity *vs* BCRP (EC_{50} ranging from 1.0 μM to 21.2 μM), belonging to the amide and the ester series. Interestingly, the potent P-gp inhibitor **76** was also the most active in BCRP inhibition (EC_{50} = 1.0 μM). Regarding MRP1 inhibition, only two ester derivatives, **87** and **89**, displayed a moderate activity. It is noteworthy that these two compounds were active also on BCRP and highly potent *vs* P-gp, showing a profile endowed with low selectivity.

Finally, as regard the P-gp interacting mechanism (see Table 3.1), all the compounds behaved as not transported substrates (category IIB3), since they had a BA/AB ratio > 2 and were not able to induce ATP cell depletion[94].

Table 3.1: MDR-reversing activity of amide and ester compounds **74-99**.



Cmpd	X	Ar	P-gp (EC_{50}) μM[a]	MRP1 (EC_{50}) μM[a]	BCRP (EC_{50}) μM[a]	ATP cell depletion	P_{app}[b]	$t_{1/2}$[c] (min)
74	NH	a	0.53±0.10	NA	21.2±4.2	no	7.0	≥ 240
75	NH	b	0.44±0.082	NA	NA	no	5.9	≥ 240
76	NH	c	0.0405±0.008	NA	1.0±0.2	no	7.4	≥ 240
77	NH	d	7.20±1.39	NA	NA	no	5.5	≥ 240
78	NH	e	0.26±0.050	NA	NA	no	5.0	≥ 240
79	NH	f	0.67±0.12	NA	NA	no	3.6	≥ 240
80	NH	g	0.12±0.022	NA	NA	no	11.8	≥ 240
81	NH	h	0.0478±0.0093	NA	NA	no	5.5	≥ 240
82	NH	i	6.40±1.26	NA	NA	no	7.3	≥ 240
83	NH	j	2.30±0.44	NA	NA	no	7.5	≥ 240
84	NH	k	1.90±0.32	NA	NA	no	4.9	≥ 240

85	NH	l	0.87±0.17	NA	NA	no	6.0	≥ 240
86	NH	m	0.66±0.13	NA	NA	no	6.5	≥ 240
87	O	a	0.36±0.069	69.7±13.9	15.1±2.89	no	5.1	≥ 240
88	O	b	0.73±0.14	NA	NA	no	9.0	63±14
89	O	c	0.10±0.02	87.0±17.0	11.0±2.0	no	5.4	88±22
90	O	d	0.15±0.03	NA	NA	no	7.1	≥ 240
91	O	e	0.34±0.07	NA	NA	no	6.2	54±9
92	O	f	0.84±0.16	NA	NA	no	4.1	≥ 240
93	O	g	0.11±0.02	NA	7.3±1.4	no	5.1	≥ 240
94	O	h	0.10±0.02	NA	NA	no	4.8	≥ 240
95	O	i	29.40±4.98	NA	NA	no	5.4	≥ 240
96	O	j	9.24±1.81	NA	NA	no	5.0	≥ 240
97	O	k	1.90±0.32	NA	NA	no	5.6	42±20
98	O	l	0.22±0.04	NA	NA	no	4.9	6±1
99	O	m	1.40±0.26	NA	NA	no	4.7	124±27
Tariq.			0.044±0.001	ND	0.010±0.005	Yes[d]	>20	ND
Elacr.			0.014±0.003	NA	10.0±2.0	Yes[e]	>20	ND

[a] Values are the mean ± SEM of two independent experiments, with samples in triplicate. [b] Apparent permeability estimation: values are from two independent experiments, with samples in duplicate. [c] The half-life ($t_{1/2}$) values were referred to the human plasma matrix. [d] 30% at a concentration of 50 μM; [e] 25% at a concentration of 10 μM. NA = not active; ND = not determined.

3.1.2.2. Enhancement of Doxorubicin cytotoxicity assay

Compounds **76** and **81**, endowing with the best P-gp inhibitory activity on the previous biological tests, were also tested alone and in co-administration with Doxorubicin, on three different cell lines that overexpress P-gp: MDCK-MDR1, HT29/DOX and A549/DOX cell lines.

In MDCK-MDR1 cells, Doxorubicin alone, at 10 μM, did not show cytotoxicity as expected, while the co-administration with **76** and **81**, used at 1 and 10 μM, was able to rehabilitate the effect of the antineoplastic agent leading to high cytotoxicity. Notably, when Doxorubicin is tested in combination with our compounds at their higher dose (10 μM) we measured cytotoxicity values of 77 % for **76** and around 50 % for **81** (Figure 3.4).

In HT29/DOX and A549/DOX cells, these two compounds were not cytotoxic. Moreover, **76** (at 10 μM) reduced the cell viability of 35% and 40%, respectively. A similar trend was observed also for **81** (Figure 3.4).

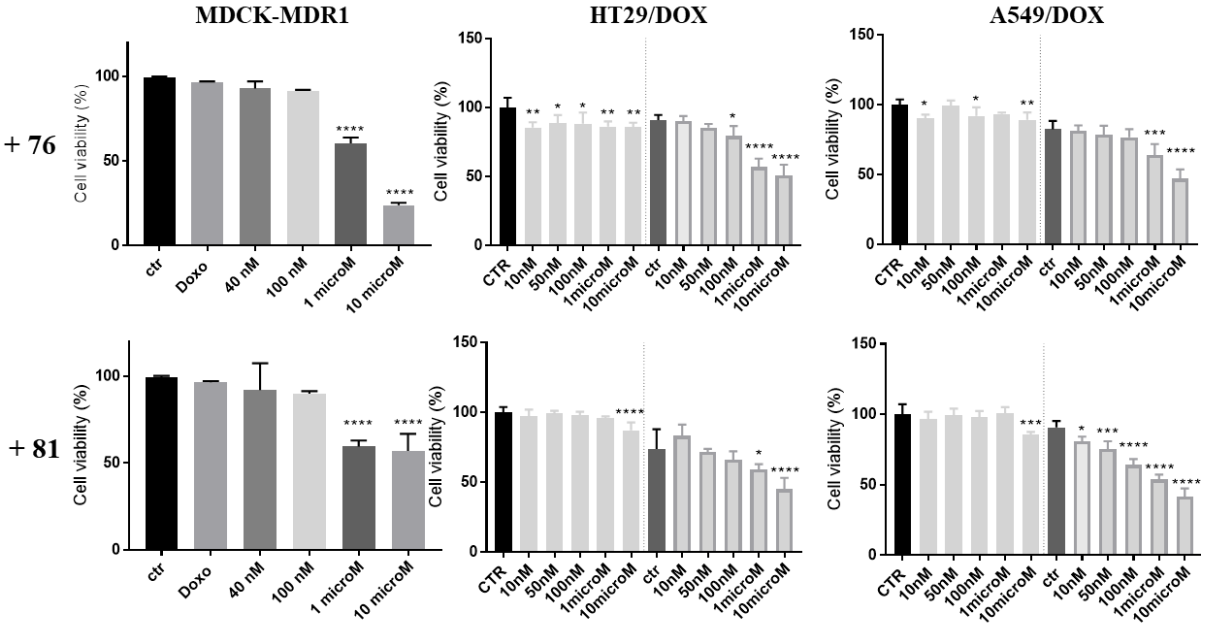


Figure 3.4: In vitro cell growth experiments performed on MDCK-MDR1, HT29/DOX and A549/DOX cells in presence of 10 μM Doxorubicin (Doxo) alone and in combination of different concentrations of the tested compounds **76** and **81**. Each bar represents the mean ± SEM of two experiments performed in triplicate. One-way ANOVA analysis: *p<0.05; **p ≤ 0.005; ***p ≤0.001; ****p < 0.0001.

3.1.2.3. Chemical stability test

Due to the presence of ester groups in the structure of derivatives **87-99**, I performed a series of chemical stability tests, following the same procedures described in Par. 2.2.2.3. All these esters were stable in PBS and most of them also in human plasma. In particular, compounds **88**, **89**, **91** and **97-99** were susceptible to enzymatic hydrolysis, showing $t_{1/2}$ values between 6 min and 124 min (Table 3.1). Derivatives **89-91** are dimethoxyphenyl isomers: interestingly, the presence of two *ortho*-methoxy substituents seemed to prevent the enzyme activity since ester **90** is stable in human plasma samples, while the other two compounds were not (Figure 3.5). The degradation profiles of all the ester derivatives are reported in Supplementary data of ref. [94].

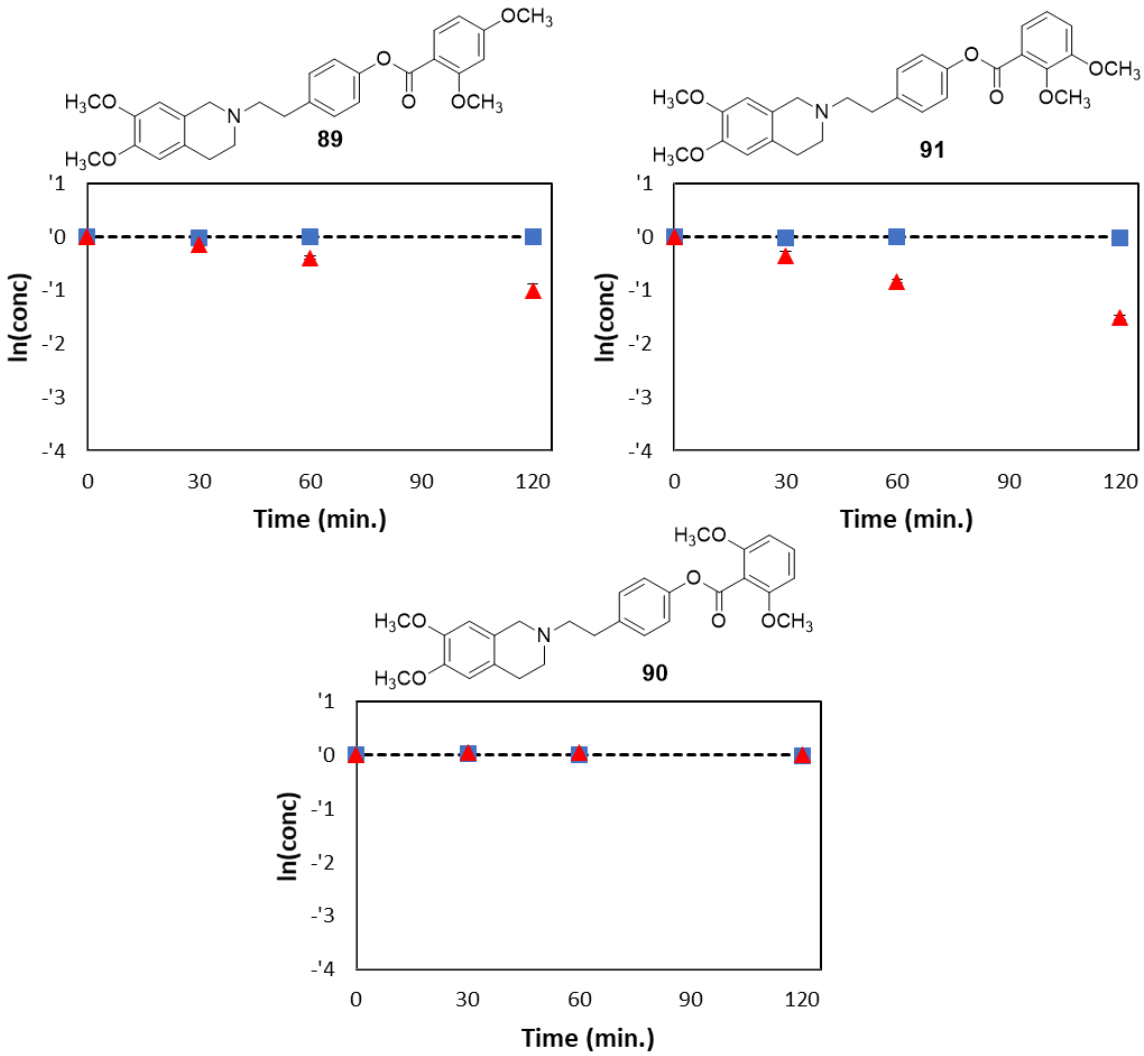


Figure 3.5: degradation profiles in PBS (blue) and human plasma (red) of hydrolyzed compounds **89** and **91** (top) and of the stable derivative **90** (bottom).

We also evaluate the susceptibility of the amide bond[94], and all the tested amides were stable both in PBS and human plasma samples (Table 3.1).

3.2. Bioisosteric heterocycles: tetrazole and oxadiazole derivatives

Since the isosteric substitution of the amide function led to ester compounds that maintained a good activity on P-gp, we decided to obtain a new series of Tariquidar analogues by replacing the amide group with two bioisosteric heterocycles: the tetrazole and the oxadiazole, based on many works that reported isosteric substitutions of the amide function of Elacridar and Tariquidar with azole rings.

In 2010, Kwak et al.[100] described HM30181 that is structurally related to Tariquidar and presents a tetrazole ring as isosteric substitution of the amide linker (Figure 3.6). HM30181 is a selective P-gp inhibitor, and in the ATPase assay on P-gp-enriched vesicles, it exhibited the highest potency, since its IC_{50} value (IC_{50} = 0.63 nM) is 50-fold, and 7.7-fold lower than those of Tariquidar and Elacridar. HM30181 did not inhibit MRP1, and partially inhibited BCRP only at very high concentrations. In 2015, Köhler et al.[101] described a series of Tariquidar derivatives which lack the 1,2,3,4-tetrahydroisoquinoline nucleus of lead compounds, and the amide function is substituted by a tetrazole ring (Figure 3.6, General structure **A**): the authors made

these modifications to increase the selectivity towards the BCRP protein. Most compounds showed only weak effects on P-gp and MRP1. Instead, all these derivatives were active on BCRP with different extent. Interestingly, two derivatives of the series, bearing a 2-methoxy substituent on the phenyl ring close to the tetrazole, displayed a dual inhibitory activity on both P-gp and BCRP. In 2018, the same authors[102] decided to further modify the chemical structure of their phenyltetrazolyl-phenylamide compounds (Figure 3.6, General structure **A**), by introducing an oxadiazole ring instead of the tetrazole one (Figure 3.6, General structure **B**). Results demonstrated that oxadiazole derivatives, carrying at least one methoxy group on the phenyl rings, were weak P-gp inhibitors, but maintained IC_{50} values on BCRP similar to those of the corresponding tetrazoles[103].

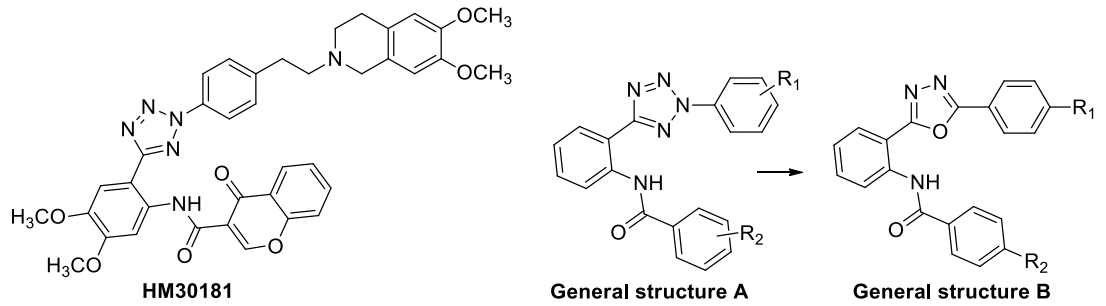


Figure 3.6: structures of HM30181[100], and of tetrazole (General structure **A**)[101] and oxadiazole (General structure **B**)[102] derivatives, synthesized by Köhler et al.

Based on these interesting results, we designed and synthesized a new series of Tariquidar analogues, where the amide function of the lead compound was substituted with two bioisosteric heterocycles: the tetrazole and the oxadiazole ones. Indeed, in this series of derivatives, the nitrogen atom of the 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline scaffold is linked by a dimethylene chain and a phenyl ring to the proper heterocycle, connected to a methoxy-substituted aryl moiety (Figure 3.7). Notably, we designed both the 2,5- (**100-105**) and the 1,5-disubstituted (**106-111**) tetrazoles, and the 2,5-disubstituted-1,3,4-oxadiazoles (**112-117**) (Figure 3.7). The methoxy-substituted aryl groups inserted in these tetrazole and oxadiazole compounds were selected since they conferred good inhibitory effects on P-gp to the previously synthesized amide and ester derivatives (see ref. [91] and Par 3.1.).

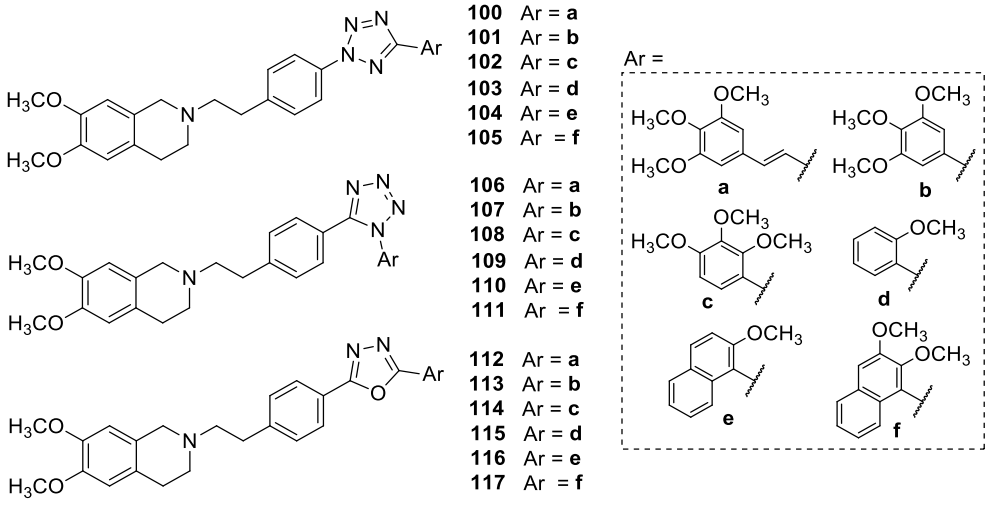


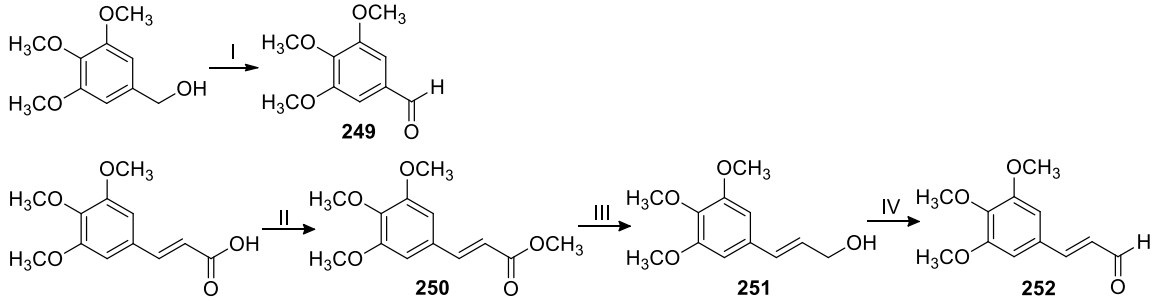
Figure 3.7: structures of the 2,5- (**100-105**) and the 1,5-disubstituted (**106-111**) tetrazoles, and of the 2,5-disubstituted-1,3,4-oxadiazoles (**112-117**) synthesized in this PhD thesis.

The activity of these new compounds on P-gp, MRP1 and BCRP was evaluated by the same biological assays described for the amide and ester series (see Par 3.1). Moreover, to further evaluate their P-gp inhibitory activity, the oxadiazoles **112-117** were also tested in combination with Doxorubicin on HT29/DOX cells, that overexpress P-gp: in this assay, we measured how our compounds affected the IC_{50} value of the co-administered drug. Moreover, the ability of all these compounds to increase the accumulation of Doxorubicin, that is a P-gp substrate, on the HT29/DOX cells was evaluated.

3.2.1. Chemistry

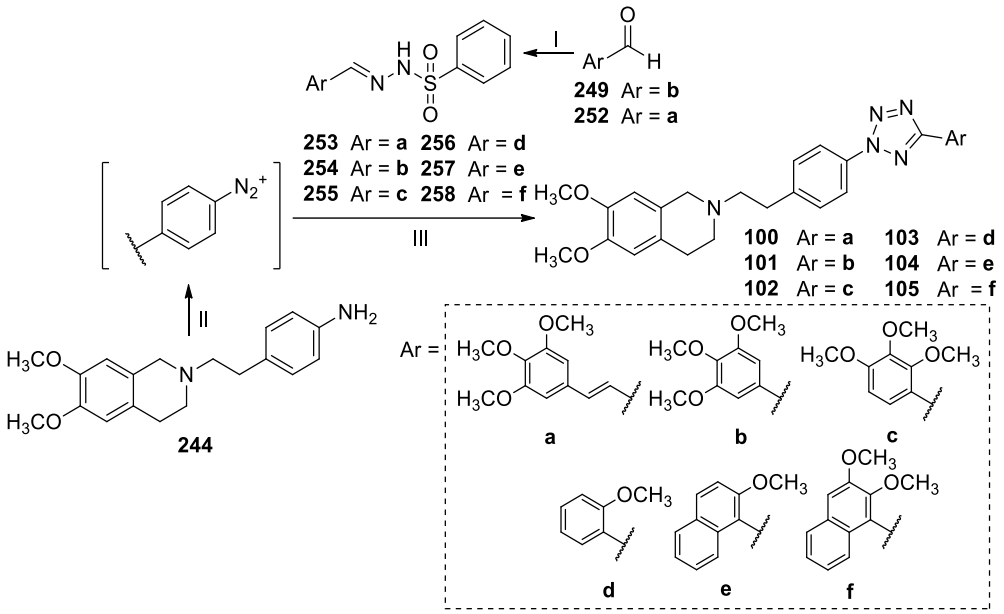
3.2.1.1. 2,5-disubstituted-2*H*-tetrazoles

The reaction pathways used to synthesize derivatives **100-105** are reported in Schemes 3.3-3.4. The key intermediates needed to achieve the 2,5-disubstituted-2*H*-tetrazoles **100-105** were the suitable methoxy-substituted aryl benzenesulfonohydrazide **253-258** and the diazonium salt of the aniline **244**[91]. The benzenesulfonohydrazides **253-258** were prepared starting from the corresponding aldehydes. 2,3,4-Trimethoxy-1-benzaldehyde, 2-methoxy-1-benzaldehyde, 2-methoxy-1-naphthaldehyde and 2,3-dimethoxy-1-naphthaldehyde are commercially available, while 3,4,5-trimethoxy-1-benzaldehyde **249** was synthesized by oxidation of the proper alcohol with pyridinium chlorochromate (PCC) and Celite in dry CH_2Cl_2 (Scheme 3.3). To synthesize the aldehyde **252**[91], the (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid was first transformed in the corresponding methyl ester **250**, then reduced with DIBAL-H in dry CH_2Cl_2, yielding the alcohol **251**[104], which was oxidated with PCC in dry CH_2Cl_2 to afford the aldehyde **252**, following the procedures reported in ref [91] (Scheme 3.3).



Scheme 3.3: *Reagents and conditions*: I) PCC, Celite, dry CH_2Cl_2, 5h; II) conc. H_2SO_4, CH_3OH; III) DIBAl-H (1 M in toluene), dry CH_2Cl_2, -78 °C, 1 h; IV) PCC, celite, dry CH_2Cl_2, 5h.

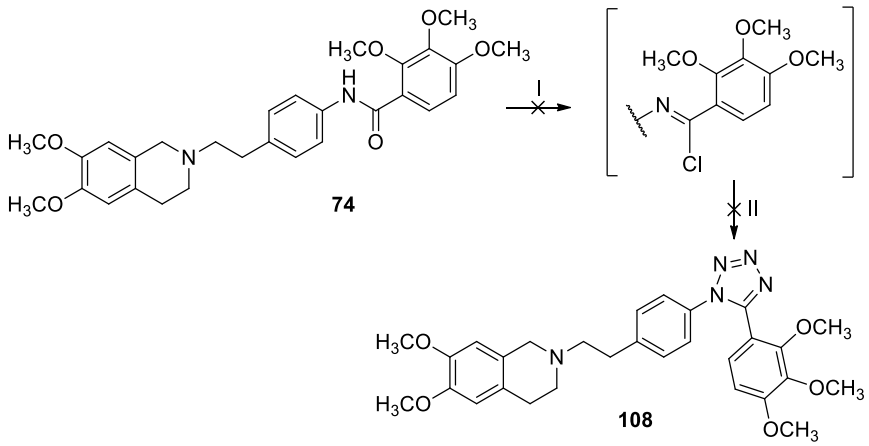
Thus, the proper aldehydes were treated with benzenesulfonyl hydrazide in EtOH[105], yielding the corresponding benzenesulfonohydrazides **253-258** (Scheme 3.4). Then, the aniline **244**[91] was reacted with sodium nitrite in an acid mixture of $EtOH/H_2O$, affording the corresponding diazonium salt[101], which was not isolated but treated with the proper benzenesulfonohydrazide in pyridine between -10 °C and -15 °C, following the procedure described by Köhler et al.[101]: in this way, the 2,5-dibustituted-2*H*-tetrazoles **100-105** were obtained in general with good yields (Scheme 3.4).



Scheme 3.4: *Reagents and conditions*: I) benzenesulfonyl hydrazide, EtOH, rt, 5 h; II) $NaNO_2$, HCl conc., EtOH, 0 °C; III) pyridine, T= -10 to -15 °C.

3.2.1.2. 1,5-disubstituted-1*H*-tetrazoles

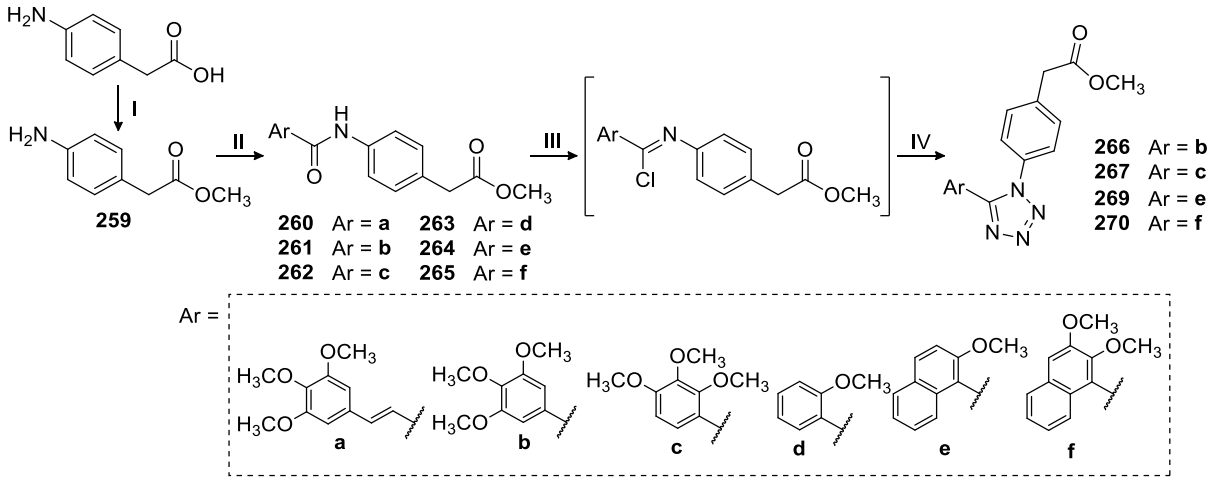
The reaction pathways used to synthesize the 1,5-disubstituted-1*H*-tetrazoles **106-111** are reported in Schemes 3.5-3.9. The first approach was to transform the amide derivatives **74-86**, shown in Par. 3.1, in the corresponding 1,5-disubstituted-1*H*-tetrazoles with a one-step procedure. For this purpose, the amide **74** was first treated with $SOCl_2$ in $CHCl_3$ (free of ethanol)[106] or with Cl_4Si in dry CH_3CN[107], to obtain the corresponding imidoyl chloride that was not isolated, but in presence of NaN_3 in dry DMF it should have led to the 2,3,4-trimethoxyphenyl-substituted-1*H*-tetrazole **108** (Scheme 3.5).



Scheme 3.5: *Reagents and conditions*: I) $SOCl_2$, $CHCl_3$ (free of ethanol), 80 °C or Cl_4Si, dry CH_3CN, 90 °C, 48 h; II) NaN_3, dry DMF, rt, 48 h.

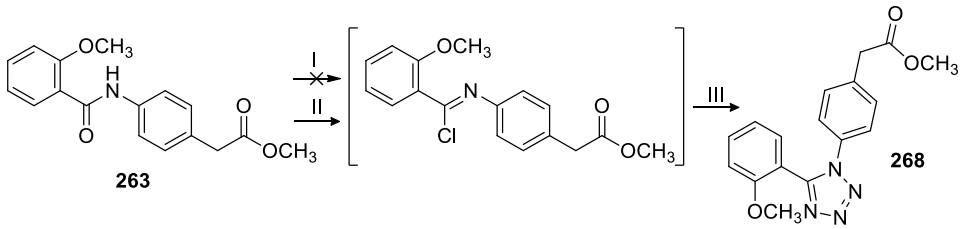
Unfortunately, this procedure did not allow obtaining the desired compound **108**, thus we decided to perform a different synthetic procedure for all the compounds.
First, the commercially available 2-(4-aminophenyl)acetic acid was transformed in the corresponding methyl ester **259** in dry methanol, then it was treated with the proper methoxy-

substituted aryl carboxylic acid, to afford the corresponding amides **260-265** (Scheme 3.6). Most of the methoxy-substituted aryl acids are commercially available, while 2-methoxy-1-naphthoic acid **246** and 2,3-dimethoxy-1-naphthoic acid **247** were synthetized as reported in Par 3.1.1. Then, the amides **261**, **262**, **264** and **265** were transformed by treatment with oxalyl chloride in dry CH_2Cl_2 in the proper imidoyl chloride, which was not isolated, but cyclized in presence of NaN_3 in dry DMF[108], affording the corresponding tetrazoles **266**, **267**, **269** and **270** (Scheme 3.6).



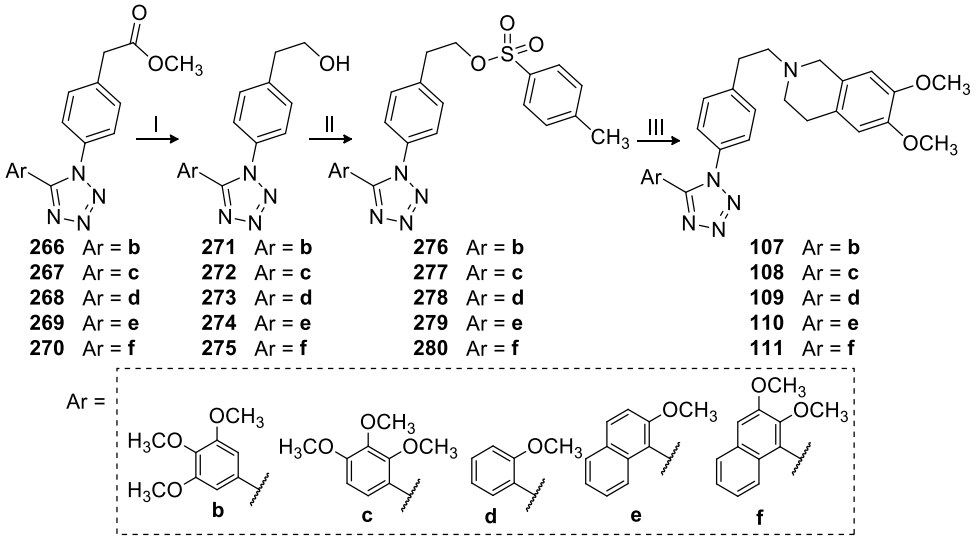
Scheme 3.6: *Reagents and conditions*: I) $SOCl_2$, dry CH_3OH, reflux, 3 h; II) ArCOCl, $CHCl_3$ (free of ethanol), rt, 18 h or ArCOOH, EDC hydrochloride, DMAP, dry CH_2Cl_2, rt, 48 h; III) oxalyl chloride, pyridine, dry CH_2Cl_2, 24 h; IV) NaN_3, dry DMF, overnight.

In the case of the tetrazole **268**, carrying the 2-methoxyphenyl moiety, different conditions were used (Scheme 3.7). First, the amide **263** was refluxed with $SOCl_2$, without obtaining the desired intermediate. Then, **263** was treated with PCl_5 (*Method A*)[109] or oxalyl chloride (*Method B*)[108] in dry CH_2Cl_2, yielding the imidoyl chloride, which was not isolated but reacted with azidotrimethylsilane (*Method A*)[109] or NaN_3 (*Method B*)[108] to afford the tetrazole **268**. Notably, the highest yield was obtained following Method B.



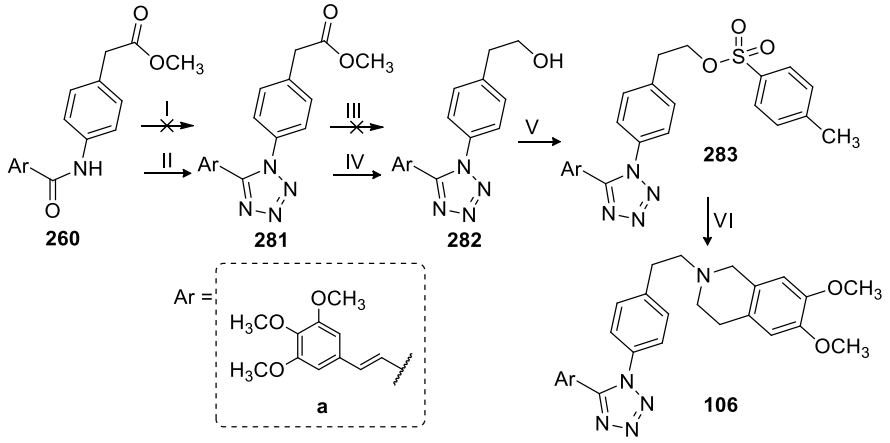
Scheme 3.7: *Reagents and conditions*: I) $SOCl_2$, reflux, 5 h; II) *Method A*: PCl_5, dry pyridine, dry CH_2Cl_2, reflux, overnight. *Method B*: oxalyl chloride, dry pyridine, dry CH_2Cl_2, rt, 24 h; III) *Method A*: azidotrimethylsilane, rt, overnight. *Method B*: NaN_3, dry DMF, 60 °C, overnight.

Once obtained the intermediates **266-270**, their ester groups were reduced using $LiAlH_4$ in dry THF, then the alcoholic function was transformed in the corresponding tosyl derivative (compounds **276-280**) by reaction with *p*-toluenesulfonyl chloride in dry CH_2Cl_2, to achieve higher yields in the following reaction. Finally, compounds **107-111** were synthesized by reaction of the tosyl derivatives **276-280** with 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride in dry CH_3CN in presence of Et_3N (Scheme 3.8).



Scheme 3.8: *Reagents and conditions*: I) $LiAlH_4$, dry THF, rt, 1 h; II) *p*-toluenesulfonyl chloride, Et_3N, dry CH_2Cl_2, rt, overnight; III) 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride, Et_3N, dry CH_3CN, rt, overnight.

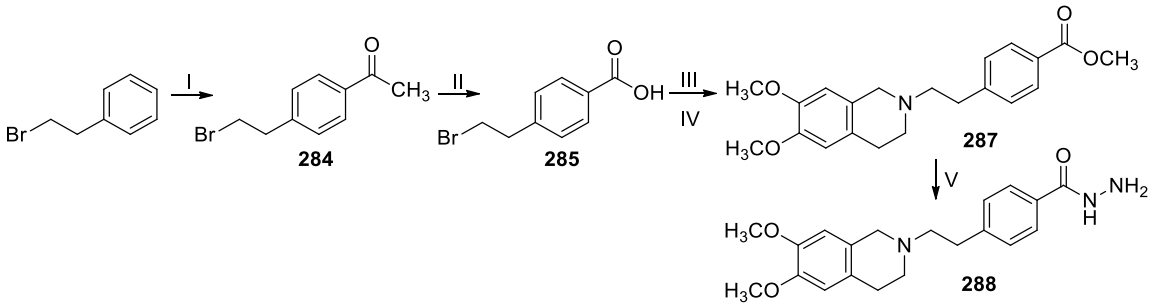
Instead, to synthesize the (*E*)-3-(3,4,5-trimethoxyphenyl)vinyl derivative **106**, a different procedure was performed (Scheme 3.9). The amide **260** was first treated with oxalyl chloride and NaN_3,[108] without obtaining the tetrazole **281**. Therefore, **260** was transformed, in presence of PCl_5, in the corresponding imidoyl chloride, that was reacted with NaN_3, affording **281** with good yields. Then, we tried to reduce the ester group of **281** with $LiAlH_4$, but this procedure did not allow us to obtain compound **282**. Thus, **281** was treated with DIBAL-H in dry CH_2Cl_2, yielding the desired intermediate **282**. Finally, **282** was transformed in the tosyl derivative **283** which was reacted with 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride to afford final compound **106**.



Scheme 3.9: *Reagents and conditions*: I) oxalyl chloride, dry pyridine, dry CH_2Cl_2, rt, 24 h, then NaN_3, dry DMF, 60 °C, overnight; II) PCl_5, dry pyridine, dry CH_2Cl_2, reflux, overnight, then NaN_3, dry DMF, 60 °C, overnight; III) $LiAlH_4$, dry THF, rt, 1 h; IV) DIBAL-H, dry CH_2Cl_2, -30 °C -15 °C, 2 h; V) p-toluenesulfonyl chloride, Et_3N, dry CH_2Cl_2, rt, overnight; VI) 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride, Et_3N, dry CH_3CN, rt, overnight.

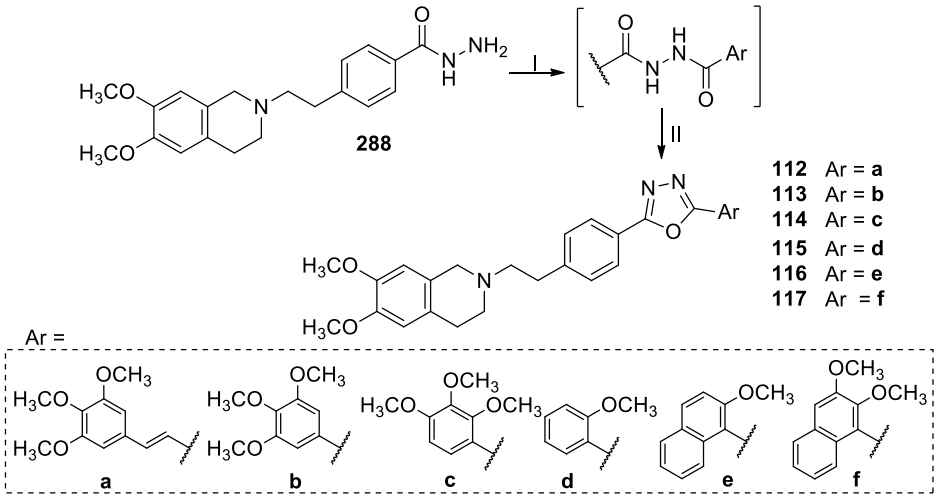
3.2.1.3. 2,5-disubstituted-1,3,4-oxadiazoles

The reaction pathways used to synthesize the oxadiazole derivatives **112-117** are reported in Schemes 3.10-3.11. The key intermediates needed to achieve the 2,5-disubstituted-oxadiazoles **112-117** were the suitable methoxy-substituted aryl acid and the benzohydrazide **288**, carrying the 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline nucleus.
To synthesize **288**, the commercially available (2-bromoethyl)benzene was first transformed in the acyl intermediate **284** in presence of $AlCl_3$ and acetyl chloride[110], by the Friedel-Crafts acylation, then in the corresponding carboxylic acid **285** with Br_2 under basic conditions[111] (Scheme 3.10). **285** was esterified with $SOCl_2$ in dry CH_3OH in compound **286** that reacted with 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline in dry CH_3CN, yielding the intermediate **287**. Finally, the desired hydrazide **288** was synthesized by reaction of **287** with hydrazine hydrate in ethanol (Scheme 3.10). First, **288** was purified by flash chromatography, but this procedure led to very low yields, probably because the hydrazide is not stable in these conditions. Then, we decided to use the reaction mixture without further purifications.



Scheme 3.10: *Reagents and conditions*: I) $AlCl_3$, CH_3COCl, an. CH_2Cl_2, rt, overnight; II) Br_2, NaOH in H_2O/dioxane, rt, 1.5 h; III) $SOCl_2$, dry CH_3OH, 65 °C, 3 h; IV) 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, K_2CO_3, an. CH_3CN, 80 °C, 18 h; V) $NH_2NH_2.H_2O$, EtOH, 80 °C, 24 h.

Following the procedure described by Stabile et al.[112], final compounds **112-117** were obtained by reaction of the methoxy-substituted aryl acid with the hydrazide **288** and successive cyclocondensation of the resulting diacylhydrazine intermediates (Scheme 3.11). Most of the carboxylic acids are commercially available, while 2-methoxy-1-naphthoic acid **246** and 2,3-dimethoxy-1-naphthoic acid **247** were synthetized as reported in Par 3.1.1. Particularly, the proper acid was first activated, using HATU as the coupling agent in presence of DIPEA, then reacted with the hydrazide **288**. In this way, we obtained the proper diacylhydrazine intermediate, which was not isolated, but cyclized in presence of *p*-toluenesulfonyl chloride as dehydrating agent, affording final compounds **112-117** (Scheme 3.11).



Scheme 3.11: *Reagents and conditions*: I) ArCOOH, HATU, DIPEA, dry CH_3CN, rt, 4 h; II) DIPEA, *p*-toluenesulfonyl chloride, dry CH_3CN, rt, 16 h.

3.2.2. Results and discussions

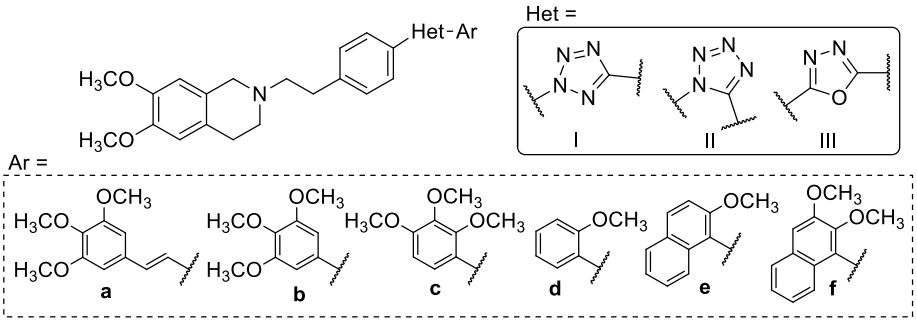
3.2.2.1. Biological activity: characterization of ABC transporters selectivity

The activity of these new compounds on P-gp, MRP1 and BCRP was evaluated by the same biological assays described for amide and ester derivatives (see Par 3.1.2.1). The obtained results are reported in Table 3.2: till now, only tests on P-gp were concluded, while those on the other two transporters are ongoing.

All these compounds were active on P-gp with different extent: the most interesting results were obtained for the 2,5-disubstituted heterocycles. As regards the tetrazoles, the 2,5-disubstituted ones **100**, **103** and **104** showed a potency in the low nanomolar range (EC_{50} = 6.8 nM, 40.8 nM and 90.3 nM, respectively). Interestingly, the oxadiazoles **112-117** were the most potent compounds since they displayed the lowest EC_{50} values. Among these, **112**, bearing the *(E)*-3-(3,4,5-trimethoxyphenyl)vinyl moiety (**a**), showed EC_{50} = 1 nM, and this result is very promising since it displayed higher activity than lead compounds Elacridar and Tariquidar. Concerning the aryl residues, the *(E)*-3-(3,4,5-trimethoxyphenyl)vinyl (**a**), 2-methoxyphenyl (**d**) and the 2-methoxynaphthalene (**e**) conferred the highest P-gp inhibitory effects to both series of 2,5-disubstituted heterocycle derivatives. The 1,5-disubstituted tetrazoles, instead, showed the lowest effects, regardless the aryl group to which the tetrazole is linked.

As regards the other transporters, at the moment only several tetrazole derivatives were tested. Compounds **100-102** and **109** were active also on BCRP and, among these, **101** displayed the highest potency (EC_{50} = 0.13 μM). Interestingly, the 1,5-disubstituted tetrazoles **107** and **108** showed a moderate activity on MRP1. So, if compared to the corresponding amide and ester derivatives (see ref. [91] and Par 3.1.), the introduction of the tetrazole ring improves the effect on BCRP and introduces a moderate inhibitory activity on MRP1.

Table 3.2: MDR-reversing activity of tetrazole and oxadiazole derivatives **100-117**.



Cmpd	Het	Ar	P-gp (EC_{50}) μM[a]	MRP1 (EC_{50}) μM[a]	BCRP (EC_{50}) μM[a]
100	I	a	0.0068±0.0028	NA	29.2±4.80
101	I	b	0.22±0.04	NA	0.13±0.01
102	I	c	0.53±0.10	NA	1.1±0.22
103	I	d	0.060±0.012	ND	ND
104	I	e	0.090±0.0037	NA	NA
105	I	f	0.23±0.02	NA	NA
106	II	a	2.15±0.43	ND	ND
107	II	b	0.61±0.06	2.5±0.50	NA
108	II	c	0.74±0.14	8.3±1.60	NA
109	II	d	1.00±0.18	NA	58.4±10.82
110	II	e	0.66±0.13	ND	ND
111	II	f	0.47±0.10	ND	ND
112	III	a	0.0010±0.00018	ND	ND
113	III	b	0.038±0.0075	ND	ND
114	III	c	0.41±0.010	ND	ND
115	III	d	0.050±0.0094	ND	ND
116	III	e	0.025±0.0030	ND	ND
117	III	f	0.036±0.0060	ND	ND
Tariq.			0.044±0.001	ND	0.010±0.005
Elacr.			0.014±0.003	NA	10.0±2.0

[a] Values are the mean ± SEM of two independent experiments, with samples in triplicate.
NA = not active; ND = not determined.

3.2.2.2. Enhancement of Doxorubicin cytotoxicity and Doxorubicin accumulation assays

To further evaluate their P-gp inhibitory activity, all the oxadiazoles were tested in combination with Doxorubicin on the chemosensitive and the resistant HT29 cell lines, and both the enhancement of drug cytotoxicity and drug intracellular accumulation in the presence of our P-gp inhibitors were measured.

In the co-administration assays, we measured how our derivatives, used at 1 μM and 10 μM, affected the IC_{50} value of Doxorubicin. All the tested compounds did not exhibit an intrinsic cytotoxicity in both the tested cell lines and did not increase the antineoplastic effect of Doxorubicin in the chemosensitive HT29 cells. In the resistant HT29/DOX cell lines, instead, compounds **112**, **113** and **115-120**, which showed the highest P-gp inhibitory effects on MDCK-MDR1 cells (EC_{50} values < 50 nM, Table 3.2), highly enhanced the cytotoxicity of Doxorubicin, as reported in Figure 3.8. Best results were obtained with the 2-methoxyphenyl derivative **115** that was able to decrease cells' viability from 70 % (with Doxorubicin alone) to

36 % when used at 10 μM. Oxadiazole **114**, instead, showed the lowest effects, as in the Calcein-AM transport assay (Table 3.2).

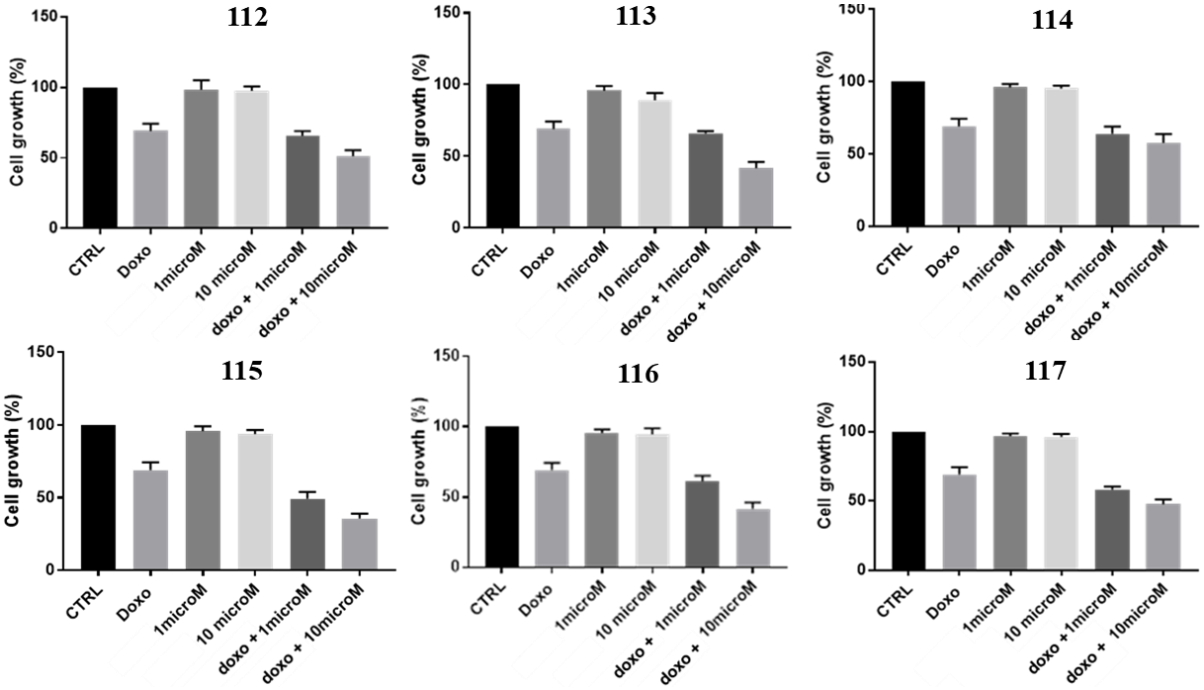


Figure 3.8: enhancement of Doxorubicin cytotoxicity assay in presence of the oxadiazole derivatives **112-117** in resistant HT29/DOX cells.

Moreover, when used at 10 μM, all these compounds were able to increase the accumulation of Doxorubicin on the HT29/DOX cells, as reported in Figure 3.9: interestingly, **115** allowed a 4 times greater accumulation of the drug.

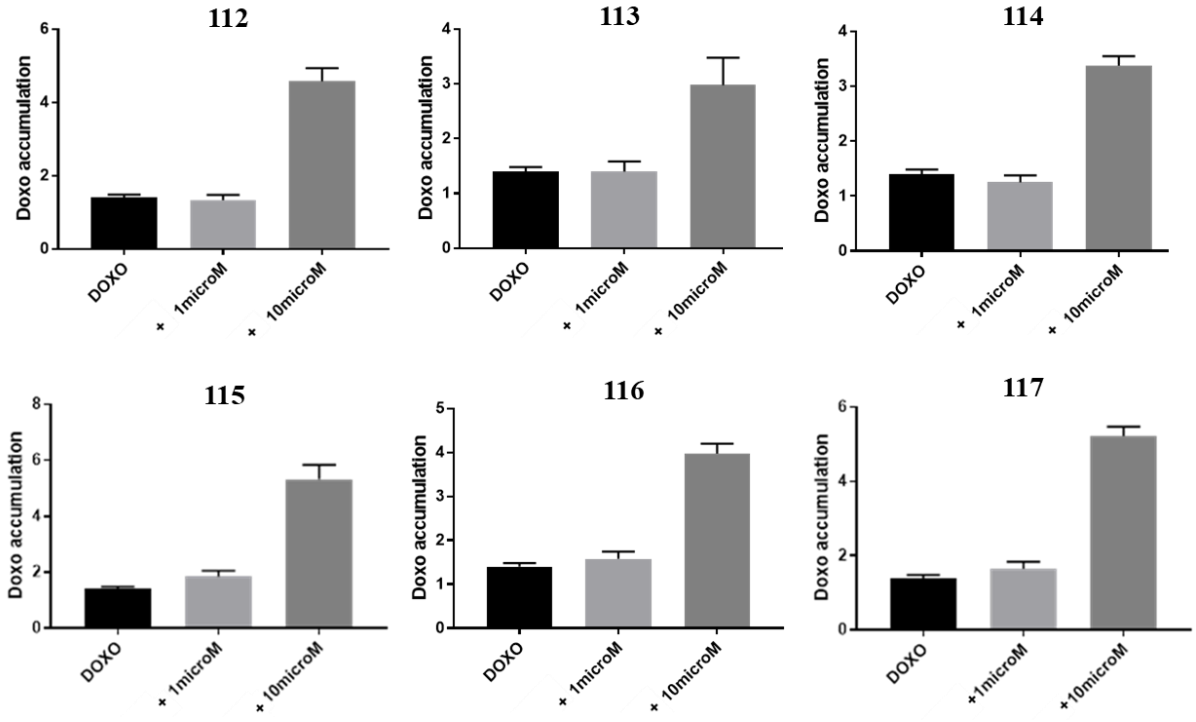


Figure 3.9: Doxorubicin accumulation assay in presence of the oxadiazole derivatives **112-117** in resistant HT29/DOX cells.

4. Quinazoline derivatives

This series of compounds is another part of the project based on the design and synthesis of potent MDR reversers with inhibitory activity both on P-gp and BCRP transporters. These new 2,4-disubstituted quinazoline derivatives present the scaffold of the quinazoline-4-amine based tyrosine kinase inhibitors (TKIs), such as Gefitinib and Erlotinib, that have been identified as potent P-gp and BCRP modulators[113,114] (Figure 4.1). Moreover, in 2017 Qiu et al.[115], described a series of quinazoline derivatives that proved to be potent P-gp inhibitors (Figure 4.1 **A**).

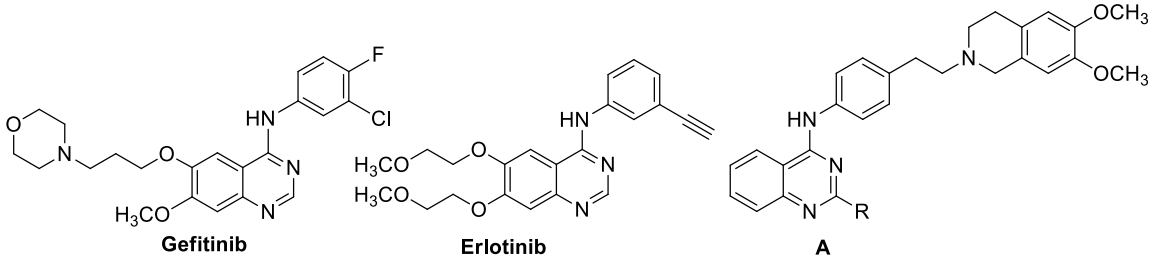


Figure 4.1: structures of Gefitinib and Erlotinib, and of the general structure (**A**) of quinazoline derivatives synthesized by Qiu et al.[115].

Thus, we designed and synthesized a new series of 2,4-disubstituted quinazolines as new P-gp and/or BCRP inhibitors (Figure 4.2). For this purpose, secondary or tertiary protonable amines (**I**-**V**) were inserted in position 4 of the quinazoline scaffold, while in position 2 we introduced aromatic groups, as anthracene or methoxy-substituted aryl moieties, found in P-gp-dependent MDR reversers[91,94]. Amine **I** was chosen since it is present in Elacridar and Tariquidar that are also able to bind the BCRP transporter, while the others (**II**-**V**) were inserted to vary both the steric hindrance and the electronic proprieties.

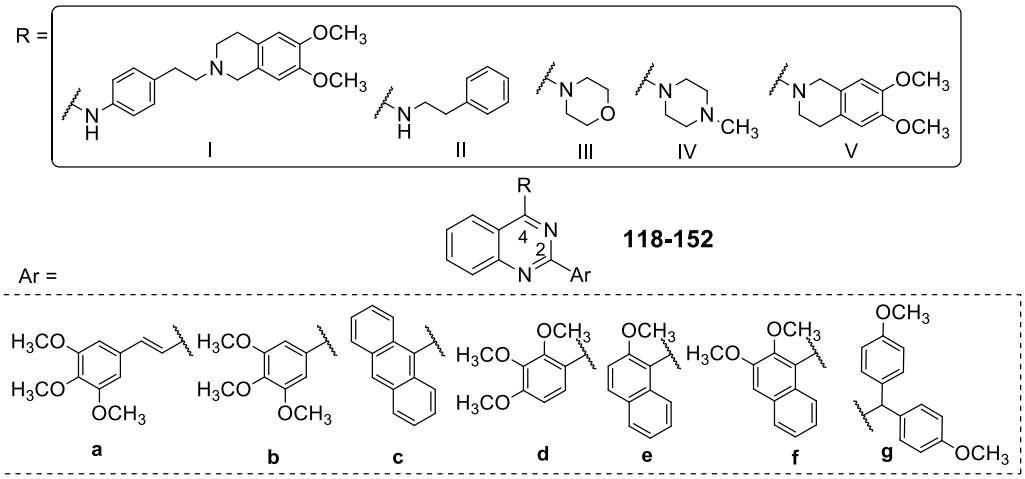


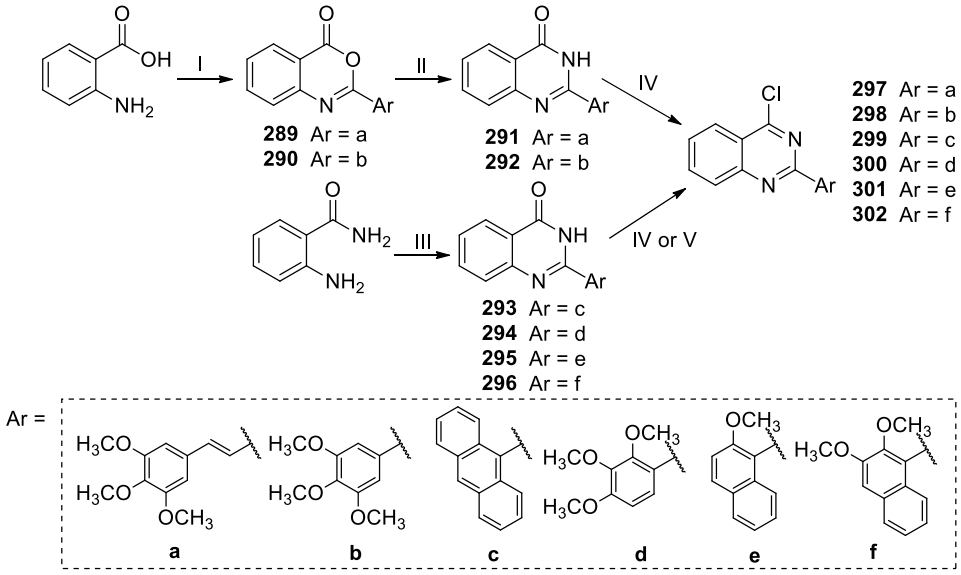
Figure 4.2: structures of quinazoline derivatives synthesized in this PhD thesis.

The ability of these quinazoline derivatives to inhibit the three ABC transporters was evaluated by the biological assays described for the Tariquidar analogues (see Par 3.1.2.1). Furthermore, the most potent P-gp inhibitor of this series was further tested alone and in co-administration with Doxorubicin in different resistant cancer cell lines, and its ability to increase the intracellular concentration of Doxorubicin in HT29/DOX cells, that overexpress P-gp, was

evaluated. Moreover, molecular docking simulation studies were performed to identify the binding mode of these compounds within the P-gp binding pocket.

4.1. Chemistry

The key intermediates needed to achieve final compounds **118-152** were the 4-chloroquinazolines **297-302** and **306**, which were synthesized by reaction of the proper quinazolin-4(3*H*)-one **291-296** and **305** with $SOCl_2$ in $CHCl_3$ (free of ethanol)[115] or $POCl_3$[116] (Scheme 4.1-4.2). Notably, while the 4-chloroquinazolines **297-299**, **301** and **302** were synthesized in presence of $SOCl_2$ as reported in ref. [115], this procedure did not allow us to obtain **300** (Ar = **d**). Indeed, the quinazolin-4(3*H*)-one **294** was transformed into the corresponding chloroderivative **300** by treatment with $POCl_3$ at 110 °C. Quinazolin-4(3*H*)-ones **291-296** were obtained following two different procedures (Scheme 4.1). On one hand, the commercially available 2-aminobenzoic acid was reacted with freshly prepared acyl chlorides in dry pyridine, affording the intermediates **289** and **290**[115], which were treated with ammonia water in ethanol,[115] to obtain the quinazolin-4(3*H*)-ones **291** and **292**[115] (Ar = **a**, **b**). On the other hand, **293-296** (Ar = **c**-**f**) were synthesized by reaction of the commercially available anthranilamide, the proper aldehyde and $CuCl_2$ in ethanol, with very good yields[117].

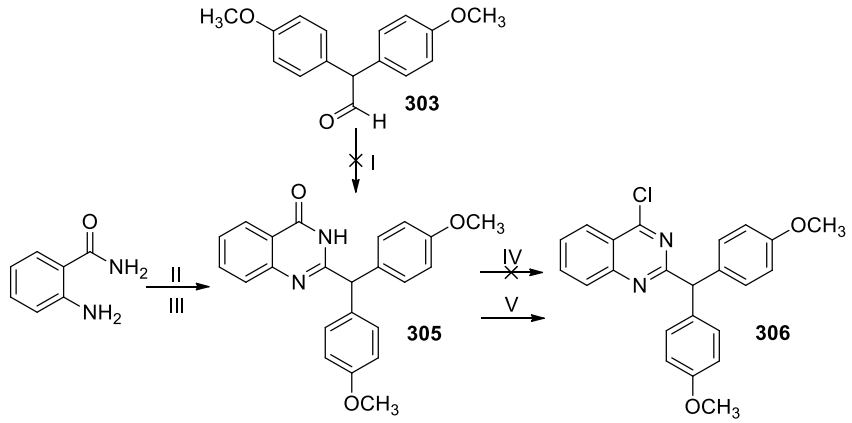


Scheme 4.1: *Reagents and conditions:* I) ArCOCl, dry pyridine, rt, 4 h; II) NH_4OH (33.0 %), EtOH, 80 °C, 20 h; III) ArCHO, $CuCl_2$, EtOH, reflux, 16 h; IV) $SOCl_2$, dry DMF, $CHCl_3$ (free of ethanol), 50 °C, 6 h; V) $POCl_3$, reflux, 12 h.

To synthesize the quinazolin-4(3*H*)-one **305** that carries the 2,2-bis(4-methoxyphenyl) moiety, the aldehyde **303**[91] and the 2,2-bis(4-methoxyphenyl)acetic acid **304**[85] were synthesized as reported in literature[85,91]. The reaction of **303**[91] with $CuCl_2$ in ethanol[117] did not provide compound **305**. Thus, to synthesize it, two different procedures were followed:

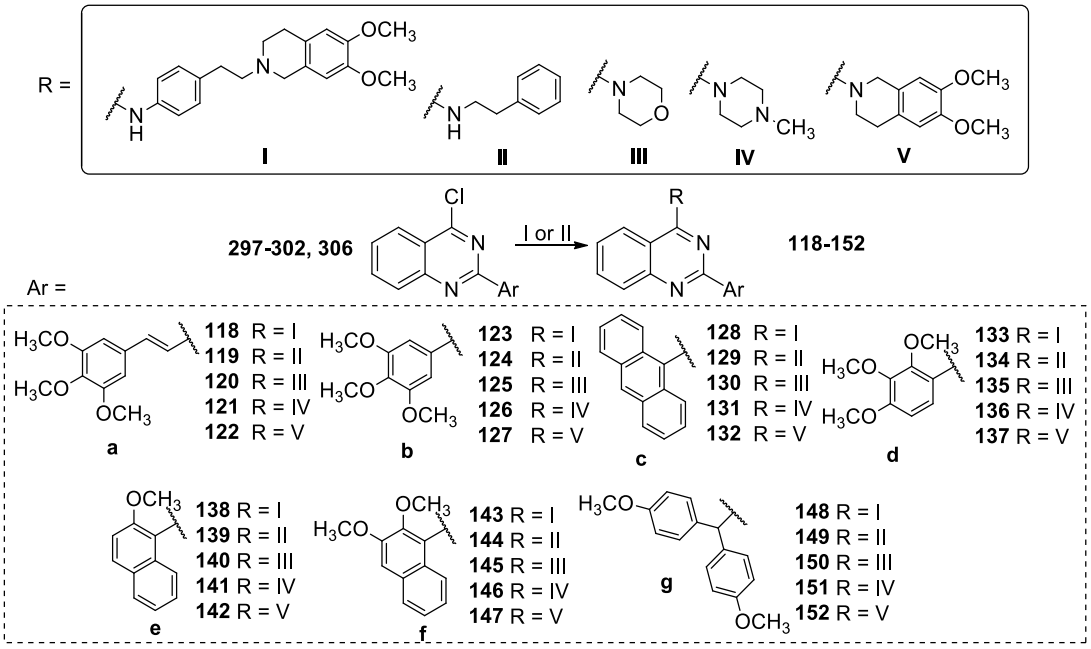
1. *Method A*: **304**[85] was transformed in the corresponding acyl chloride that was reacted with anthranilamide, yielding **305** with good yields (Scheme 4.2).
2. *Method B*[118]: through a coupling reaction between anthranilamide and **304**[85], by using HATU as the activating agent in presence of DIPEA, quinazolin-4(3*H*)-one **305** was obtained with the highest yields (Scheme 4.2).

Then, to synthesize the 4-chloroquinazoline **306**, the intermediate **305** was treated with $SOCl_2$ as reported in ref. [115], but this procedure did not provide the desired compound. Thus, quinazolin-4(3*H*)-one **305** was reacted with $POCl_3$, obtaining **306** with very good yields[116] (Scheme 4.2).



Scheme 4.2: *Reagents and conditions:* I) $CuCl_2$, EtOH, reflux, 16 h; II) *Method A*: 2,2-bis(4-methoxyphenyl)acetyl chloride, K_2CO_3, dry CH_3CN, 80 °C, 18 h, then NaOH (10.0 M), EtOH, rt; III) *Method B*: **304**[85], HATU, DIPEA, dry CH_2Cl_2, 50 °C, 16 h, then NaOH (10.0 M), EtOH, rt, 2 h; IV) $SOCl_2$, dry DMF, $CHCl_3$ (free of ethanol), 50 °C, 6 h; V) $POCl_3$, reflux, 5 h.

The reaction pathway used to synthesize final compounds **118-152** is reported in Scheme 4.3: the proper 4-chloroquinazoline (**297-302** and **306**) was reacted with the suitable amine in presence of methanesulfonic acid in abs. ethanol (*Method A*)[115], or in the presence of K_2CO_3 in dry DMF (*Method B*) (for details, see the Experimental Section). Most of the used amines are commercially available (2-phenylethanamine, morpholine, 1-methylpiperazine and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) while **244**[91] was synthesized as reported in ref. [91].



Scheme 4.3: *Reagents and conditions*: I) *Method A*: amines, CH_3SO_3H, abs. EtOH, reflux, 4 h; II) *Method B*: amines, K_2CO_3, dry DMF, 60 °C, 5 h.

4.2. Results and discussions

4.2.1. Biological activity: characterization of P-gp interacting profile and ABC transporters selectivity

The activity of these new compounds on P-gp, MRP1 and BCRP was evaluated by the same biological assays described for Tariquidar analogues (see Par 3.1.2.1), and the obtained results are reported in Table 4.1.

All compounds were able to inhibit the P-gp-mediated Calcein-AM transport, except for compounds **129** and **130**, carrying the aryl residue **c**, which were inactive. Most compounds showed EC_{50} values below 1 μM reaching also the nanomolar range as in the case of compounds **121**, **122**, **123**, **125** and **127** (EC_{50} = 36.0 nM, 31.3 nM, 50.0 nM, 85.6 nM and 58.9 nM, respectively). A thorough evaluation of the P-gp inhibition values indicated that the activity of these compounds was influenced by both the substituents in positions 2 and 4. Best results were obtained for the (*E*)-3-(3,4,5-trimethoxyphenyl)vinyl (**a**) and 3,4,5-trimethoxyphenyl (**b**) derivatives: indeed, all compounds of these two sets showed EC_{50} values in the submicromolar or nanomolar range, except for compound **126**. Otherwise, compounds bearing the aryl moieties **c**-**f**, showed low inhibitory effect on P-gp. Lastly, all the 2,2-bis(4-methoxyphenyl) (**g**) derivatives showed EC_{50} values below 1 μM, except for compound **150**. As regards the substitution in position 4 of the quinazoline scaffold, best results, within each set of the series, were obtained for derivatives carrying amines **I** and **V**: all these compounds showed in general EC_{50} values in the submicromolar or nanomolar range.

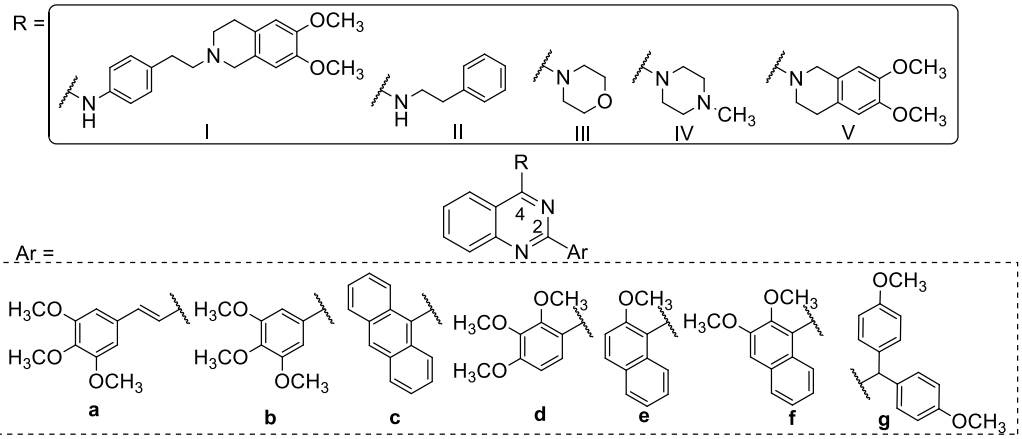
Moreover, only few compounds displayed a good/moderate activity on MRP1: the most potent compounds were **125-127**, carrying the aryl residue **b**, and showing EC_{50} = 3.97 μM, 2.77 μM and 2.10 μM, respectively.

As regard the BCRP inhibition, compounds **119** and **120** (Ar = **a**), and **123** and **124** (Ar = **b**) displayed the highest EC_{50} values (EC_{50} = 0.40 μM, 0.96 μM, 0.26 μM and 0.31 μM, respectively). Moreover, **121**, **125**, **126**, **131** and **151** showed a moderate activity on BCRP, while all the other compounds were inactive.

Interestingly, most of the compounds able to modulate the BCRP activity also showed a significant effect on P-gp and MRP1 except for compounds **123** and **131** that were inactive towards MRP1. Therefore, compound **123** showed the best combination of activity on P-gp and BCRP (EC_{50} = 0.05 μM on P-gp and EC_{50} = 0.26 μM on BCRP) and compound **122** was the most active and selective P-gp ligand.

As regards the P-gp interacting profile, only compound **150**, having a P_{app} = 1.5, inhibiting Calcein-AM transport and do not inducing ATP cell depletion, may be defined as P-gp inhibitor. Moreover, **149** may be defined as a P-gp unambiguous substrate (category I) since it was able to induce ATP cell depletion and to inhibit Calcein-AM transport with a $P_{app} > 2$. The other compounds behaved as not transported substrates (category IIB3).

Table 4.1: Biological results of compounds **118-152**: inhibition activity on MDCK-MDR1, MDCK-MRP1 and MDCK-BCRP cells, overexpressing P-gp, MRP1 and BCRP, respectively.



Cmpd	R	Ar	P-gp (EC_{50}) µM[a]	MRP1 (EC_{50}) µM[a]	BCRP (EC_{50}) µM[a]	ATP cell depletion	P_{app}[b]
118	I	a	0.14±0.02	NA	NA	No	23.4
119	II	a	0.27±0.04	40.0±8.0	0.40±0.08	No	13.5
120	III	a	0.37±0.06	7.66±1.50	0.96±0.18	No	9.9
121	IV	a	0.0360±0.006	52.5±10.0	4.80±0.96	No	9.4
122	V	a	0.0313±0.005	23.0±4.6	NA	No	23.0
123	I	b	0.0500±0.001	NA	0.26±0.050	No	16.1
124	II	b	0.38± 0.06	10.00±1.89	0.31±0.06	No	3.6
125	III	b	0.0856±0.014	3.97±0.66	3.62±0.60	No	6.1
126	IV	b	1.21±0.22	2.77±0.50	7.70±1.50	No	7.4
127	V	b	0.0589±0.011	2.10±0.40	NA	No	9.3
128	I	c	0.38±0.06	NA	NA	No	17.0
129	II	c	NA	NA	NA	ND	10.5
130	III	c	NA	NA	NA	ND	20.0
131	IV	c	8.95±1.60	NA	7.2±1.40	No	9.8
132	V	c	1.14±0.20	NA	NA	No	18.5
133	I	d	0.24±0.03	NA	NA	No	6.3
134	II	d	1.41±0.25	52.0±10.1	NA	No	14.8
135	III	d	9.55±1.80	NA	NA	No	3.8
136	IV	d	8.64±1.50	NA	NA	No	3.9
137	V	d	0.87±0.16	8.47±1.60	NA	No	6.6
138	I	e	0.20±0.04	NA	NA	No	16.6
139	II	e	4.00±0.6	NA	NA	No	3.9
140	III	e	1.25±0.20	NA	NA	No	5.2
141	IV	e	6.06±1.20	NA	NA	No	5.4
142	V	e	0.40±0.08	NA	NA	No	5.1
143	I	f	0.20±0.04	NA	NA	No	19.2
144	II	f	2.64±0.50	NA	NA	No	6.2
145	III	f	10.0±1.88	NA	NA	No	5.4
146	IV	f	1.46±0.28	NA	NA	No	3.4
147	V	f	0.27±0.05	NA	NA	No	11.8
148	I	g	0.11±0.02	NA	NA	No	32.9
149	II	g	0.89±0.16	NA	NA	Yes[c]	3.4
150	III	g	2.13±0.40	NA	NA	No	1.5

151	IV	g	0.96±0.18	43.2±8.50	8.62±1.60	No	6.9
152	V	g	0.16±0.026	NA	NA	No	8.4
Tariq.			0.044±0.001	ND	0.010±0.005	Yes[d]	>20
Elacr.			0.014±0.003	NA	10.0±2.0	Yes[e]	>20

[a] Values are the mean ±SEM of two independent experiments, with samples in triplicate. [b] Apparent permeability estimation: values are from two independent experiments, with samples in duplicate. [c] 50% at a concentration of 1 μM; [d] 30% at a concentration of 50 μM; [e] 25% at a concentration of 10 μM. NA = not active. ND = not determined

4.2.2. Enhancement of Doxorubicin cytotoxicity and Doxorubicin accumulation assays

The highly active and selective P-gp ligand, compound **122**, was further studied to evaluate its ability to restore the cytotoxic activity of Doxorubicin in a co-administration assay on the transfected MDCK-MDR1, and HT29 cells, both on the sensible and resistant cell lines. In these assays, Doxorubicin was tested at 10 μM, while **122** was used at different concentrations.

In MDCK-MDR1 cells, **122** alone shows an intrinsic cytotoxicity of around 20-30% at each tested dose, and in the co-administration assay, it enhanced the Doxorubicin cytotoxicity by 50% already at the dose of 500 nM, reaching an increase of 80 % when tested at 10 μM, as depicted in Figure 4.3.

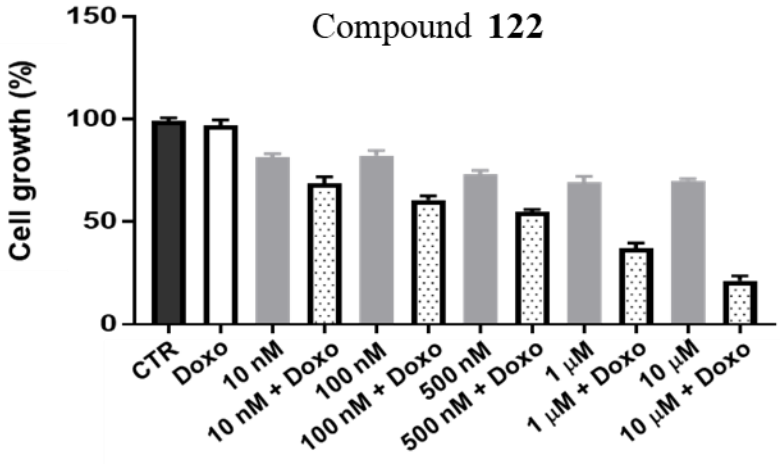


Figure 4.3: Antiproliferative activity on MDCK-MDR1 cells of Doxorubicin at 10 μM and of compound **122** at different concentrations, alone and in co-administration with Doxorubicin. Each bar represents the mean ± SEM of two experiments performed in triplicate.

In the HT29/DOX cell line, compound **122** increase the Doxorubicin cytotoxic effect in a dose-dependent manner, and at a concentration of 10 μM it reduces the cells viability at the same extent of Doxorubicin-sensitive cells (Figure 4.4).

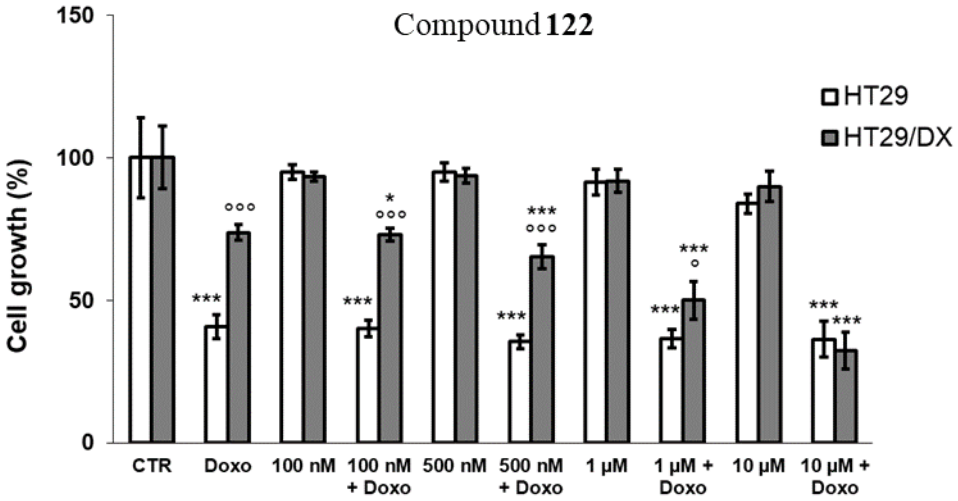


Figure 4.4: Antiproliferative activity on HT29 and HT29/DOX cells of Doxorubicin, alone and in co-administration with compound **122** at different concentrations. Each bar represents the mean ± SEM of two experiments performed in triplicate. One-way analysis of variance (ANOVA) analysis: *$p < 0.05$; ***$p < 0.001$ *vs* control; °$p < 0.05$; °°°$p < 0.001$: HT29/DOX cells *vs* respective HT29 cells

To investigate whether the reduced viability measured in the previous assay was due to a different retention of the anthracycline within the cells, we evaluated the intracellular concentration of Doxorubicin in cells treated with increasing concentrations of **122**. Interestingly, **122** progressively increased the intracellular concentration of Doxorubicin only on HT29/DOX cells, without modify it on the sensible HT29 cell lines, as shown in Figure 4.5. This difference is likely due to the P-gp inhibitory activity of **122**.

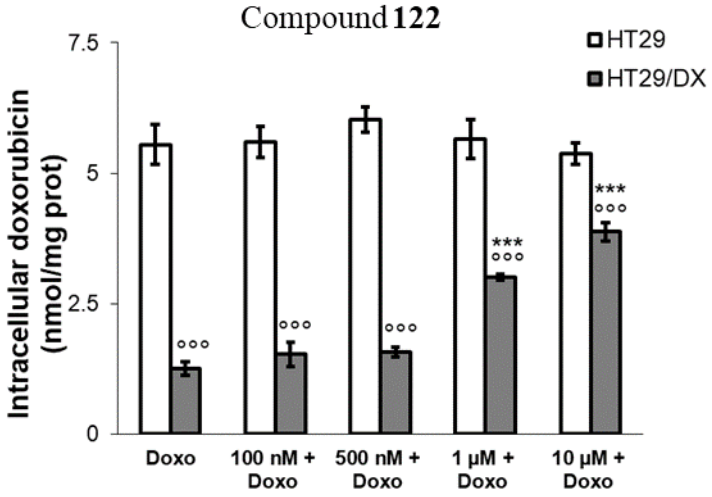


Figure 4.5: Intracellular accumulation of Doxorubicin in HT29 and HT29/DOX cells, incubated 24 h with Doxorubicin (Doxo) at 10 µM, alone and in co-administration with compound **122** at different concentrations. Each bar represents the mean ± SEM of two experiments performed in triplicate. One-way analysis of variance (ANOVA) analysis: ***$p < 0.001$: HT29/DOX *vs* HT29 cells; °°°$p < 0.001$: *vs* Doxo alone.

4.2.3. Molecular Modeling studies

To give a sensible explanation of the activity profile of target compounds towards P-gp, in the laboratory of Prof. Guglielmo from the University of Turin, a molecular docking study was performed using the crystal structure of P-gp in its inward conformation (PDB code 4XWK)[119].

For all the compounds, the quinazoline ring is placed as a "pivot". Depending on the specific position of this moiety, two different patterns can be identified for **121**, **122** and **123**, and for **125** and **127** (Figure 4.6). The 3,4,5-trimethoxyphenyl ring is projected towards the lower limit of the transmembrane region and establishes contacts with TM7 and TM12 (**121**, **125** and **127**) and with TM6 (**122**) and TM12 (**123**). In case of **121** and, to a lesser extent, **127**, this moiety can give polar contacts with Gln986 side chain. The other "arm" of these two molecules is kept in the apical part of the binding region and gives additional hydrophobic contacts (cation-π in case of **121**). Moreover, compounds **123** and **127** display a slightly different binding mode: the two "arms" are both directed downward in a nutcracker fashion, giving hydrophobic interactions and, in case of **123**, polar contacts with Gln986 and with Gln343.

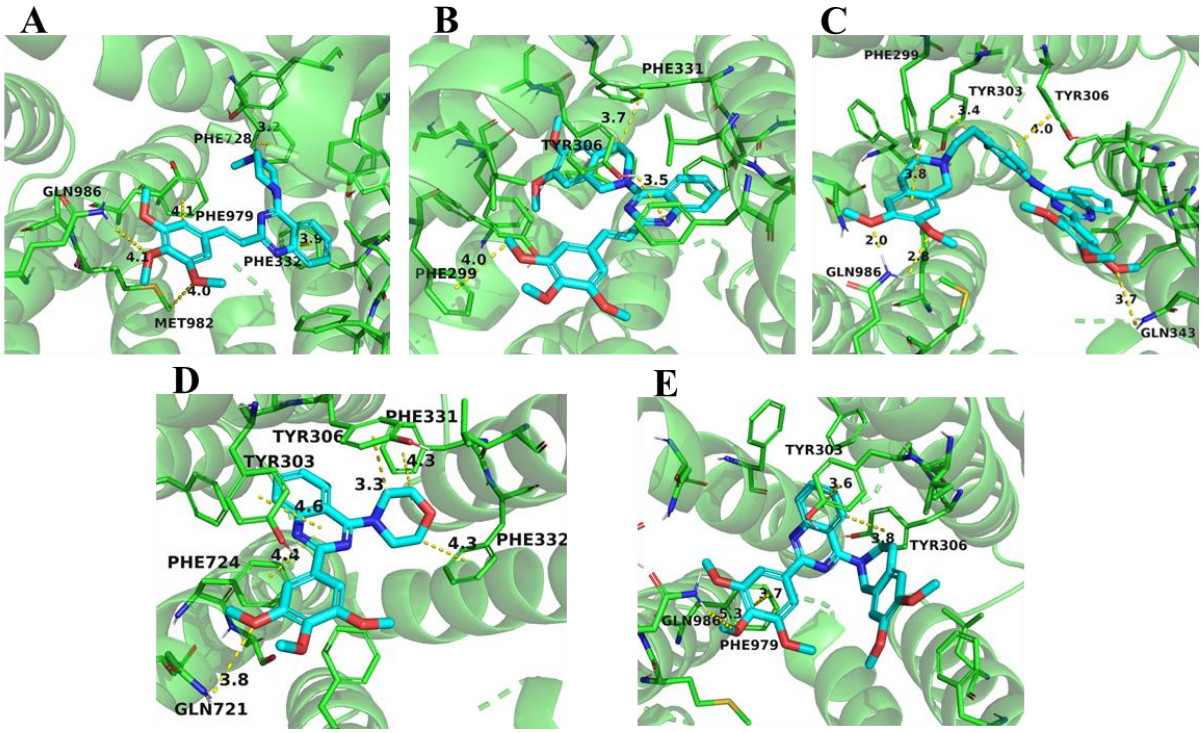


Figure 4.6: Binding poses of the most active compounds **121** (**A**), **122** (**B**), **123** (**C**), **125** (**D**) and **127** (**E**) within the P-gp binding region.

Compound **150**, which is the only ligand of the set showing a behavior of pure inhibitor, with a BA/AB < 2 (Table 4.1), is characterized by a binding pose in a very apical position, inside the internal cavity of P-gp (Figure 4.7). This localization enables the compound to reach three different domains (TM1, TM6 and TM12) with weak hydrophobic interactions. The peculiar pose of the compound and its "cross-linking" ability could be an explanation of the functional profile of this ligand that is able to block the protein, rather than being transported.

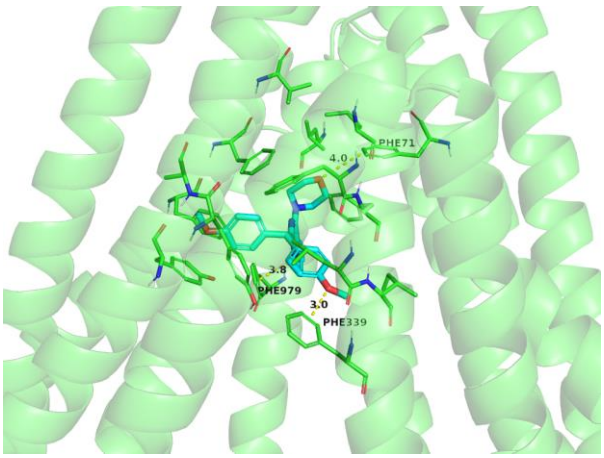


Figure 4.7: Binding pose within the P-gp binding region of the pure inhibitor **150**.

5. P-gp modulators: linear and chiral *N*,*N*-bis(alkanol)amine aryl diesters

Verapamil is the first described compound showing P-gp modulating activity, and it has become a lead compound, on which several modifications were carried out, to identify more potent and selective MDR reversers. Also Pervilleine A, a tropane alkaloid obtained from the roots of *Erythroxylum pervillei*, displayed P-gp inhibitory effects. Comparing the chemical structures of Verapamil and Pervilleine A (Figure 5.1), these two molecules showed a common structure, in which a basic nitrogen atom is connected by linkers (L) to two aromatic groups (H_1 and H_2). Based on these observations, over the years, our research group synthesized a wide series of compounds, carrying the structural features, of Verapamil and Pervilleine A, considered important for the interaction with P-gp.

During my PhD thesis, I also performed a series of chemical stability tests on linear and chiral *N*,*N*-bis(alkanol)amine aryl diester derivatives (Figure 5.1, for details see refs. [80,83]), bearing liable ester groups: all these compounds were designed based on the chemical structures of Verapamil and Pervilleine A. Linear diester compounds (**153-180**) are characterized by a *N*-methylated basic portion linked, by flexible polimethylene chains of variable length, to two aryl ester residues, as the (*E*)-3-(3,4,5-trimethoxyphenyl)vinyl (**a**), 3,4,5-trimethoxyphenyl (**b**) and the anthracene (**c**) ones[80]. The chiral derivatives (**181-192**) are branched homologues which carry a methyl group on a 3-methylene chain[83].

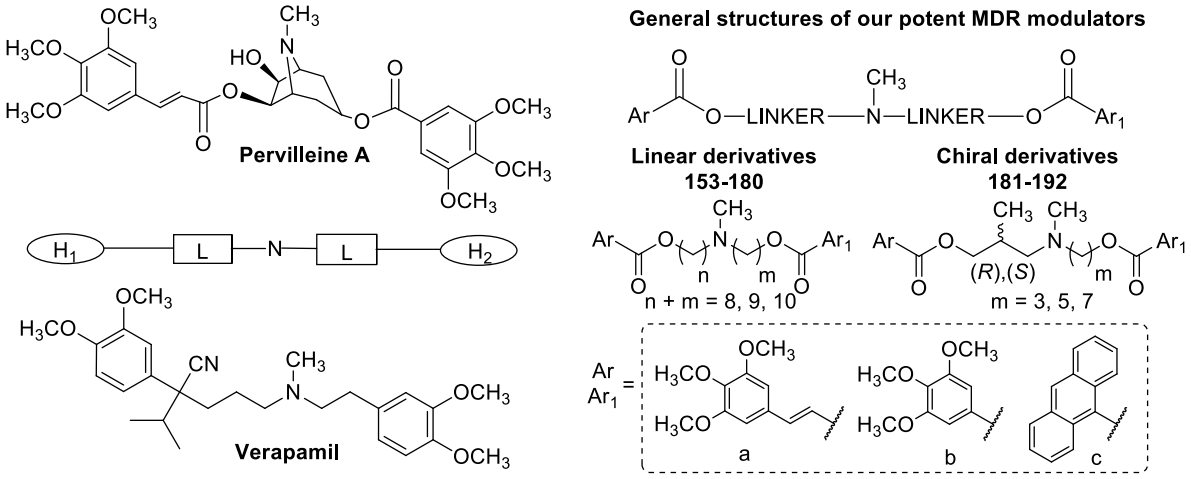


Figure 5.1: structures of Pervilleine A and Verapamil, two known P-gp modulators (L=linker, H_1,H_2=aryl groups), and of the previously synthesized linear and chiral derivatives[80,83].

These linear and chiral compounds showed high P-gp-dependent MDR reversing activity[80,83], and since they have two ester groups in their structures, I performed a series of chemical stability tests in both PBS and human plasma samples. Briefly, tests demonstrated that in most cases the ester groups were not susceptible to chemical or enzymatic hydrolysis, confirming the validity of this scaffold.

Moreover, we also developed a valid method to evaluate the enantiomeric excess of (*R*) and (*S*) enantiomers of chiral compounds, by enantioselective liquid chromatography coupled with diode array detector (LC-DAD) analysis.

5.1. Chemical stability tests

During my first PhD year, I performed a series of chemical stability tests on these 40 *N*,*N*-bis(alkanol)amine aryl diester compounds: the analyses were performed, following the procedures described in Par. 2.2.2.3. The LC-MS/MS system and parameters used were reported in Par. 7.2.1.

The obtained results demonstrated that all these compounds were stable in PBS and most of them also in human plasma samples. Indeed, only linear derivatives **153**, **154** and **155** and enantiomers *(R)*-**181**, *(S)*-**181**, *(R)*-**183**, *(R)*-**185**, *(S)*-**185**, *(R)*-**189** and *(S)*-**189**, reported in Figure 5.2, underwent enzymatic hydrolysis, showing $t_{1/2}$ values between 18 and 123 minutes (for details see refs. [80,83]).

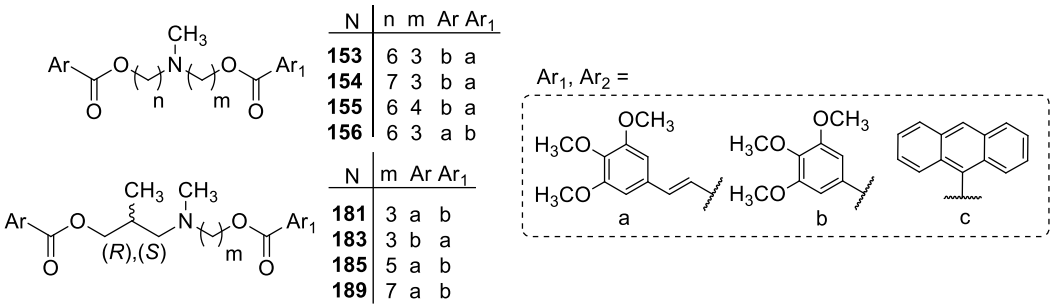


Figure 5.2: structures of linear and chiral derivatives which underwent enzymatic hydrolysis[80,83].

Interestingly, the hydrolysis occurs only when the *(E)*-3-(3,4,5-trimethoxyphenyl)vinyl ester group (**a**) is combined with the 3,4,5-trimethoxyphenyl moiety (**b**), while the anthracene residue (**c**) prevents the enzymatic activity. Furthermore, hydrolysis occurs only when the *N*-alkyl chain length of the *(E)*-3-(3,4,5-trimethoxyphenyl)vinyl portion is of three methylenes. Only **155**, bearing a *N*-alkyl chain length of four methylenes linked to residue **a**, shows an appraisable degradation with $t_{1/2}$ values of 123 minutes, that is three times higher than those of derivatives **153** and **154** ($t_{1/2}$ = 39 and 45 minutes, respectively[80]). Interestingly, for *(R)*-**183** and *(S)*-**183**, the configuration of the stereogenic center influenced the enzymatic hydrolysis: *(R)*-**183** suffers a remarkable degradation ($t_{1/2}$ = 54 minutes), while *(S)*-**183** is stable in human plasma samples[83]. As an example, in Figure 5.3 the human plasma degradation profiles of compound **153** and *(R)*-**183** compared to those of their stable isomers (**156** and *(S)*-**183**, respectively) were reported. The degradation profiles of all the other linear and chiral derivatives are reported in Supplementary data of refs. [80,83].

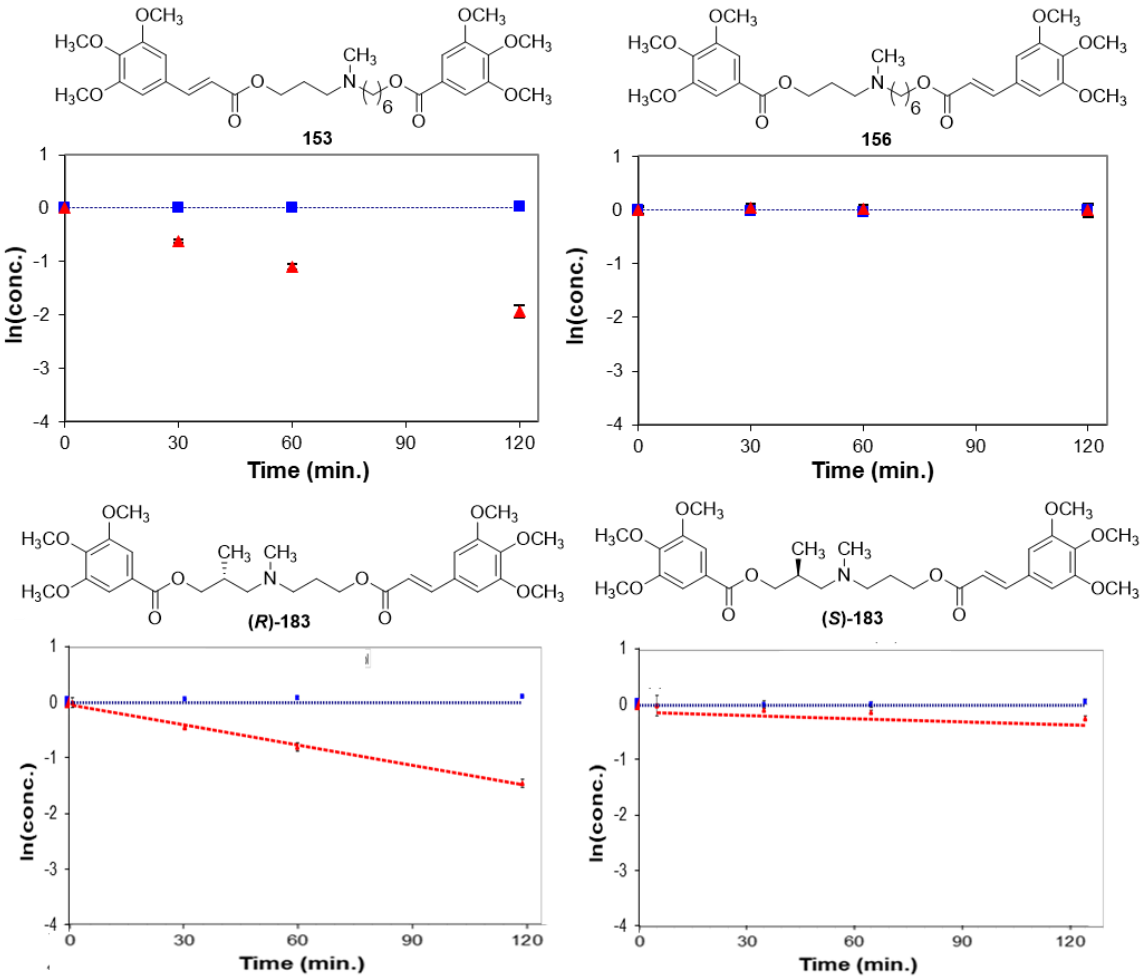


Figure 5.3: Degradation profiles in PBS (blue) and human plasma (red) of compounds **153** and **156** (top), and *(R)*-**183** and *(S)*-**183** (bottom).

Moreover, we discovered that both linear and chiral derivatives were degraded for hydrolysis of the ester group linked to the *(E)*-3-(3,4,5-trimethoxyphenyl)vinyl moiety, with formation of the corresponding *N*-alkyl alcohol and of the free (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid. As an example, the chromatographic profiles of compound **154** in human plasma samples at two incubation times (0 and 120 minutes) are reported in Figure 5.4.

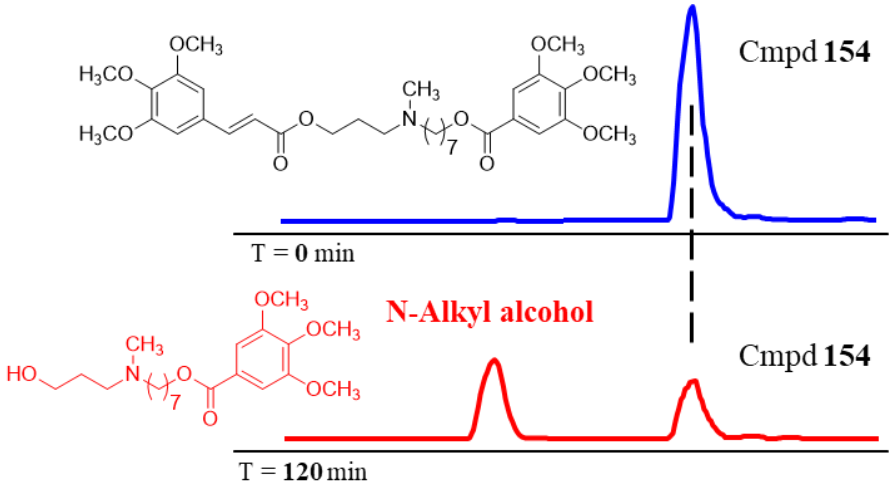


Figure 5.4: LC-MS/MS chromatographic profiles of compound **154** in human plasma at the initial (up) and final (bottom) incubation times.

5.2. Enantiomeric excess (ee) of (*R*) and (*S*) enantiomers evaluation

In the laboratory of Prof. Bartolucci from the University of Florence, I performed a series of experiments that allow us to develop a valid method to calculate the enantiomeric excess (ee) of (*R*) and (*S*) enantiomers of our chiral compounds, by enantioselective liquid chromatography coupled with diode array detector (LC-DAD) analysis.

To perform this analysis, different elution conditions were employed (for details see Par. 7.2.2). By using these conditions, we calculated the Retention Time (RT), ee values and the resolution (R) between the enantiomers of the same compounds: results are reported in Table 5.1. All enantiomers showed ee ≥ 95%, that is the maximal evaluable value with the used method, except for compounds **184**, **187** and **192** whose enantiomer pairs (*R*)/(*S*) did not reach a sufficient resolution to assess their ee values; anyway, since the synthetic pathway and the used enantiomeric reagents were common for all products, it was reasonable that also these compounds maintained the same enantiomeric excess (≥ 95%).

Table 5.1: Retention time (RT), ee values and resolution (R) between the enantiomers of compounds **181-183**, **185**, **186** and **188-191**.

Compounds	RT (min)	ee %	R value
(R)-**181**	22.28 ± 0.02	≥ 95	2.18 ± 0.04
(S)-**181**	19.36 ± 0.03	≥ 95	
(R)-**182**	16.47 ± 0.05	≥ 95	1.22 ± 0.04
(S)-**182**	14.73 ± 0.04	≥ 95	
(R)-**183**	19.7 ± 0.2	≥ 95	1.43 ± 0.03
(S)-**183**	21.7 ± 0.2	≥ 95	
(R)-**185**	22.7 ± 0.6	≥ 95	2.78 ± 0.05
(S)-**185**	19.1 ± 0.5	≥ 95	
(R)-**186**	12.02 ± 0.01	≥ 95	3.88 ± 0.01
(S)-**186**	9.27 ± 0.01	≥ 95	
(R)-**188**	23.22 ± 0.06	≥ 95	1.18 ± 0.01
(S)-**188**	24.80 ± 0.07	≥ 95	
(R)-**189**	22.72 ± 0.02	≥ 95	1.89 ± 0.05
(S)-**189**	20.13 ± 0.02	≥ 95	
(R)-**190**	11.01 ± 0.01	≥ 95	3.12 ± 0.01
(S)-**190**	9.11 ± 0.01	≥ 95	
(R)-**191**	23.5 ± 0.3	≥ 95	1.32 ± 0.01
(S)-**191**	21.5 ± 0.2	≥ 95	

As an example, Figure 5.5 reported the chromatographic profiles of each enantiomer of compound **186** and of their racemic mixture, since **186** displayed the highest resolution value (R = 3.88).

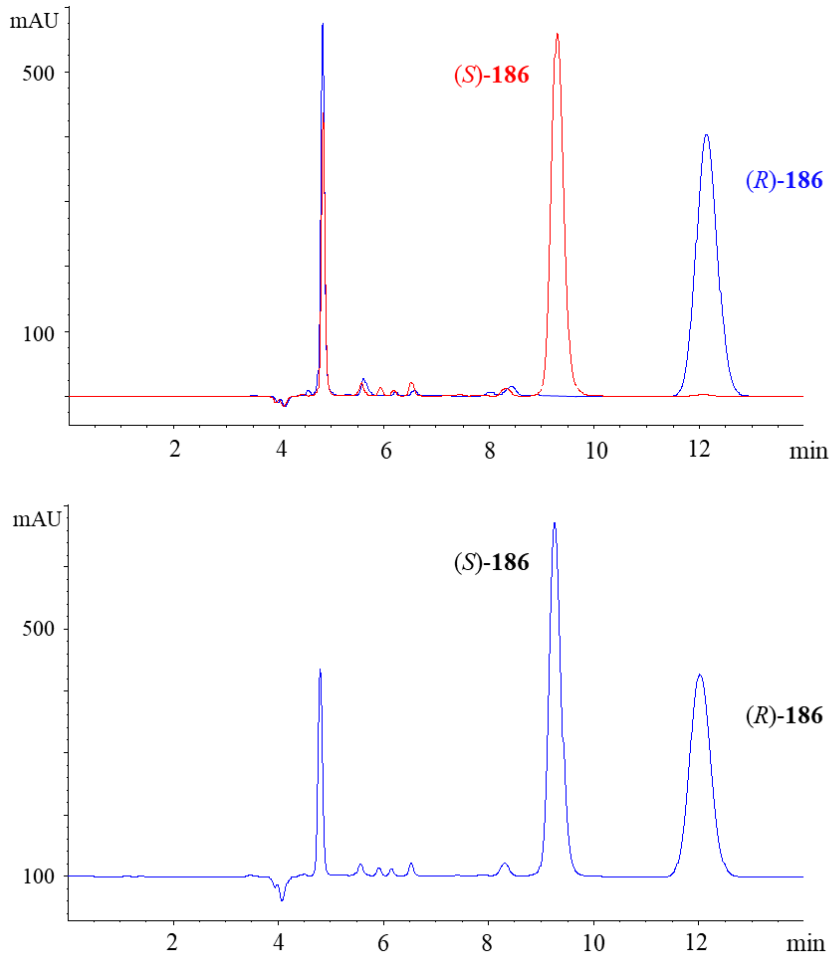


Figure 5.5: chromatographic profiles of each enantiomer (top: *(R)*-**186** in blue *(S)*-**186** in red) and of their racemic mixture (bottom).

6. Conclusions

This PhD thesis consisted in two main projects: the design and synthesis of compounds with dual P-gp/hCA XII inhibitory effects, and of MDR reversers active as ABC modulators.
The dual P-gp/hCA XII inhibitors were synthesized to reverse the P-gp-mediated MDR in cancer cells which overexpress both the transmembrane P-gp and hCA XII proteins. Therefore, in this project we designed hybrid inhibitors characterized by the presence of both P-gp and hCA XII binding moieties to maintain a high potency on P-gp and introduce a selectivity towards hCA XII. For this purpose, we incorporated in a typical scaffold of potent P-gp modulators a residue to target hCA XII. All these molecules were able to enhance the intracellular accumulation of two P-gp substrate, Rhodamine-123 and Doxorubicin, in K562/DOX cells that overexpress only P-gp. As regard the hCA inhibitory activity, coumarin derivatives were selective inhibitors of the tumor-associated hCA IX and hCA XII isoforms. Interestingly, most of our compounds displayed the highest MDR reverser effects on the tested resistant cell lines (LoVo/DOX, HT29/DOX and A549/DOX), that overexpress both P-gp and hCA XII proteins, showing an interesting synergistic effect.
MDR reversers, active as ABC modulators, are part of a wide project based on the design and synthesis of molecules that display potent inhibitory effects both on P-gp and BCRP transporters. Indeed, these two proteins are mainly involved in MDR, and they are co-overexpressed in several resistant cancer cells: thus, compounds able to inhibit both P-gp and BCRP could be very useful to overcome MDR. For this aim, we synthesized some series of analogues of the third-generation chemosensitizer Tariquidar, that contain a 6,7-dimethoxy-2-phenethyl-1,2,3,4-tetrahydroisoquinoline moiety linked to an aryl-substituted amide function, and 2,4-disubstituted quinazoline derivatives, containing the quinazoline-4-amine scaffold of the tyrosine kinase inhibitors (TKIs) Gefitinib and Erlotinib, that have been identified as potent P-gp and BCRP modulators. To deepen the structure-activity relationships of Tariquidar analogues, we designed and synthesized new series of amide and ester derivatives, and compounds modified at the amide function by introducing two bioisosteric heterocycles, the tetrazole and the oxadiazole ones. All these compounds carry different aryl o methoxy-substituted aryl groups to increase the activity against P-gp. In the series of quinazoline derivatives, we introduced secondary or tertiary protonable amines in position 4 of quinazoline scaffold, while in position 2 we inserted the anthracene or methoxy-substituted aryl moieties.
All these MDR modulators were studied on three different transfected cell lines (MDCK-MDR1, MDCK-MRP1 and MDCK-BCRP that overexpress P-gp, MRP1 and BCRP, respectively) to evaluate their activity on these ABC proteins, by measuring the inhibition of the transport of two fluorescent probes. In general, all these derivatives showed high inhibitory effects on P-gp and some compounds displayed activity on the other two transporters MRP1 and BCRP. Some compounds were also studied to evaluate their ability to enhance the cytotoxicity of the co-administered Doxorubicin, on MDCK-MDR1 cells and on the resistant HT29/DOX and A549/DOX cell lines that overexpressed P-gp.
Moreover, during my PhD thesis, I also performed a series of chemical stability tests on derivatives bearing liable ester groups: these experiments were carried out to evaluate the susceptibility of our ester molecules towards spontaneous and enzymatic hydrolysis. Stability analyses were performed by liquid chromatography coupled with mass spectrometry (LC-MS/MS) methods.

7. Experimental section

7.1. Chemistry

All melting points were taken on a Büchi apparatus and are uncorrected. NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz for ^{1}H-NMR, 100 MHz for ^{13}C-NMR). ^{1}H and ^{13}C NMR spectra were measured at room temperature (25 °C) in an appropriate solvent. ^{1}H and ^{13}C chemical shifts are expressed in ppm (δ) referenced to TMS. Spectral data are reported using the following abbreviations: bs = broad singlet, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, td = triplet of doublets, and coupling constants are reported in Hz, followed by integration. Assignments of the ^{13}C signals were performed using the attached proton test (APT) technique.
Chromatographic separations were performed on a silica gel column by flash chromatography (Kieselgel 40, 0.040-0.063 mm; Merck). Yields are given after purification, unless otherwise stated. The high-resolution mass spectrometry (HRMS) analysis was performed with a Thermo Finnigan LTQ Orbitrap mass spectrometer equipped with an electrospray ionization source (ESI). The accurate mass measure was carried out by introducing, via syringe pump at 10 μL min^{-1}, the sample solution (1.0 μg mL^{-1} in mQ water: acetonitrile 50:50), and the signal of the positive ions was acquired. The proposed experimental conditions allowed to monitoring the protonated molecules of studied compounds ($[M+H]^+$ species), that they were measured with a proper dwell time to achieve 60 000 units of resolution at Full Width at Half Maximum (FWHM). The elemental composition of compounds was calculated on the basis of their measured accurate masses, accepting only results with an attribution error less than 2.5 ppm and a not integer RDB (double bond/ring equivalents) value, in order to consider only the protonated species[120]. The LC-MS spectra were acquired with a triple quadrupole analyzer (VARIAN 1200 L) equipped with an electrospray ion source (ESI); spectra were recorded in positive and negative in the proper m/z range.
Compounds were named following IUPAC rules as applied by ChemBioDraw Ultra 14.0 software. When reactions were performed in dry conditions, the mixtures were maintained under nitrogen. Free bases were transformed into the hydrochloride by treatment with a solution of acetyl chloride (1.2 equiv./N atom) in dry CH_3OH. The salts were crystallized from abs. ethanol/petroleum ether.

DMAP: 4-dimethylaminopyridine
EDC hydrochloride: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
HATU: 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate
DIPEA: *N*,*N*-Diisopropylethylamine
CHX: cyclohexane
DMF: *N*,*N*-dimethylformamide
THF: tetrahydrofuran
WE7: NH_4OH/abs. ethanol/Et_2O/CH_2Cl_2/petroleum ether 2.5:45:180:180:450.
HOBt: 1-hydroxybenzotriazole hydrate

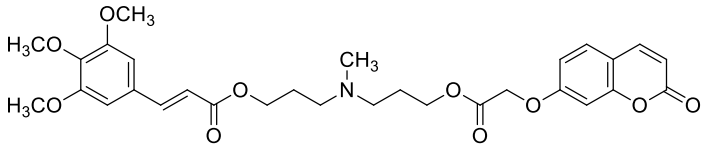
7.1.1. Final compounds

7.1.1.1. P-gp/hCAXII inhibitors

7.1.1.1.1. Coumarin and sulfamoyl benzoate diester compounds

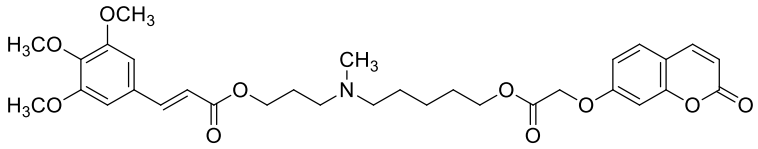
General procedures for the synthesis of coumarin compounds 1-14.
To a solution of **212** (1.3 equiv.) in dry CH_3CN, EDC hydrochloride (1 equiv.) and HOBt (1 equiv.) were added. The mixture was stirred at rt for 1 h, then the suitable (hydroxyalkyl)methylaminoester **197-210** (1 equiv.) dissolved in dry CH_3CN was added. The reaction was stirred at rt for 4 h, and the solvent was removed under reduce pressure. Then CH_2Cl_2 was added, and the organic layer was washed twice with a saturated solution of $NaHCO_3$, dried over Na_2SO_4, and concentrated under vacuum. The residue was then purified by flash chromatography using the proper eluting system, yielding the desired compound as an oil.

(*E*)-3-(Methyl(3-(2-((2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)propyl)amino)propyl 3-(3,4,5-trimethoxyphenyl)acrylate 1 (CRF9)



Following the general procedure, compound **1** (0.047 g, yield: 37.0 %) was synthesized as a yellow oil, starting from **199**[84] (0.082 g, 0.22 mmol) and **212** (0.064 g, 0.29 mmol) in 25.0 mL of dry CH_3CN.
Chromatographic eluent: CH_2Cl_2/CH_3OH 95:5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.63 (d, *J* = 9.4 Hz, 1H, CH); 7.58 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.38 (d, *J* = 8.4 Hz, 1H, CH); 6.87 (dd, *J* = 8.4, 2.2 Hz, 1H, CH); 6.77 (d, *J* = 2.2 Hz, 1H, CH); 6.74 (s, 2H, CH); 6.33 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.26 (d, *J* = 9.4 Hz, 1H, CH); 4.68 (s, 2H, OCH_2); 4.30-4.22 (m, 4H, OCH_2); 3.88 (s, 6H, OCH_3); 3.87 (s, 3H, OCH_3); 2.47-2.35 (m, 4H, NCH_2); 2.22 (s, 3H, NCH_3); 1.87-1.81 (m, 4H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.97 (C); 166.95 (C); 160.84 (C); 155.62 (C); 153.44 (C); 144.73 (CH); 143.16 (CH); 129.88 (C); 128.97 (CH); 117.31 (CH); 113.78 (CH); 133.33 (C); 112.80 (CH); 105.27 (CH); 101.70 (CH); 65.34 (CH_2); 64.02 (CH_2); 62.85 (CH_2); 60.95 (OCH_3); 56.17 (OCH_3); 54.20 (CH_2); 53.85 (CH_2); 41.95 (NCH_3); 26.67 (CH_2); 26.47 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{30}H_{36}NO_{10}$ = 570.2334, found 570.2340.

(*E*)-3-(Methyl(5-(2-((2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)pentyl)amino)propyl 3-(3,4,5-trimethoxyphenyl)acrylate 2 (CRF10)



Following the general procedure, compound **2** (0.043 g, yield: 29.0 %) was synthesized as a yellow oil, starting from **200**[86] (0.099 g, 0.25 mmol) and **212** (0.066 g, 0.30 mmol) in 20.0 mL of dry CH_3CN.

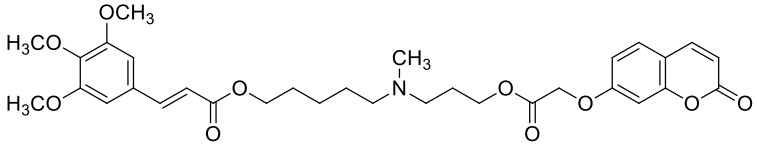
Chromatographic eluent: CH_2Cl_2/CH_3OH 93:7.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.60 (d, *J* = 9.6 Hz, 1H, CH); 7.56 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.36 (d, *J* = 8.4 Hz, 1H, CH); 6.85 (dd, *J* = 8.4, 2.2 Hz, 1H, CH); 6.74 (d, *J* = 2.2 Hz, 1H, CH); 6.72 (s, 2H, CH); 6.31 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.23 (d, *J* = 9.6 Hz, 1H, CH); 4.66 (s, 2H, OCH_2); 4.23-4.16 (m, 4H, OCH_2); 3.85 (s, 6H, OCH_3); 3.84 (s, 3H, OCH_3); 2.42 (t, *J* = 6.8 Hz, 2H, NCH_2); 2.30 (t, *J* = 6.8 Hz, 2H, NCH_2); 2.19 (s, 3H, NCH_3); 1.88-1.83 (m, 2H, CH_2); 1.71-1.60 (m, 2H, CH_2); 1.50-1.40 (m, 2H, CH_2); 1.38-1.28 (m, 2H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.98 (C); 166.92 (C); 160.81 (C); 155.63 (C); 153.41 (C); 144.65 (CH); 143.18 (CH); 129.87 (C); 128.95 (CH); 117.33 (CH); 113.70 (CH); 113.29 (C); 112.79 (CH); 105.26 (CH); 101.71 (CH); 65.63 (CH_2); 65.32 (CH_2); 62.98 (CH_2); 60.92 (OCH_3); 57.45 (CH_2); 56.15 (OCH_3); 54.19 (CH_2); 42.08 (NCH_3); 28.42 (CH_2); 26.87 (CH_2); 26.67 (CH_2); 23.67 (CH_2) ppm.

ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{32}H_{40}NO_{10}$ = 598.2647, found 598.2651.

(*E*)-5-(Methyl(3-(2-((2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)propyl)amino)pentyl 3-(3,4,5-trimethoxyphenyl)acrylate 3 (GG2)



Following the general procedure, compound **3** (0.070 g, yield: 33.0 %) was synthesized as a yellow oil, starting from **201**[86] (0.14 g, 0.36 mmol) and **212** (0.095 g, 0.43 mmol) in 20.0 mL of dry CH_3CN.

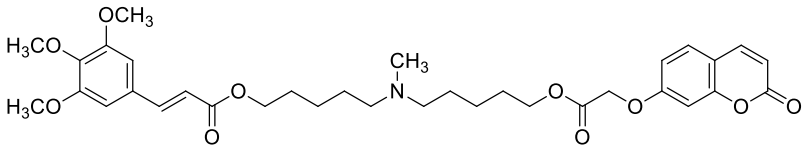
Chromatographic eluent: CH_2Cl_2/CH_3OH 95:5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.59 (d, *J* = 9.6 Hz, 1H, CH); 7.53 (d, *J* = 15.6 Hz, 1H, C*H*=CH); 7.34 (d, *J* = 8.4 Hz, 1H, CH); 6.82 (dd, *J* = 8.4, 2.2 Hz, 1H, CH); 6.72 (d, *J* = 2.2 Hz, 1H, CH); 6.70 (s, 2H, CH); 6.30 (d, *J* = 15.6 Hz, 1H, C*H*=CH); 6.21 (d, *J* = 9.6 Hz, 1H, CH); 4.64 (s, 2H, OCH_2); 4.22 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.15 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.83 (s, 6H, OCH_3); 3.82 (s, 3H, OCH_3); 2.34 (t, *J* = 6.8 Hz, 2H, NCH_2); 2.29 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.15 (s, 3H, NCH_3); 1.82-1.75 (m, 2H, CH_2); 1.69-1.64 (m, 2H, CH_2); 1.52-1.32 (m, 4H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.95 (C); 166.97 (C); 160.77 (C); 155.61 (C); 153.38 (C); 144.58 (CH); 143.20 (CH); 140.04 (C); 129.90 (C); 128.98 (CH); 117.41 (CH); 113.68 (CH); 113.29 (C); 112.73 (CH); 105.21 (CH); 101.68 (CH); 65.29 (CH_2); 64.47 (CH_2); 64.06 (CH_2); 60.90 (OCH_3); 57.51 (CH_2); 56.12 (OCH_3); 53.88 (CH_2); 41.97 (NCH_3); 28.64 (CH_2); 26.81 (CH_2); 26.37 (CH_2); 23.81 (CH_2) ppm.

ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{32}H_{40}NO_{10}$ = 598.2647, found 598.2642.

(*E*)-5-(Methyl(5-(2-((2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)pentyl)amino)pentyl 3-(3,4,5-trimethoxyphenyl)acrylate 4 (CRF32)



Following the general procedure, compound **4** (0.054 g, yield: 46.0 %) was synthesized as a yellow oil, starting from **202**[84] (0.080 g, 0.19 mmol) and **212** (0.050 g, 0.23 mmol) in 19.0 mL of dry CH_3CN.

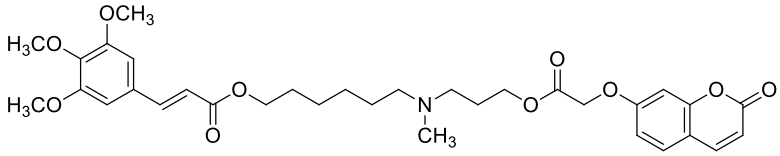
Chromatographic eluent: CH_2Cl_2/CH_3OH 95:5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.62 (d, *J* = 9.6 Hz, 1H, CH); 7.57 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.38 (d, *J* = 8.8 Hz, 1H, CH); 6.87 (dd, *J* = 8.8, 2.2 Hz, 1H, CH); 6.76 (d, *J* = 2.2 Hz, 1H, CH); 6.74 (s, 2H, CH); 6.33 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.25 (d, *J* = 9.6 Hz, 1H, CH); 4.67 (s, 2H, OCH_2); 4.22-4.15 (m, 4H, OCH_2); 3.87 (s, 6H, OCH_3); 3.86 (s, 3H, OCH_3); 2.35-2.29 (m, 4H, NCH_2); 2.20 (s, 3H, NCH_3); 1.75-1.63 (m, 4H, CH_2); 1.55-1.30 (m, 8H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.99 (C); 167.01 (C); 160.86 (C); 160.82 (C); 155.66 (C); 153.43 (C); 144.61 (CH); 143.20 (CH); 140.21 (C); 129.93 (C); 128.97 (CH); 117.44 (CH); 113.76 (CH); 113.32 (C); 112.85 (CH); 105.24 (CH); 101.70 (CH); 65.66 (CH_2); 65.34 (CH_2); 64.54 (CH_2); 60.95 (OCH_3); 57.64 (CH_2); 57.54 (CH_2); 56.16 (OCH_3); 42.15 (NCH_3); 28.70 (CH_2); 28.45 (CH_2); 26.88 (CH_2); 26.80 (CH_2); 23.97 (CH_2); 23.77 (CH_2) ppm.

ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{34}H_{44}NO_{10}$ = 626.2960, found 626.2951.

(*E*)-6-(Methyl(3-(2-((2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)propyl)amino)hexyl 3-(3,4,5-trimethoxyphenyl)acrylate 5 (CRF21)



Following the general procedure, compound **5** (0.058 g, yield: 80.0 %) was synthesized as a yellow oil, starting from **203**[80] (0.048 g, 0.12 mmol) and **212** (0.033 g, 0.14 mmol) in 18.0 mL of dry CH_3CN.

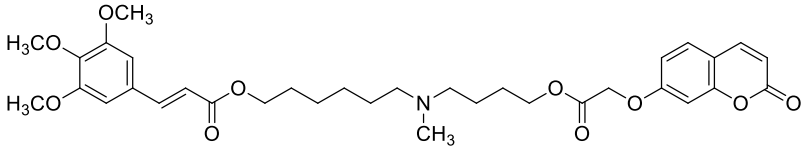
Chromatographic eluent: CH_2Cl_2/CH_3OH 96:4.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, *J* = 9.6 Hz, 1H, CH); 7.56 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.36 (d, *J* = 8.4 Hz, 1H, CH); 6.85 (dd, *J* = 8.4, 2.2 Hz, 1H, CH); 6.74 (d, *J* = 2.2 Hz, 1H, CH); 6.72 (s, 2H, CH); 6.32 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.24 (d, *J* = 9.6 Hz, 1H, CH,); 4.66 (s, 2H, OCH_2); 4.24 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.16 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.85 (s, 6H, OCH_3); 3.84 (s, 3H, OCH_3); 2.35 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.28 (t, *J* = 7.6 Hz, 2H, NCH_2); 2.16 (s, 3H, NCH_3); 1.84-1.77 (m, 2H, CH_2); 1.70-1.64 (m, 2H, CH_2); 1.47-1.30 (m, 6H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.96 (C); 167.01 (C); 160.84 (C); 160.79 (C); 155.64 (C); 153.41 (C); 144.57 (CH); 143.20 (CH); 140.06 (C); 129.93 (C); 128.98 (CH); 117.45 (CH); 113.73 (CH); 113.31 (C); 112.77 (CH); 105.21 (CH); 101.70 (CH); 65.31 (CH_2); 64.55 (CH_2); 64.15 (CH_2); 60.93 (OCH_3); 57.62 (CH_2); 56.14 (OCH_3); 53.91 (CH_2); 42.01 (NCH_3); 28.71 (CH_2); 27.09 (CH_2); 26.40 (CH_2); 25.90 (CH_2) ppm.

ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{33}H_{42}NO_{10}$ = 612.2803, found 612.2794.

(*E*)-6-(Methyl(4-(2-((2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)butyl)amino)hexyl 3-(3,4,5-trimethoxyphenyl)acrylate 6 (GG3)



Following the general procedure, compound **6** (0.15 g, yield: 100.0 %) was synthesized as a yellow oil, starting from **204**[80] (0.10 g, 0.24 mmol) and **212** (0.063 g, 0.29 mmol) in 15.0 mL of dry CH_3CN.

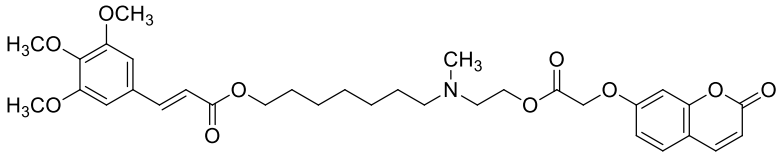
Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, *J* = 9.6 Hz, 1H, CH); 7.55 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.36 (d, *J* = 8.4 Hz, 1H, CH); 6.84 (dd, *J* = 8.4, 2.2 Hz, 1H, CH); 6.73 (d, *J* = 2.2 Hz, 1H, CH); 6.71 (s, 2H, CH); 6.31 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.23 (d, *J* = 9.6 Hz, 1H, CH); 4.65 (s, 2H, OCH_2); 4.21-4.14 (m, 4H, OCH_2); 3.85 (s, 6H, OCH_3); 3.84 (s, 3H, OCH_3) 2.32-2.27 (m, 4H, NCH_2); 2.16 (s, 3H, NCH_3); 1.69-1.62 (m, 4H, CH_2); 1.51-1.29 (m, 8H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.98 (C); 166.98 (C); 160.85 (C); 160.77 (C); 155.59 (C); 153.38 (C); 144.54 (CH); 143.26 (CH); 139.99 (C); 129.91 (C); 129.00 (CH); 117.44 (CH); 113.66 (CH); 113.29 (C); 112.73 (CH); 105.16 (CH); 101.69 (CH); 65.50 (CH_2); 65.28 (CH_2); 64.53 (CH_2); 60.92 (OCH_3); 57.57 (CH_2); 56.96 (CH_2); 56.12 (OCH_3); 41.88 (NCH_3); 28.68 (CH_2); 27.11 (CH_2); 26.44 (CH_2); 25.89 (CH_2) ppm.

ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{34}H_{44}NO_{10}$ = 626.2960, found 626.2966.

(*E*)-7-(Methyl(2-(2-((2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)ethyl)amino)heptyl 3-(3,4,5-trimethoxyphenyl)acrylate 7 (CRF14)



Following the general procedure, compound **7** (0.015 g, yield: 10.0 %) was synthesized as a yellow oil, starting from **208**[80] (0.055 g, 0.19 mmol) and **212** (0.079 g, 0.25 mmol) in 20.0 mL of dry CH_3CN.

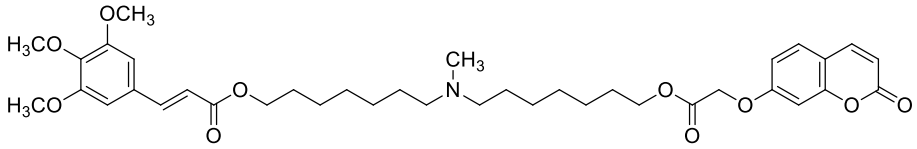
Chromatographic eluent: CH_2Cl_2/CH_3OH 95:5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.63 (d, *J* = 9.6 Hz, 1H, CH); 7.58 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.38 (d, *J* = 8.8 Hz, 1H, CH); 6.88 (dd, *J* = 8.8, 2.2 Hz, 1H, CH); 6.78 (d, *J* = 2.2 Hz, 1H, CH); 6.74 (s, 2H, CH); 6.33 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.27 (d, *J* = 9.6 Hz, 1H, CH); 4.70 (s, 2H, OCH_2); 4.31 (t, *J* = 5.6 Hz, 2H, OCH_2); 4.18 (t, *J* = 5.6 Hz, 2H, OCH_2); 3.88 (s, 6H, OCH_3); 3.87 (s, 3H, OCH_3); 2.64 (t, *J* = 5.6 Hz 2H, NCH_2); 2.38 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.26 (s, 3H, NCH_3); 1.71-1.65 (m, 4H, CH_2); 1.47-1.30 (m, 6H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.93 (C); 167.05 (C); 160.89 (C); 160.74 (C); 153.43 (C); 144.64 (CH); 143.21 (CH); 129.93 (C); 129.03 (CH); 117.43 (CH); 113.80 (CH); 113.38 (C); 112.79 (CH); 105.22 (CH); 101.84 (CH); 65.35 (CH_2); 65.26 (CH_2); 64.52 (CH_2); 60.97 (OCH_3); 57.53 (CH_2); 56.17 (OCH_3); 54.99 (CH_2); 52.50 (NCH_3); 29.02 (CH_2); 28.66 (CH_2); 27.07 (CH_2); 25.86 (CH_2) ppm.

ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{33}H_{42}NO_{10}$ = 612.2803, found 612.2794.

(*E*)-7-(Methyl(7-(2-((2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)heptyl)amino)heptyl 3-(3,4,5-trimethoxyphenyl)acrylate 8 (CRF16)



Following the general procedure, compound **8** (0.043 g, yield: 31.0 %) was synthesized as a yellow oil, starting from **205**[84] (0.088 g, 0.18 mmol) and **212** (0.049 g, 0.22 mmol) in 20.0 mL of dry CH_3CN.

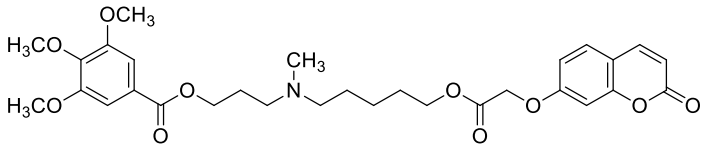
Chromatographic eluent: CH_2Cl_2/CH_3OH 95:5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, *J* = 9.6 Hz, 1H, CH); 7.56 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.37 (d, *J* = 8.8 Hz, 1H, CH); 6.84 (dd, *J* = 8.8, 2.2 Hz, 1H, CH); 6.75 (d, *J* = 2.2 Hz, 1H, CH); 6.73 (s, 2H, CH); 6.33 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.24 (d, *J* = 9.6 Hz, 1H, CH); 4.66 (s, 2H, OCH_2); 4.20-4.15 (m, 4H, OCH_2); 3.86 (s, 6H, OCH_3); 3.85 (s, 3H, OCH_3); 2.34-2.28 (m, 4H, NCH_2); 2.20 (s, 3H, NCH_3); 1.70-1.62 (m, 4H, CH_2); 1.50-1.20 (m, 16H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 168.00 (C); 167.03 (C); 160.87 (C); 160.82 (C); 155.65 (C); 153.41 (C); 144.54 (CH); 143.22 (CH); 140.06 (C); 129.94 (C); 128.97 (CH); 117.48 (CH); 113.72 (CH); 113.30 (C); 112.82 (CH); 105.21 (CH); 101.70 (CH); 65.73 (CH_2); 65.33 (CH_2); 64.63 (CH_2); 60.94 (OCH_3); 57.77 (CH_2); 57.74 (CH_2); 56.14 (OCH_3); 42.15 (NCH_3); 29.20 (CH_2); 29.08 (CH_2); 28.69 (CH_2); 28.43 (CH_2); 27.44 (CH_2); 27.38 (CH_2); 27.04 (CH_2); 27.02 (CH_2); 25.92 (CH_2); 25.72 (CH_2) ppm.

ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{38}H_{52}NO_{10}$ = 682.3586, found 682.3573.

3-(Methyl(5-(2-((2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)pentyl)amino)propyl 3,4,5-trimethoxy benzoate 9 (GG8)



Following the general procedure, compound **9** (0.076 g, yield: 86.0 %) was synthesized as a pale-yellow oil, starting from **206**[86] (0.055 g, 0.15 mmol) and **212** (0.041 g, 0.19 mmol) in 14.0 mL of dry CH_3CN.

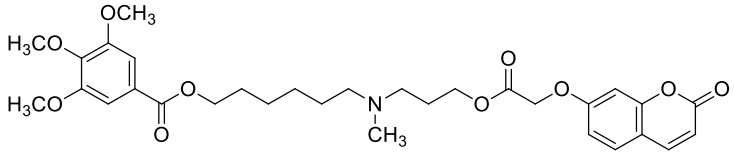
Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 90:10:1.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, *J* = 9.2 Hz, 1H, CH); 7.36 (d, *J* =8.4 Hz, 1H, CH); 7.25 (s, 2H, CH); 6.85 (dd, *J* = 8.4, 2.2 Hz, 1H, CH); 6.73 (d, *J* = 2.2 Hz, 1H, CH); 6.23 (d, *J* = 9.2 Hz, 1H, CH); 4.66 (s, 2H, OCH_2); 4.33 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.16 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.86 (s, 9H, OCH_3); 2.56 (t, *J* = 6.8 Hz, 2H, NCH_2); 2.41 (t, *J* =7.2 Hz, 2H, NCH_2); 2.29 (s, 3H, NCH_3); 2.00-1.95 (m, 2H, CH_2); 1.67-1.60 (m, 2H, CH_2); 1.52-1.46 (m, 2H, CH_2); 1.35-1.27 (m, 2H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 168.01 (C); 166.15 (C); 160.88 (C); 160.80 (C); 155.61 (C); 152.91 (C); 143.27 (CH); 142.19 (C); 129.00 (CH); 125.24 (C); 113.69 (CH); 113.28 (C); 112.86 (CH); 106.78 (CH); 101.63 (CH); 65.50 (CH_2); 65.30 (CH_2); 63.30 (CH_2); 60.90 (OCH_3); 57.19 (CH_2); 56.24 (OCH_3); 53.96 (CH_2); 41.70 (NCH_3); 28.36 (CH_2); 26.37 (CH_2); 26.18 (CH_2); 23.62 (CH_2) ppm.

ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{30}H_{38}NO_{10}$ = 572.2490, found 572.2479.

6-(Methyl(3-(2-((2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)propyl)amino)hexyl 3,4,5-trimethoxy benzoate 10 (LB49)



Following the general procedure, compound **10** (0.10 g, yield: 83.0 %) was synthesized as a yellow oil, starting from **197** (0.067 g, 0.18 mmol) and **212** (0.046 g, 0.21 mmol) in 15.0 mL of dry CH_3CN.

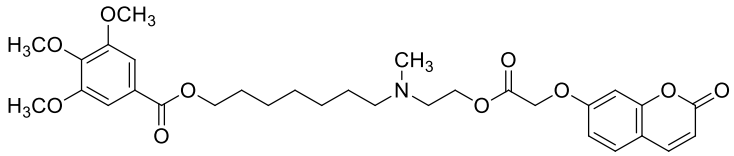
Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 90:10:1.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, *J* = 9.6 Hz, 1H, CH); 7.37 (d, *J* = 8.4 Hz, 1H, CH); 7.27 (s, 2H, CH); 6.85 (d, *J* = 8.4 Hz, 1H, CH); 6.75 (s, 1H, CH); 6.24 (d, *J* = 9.6 Hz, 1H, CH); 4.67 (s, 2H, OCH_2); 4.29-4.23 (m, 4H, OCH_2); 3.88 (s, 9H, OCH_3); 2.40 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.33 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.20 (s, 3H, NCH_3); 1.83-1.73 (m, 4H, CH_2); 1.47-1.34 (m, 6H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.92 (C); 166.18 (C); 160.77 (C); 155.60 (C); 152.87 (C); 143.18 (CH); 142.14 (C); 128.97 (CH); 125.45 (C); 113.69 (CH); 113.29 (C); 112.72 (CH); 106.80 (CH); 101.68 (CH); 65.29 (CH_2); 65.09 (CH_2); 63.99 (CH_2); 60.85 (OCH_3); 57.49 (CH_2); 56.22 (OCH_3); 53.85 (CH_2); 41.81 (NCH_3); 28.68 (CH_2); 27.02 (CH_2); 26.89 (CH_2); 26.22 (CH_2); 25.89 (CH_2) ppm.

ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{31}H_{40}NO_{10}$ = 586.2647, found 586.2643.

7-(Methyl(2-(2-((2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)ethyl)amino)heptyl 3,4,5-trimethoxy benzoate 11 (GG11)



Following the general procedure, compound **11** (0.20 g, yield: 89.0 %) was synthesized as a pale-yellow oil, starting from **209**[80] (0.15 g, 0.39 mmol) and **212** (0.10 g, 0.47 mmol) in 20.0 mL of dry CH_3CN.

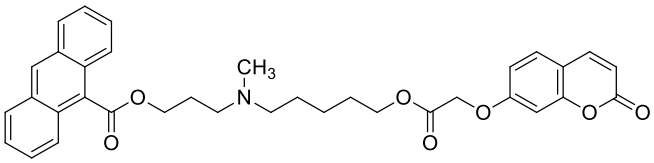
Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 93:7:0.3.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.58 (d, *J* = 9.6 Hz, 1H, CH); 7.33 (d, *J* = 8.4 Hz, 1H, CH); 7.24 (s, 2H, CH); 6.83 (d, *J* = 8.4 Hz, 1H, CH); 6.73 (s, 1H, CH); 6.20 (d, *J* = 9.6 Hz, 1H, CH); 4.66 (s, 2H, OCH_2); 4.29 (t, *J* = 5.6 Hz, 2H, OCH_2); 4.24 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.85 (s, 9H, OCH_3); 2.67 (t, *J* = 5.2 Hz, 2H, NCH_2); 2.40 (t, *J* =7.6 Hz, 2H, NCH_2); 2.27 (s, 3H, NCH_3); 1.73-1.69 (m, 2H, CH_2); 1.45-1.26 (m, 8H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.86 (C); 166.08 (C); 160.70 (C); 155.52 (C); 152.82 (C); 143.21 (CH); 142.07 (C); 128.96 (CH); 125.43 (C); 113.54 (CH); 113.23 (C); 112.60 (CH); 106.74 (CH); 101.72 (CH); 65.21 (CH_2); 65.06 (CH_2); 62.40 (CH_2); 60.76 (OCH_3); 57.53 (CH_2); 56.15 (OCH_3); 55.10 (CH_2); 42.00 (NCH_3); 29.01 (CH_2); 28.58 (CH_2); 27.08 (CH_2); 26.46 (CH_2); 25.84 (CH_2) ppm.

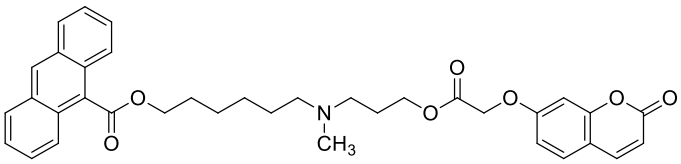
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{31}H_{40}NO_{10}$ = 586.2647, found 586.2638.

3-(Methyl(5-(2-((2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)pentyl)amino)propyl anthracene-9-carboxylate 12 (GG10)



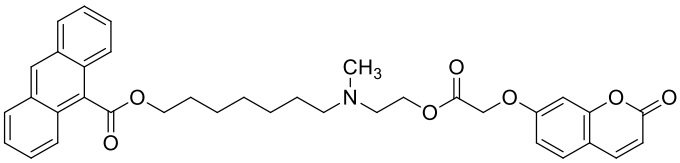
Following the general procedure, compound **12** (0.062 g, yield: 81.0 %) was synthesized as a yellow oil, starting from **207**[86] (0.050 g, 0.13 mmol) and **212** (0.035 g, 0.16 mmol) in 13.0 mL of dry CH_3CN.
Chromatographic eluent: CH_2Cl_2/CH_3OH 95:5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.51 (s, 1H, CH); 8.05-8.00 (m, 4H, CH); 7.56-7.46 (m, 5H, CH); 7.30 (d, *J* = 8.8 Hz, 1H, CH); 6.82 (dd, *J* = 8.8, 2.2 Hz, 1H, CH); 6.73 (d, *J* = 2.2 Hz, 1H, CH); 6.23 (d, *J* = 9.2 Hz, 1H, CH); 4.68 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.64 (s, 2H, OCH_2); 4.19 (t, *J* = 6.4 Hz, 2H, OCH_2); 2.54 (t, *J* =7.2 Hz, 2H, NCH_2); 2.35 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.25 (s, 3H, NCH_3); 2.11-2.02 (m, 2H, CH_2); 1.68-1.61 (m, 2H, CH_2); 1.53-1.45 (m, 2H, CH_2); 1.39-1.30 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.60 (C); 167.99 (C); 160.87 (C); 160.77 (C); 155.59 (C); 143.19 (CH); 130.97 (C); 129.29 (CH); 128.93 (CH); 128.64 (CH); 128.38 (C); 127.98 (C); 126.96 (CH); 125.48 (CH); 124.97 (CH); 113.66 (CH); 113.24 (C); 112.74 (CH); 101.68 (CH); 65.55 (CH_2); 65.31 (CH_2); 64.04 (CH_2); 57.40 (CH_2); 54.13 (CH_2); 41.89 (NCH_3); 28.38 (CH_2); 26.59 (CH_2); 26.39 (CH_2); 23.67 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{35}H_{36}NO_7$ = 582.2486, found 582.2489.

6-(Methyl(3-(2-((2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)propyl)amino)hexyl anthracene-9-carboxylate 13 (LB46)



Following the general procedure, compound **13** (0.16 g, yield: 98.0 %) was synthesized as a yellow oil, starting from **198** (0.11 g, 0.28 mmol) and **212** (0.072 g, 0.33 mmol) in 20.0 mL of dry CH_3CN.
Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 90:10:1.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.38 (s, 1H, CH); 7.96 (d, *J* = 8.4 Hz, 2H, CH); 7.90 (d, *J* = 8.4 Hz, 2H, CH); 7.47-7.37 (m, 5H, CH); 7.16 (d, *J* = 8.8 Hz, 1H, CH); 6.70 (dd, *J* = 8.8, 2.2 Hz, 1H, CH); 6.64 (d, *J* = 2.2 Hz, 1H, CH); 6.10 (d, *J* = 9.6 Hz, 1H, CH); 4.57-4.52 (m, 4H, OCH_2); 4.17 (t, *J* = 6.4 Hz, 2H, OCH_2); 2.27 (t, *J* = 6.8 Hz, 2H, NCH_2); 2.21 (t, *J* = 6.8 Hz, 2H, NCH_2); 2.09 (s, 3H, NCH_3); 1.85-1.69 (m, 4H, CH_2); 1.46-1.35 (m, 4H, CH_2); 1.33-1.26 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.71 (C); 167.94 (C); 160.85 (C); 160.75 (C); 155.60 (C); 143.16 (CH); 142.69 (C); 141.90 (C); 130.98 (C); 129.22 (CH); 128.95 (CH); 128.63 (CH); 128.36 (C); 128.16 (C); 126.93 (CH); 125.47 (CH); 124.99 (CH); 113.70 (CH); 113.28 (C); 112.69 (CH); 101.70 (CH); 65.84 (CH_2); 65.30 (CH_2); 64.06 (CH_2); 57.52 (CH_2); 53.87 (CH_2); 41.91 (NCH_3); 28.75 (CH_2); 27.04 (CH_2); 26.95 (CH_2); 26.30 (CH_2); 26.04 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{36}H_{38}NO_7$ = 596.2643, found 596.2652.

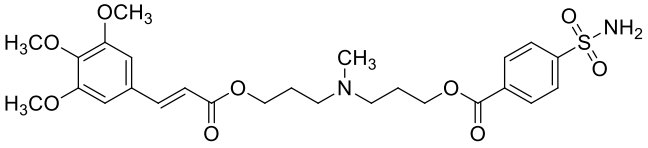
7-(Methyl(2-(2-((2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)ethyl)amino)heptyl anthracene-9-carboxylate 14 (GG14)



Following the general procedure, compound **14** (0.080 g, yield: 68.0 %) was synthesized as a yellow oil, starting from **210**[80] (0.078 g, 0.20 mmol) and **212** (0.052 g, 0.14 mmol) in 16.0 mL of dry CH_3CN.
Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 96:4:0.4.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.48 (s, 1H, CH); 8.03-7.98 (m, 4H, CH); 7.54-7.44 (m, 5H, CH); 7.28 (d, *J* = 8.4 Hz, 1H, CH); 6.80 (dd, *J* = 8.4, 2.2 Hz, 1H, CH); 6.73 (d, *J* = 2.2 Hz, 1H, CH); 6.20 (d, *J* = 9.6 Hz, 1H, CH); 4.64 (s, 2H, OCH_2); 4.60 (t, *J* = 6.8 Hz, 2H, OCH_2); 4.29 (t, *J* = 5.6 Hz, 2H, OCH_2); 2.62 (t, *J* = 5.6 Hz, 2H, NCH_2); 2.36 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.24 (s, 3H, NCH_3); 1.90-1.83 (m, 2H, CH_2); 1.50-1.25 (m, 8H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.71 (C); 167.94 (C); 160.85 (C); 160.73 (C); 155.59 (C); 143.15 (CH); 130.98 (C); 129.19 (CH); 128.91 (CH); 128.61 (CH); 128.35 (C); 126.90 (CH); 125.45 (CH); 124.99 (CH); 113.68 (CH); 113.26 (C); 112.71 (CH); 101.75 (CH); 65.87 (CH_2); 65.29 (CH_2); 63.11 (CH_2); 57.91 (CH_2); 55.48 (CH_2); 42.49 (NCH_3); 29.14 (CH_2); 28.72 (CH_2); 27.24 (CH_2); 27.07 (CH_2); 26.07 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{36}H_{38}NO_7$ = 596.2643, found 596.2631.

General procedures for the synthesis of sulfamoyl benzoate compounds 15-28.
4-sulfamoylbenzoic acid (1 equiv.) was transformed into the acyl chloride by reaction with $SOCl_2$ (2 equiv.) in the adequate amount of $CHCl_3$ (free of ethanol) at 60 °C for 5 h. The reaction was cooled to rt, and the solvent was removed under reduced pressure; the mixture was then treated twice with CHX, and the solvent removed under vacuum, to eliminate the excess of $SOCl_2$. The obtained acyl chloride was dissolved in $CHCl_3$ (free of ethanol), and the suitable (hydroxyalkyl)methylaminoester **197-210** (1 equiv.) was added. The solution was stirred at rt for 17 h, then the solvent was removed under reduce pressure. The residue was treated with CH_2Cl_2 and the organic layer was washed twice with a saturated solution of $NaHCO_3$, dried over Na_2SO_4, and concentrated under vacuum. The crude product was then purified by flash chromatography using the proper eluting system, yielding the desired compound as an oil. Final compounds were transformed into the corresponding hydrochloride as solid. The salts were crystallized from abs. ethanol/petroleum ether.

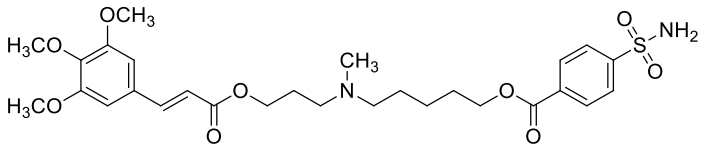
(*E*)-3-(Methyl(3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)propyl 4-sulfamoyl benzoate 15 (CRF25)



Following the general procedure, compound **15** (0.064 g, yield: 63.0 %) was synthesized as a yellow oil, starting from **199**[84] (0.068 g, 0.18 mmol) and 4-sulfamoylbenzoyl chloride (0.071 g, 0.37 mmol) in 10.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 93:7:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.02 (d, *J* = 8.4 Hz, 2H, CH); 7.88 (d, *J* = 8.4 Hz, 2H, CH); 7.54 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.69 (s, 2H, CH); 6.30 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 4.35

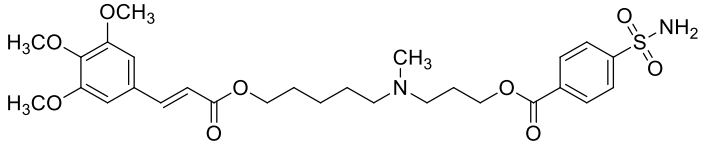
(t, *J* = 6.4 Hz, 2H, OCH_2); 4.22 (t, *J* = 6.0 Hz, 2H, OCH_2); 3.83 (s, 6H, OCH_3); 3.82 (s, 3H, OCH_3); 2.51-2.46 (m, 4H, NCH_2); 2.23 (s, 3H, NCH_3); 1.95-1.81 (m, 4H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.04 (C); 165.14 (C); 153.31 (C); 146.14 (C); 144.75 (CH); 139.89 (C); 133.72 (C); 130.10 (CH); 129.94 (C); 126.29 (CH); 117.29 (CH); 105.28 (CH); 64.04 (CH_2); 62.70 (CH_2); 60.95 (OCH_3); 56.16 (OCH_3); 54.00 (CH_2); 53.69 (CH_2); 41.99 (NCH_3); 26.47 (CH_2); 26.43 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{26}H_{35}N_2O_9S$ = 551.2058, found 551.2050.
Hydrochloride: low melting solid.

(*E*)-5-(Methyl(3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)pentyl 4-sulfamoyl benzoate 16 (CRF22)



Following the general procedure, compound **16** (0.025 g, yield: 21.0 %) was synthesized as a yellow oil, starting from **200**[86] (0.080 g, 0.21 mmol) and 4-sulfamoylbenzoyl chloride (0.051 g, 0.27 mmol) in 10.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.09 (d, *J* = 8.4 Hz, 2H, CH); 7.95 (d, *J* = 8.4 Hz, 2H, CH); 7.58 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.73 (s, 2H, CH); 6.33 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 4.33 (t, *J* = 6.8 Hz, 2H, OCH_2); 4.22 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.86 (s, 6H, OCH_3); 3.85 (s, 3H, OCH_3); 2.48 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.39 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.24 (s, 3H, NCH_3); 1.89-1.74 (m, 4H, CH_2); 1.59-1.41 (m, 4H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.06 (C); 165.15 (C); 153.39 (C); 146.05 (C); 144.83 (CH); 140.09 (C); 133.97 (C); 130.17 (CH); 129.90 (C); 126.37 (CH); 117.25 (CH); 105.35 (CH); 65.67 (CH_2); 62.88 (CH_2); 60.94 (OCH_3); 57.43 (CH_2); 56.18 (OCH_3); 53.93 (CH_2); 41.99 (NCH_3); 28.48 (CH_2); 26.76 (CH_2); 26.48 (CH_2); 23.84 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{28}H_{39}N_2O_9S$ = 579.2371, found 579.2380. **Hydrochloride:** mp 98-100 °C.

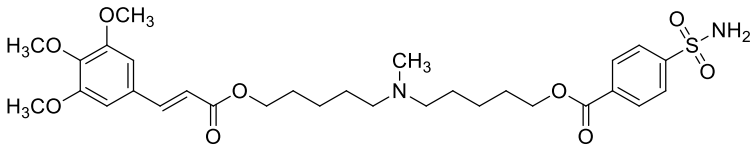
(*E*)-3-(Methyl(5-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)pentyl)amino)propyl 4-sulfamoyl benzoate 17 (CRF35)



Following the general procedure, compound **17** (0.16 g, yield: 100.0 %) was synthesized as a yellow oil, starting from **201**[86] (0.11 g, 0.27 mmol) and 4-sulfamoylbenzoyl chloride (0.10 g, 0.53 mmol) in 10.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.10 (d, *J* = 8.4 Hz, 2H, CH); 7.96 (d, *J* = 8.4 Hz, 2H, CH); 7.58 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.73 (s, 2H, CH); 6.34 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 4.40 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.15 (t, *J* = 6.8 Hz, 2H, OCH_2); 3.87 (s, 6H, OCH_3); 3.86 (s, 3H, OCH_3); 2.48 (t, *J* = 6.8 Hz, 2H, NCH_2); 2.35 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.22 (s, 3H, NCH_3); 1.96-1.90 (m, 2H, CH_2); 1.71-1.64 (m, 2H, CH_2); 1.51-1.40 (m, 4H, CH_2) ppm.

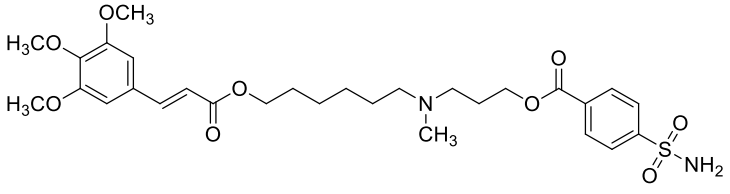
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.23 (C); 165.14 (C); 153.35 (C); 146.22 (C); 144.81 (CH); 139.97 (C); 133.79 (C); 130.14 (CH); 129.93 (C); 126.35 (CH); 117.33 (CH); 105.27 (CH); 64.53 (CH_2); 64.06 (CH_2); 60.94 (OCH_3); 57.50 (CH_2); 56.15 (OCH_3); 53.75 (CH_2); 42.14 (NCH_3); 28.66 (CH_2); 26.87 (CH_2); 26.39 (CH_2); 23.81 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{28}H_{39}N_2O_9S$ = 579.2371, found 579.2364. **Hydrochloride:** mp 83-86 °C.

(*E*)-5-(Methyl(5-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)pentyl)amino)pentyl 4-sulfamoyl benzoate 18 (CRF33)



Following the general procedure, compound **18** (0.036 g, yield: 19.0 %) was synthesized as a yellow oil, starting from **202**[84] (0.14 g, 0.33 mmol) and 4-sulfamoylbenzoyl chloride (0.12 g, 0.65 mmol) in 10.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 97:3:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.11 (d, *J* = 8.4 Hz, 2H, CH); 7.96 (d, *J* = 8.4 Hz, 2H, CH); 7.58 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.74 (s, 2H, CH); 6.33 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 4.33 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.17 (t, *J* = 6.8 Hz, 2H, OCH_2); 3.87 (s, 6H, OCH_3); 3.85 (s, 3H, OCH_3); 2.40-2.32 (m, 4H, NCH_2); 2.22 (s, 3H, NCH_3); 1.83-1.66 (m, 4H, CH_2); 1.58-1.37 (m, 8H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.15 (C); 165.17 (C); 153.41 (C); 146.25 (C); 144.76 (CH); 140.14 (C); 133.94 (C); 130.17 (CH); 129.92 (C); 126.36 (CH); 117.36 (CH); 105.36 (CH); 65.79 (CH_2); 65.63 (CH_2); 64.53 (CH_2); 60.92 (OCH_3); 57.41 (CH_2); 57.38 (CH_2); 56.17 (OCH_3); 42.05 (NCH_3); 28.65 (CH_2); 28.53 (CH_2); 26.64 (CH_2); 23.96 (CH_2); 23.90 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{30}H_{43}N_2O_9S$ = 607.2684, found 607.2672.
Hydrochloride: mp 83-85 °C.

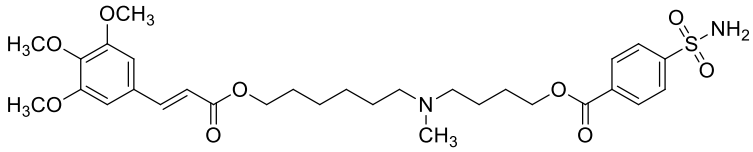
(*E*)-3-(Methyl(6-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)hexyl)amino)propyl 4-sulfamoyl benzoate 19 (GG1)



Following the general procedure, compound **19** (0.057 g, yield: 40.0 %) was synthesized as a yellow oil, starting from **203**[80] (0.098 g, 0.23 mmol) and 4-sulfamoylbenzoyl chloride (0.090 g, 0.48 mmol) in 10.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 90:10:1. Yield: %.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.07 (d, *J* = 8.4 Hz, 2H, CH); 7.94 (d, *J* = 8.4 Hz, 2H, CH); 7.57 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.73 (s, 2H, CH); 6.33 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 4.99 (bs, 2H, NH_2); 4.35 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.12 (t, *J* = 6.8 Hz, 2H, OCH_2); 3.85 (s, 6H, OCH_3); 3.83 (s, 3H, OCH_3); 2.49 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.35 (t, *J* = 7.2 Hz, 2H, NCH_2);

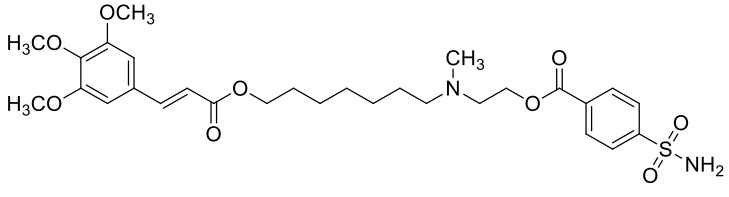
2.22 (s, 3H, NCH_3); 1.95-1.88 (m, 2H, CH_2); 1.69-1.60 (m, 2H, CH_2); 1.49-1.32 (m, 6H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.28 (C); 165.16 (C); 153.35 (C); 146.38 (C); 144. 83 (CH); 139.98 (C); 133.70 (C); 130.15 (CH); 129.91 (C); 126.34 (CH); 117.31 (CH); 105.26 (CH); 64.63 (CH_2); 63.96 (CH_2); 60.93 (OCH_3); 57.51 (CH_2); 56.15 (OCH_3); 53.69 (CH_2); 42.00 (NCH_3); 28.63 (CH_2); 27.01 (CH_2); 26.93 (CH_2); 26.22 (CH_2); 25.87 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{29}H_{41}N_2O_9S$ = 593.2527, found 593.2522. **Hydrochloride:** mp 73-76 °C.

(*E*)-4-(Methyl(6-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)hexyl)amino)butyl 4-sulfamoyl benzoate 20 (GG4)



Following the general procedure, compound **20** (0.078 g, yield: 49.0 %) was synthesized as a yellow oil, starting from **204**[80] (0.11 g, 0.26 mmol) and 4-sulfamoylbenzoyl chloride (0.098 g, 0.52 mmol) in 10.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.06 (d, *J* = 8.8 Hz, 2H, CH); 7.92 (d, *J* = 8.8 Hz, 2H, CH); 7.57 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.73 (s, 2H, CH); 6.33 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 5.50 (bs, 2H, NH_2); 4.32 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.15 (t, *J* = 6.8 Hz, 2H, OCH_2); 3.85 (s, 6H, OCH_3); 3.84 (s, 3H, OCH_3) 2.40-2.31 (m, 4H, NCH_2); 2.20 (s, 3H, NCH_3); 1.79-1.72 (m, 2H, CH_2); 1.70-1.55 (m, 4H, CH_2); 1.50-1.30 (m, 6H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.22 (C); 165.21 (C); 153.35 (C); 146.39 (C); 144.75 (CH); 139.95 (C); 133.72 (C); 130.15 (CH); 129.92 (C); 126.31 (CH); 117.36 (CH); 105.21 (CH); 65.53 (CH_2); 64.61 (CH_2); 60.94 (OCH_3); 57.45 (CH_2); 57.03 (CH_2); 56.14 (OCH_3); 42.05 (NCH_3); 28.66 (CH_2); 27.14 (CH_2); 26.84 (CH_2); 26.60 (CH_2); 25.86 (CH_2); 23.62 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{30}H_{43}N_2O_9S$ = 607.2684, found 607.2683.
Hydrochloride: mp 89-91 °C.

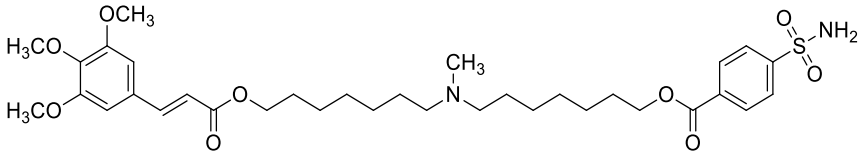
(*E*)-2-(Methyl(7-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)heptyl)amino)ethyl 4-sulfamoyl benzoate 21 (GG5)



Following the general procedure, compound **21** (0.087 g, yield: 45.0 %) was synthesized as a yellow oil, starting from **208**[80] (0.13 g, 0.33 mmol) and 4-sulfamoylbenzoyl chloride (0.12 g, 0.65 mmol) in 10.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.11 (d, *J* = 8.8 Hz, 2H, CH); 7.95 (d, J = 8.8 Hz, 2H, CH); 7.59 (d, *J* = 15.6 Hz, 1H, C*H*=CH); 6.75 (s, 2H, CH); 6.34 (d, *J* = 15.6 Hz, 1H, C*H*=CH); 4.43 (t, *J* = 5.6 Hz, 2H, OCH_2); 4.14 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.87 (s, 6H, OCH_3); 3.86 (s, 3H, OCH_3); 2.76 (t, *J* = 5.6 Hz, 2H, NCH_2); 2.43 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.31 (s, 3H, NCH_3); 1.66-1.62 (m, 2H, CH_2); 1.49-1.45 (m, 2H, CH_2); 1.33-1.25 (m, 6H, CH_2) ppm.

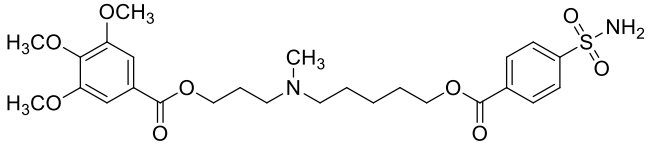
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.37 (C); 165.14 (C); 153.40 (C); 146.19 (C); 144.92 (CH); 140.07 (C); 133.83 (C); 130.29 (CH); 129.90 (C); 126.36 (CH); 117.29 (CH); 105.29 (CH); 64.73 (CH_2); 63.46 (CH_2); 60.96 (OCH_3); 57.80 (CH_2); 56.17 (OCH_3); 55.47 (CH_2); 42.73 (NCH_3); 29.13 (CH_2); 28.66 (CH_2); 27.20 (CH_2); 27.17 (CH_2); 25.92 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{29}H_{41}N_2O_9S$ = 593.2527, found 593.2524.
Hydrochloride: mp 70-73 °C.

(*E*)-7-(Methyl(7-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)heptyl)amino)heptyl 4-sulfamoyl benzoate 22 (GG6)



Following the general procedure, compound **22** (0.018 g, yield: 22.0 %) was synthesized as a yellow oil, starting from **205**[84] (0.060 g, 0.13 mmol) and 4-sulfamoylbenzoyl chloride (0.047 g, 0.25 mmol) in 8.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.15 (d, *J* = 8.4 Hz, 2H, CH); 8.00 (d, *J* = 8.4 Hz, 2H, CH); 7.61 (d, *J* = 15.6 Hz, 1H, C*H*=CH); 6.77 (s, 2H, CH); 6.37 (d, J = 15.6 Hz, 1H, C*H*=CH); 4.36 (t, *J* = 6.8 Hz, 2H, OCH_2); 4.20 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.90 (s, 6H, OCH_3); 3.89 (s, 3H, OCH_3); 2.40-2.35 (m, 4H, NCH_2); 2.26 (s, 3H, NCH_3); 1.82-1.65 (m, 4H, CH_2); 1.52-1.30 (m, 16H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.13 (C); 165.18 (C); 153.42 (C); 146.07 (C); 144.64 (CH); 139.96 (C); 134.09 (C); 130.22 (CH); 129.96 (C); 126.42 (CH); 117.46 (CH); 105.29 (CH); 65.78 (CH_2); 64.66 (CH_2); 60.95 (OCH_3); 57.57 (CH_2); 56.17 (OCH_3); 42.02 (NCH_3); 29.14 (CH_2); 28.67 (CH_2); 28.53 (CH_2); 27.37 (CH_2); 27.36 (CH_2); 26.77 (CH_2); 25.95 (CH_2); 25.89 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{34}H_{51}N_2O_9S$ = 663.3310, found 663.3298.
Hydrochloride: mp 68-70 °C.

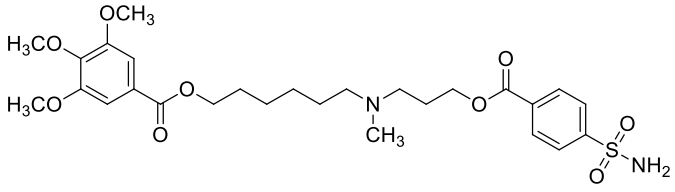
3-(Methyl(5-((4-sulfamoylbenzoyl)oxy)pentyl)amino)propyl 3,4,5-trimethoxybenzoate 23 (GG9)



Following the general procedure, compound **23** (0.018 g, yield: 22.0 %) was synthesized as a yellow oil, starting from **206**[86] (0.054 g, 0.15 mmol) and 4-sulfamoylbenzoyl chloride (0.057 g, 0.30 mmol) in 7.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.11 (d, *J* = 8.8 Hz, 2H, CH); 7.97 (d, *J* = 8.8 Hz, 2H, CH); 7.28 (s, 2H, CH); 4.37-4.32 (m, 4H, CH_2); 3.90 (s, 9H, OCH_3); 2.62 (t, *J* = 6.8 Hz, 2H, NCH_2); 2.51 (t, *J* = 6.8 Hz, 2H, NCH_2); 2.35 (s, 3H, NCH_3); 2.05-1.98 (m, 2H, CH_2); 1.83-1.76 (m, 2H, CH_2); 1.65-1.58 (m, 2H, CH_2); 1.51-1.43 (m, 2H, CH_2) ppm.

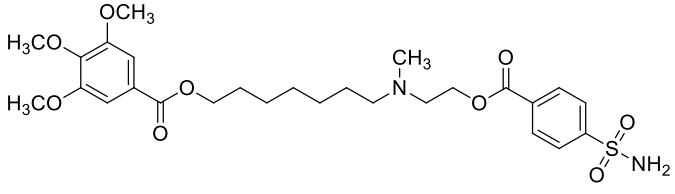
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 166.25 (C); 165.15 (C); 152.91 (C); 146.07 (C); 133.93 (C); 130.21 (CH); 126.41 (CH); 125.20 (C); 106.87 (CH); 65.56 (CH_2); 63.28 (CH_2); 60.92 (OCH_3); 57.29 (CH_2); 56.28 (OCH_3); 54.00 (CH_2); 41.74 (NCH_3); 28.42 (CH_2); 26.33 (CH_2); 26.16 (CH_2); 23.82 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{26}H_{37}N_2O_9S$ = 553.2214, found 553.2218.
Hydrochloride: mp 52-55 °C.

6-(Methyl(3-((4-sulfamoylbenzoyl)oxy)propyl)amino)hexyl 3,4,5-trimethoxybenzoate 24 (LB48)



Following the general procedure, compound **24** (0.080 g, yield: 55.0 %) was synthesized as a yellow oil, starting from **197** (0.098 g, 0.26 mmol) and 4-sulfamoylbenzoyl chloride (0.14 g, 0.77 mmol) in 6.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.01 (d, *J* = 8.4 Hz, 2H, CH); 7.88 (d, *J* = 8.4 Hz, 2H, CH); 7.21 (s, 2H, CH); 5.56 (bs, 2H, NH_2); 4.31 (t, *J* = 6.0 Hz, 2H, OCH_2); 4.19 (t, *J* = 6.8 Hz, 2H, OCH_2); 3.82 (s, 6H, OCH_3); 3.81 (s, 3H, OCH_3); 2.45 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.31 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.18 (s, 3H, NCH_3); 1.90-1.85 (m, 2H, CH_2); 1.69-1.65 (m, 2H, CH_2); 1.42-1.28 (m, 6H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 166.39 (C); 165.13 (C); 152.86 (C); 146.37 (C); 142.11 (C); 133.70 (C); 130.13 (CH); 126.33 (CH); 125.41 (C); 106.85 (CH); 65.20 (CH_2); 63.98 (CH_2); 60.87 (OCH_3); 57.50 (CH_2); 56.25 (OCH_3); 53.77 (CH_2); 41.94 (NCH_3); 28.64 (CH_2); 27.03 (CH_2); 26.89 (CH_2); 26.23 (CH_2); 25.90 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{27}H_{39}N_2O_9S$ = 567.2371, found 567.2380.
Hydrochloride: mp 53-56 °C.

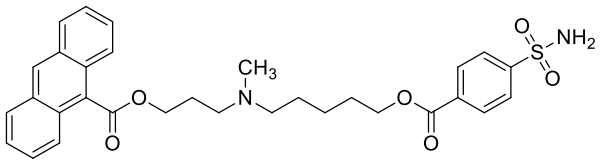
7-(Methyl(2-((4-sulfamoylbenzoyl)oxy)ethyl)amino)heptyl 3,4,5-trimethoxybenzoate 25 (GG13)



Following the general procedure, compound **25** (0.10 g, yield: 40.0 %) was synthesized as a yellow oil, starting from **209**[80] (0.17 g, 0.44 mmol) and 4-sulfamoylbenzoyl chloride (0.25 g, 1.33 mmol) in 15.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.08 (d, *J* = 8.4 Hz, 2H, CH); 7.93 (d, *J* = 8.4 Hz, 2H, CH); 7.27 (s, 2H, CH); 4.43 (t, *J* = 5.6 Hz, 2H, OCH_2); 4.26 (t, *J* = 6.8 Hz, 2H, OCH_2); 3.89 (s, 6H, OCH_3); 3.87 (s, 3H, OCH_3); 2.78 (t, *J* = 5.6 Hz, 2H, NCH_2); 2.44 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.32 (s, 3H, NCH_3); 1.74-1.71 (m, 2H, CH_2); 1.50-1.46 (m, 2H, CH_2); 1.40-1.30 (m, 6H, CH_2) ppm.

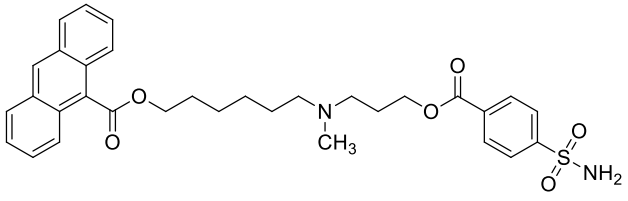
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 166.48 (C); 165.14 (C); 152.88 (C); 146.32 (C); 142.14 (C); 133.65 (C); 130.24 (CH); 126.31 (CH); 125.40 (C); 106.86 (CH); 65.29 (CH_2); 63.35 (CH_2); 60.88 (OCH_3); 57.81 (CH_2); 56.26 (OCH_3); 55.40 (CH_2); 42.63 (NCH_3); 29.12 (CH_2); 28.63 (CH_2); 27.19 (CH_2); 27.02 (CH_2); 25.93 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{27}H_{39}N_2O_9S$ = 567.2371, found 567.2368.
Hydrochloride: mp 60-63 °C.

3-(Methyl(5-((4-sulfamoylbenzoyl)oxy)pentyl)amino)propyl anthracene-9-carboxylate 26 (GG7)



Following the general procedure, compound **26** (0.030 g, yield: 41.0 %) was synthesized as a yellow oil, starting from **207**[86] (0.050 g, 0.13 mmol) and 4-sulfamoylbenzoyl chloride (0.074 g, 0.39 mmol) in 6.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 93:7:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.52 (s, 1H, CH); 8.10 (d, *J* = 8.4 Hz, 2H, CH); 8.02 (d, *J* = 8.4 Hz, 4H, CH); 7.93 (d, *J* = 8.4 Hz, 2H, CH); 7.55-7.47 (m, 4H, CH); 4.66 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.32 (t, *J* = 6.4 Hz, 2H, OCH_2); 2.60 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.44 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.29 (s, 3H, NCH_3); 2.12-2.05 (m, 2H, CH_2); 1.79-1.72 (m, 2H, CH_2); 1.60-1.40 (m, 4H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.68 (C); 165.17 (C); 146.00 (C); 133.88 (C); 130.94 (C); 130.20 (CH); 129.39 (CH); 128.67 (CH); 128.35 (C); 127.79 (C); 127.05 (CH); 126.37 (CH); 125.52 (CH); 124.89 (CH); 65.56 (CH_2); 63.95 (CH_2); 57.32 (CH_2); 53.98 (CH_2); 41.73 (NCH_3); 28.42 (CH_2); 26.06 (CH_2); 23.82 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{31}H_{35}N_2O_6S$ = 563.2210, found 563.2211.
Hydrochloride: mp 82-84 °C.

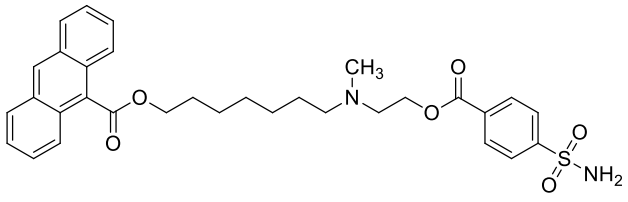
6-(Methyl(3-((4-sulfamoylbenzoyl)oxy)propyl)amino)hexyl anthracene-9-carboxylate 27 (GG12)



Following the general procedure, compound **27** (0.052 g, yield: 29.0 %) was synthesized as a yellow oil, starting from **198** (0.12 g, 0.31 mmol) and 4-sulfamoylbenzoyl chloride (0.18 g, 0.93 mmol) in 7.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 93:7:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.51 (s, 1H, CH); 8.08 (d, *J* = 8.4 Hz, 2H, CH); 8.01 (d, *J* = 8.4 Hz, 4H, CH); 7.93 (d, *J* = 8.4 Hz, 2H, CH); 7.55-7.46 (m, 4H, CH); 5.08 (bs, 2H, NH_2); 4.59 (t, *J* = 6.8 Hz, 2H, OCH_2); 4.37 (t, *J* = 6.4 Hz, 2H, OCH_2); 2.51 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.38 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.24 (s, 3H, NCH_3); 1.97-1.82 (m, 4H, CH_2); 1.51-1.35 (m, 6H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.85 (C); 165.09 (C); 146.22 (C); 142.61 (C); 141.80 (C); 133.63 (C); 130.96 (C); 130.18 (CH); 129.28 (CH); 128.64 (CH); 128.32 (C); 128.01 (C); 127.00 (CH); 126.37 (CH); 125.50 (CH); 124.91 (CH); 65.85 (CH_2); 63.71 (CH_2); 57.23 (CH_2); 53.71 (CH_2); 41.60 (NCH_3); 28.62 (CH_2); 26.86 (CH_2); 26.39 (CH_2); 25.92 (CH_2); 25.84 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{32}H_{37}N_2O_6S$ = 577.2367, found 577.2361.
Hydrochloride: mp 81-84 °C.

7-(Methyl(2-((4-sulfamoylbenzoyl)oxy)ethyl)amino)heptyl anthracene-9-carboxylate 28 (GG15)



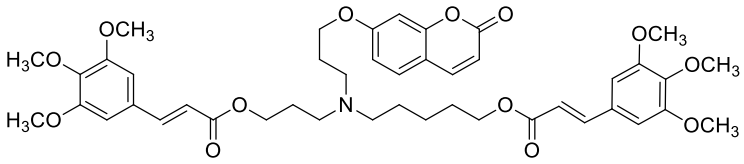
Following the general procedure, compound **28** (0.049 g, yield: 33.0 %) was synthesized as a yellow oil, starting from **210**[80] (0.10 g, 0.25 mmol) and 4-sulfamoylbenzoyl chloride (0.14 g, 0.76 mmol) in 8.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.52 (s, 1H, CH); 8.11 (d, *J* = 8.0 Hz, 2H, CH); 8.01 (d, *J* = 8.0 Hz, 4H, CH); 7.93 (d, *J* = 8.0 Hz, 2H, CH); 7.56-7.47 (m, 4H, CH); 5.31 (bs, 2H, NH_2); 4.60 (t, *J* = 6.8 Hz, 2H, OCH_2); 4.46 (t, *J* = 5.6 Hz, 2H, OCH_2); 2.80 (t, *J* = 5.6 Hz, 2H, NCH_2); 2.47 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.35 (s, 3H, NCH_3); 1.88-1.82 (m, 2H, CH_2); 1.54-1.30 (m, 8H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.93 (C); 165.08 (C); 146.27 (C); 133.47 (C); 130.96 (C); 130.28 (CH); 129.28 (CH); 128.64 (CH); 128.32 (C); 128.01 (C); 127.00 (CH); 126.31 (CH); 125.50 (CH); 124.92 (CH); 65.97 (CH_2); 62.86 (CH_2); 57.65 (CH_2); 55.18 (CH_2); 42.34 (NCH_3); 29.03 (CH_2); 28.64 (CH_2); 27.10 (CH_2); 26.53 (CH_2); 26.00 (CH_2) ppm.
ESI-HRMS (m/z) calculated for $[M+H]^+$ ion species $C_{32}H_{37}N_2O_6S$ = 577.2367, found 577.2374. **Hydrochloride:** mp 71-73 °C.

7.1.1.1.2. (*N*-Alkylcoumarin)aminoaryl diester compounds

General procedures for the synthesis of diester compounds 29-55.
Diester compounds were synthesized using two different general procedures.
Method A: In an ice-bath, to a solution of the suitable ((hydroxyalkyl)alkylcoumarin)amino ester **224-233** (1 equiv.) in the adequate amount of dry CH_2Cl_2, the proper carboxylic acid (1.5 equiv.), DMAP (0.8 equiv.) and EDC hydrochloride (1.8 equiv.) were added in this order. The reaction mixture was stirred at 0 °C for 1 h, then at rt for 48 h. Then, the residue was treated with CH_2Cl_2, and the organic layer was washed twice with water and with a saturated solution of $NaHCO_3$, dried over Na_2SO_4 and concentrated under reduced pressure. Finally, the residue was purified by flash chromatography using $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3 as the proper eluting system, obtaining the desired compound as an oil. Final compounds were transformed into the corresponding hydrochloride as solid. The salts were crystallized from abs. ethanol/petroleum ether.

Method B: The proper carboxylic acid (1.5 equiv.) was transformed into the corresponding acyl chloride by treatment with $SOCl_2$ (15 equiv.) in the adequate amount of $CHCl_3$ (free of ethanol) at 60 °C for 4-6 h. Upon completion of the reaction, the mixture was cooled to rt, and the solvent was removed under reduced pressure. The residue was treated twice with CHX and the solvent was removed under vacuum. The obtained acyl chloride was dissolved in the proper amount of $CHCl_3$ (free of ethanol) and the suitable ((hydroxyalkyl)alkylcoumarin)amino ester **224-233** (1 equiv.) was added. The mixture was stirred at rt for 24 h, then the organic layer was washed twice with a saturated solution of $NaHCO_3$, dried over Na_2SO_4 and concentrated under reduced pressure. Finally, the residue was purified by flash chromatography, using $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3 as the proper eluting system, yielding the desired compound as an oil. Final compounds were transformed into the corresponding hydrochloride as solid. The salts were crystallized from abs. ethanol/petroleum ether.

(*E*)-5-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(3-(((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)pentyl 3-(3,4,5-trimethoxyphenyl)acrylate 29 (KIS 7)



Following method **A**, compound **29** (0.11 g, yield: 72.8 %) was synthesized as a pale yellow oil, starting from **224** (0.11 g, 0.19 mmol) and (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (0.067 g, 0.28 mmol) in 4.0 mL of dry CH_2Cl_2.

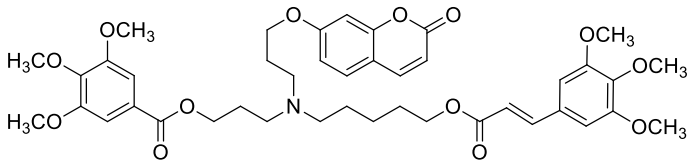
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.54-7.50 (m, 3H, C*H*=CH); 7.26 (d, *J* = 9.6 Hz, 1H, CH arom.); 6.77-6.73 (m, 2H, CH arom.); 6.69 (s, 2H, CH arom.); 6.68 (s, 2H, CH arom.); 6.28 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.25 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.13 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.18 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.10 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.02 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.81 (s, 18H, OCH_3); 2.70-2.49 (m, 4H, NCH_2); 2.48-2.35 (m, 2H, NCH_2); 1.97-1.86 (m, 2H, CH_2); 1.86-1.76 (m, 2H, CH_2); 1.66-1.59 (m, 2H, CH_2); 1.52-1.41 (m, 2H, CH_2); 1.39-1.29 (m, 2H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.93 (C); 166.83 (C); 162.14 (C); 161.06 (C); 155.86 (C); 153.43 (C); 144.82 (CH); 144.65 (CH); 143.29 (CH); 140.25 (C); 140.18 (C); 129.89 (C); 129.81 (C); 128.76 (CH); 117.36 (CH); 117.12 (CH); 113.00 (CH); 112.60 (CH); 112.52 (C); 105.34 (CH); 104.54 (C); 101.50 (CH); 66.39 (OCH_2); 64.35 (OCH_2); 62.63 (OCH_2); 60.91 (OCH_3); 56.17 (OCH_3); 53.91 (NCH_2); 50.48 (NCH_2); 50.22 (NCH_2); 28.65 (CH_2); 23.81 (CH_2) ppm.

Hydrochloride: white solid; mp 81-84 °C.

(*E*)-3-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(5-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)pentyl)amino)propyl 3,4,5-trimethoxybenzoate
30 (LB63)



Following method **B**, compound **30** (0.058 g, yield: 77.9 %) was synthesized as a pale yellow oil, starting from (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (0.034 g, 0.14 mmol) and **225** (0.053 g, 0.096 mmol).

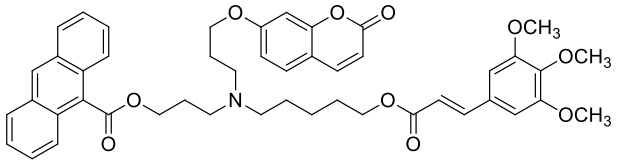
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.52 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 7.52 (d, *J* = 15.6 Hz, 1H, C*H*=CH); 7.25 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.20 (s, 2H, CH arom.); 6.75-6.72 (m, 2H, CH arom.); 6.69 (s, 2H, CH arom.); 6.28 (d, *J* = 15.6 Hz, 1H, C*H*=CH); 6.14 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.28 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.08 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.01 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.83 (s, 3H, OCH_3); 3.82 (s, 6H, OCH_3); 3.81 (s, 6H, OCH_3); 3.81 (s, 3H, OCH_3); 2.62-2.49 (m, 4H, NCH_2); 2.41 (t, *J* = 6.4 Hz, 2H, NCH_2); 1.95-1.82 (m, 4H, CH_2); 1.63-1.56 (m, 2H, CH_2); 1.50-1.38 (m, 2H, CH_2); 1.37-1.30 (m, 2H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.96 (C); 166.09 (C); 162.16 (C); 161.13 (C); 155.84 (C); 153.40 (C); 152.90 (C); 144.66 (CH); 143.35 (CH); 142.21 (C); 140.08 (C); 129.89 (C); 128.76 (CH); 125.25 (C); 117.35 (CH); 112.96 (CH); 112.62 (CH); 112.48 (C); 106.76 (CH); 105.22 (CH); 101.38 (CH); 66.36 (OCH_2); 64.40 (OCH_2); 63.28 (OCH_2); 60.93 (OCH_3); 60.88 (OCH_3); 56.22 (OCH_3); 56.14 (OCH_3); 53.94 (NCH_2); 50.37 (NCH_2); 50.13 (NCH_2); 28.67 (CH_2); 26.80 (CH_2); 26.44 (CH_2); 23.82 (CH_2) ppm.

Hydrochloride: white solid; mp 128-131 °C.

(*E*)-3-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(5-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)pentyl)amino)propyl anthracene-9-carboxylate
31 (LB59)



Following method **B**, compound **31** (0.046 g, yield: 74.0 %) was synthesized as a pale yellow oil, starting from (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (0.028 g, 0.12 mmol) and **226** (0.045 g, 0.079 mmol).

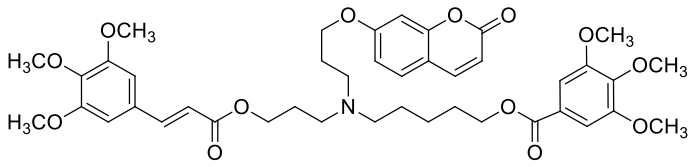
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.46 (s, 1H, CH arom.); 7.97 (t, *J* = 7.2 Hz, 4H, CH arom.); 7.54 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.50-7.41 (m, 5H, CH arom. and C*H*=CH); 7.20 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.73-6.69 (m, 4H, CH arom.); 6.30 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.15 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.62 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.10 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.98 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.83 (s, 3H, OCH_3); 3.80 (s, 6H, OCH_3); 2.62-2.53 (m, 4H, NCH_2); 2.44 (t, *J* = 6.4 Hz, 2H, NCH_2); 2.06-1.95 (m, 2H, CH_2); 1.94-1.82 (m, 2H, CH_2); 1.65-1.55 (m, 2H, CH_2); 1.48-1.40 (m, 2H, CH_2); 1.39-1.30 (m, 2H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.60 (C); 167.00 (C); 162.17 (C); 161.17 (C); 155.82 (C); 153.41 (C); 144.66 (CH); 143.33 (CH); 130.97 (C); 129.91 (C); 129.29 (CH); 128.71 (CH); 128.65 (CH); 128.36 (C); 127.98 (C); 126.95 (CH); 125.48 (CH); 124.92 (CH); 117.40 (CH); 112.94 (CH); 112.64 (CH); 112.44 (C); 105.27 (CH); 101.40 (CH); 66.42 (OCH_2); 64.44

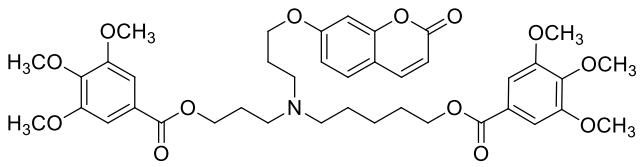
(OCH_2); 64.04 (OCH_2); 60.94 (OCH_3); 56.14 (OCH_3); 53.96 (NCH_2); 50.66 (NCH_2); 50.21 (NCH_2); 28.67 (CH_2); 26.79 (CH_2); 26.69 (CH_2); 23.86 (CH_2) ppm.
Hydrochloride: pale yellow solid; mp 94-97 °C.

(*E*)-5-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)pentyl 3,4,5-trimethoxybenzoate 32 (KIS 9)



Following method **A**, compound **32** (0.10 g, yield: 83.5 %) was synthesized as a pale yellow oil, starting from **224** (0.090 g, 0.15 mmol) and 3,4,5-trimethoxybenzoic acid (0.049 g, 0.23 mmol) in 4.0 mL of dry CH_2Cl_2.
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.53 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.53 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 7.28 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.23 (s, 2H, CH arom.); 6.78-6.75 (m, 2H, CH arom.); 6.69 (s, 2H, CH arom.); 6.27 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.15 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.23 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.19 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.03 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.85 (s, 9H, OCH_3); 3.83 (s, 9H, OCH_3); 2.72-2.49 (m, 4H, NCH_2); 2.48-2.35 (m, 2H, NCH_2); 2.01-1.87 (m, 2H, CH_2); 1.86-1.77 (m, 2H, CH_2); 1.76-1.67 (m, 2H, CH_2); 1.58-1.45 (m, 2H, CH_2); 1.43-1.31 (m, 2H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.86 (C); 166.19 (C); 162.15 (C); 161.10 (C); 155.86 (C); 153.43 (C); 152.92 (C); 144.85 (CH); 143.32 (CH); 142.24 (C); 140.21 (C); 129.81 (C); 128.76 (CH); 125.39 (C); 117.11 (CH); 113.00 (CH); 112.65 (CH); 112.52 (C); 106.86 (CH); 105.30 (CH); 101.46 (CH); 66.39 (OCH_2); 64.97 (OCH_2); 62.67 (OCH_2); 60.94 (OCH_3); 60.88 (OCH_3); 56.26 (OCH_3); 56.17 (OCH_3); 53.94 (NCH_2); 50.50 (NCH_2); 50.22 (NCH_2); 28.69 (CH_2); 26.60 (CH_2); 23.84 (CH_2) ppm.
Hydrochloride: white solid; mp 74-77 °C.

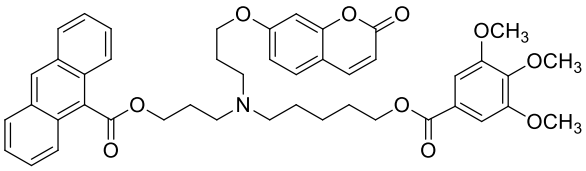
5-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(3-((3,4,5-trimethoxybenzoyl)oxy)propyl)amino)pentyl 3,4,5-trimethoxybenzoate 33 (LB65)



Following method **A**, compound **33** (0.055 g, yield: 74.2 %) was synthesized as a pale yellow oil, starting from **225** (0.054 g, 0.098 mmol) and 3,4,5-trimethoxybenzoic acid (0.031 g, 0.15 mmol) in 5.0 mL of dry CH_2Cl_2.
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.52 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 7.26 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.22 (s, 2H, CH arom.); 7.20 (s, 2H, CH arom.); 6.75-6.72 (m, 2H, CH arom.); 6.14 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.28 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.21 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.01 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.84 (s, 6H, OCH_3); 3.84 (s, 6H, OCH_3); 3.83 (s, 6H, OCH_3); 2.70-2.51 (m, 4H, NCH_2); 2.50-2.35 (m, 2H, NCH_2); 1.97-1.80 (m, 4H, CH_2); 1.72-1.64 (m, 2H, CH_2); 1.56-1.42 (m, 2H, CH_2); 1.41-1.32 (m, 2H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.18 (C); 166.09 (C); 162.14 (C); 161.12 (C); 155.83 (C); 152.90 (C); 143.34 (CH); 142.19 (C); 128.75 (CH); 125.39 (C); 125.24 (C); 112.97 (CH);

112.65 (CH); 112.48 (C); 106.81 (CH); 106.77 (CH); 101.35 (CH); 66.36 (OCH_2); 64.99 (OCH_2); 63.28 (OCH_2); 60.88 (OCH_3); 56.23 (OCH_3); 53.97 (NCH_2); 50.39 (NCH_2); 50.16 (NCH_2); 28.69 (CH_2); 26.78 (CH_2); 23.85 (CH_2) ppm.
Hydrochloride: white solid; mp 84-87 °C.

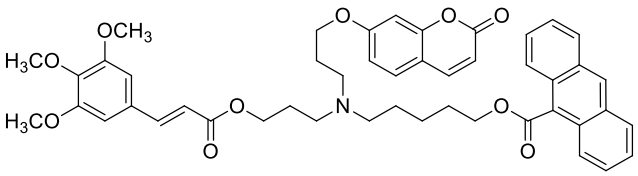
3-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(5-((3,4,5-trimethoxybenzoyl)oxy)pentyl)amino)propyl anthracene-9-carboxylate 34 (LB61)



Following method **A**, compound **34** (0.044g, yield: 59.3 %) was synthesized as a pale yellow oil, starting from **226** (0.054 g, 0.095 mmol) and 3,4,5-trimethoxybenzoic acid (0.030 g, 0.14 mmol) in 5.0 mL of dry CH_2Cl_2.

Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.45 (s, 1H, CH arom.); 7.97 (t, *J* = 8.0 Hz, 4H, CH arom.); 7.50-7.40 (m, 5H, CH arom. and C*H*=CH); 7.24 (s, 2H, CH arom.); 7.19 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.73-6.70 (m, 2H, CH arom.); 6.14 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.62 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.20 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.97 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.86 (s, 3H, OCH_3); 3.84 (s, 6H, OCH_3); 2.62-2.55 (m, 4H, NCH_2); 2.43 (t, *J* = 6.4 Hz, 2H, NCH_2); 2.02-1.94 (m, 2H, CH_2); 1.90-1.83 (m, 2H, CH_2); 1.70-1.63 (m, 2H, CH_2); 1.49-1.43 (m, 2H, CH_2); 1.42-1.33 (m, 2H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.58 (C); 166.19 (C); 162.20 (C); 161.13 (C); 155.85 (C); 152.94 (C); 143.29 (CH); 142.29 (C); 130.98 (C); 129.27 (CH); 128.69 (CH); 128.64 (CH); 128.37 (C); 128.00 (C); 126.92 (CH); 125.46 (CH); 124.93 (CH); 112.94 (CH); 112.68 (CH); 112.43 (C); 106.93 (CH); 101.39 (CH); 66.46 (OCH_2); 65.03 (OCH_2); 64.06 (OCH_2); 60.89 (OCH_3); 56.27 (OCH_3); 54.06 (NCH_2); 50.71 (NCH_2); 50.25 (NCH_2); 28.70 (CH_2); 26.91 (CH_2); 26.77 (CH_2); 23.87 (CH_2) ppm.
Hydrochloride: yellow solid; mp 114-117 °C.

(*E*)-5-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)pentyl anthracene-9-carboxylate 35 (KIS 8)

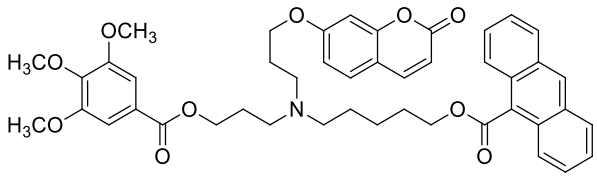


Following method **B**, compound **35** (0.020 g, yield: 18.5 %) was synthesized as a pale-yellow oil, starting from anthracene-9-carboxylic acid (0.046 g, 0.21 mmol) and **224** (0.080 g, 0.14 mmol).

Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.47 (s, 1H, CH arom.); 7.97 (d, *J* = 9.2 Hz, 4H, CH arom.); 7.54 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.51-7.42 (m, 5H, CH arom. and C*H*=CH); 7.24 (d, *J* = 9.2 Hz, 1H, CH arom.); 6.74-6.71 (m, 2H, CH arom.); 6.69 (s, 2H, CH arom.); 6.26 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.15 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.56 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.18 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.98 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.83 (s, 3H, OCH_3); 3.82 (s, 6H, OCH_3); 2.80-2.34 (m, 6H, NCH_2); 1.91-1.78 (m, 4H, CH_2); 1.66-1.42 (m, 6H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 160.91 (C); 155.68 (C); 153.46 (C); 145.97 (CH); 143.21 (CH); 130.94 (C); 129.46 (CH); 129.00 (CH); 128.74 (CH); 128.34 (C); 127.17 (CH); 125.58 (CH); 124.78 (CH); 116.11 (CH); 113.64 (CH); 113.09 (CH); 112.15 (CH); 105.39 (CH); 101.70 (CH); 65.20 (OCH_2); 61.04 (OCH_2); 60.99 (OCH_3); 56.20 (OCH_3); 52.72 (NCH_2); 50.26 (NCH_2); 28.06 (CH_2); 23.73 (CH_2); 23.51 (CH_2); 23.03 (CH_2); 22.91 (CH_2) ppm.
Hydrochloride: yellow solid.

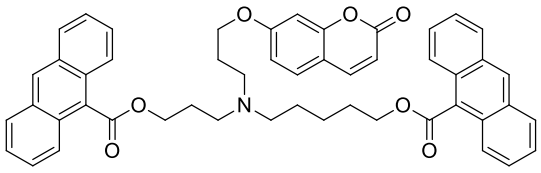
5-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(3-((3,4,5-trimethoxybenzoyl)oxy)propyl)amino)pentyl anthracene-9-carboxylate 36 (LB64)



Following method **B**, compound **36** (0.063 g, yield: 79.7 %) was synthesized as a yellow oil, starting from anthracene-9-carboxylic acid (0.034 g, 0.15 mmol) and **225** (0.058 g, 0.10 mmol).

Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.46 (s, 1H, CH arom.); 7.98-7.95 (m, 4H, CH arom.); 7.51-7.41 (m, 5H, CH arom. and C*H*=CH); 7.22 (s, 2H, CH arom.); 7.20 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.71-6.68 (m, 2H, CH arom.); 6.13 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.54 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.27 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.94 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.85 (s, 3H, OCH_3); 3.83 (s, 6H, OCH_3); 2.61-2.49 (m, 4H, NCH_2); 2.48-2.38 (m, 2H, NCH_2); 1.90-1.76 (m, 6H, CH_2); 1.57-1.40 (m, 4H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.66 (C); 166.10 (C); 162.07 (C); 161.16 (C); 155.80 (C); 152.92 (C); 143.33 (CH); 130.96 (C); 129.26 (CH); 128.73 (CH); 128.65 (CH); 128.34 (C); 128.05 (C); 126.96 (CH); 125.48 (CH); 125.23 (C); 124.93 (CH); 112.96 (CH); 112.60 (CH); 112.46 (C); 106.78 (CH); 101.33 (CH); 66.27 (OCH_2); 65.71 (OCH_2); 63.25 (OCH_2); 60.92 (OCH_3); 56.24 (OCH_3); 53.86 (NCH_2); 50.38 (NCH_2); 50.11 (NCH_2); 28.65 (CH_2); 24.01 (CH_2) ppm.
Hydrochloride: pale yellow solid; mp 120-123 °C.

3-((5-((Anthracene-9-carbonyl)oxy)pentyl)(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)propyl anthracene-9-carboxylate 37 (LB60)

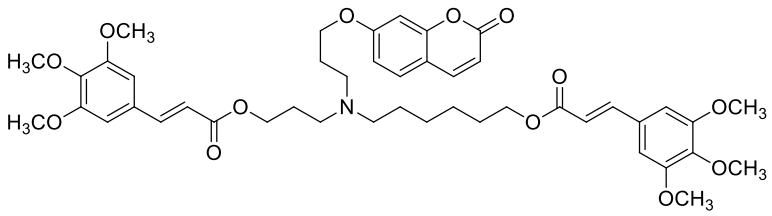


Following method **B**, compound **37** (0.045 g, yield: 66.8 %) was synthesized as a yellow oil, starting from anthracene-9-carboxylic acid (0.029 g, 0.13 mmol) and **226** (0.049 g, 0.086 mmol).

Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.46 (s, 2H, CH arom.); 8.00-7.95 (m, 8H, CH arom.); 7.51-7.40 (m, 9H, CH arom. and C*H*=CH); 7.12 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.68-6.65 (m, 2H, CH arom.); 6.12 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.60 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.52 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.91 (t, *J* = 6.4 Hz, 2H, OCH_2); 2.59-2.53 (m, 4H, NCH_2); 2.42 (t, *J* = 6.4 Hz, 2H, NCH_2); 2.01-1.92 (m, 2H, CH_2); 1.86-1.73 (m, 4H, CH_2); 1.55-1.40 (m, 4H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.68 (C); 162.13 (C); 161.22 (C); 155.79 (C); 143.33 (CH); 142.61 (C); 141.89 (C); 130.98 (C); 129.31 (CH); 129.25 (CH); 128.64 (CH); 128.37 (C);

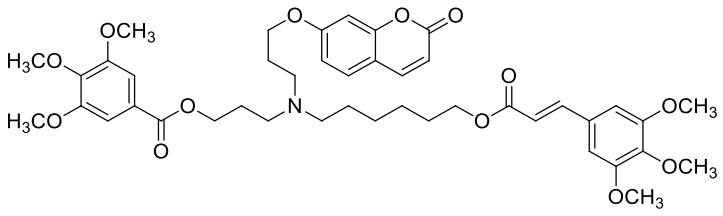
128.11 (C); 127.97 (C); 126.95 (CH); 125.48 (CH); 124.97 (CH); 124.93 (CH); 112.88 (CH); 112.65 (CH); 112.39 (C); 101.33 (CH); 66.35 (OCH_2); 65.74 (OCH_2); 64.03 (OCH_2); 53.93 (NCH_2); 50.63 (NCH_2); 50.15 (NCH_2); 28.67 (CH_2); 26.75 (CH_2); 26.60 (CH_2); 24.03 (CH_2) ppm.
Hydrochloride: yellow solid; mp 118-121 (dec) °C.

(*E*)-6-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(3-(((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)hexyl 3-(3,4,5-trimethoxyphenyl)acrylate 38 (KIS 3)



Following method **A**, compound **38** (0.082 g, yield: 100.0 %) was synthesized as a pale-yellow oil, starting from **227** (0.060 g, 0.10 mmol) and *E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (0.036 g, 0.15 mmol) in 4.0 mL of dry CH_2Cl_2.
Free base: TLC: CH_2Cl_2/CH_3OH/NH_4OH 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.55-7.51 (m, 3H, C*H*=CH); 7.28 (d, *J* = 9.2 Hz, 1H, CH arom.); 6.80- 6.76 (m, 2H, CH arom.); 6.71 (s, 2H, CH arom.); 6.70 (s, 2H, CH arom.); 6.30 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.27 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.16 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.19 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.11 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.04 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.84 (s, 9H, OCH_3); 3.84 (s, 9H, OCH_3); 2.56 (t, *J* = 6.4 Hz, 2H, NCH_2); 2.50 (t, *J* = 6.4 Hz, 2H, NCH_2) 2.39 (t, *J* = 6.4 Hz, 2H, NCH_2); 1.93-1.86 (m, 2H, CH_2); 1.82-1.77 (m, 2H, CH_2); 1.64-1.57 (m, 2H, CH_2); 1.43-1.29 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.03 (C); 162.31 (C); 155.91 (C); 153.43 (C); 144.73 (CH); 144.62 (CH); 143.37 (CH); 140.08 (C); 129.93 (C); 129.86 (C); 128.71 (CH); 117.43 (CH); 117.24 (CH); 112.95 (CH); 112.83 (CH); 112.43 (C); 105.22 (CH); 101.38 (CH); 66.52 (OCH_2); 64.56 (OCH_2); 62.90 (OCH_2); 60.97 (OCH_3); 56.17 (OCH_3); 54.10 (NCH_2); 50.52 (NCH_2); 50.16 (NCH_2); 28.74 (CH_2); 27.23 (CH_2); 27.17 (CH_2); 27.03 (CH_2); 26.71 (CH_2); 25.93 (CH_2) ppm.
Hydrochloride: white solid; mp 87-90 °C.

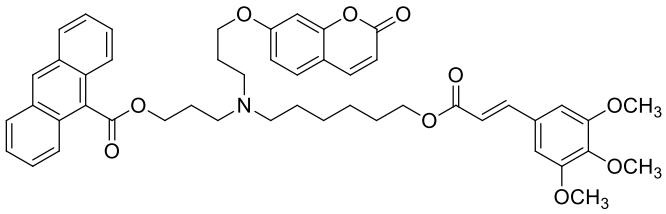
(*E*)-3-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(6-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)hexyl)amino)propyl 3,4,5-trimethoxybenzoate 39 (KIS 2)



Following method **B**, compound **39** (0.043 g, yield: 40.6 %) was synthesized as a pale-yellow oil, starting from 3,4,5-trimethoxybenzoic acid (0.043 g, 0.20 mmol) and **227** (0.080 g, 0.13 mmol).
Free base: TLC: CH_2Cl_2/CH_3OH/NH_4OH 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.55 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 7.54 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.28 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.22 (s, 2H, CH arom.); 6.78-6.75 (m, 2H, CH

arom.); 6.71 (s, 2H, CH arom.); 6.30 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.17 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.30 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.10 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.03 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.86 (s, 3H, OCH_3); 3.85 (s, 6H, OCH_3); 3.84 (s, 6H, OCH_3); 3.83 (s, 3H, OCH_3); 2.59-2.52 (m, 4H, NCH_2); 2.40 (t, *J* = 6.4 Hz, 2H, NCH_2); 1.91-1.84 (m, 4H, CH_2); 1.64-1.56 (m, 2H, CH_2); 1.44-1.36 (m, 2H, CH_2); 1.35-1.25 (m, 4H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.02 (C); 166.12 (C); 162.27 (C); 161.17 (C); 155.89 (C); 153.42 (C); 152.90 (C); 144.62 (CH); 143.36 (CH); 142.18 (C); 140.07 (C); 129.93 (C); 128.71 (CH); 125.34 (C); 117.43 (CH); 112.96 (CH); 112.80 (CH); 112.43 (C); 106.75 (CH); 105.22 (CH); 101.30 (CH); 66.45 (OCH_2); 64.53 (OCH_2); 63.43 (OCH_2); 60.96 (OCH_3); 60.91 (OCH_3); 56.23 (OCH_3); 56.16 (OCH_3); 54.11 (NCH_2); 50.41 (NCH_2); 50.13 (NCH_2); 28.74 (CH_2); 27.24 (CH_2); 27.17 (CH_2); 27.02 (CH_2); 26.66 (CH_2); 25.91 (CH_2) ppm.
Hydrochloride: white solid; mp 71-74 °C.

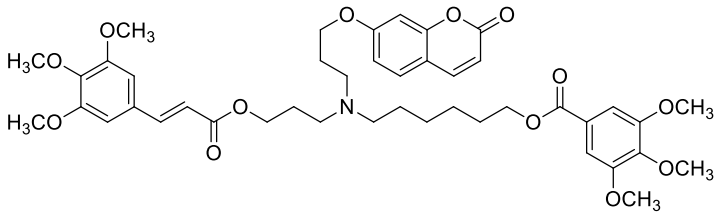
(*E*)-3-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(6-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)hexyl)amino)propyl anthracene-9-carboxylate 40 (KIS 4)



Following method **B**, compound **40** (0.068 g, yield: 56.5 %) was synthesized as a pale-yellow oil, starting from anthracene-9-carboxylic acid (0.050 g, 0.23 mmol) and **227** (0.090 g, 0.15 mmol).

Free base: TLC: CH_2Cl_2/CH_3OH/NH_4OH 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.47 (s, 1H, CH arom.); 8.01-7.94 (m, 4H, CH arom.); 7.55 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.52-7.43 (m, 5H, CH arom. and C*H*=CH); 7.21 (d, *J* = 9.2 Hz, 1H, CH arom.); 6.74-6.72 (m, 2H, CH arom.); 6.71 (s, 2H, CH arom.); 6.30 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.16 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.62 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.10 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.99 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.84 (s, 3H, OCH_3); 3.83 (s, 6H, OCH_3); 2.60-2.55 (m, 4H, NCH_2); 2.40 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.01-1.95 (m, 2H, CH_2); 1.89.1.83 (m, 2H, CH_2); 1.62-1.55 (m, 2H, CH_2); 1.45-1.36 (m, 2H, CH_2); 1.34.1.25 (m, 4H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.64 (C); 167.04 (C); 162.21 (C); 161.22 (C); 155.84 (C); 153.42 (C); 144.62 (CH); 143.36 (CH); 140.06 (C); 130.97 (C); 129.93 (C); 129.30 (CH); 128.66 (CH); 128.36 (C); 127.98 (C); 126.96 (CH); 125.49 (CH); 124.93 (CH); 117.44 (CH); 112.95 (CH); 112.78 (CH); 112.41 (C); 105.20 (CH); 101.32 (CH); 66.42 (OCH_2); 64.55 (OCH_2); 64.08 (OCH_2); 60.98 (OCH_3); 56.15 (OCH_3); 54.08 (NCH_2); 50.63 (NCH_2); 50.20 (NCH_2); 28.72 (CH_2); 27.15 (CH_2); 26.83 (CH_2); 26.65 (CH_2); 25.90 (CH_2) ppm.
Hydrochloride: pale yellow solid; mp 99-102 °C.

(*E*)-6-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)hexyl 3,4,5-trimethoxybenzoate 41 (LB52)



Following method **B**, compound **41** (0.096 g, yield: 97.7 %) was synthesized as a pale-yellow oil, starting from (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (0.044 g, 0.19 mmol) and **228** (0.071 g, 0.12 mmol).

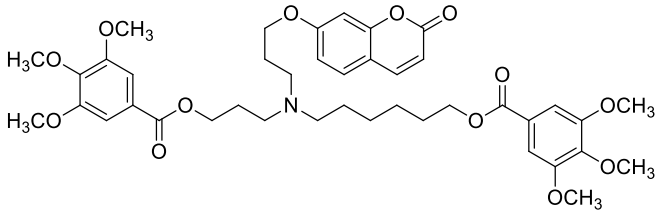
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.51 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 7.50 (d, *J* =15.6 Hz, 1H, C*H*=CH); 7.25 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.21 (s, 2H, CH arom.); 6.75-6.72 (m, 2H, CH arom.); 6.66 (s, 2H, CH arom.); 6.24 (d, *J* =15.6 Hz, 1H, C*H*=CH); 6.11 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.20-4.14 (m, 4H, OCH_2); 4.00 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.82 (s, 9H, OCH_3); 3.80 (s, 6H, OCH_3); 3.80 (s, 3H, OCH_3); 2.53 (t, *J* = 6.4 Hz, 2H, NCH_2); 2.48 (t, *J* = 6.4 Hz, 2H, NCH_2); 2.36 (t, *J* =6.4 Hz, 2H, NCH_2); 1.90-1.82 (m, 2H, CH_2); 1.81-1.72 (m, 2H, CH_2); 1.71-1.62 (m, 2H, CH_2); 1.45-1.23 (m, 6H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.87 (C); 166.18 (C); 162.26 (C); 161.11 (C); 155.86 (C); 153.39 (C); 152.88 (C); 144.69 (CH); 143.36 (CH); 142.13 (C); 140.10 (C); 129.84 (C); 128.72 (CH); 125.45 (C); 117.21 (CH); 112.87 (CH); 112.74 (CH); 112.41 (C); 106.78 (CH); 105.22 (CH); 101.35 (CH); 66.48 (OCH_2); 65.10 (OCH_2); 62.82 (OCH_2); 60.91 (OCH_3); 60.86 (OCH_3); 56.22 (OCH_3); 56.14 (OCH_3); 54.06 (NCH_2); 50.47 (NCH_2); 50.14 (NCH_2); 28.72 (CH_2); 27.13 (CH_2); 26.94 (CH_2); 26.63 (CH_2); 25.93 (CH_2) ppm.

Hydrochloride: white solid; mp 78-81 °C.

6-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(3-((3,4,5-trimethoxybenzoyl)oxy)propyl)amino)hexyl 3,4,5-trimethoxybenzoate 42 (LB58)



Following method **A**, compound **42** (0.089 g, yield: 91.7 %) was synthesized as a pale-yellow oil, starting from **228** (0.072 g, 0.13 mmol) and 3,4,5-trimethoxybenzoic acid (0.040 g, 0.19 mmol) in 5.0 mL of dry CH_2Cl_2.

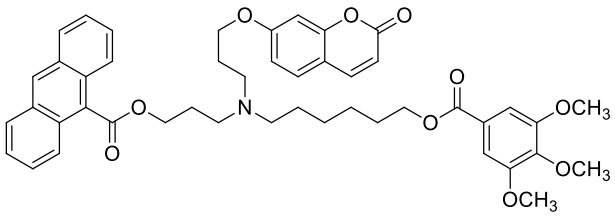
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.52 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 7.25 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.22 (s, 2H, CH arom.); 7.20 (s, 2H, CH arom.); 6.75-6.72 (m, 2H, CH arom.); 6.14 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.27 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.20 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.00 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.84 (s, 9H, OCH_3); 3.83 (s, 9H, OCH_3); 2.58-2.49 (m, 4H, NCH_2); 2.38 (t, *J* = 6.4 Hz, 2H, NCH_2); 1.89-1.81 (m, 4H, CH_2); 1.69-1.62 (m, 2H, CH_2); 1.45-1.23 (m, 6H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.19 (C); 166.08 (C); 162.23 (C); 161.10 (C); 155.86 (C); 152.90 (C); 143.33 (CH); 142.19 (C); 128.72 (CH); 125.46 (C); 125.31 (C); 112.93 (CH); 112.73 (CH); 112.42 (C); 106.83 (CH); 106.77 (CH); 101.30 (CH); 66.44 (OCH_2); 65.09 (OCH_2); 63.38 (OCH_2); 60.87 (OCH_3); 56.24 (OCH_3); 56.22 (OCH_3); 54.09 (NCH_2); 50.40

(NCH_2); 50.15 (NCH_2); 28.74 (CH_2); 27.15 (CH_2); 26.95 (CH_2); 26.61 (CH_2); 25.93 (CH_2) ppm.
Hydrochloride: pale yellow solid; mp 83-85 °C.

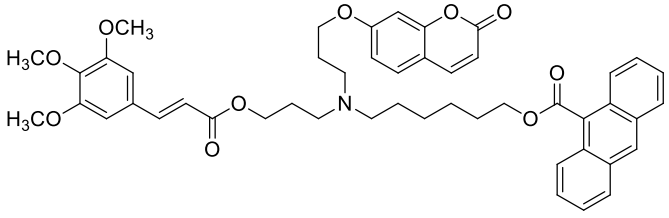
3-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(6-((3,4,5-trimethoxybenzoyl)oxy)hexyl)amino)propyl anthracene-9-carboxylate 43 (LB54)



Following method **B**, compound **43** (0.016 g, yield: 14.2 %) was synthesized as a yellow oil, starting from anthracene-9-carboxylic acid (0.049 g, 0.21 mmol) and **228** (0.084 g, 0.15 mmol).

Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.47 (s, 1H, CH arom.); 7.99-7.96 (m, 4H, CH arom.); 7.52-7.42 (m, 5H, CH arom. and C*H*=CH); 7.24 (s, 2H, CH arom.); 7.21 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.73-6.71 (m, 2H, CH arom.); 6.16 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.62 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.20 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.98 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.86 (s, 3H, OCH_3); 3.85 (s, 6H, OCH_3); 2.71-2.57 (m, 4H, NCH_2); 2.52-2.40 (m, 2H, NCH_2); 2.10-1.95 (m, 2H, CH_2); 1.94-1.85 (m, 2H, CH_2); 1.71-1.62 (m, 2H, CH_2); 1.50-1.39 (m, 2H, CH_2); 1.37-1.25 (m, 4H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.24 (C); 162.15 (C); 161.14 (C); 152.94 (C); 143.30 (CH); 142.25 (C); 130.98 (C); 129.32 (CH); 128.66 (CH); 128.38 (C); 126.96 (CH); 125.48 (CH); 124.92 (CH); 113.00 (CH); 112.73 (CH); 112.46 (C); 106.89 (CH); 101.37 (CH); 66.39 (OCH_2); 65.09 (OCH_2); 63.97 (OCH_2); 60.91 (OCH_3); 56.28 (OCH_3); 54.06 (NCH_2); 50.63 (NCH_2); 50.26 (NCH_2); 28.72 (CH_2); 27.13 (CH_2); 25.89 (CH_2) ppm.
Hydrochloride: yellow solid; mp 148-151 °C.

(*E*)-6-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)hexyl anthracene-9-carboxylate 44 (LB55)

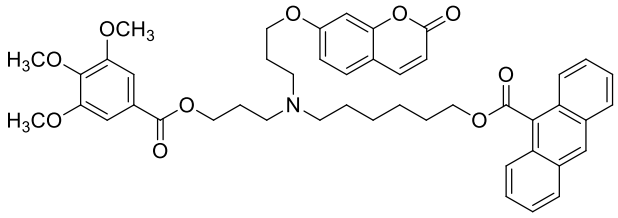


Following method **B**, compound **44** (0.060 g, yield: 100.0 %) was synthesized as a yellow oil, starting from (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (0.027 g, 0.11 mmol) and **229** (0.043 g, 0.074 mmol).

Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.44 (s, 1H, CH arom.); 7.98 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.95 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.54 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.50-7.40 (m, 5H, CH arom. and C*H*=CH); 7.17 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.71-6.69 (m, 2H, CH arom.); 6.68 (s, 2H, CH arom.); 6.27 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.09 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.54 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.18 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.96 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.83 (s, 3H, OCH_3); 3.80 (s, 6H, OCH_3); 2.54 (t, *J* = 6.4 Hz, 2H, NCH_2); 2.49 (t, *J* = 6.4 Hz, 2H, NCH_2); 2.38 (t, *J* = 6.4 Hz, 2H, NCH_2); 1.86-1.75 (m, 6H, CH_2); 1.49-1.27 (m, 6H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.64 (C); 166.88 (C); 162.23 (C); 161.12 (C); 155.85 (C); 153.44 (C); 144.72 (CH); 143.31 (CH); 141.92 (C); 140.22 (C); 130.98 (C); 129.86 (C); 129.20 (CH); 128.70 (CH); 128.62 (CH); 128.36 (C); 128.18 (C); 126.90 (CH); 125.45 (CH); 124.99 (CH); 117.25 (CH); 112.86 (CH); 112.68 (CH); 112.41 (C); 105.34 (CH); 101.39 (CH); 66.46 (OCH_2); 65.78 (OCH_2); 62.82 (OCH_2); 60.93 (OCH_3); 56.17 (OCH_3); 54.01 (NCH_2); 50.54 (NCH_2); 50.17 (NCH_2); 28.75 (CH_2); 27.06 (CH_2); 26.88 (CH_2); 26.62 (CH_2); 26.05 (CH_2) ppm.
Hydrochloride: pale yellow solid; mp 92-95 °C.

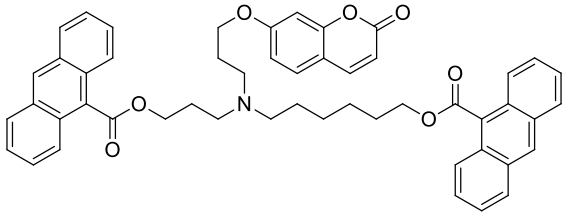
6-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(3-((3,4,5-trimethoxybenzoyl)oxy)propyl)amino)hexyl anthracene-9-carboxylate 45 (LB56)



Following method **A**, compound **45** (0.076 g, yield: 77.7 %) was synthesized as a yellow oil, starting from **229** (0.074 g, 0.13 mmol) and 3,4,5-trimethoxybenzoic acid (0.040 g, 0.19 mmol) in 5.0 mL of dry CH_2Cl_2.

Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.44 (s, 1H, CH arom.); 7.98 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.95 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.51-7.40 (m, 5H, CH arom. and C*H*=CH); 7.22 (s, 2H, CH arom.); 7.16 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.70-6.67 (m, 2H, CH arom.); 6.11 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.54 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.27 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.95 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.85 (s, 3H, OCH_3); 3.83 (s, 6H, OCH_3); 2.56-2.48 (m, 4H, NCH_2); 2.37 (t, *J* = 6.4 Hz, 2H, NCH_2); 1.89-1.74 (m, 6H, CH_2); 1.48-1.28 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.66 (C); 166.11 (C); 162.21 (C); 161.14 (C); 155.84 (C); 152.93 (C); 143.31 (CH); 142.27 (C); 130.99 (C); 129.21 (CH); 128.69 (CH); 128.62 (CH); 128.37 (C); 128.17 (C); 126.91 (CH); 125.46 (CH); 125.34 (C); 124.99 (CH); 112.90 (CH); 112.69 (CH); 112.41 (C); 106.83 (CH); 101.31 (CH); 66.41 (OCH_2); 65.78 (OCH_2); 63.40 (OCH_2); 60.90 (OCH_3); 56.24 (OCH_3); 54.03 (NCH_2); 50.44 (NCH_2); 50.14 (NCH_2); 28.75 (CH_2); 27.09 (CH_2); 26.92 (CH_2); 26.61 (CH_2); 26.06 (CH_2) ppm.
Hydrochloride: yellow solid; mp 87-90 °C.

3-((6-((Anthracene-9-carbonyl)oxy)hexyl)(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)propyl anthracene-9-carboxylate 46 (KIS 5)



Following method **B**, compound **46** (0.030 g, yield: 44.3 %) was synthesized as a pale-yellow oil, starting from anthracene-9-carboxylic acid (0.029 g, 0.13 mmol) and **229** (0.050 g, 0.086 mmol).

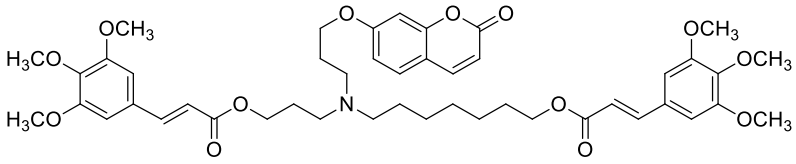
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.47 (s, 1H, CH arom.); 8.45 (s, 1H, CH arom.); 8.00-7.94 (m, 8H, CH arom.); 7.51-7.40 (m, 9H, CH arom. and C*H*=CH); 7.12 (d, *J* = 9.2 Hz, 1H, CH arom.); 6.68-6.66 (m, 2H, CH arom.); 6.12 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.60 (t, *J* = 6.4 Hz, 2H,

OCH_2); 4.53 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.93 (t, *J* = 6.0 Hz, 2H, OCH_2); 2.58-2.52 (m, 4H, NCH_2); 2.38 (t, *J* = 7.2 Hz, 2H, NCH_2); 1.99-1.92 (m, 2H, CH_2); 1.83-1.73 (m, 4H, CH_2); 1.44-1.30 (m, 6H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.72 (C); 169.66 (C); 162.20 (C); 161.24 (C); 155.79 (C); 143.37 (CH); 142.70 (C); 141.91 (C); 130.98 (C); 129.29 (CH); 129.24 (CH); 128.65 (CH); 128.37 (C); 128.17 (C); 128.03 (C); 126.94 (CH); 125.48 (CH); 125.00 (CH); 124.95 (CH); 112.83(CH); 112.72 (CH); 112.34 (C); 101.28 (CH); 66.42 (OCH_2); 65.84 (OCH_2); 64.13 (OCH_2); 54.03 (NCH_2); 50.63 (NCH_2); 50.16 (NCH_2); 28.73 (CH_2); 27.12 (CH_2); 26.86 (CH_2); 26.72 (CH_2); 26.06 (CH_2) ppm.

Hydrochloride: yellow solid, mp 136-139 °C.

(*E*)-7-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(3-(((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)heptyl 3-(3,4,5-trimethoxyphenyl)acrylate 47 (KIS 11)



Following method **B**, compound **47** (0.050 g, yield: 56.6 %) was synthesized as a pale-yellow oil, starting from (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (0.029 g, 0.12 mmol) and **230** (0.050 g, 0.080 mmol).

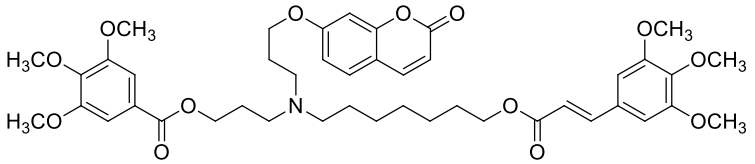
Free base: TLC: CH_2Cl_2/CH_3OH/NH_4OH 95:5:0.5.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.55-7.51 (m, 3H, C*H*=CH); 7.28 (d, *J* = 9.2 Hz, 1H, CH arom.); 6.78-6.75 (m, 2H, CH arom.); 6.70 (s, 2H, CH arom.); 6.68 (s, 2H, CH arom.); 6.29 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.26 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.15 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.18 (t, *J* = 6.0 Hz, 2H, OCH_2); 4.11 (t, *J* = 6.0 Hz, 2H, OCH_2); 4.03 (t, *J* = 6.0 Hz, 2H, OCH_2); 3.83 (s, 18H, OCH_3); 2.67-2.47 (m, 4H, NCH_2); 2.44-2.32 (m, 2H, NCH_2); 1.95-1.85 (m, 2H, CH_2); 1.84-1.74 (m, 2H, CH_2); 1.66-1.56 (m, 2H, CH_2); 1.44-1.35 (m, 2H, CH_2); 1.34-1.17 (m, 6H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.03 (C); 166.89 (C); 162.20 (C); 161.16 (C); 155.86 (C); 153.42 (C); 144.81 (CH); 144.59 (CH); 143.37 (CH); 140.14 (C); 140.06 (C); 129.93 (C); 129.83 (C); 128.75 (CH); 117.44 (CH); 117.14 (CH); 112.98 (CH); 112.70 (CH); 112.49 (C); 105.30 (CH); 105.21 (CH); 101.44 (CH); 66.43 (OCH_2); 64.56 (OCH_2); 62.71 (OCH_2); 60.95 (OCH_3); 56.15 (OCH_3); 54.03 (NCH_2); 50.48 (NCH_2); 50.18 (NCH_2); 29.71 (CH_2); 29.21 (CH_2); 28.69 (CH_2); 27.35 (CH_2); 25.92 (CH_2) ppm.

Hydrochloride: white solid, mp 90-92 °C.

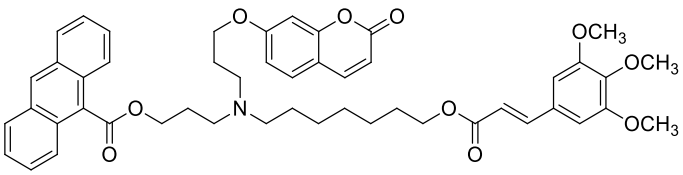
(*E*)-3-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(7-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)heptyl)amino)propyl 3,4,5-trimethoxybenzoate 48 (KIS 16)



Following method **B**, compound **48** (0.026 g, yield: 33.0 %) was synthesized as a pale-yellow oil, starting from (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (0.035 g, 0.15 mmol) and **231** (0.058 g, 0.10 mmol).

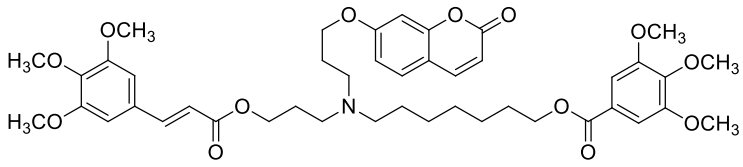
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.59 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 7.58 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.32 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.26 (s, 2H, CH arom.); 6.81-6.78 (m, 2H, CH arom.); 6.75 (s, 2H, CH arom.); 6.34 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.21 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.33 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.15 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.06 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.89 (s, 3H, OCH_3); 3.88 (s, 6H, OCH_3); 3.87 (s, 6H, OCH_3); 3.86 (s, 3H, OCH_3); 2.70-2.50 (m, 4H, NCH_2); 2.49-2.31 (m, 2H, NCH_2); 2.03-1.82 (m, 4H, CH_2); 1.75- 1.57 (m, 2H, CH_2); 1.51-1.39 (m, 2H, CH_2); 1.33-1.22 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.03 (C); 166.12 (C); 162.10 (C); 161.14 (C); 155.85 (C); 153.42 (C); 152.92 (C); 144.61 (CH); 143.34 (CH); 142.24 (C); 140.08 (C); 129.93 (C); 128.76 (CH); 125.21 (C); 117.44 (CH); 113.06 (CH); 112.66 (CH); 112.53 (C); 106.78 (CH); 105.22 (CH); 101.40 (CH); 66.33 (OCH_2); 64.54 (OCH_2); 63.20 (OCH_2); 60.96 (OCH_3); 60.91 (OCH_3); 56.25 (OCH_3); 56.16 (OCH_3); 53.99 (NCH_2); 50.38 (NCH_2); 50.18 (NCH_2); 29.18 (CH_2); 28.69 (CH_2); 28.53 (CH_2); 27.33 (CH_2); 25.92 (CH_2) ppm.
Hydrochloride: white solid, mp 84-87 °C.

(*E*)-3-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(7-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)heptyl)amino)propyl anthracene-9-carboxylate
49 (KIS 13)



Following method **B**, compound **49** (0.080 g, yield: 86.0 %) was synthesized as a pale-yellow oil, starting from anthracene-9-carboxylic acid (0.038 g, 0.17 mmol) and **230** (0.070 g, 0.11 mmol).
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.48 (s, 1H, CH arom.); 7.98 (d, *J* = 8.4 Hz, 4H, CH arom.); 7.57-7.42 (m, 6H, CH arom. and C*H*=CH); 7.21 (d, *J* = 9.2 Hz, 1H, CH arom.); 6.73-6.70 (m, 4H, CH arom.); 6.30 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.16 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.62 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.12 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.98 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.84 (s, 9H, OCH_3); 2.70-2.53 (m, 4H, NCH_2); 2.50-2.32 (m, 2H, NCH_2); 2.10-1.96 (m, 2H, CH_2); 1.95-1.79 (m, 2H, CH_2); 1.66-1.56 (m, 2H, CH_2); 1.44-1.35 (m, 2H, CH_2); 1.29-1.12 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.59 (C); 167.04 (C); 162.15 (C); 161.21 (C); 155.81 (C); 153.41 (C); 144.59 (CH); 143.37 (CH); 130.95 (C); 129.94 (C); 129.31 (CH); 128.65 (CH); 128.35 (C); 126.96 (CH); 125.48 (CH); 124.92 (CH); 117.47 (CH); 112.93 (CH); 112.69 (CH); 112.43 (C); 105.19 (CH); 101.36 (CH); 66.38 (OCH_2); 64.59 (OCH_2); 64.03 (OCH_2); 60.97 (OCH_3); 56.14 (OCH_3); 54.08 (NCH_2); 50.64 (NCH_2); 50.21 (NCH_2); 29.72 (CH_2); 29.19 (CH_2); 28.70 (CH_2); 27.36 (CH_2); 26.12 (CH_2); 25.93 (CH_2) ppm.
Hydrochloride: pale yellow solid, mp 100-102 °C.

(*E*)-7-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)heptyl 3,4,5-trimethoxybenzoate 50 (KIS 21)



Following method **A**, compound **50** (0.054 g, yield: 74.5 %) was synthesized as a pale-yellow oil, starting from **232** (0.055 g, 0.090 mmol) and 3,4,5-trimethoxybenzoic acid (0.029 g, 0.14 mmol).

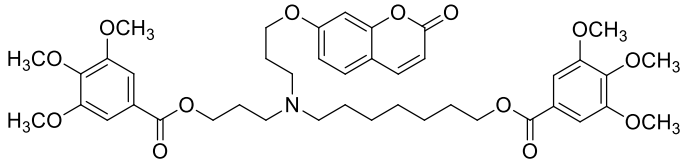
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.53 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 7.52 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.27 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.23 (s, 2H, CH arom.); 6.77-6.74 (m, 2H, CH arom.); 6.68 (s, 2H, CH arom.); 6.26 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.14 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.21 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.17 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.02 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.84 (s, 6H, OCH_3); 3.83 (s, 3H, OCH_3); 3.82 (s, 6H, OCH_3); 3.81 (s, 3H, OCH_3); 2.55 (t, *J* = 6.4 Hz, 2H, NCH_2); 2.49 (t, *J* = 6.4 Hz, 2H, NCH_2); 2.37 (t, *J* = 6.4 Hz, 2H, NCH_2); 1.90-1.85 (m, 2H, CH_2); 1.80-1.76 (m, 2H, CH_2); 1.70-1.63 (m, 2H, CH_2); 1.43-1.22 (m, 8H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.90 (C); 166.24 (C); 162.25 (C); 161.17 (C); 155.87 (C); 153.42 (C); 152.90 (C); 144.75 (CH); 143.38 (CH); 142.12 (C); 140.11 (C); 129.85 (C); 128.73 (CH); 125.50 (C); 117.20 (CH); 112.95 (CH); 112.73 (CH); 112.45 (C); 106.77 (CH); 105.22 (CH); 101.42 (CH); 66.49 (OCH_2); 65.18 (OCH_2); 62.82 (OCH_2); 60.96 (OCH_3); 60.90 (OCH_3); 56.24 (OCH_3); 56.16 (OCH_3); 54.10 (NCH_2); 50.49 (NCH_2); 50.16 (NCH_2); 29.26 (CH_2); 28.70 (CH_2); 27.41 (CH_2); 26.86 (CH_2); 25.98 (CH_2) ppm.

Hydrochloride: white solid, mp 73-76 °C.

7-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(3-((3,4,5-trimethoxybenzoyl)oxy)propyl)amino)heptyl 3,4,5-trimethoxybenzoate 51 (KIS 17)



Following method **A**, compound **51** (0.052 g, yield: 69.2 %) was synthesized as a pale yellow oil, starting from **231** (0.056 g, 0.10 mmol) and 3,4,5-trimethoxybenzoic acid (0.031 g, 0.14 mmol).

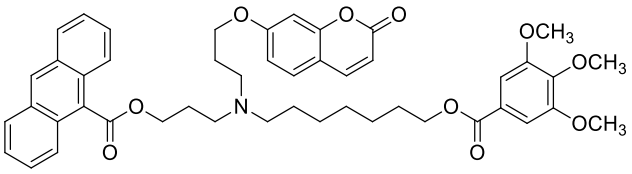
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.57 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 7.30 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.27 (s, 2H, CH arom.); 7.25 (s, 2H, CH arom.); 6.79-6.76 (m, 2H, CH arom.); 6.19 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.32 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.25 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.05 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.88 (s, 12H, OCH_3); 3.87 (s, 6H, OCH_3); 2.64-2.52 (m, 4H, NCH_2); 2.46-2.35 (m, 2H, NCH_2); 1.97-1.84 (m, 4H, CH_2); 1.75-1.66 (m, 2H, CH_2); 1.46-1.24 (m, 8H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.24 (C); 166.10 (C); 162.21 (C); 161.17 (C); 155.85 (C); 152.89 (C); 143.38 (CH); 142.14 (C); 142.09 (C); 128.74 (CH); 125.49 (C); 125.29 (C); 112.95 (CH); 112.71 (CH); 112.44 (C); 106.74 (CH); 106.71 (CH); 101.33 (CH); 66.42 (OCH_2); 65.17 (OCH_2); 63.36 (OCH_2); 60.91 (OCH_3); 56.23 (OCH_3); 54.10 (NCH_2); 50.37 (NCH_2); 50.13 (NCH_2); 29.26 (CH_2); 28.70 (CH_2); 27.42 (CH_2); 26.88 (CH_2); 26.48 (CH_2); 25.98 (CH_2) ppm.

Hydrochloride: pale yellow solid, mp 74-77 °C.

3-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(7-((3,4,5-trimethoxybenzoyl)oxy)heptyl)amino)propyl anthracene-9-carboxylate 52 (LB121)



Following method **A**, compound **52** (0.11 g, yield: 92.3 %) was synthesized as a yellow oil, starting from **233** (0.090 g, 0.15 mmol) and 3,4,5-trimethoxybenzoic acid (0.048 g, 0.23 mmol).

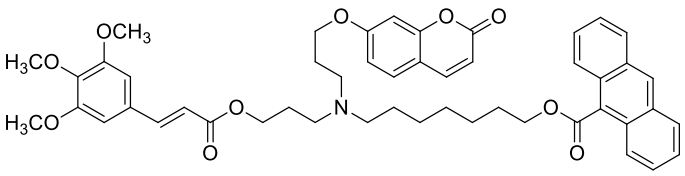
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.51 (s, 1H, CH arom.); 8.01 (d, *J* = 8.8 Hz, 4H, CH arom.); 7.55-7.45 (m, 5H, CH arom. and C*H*=CH); 7.28 (s, 2H, CH arom.); 7.25 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.76-6.74 (m, 2H, CH arom.); 6.19 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.65 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.25 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.01 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.89 (s, 3H, OCH_3); 3.89 (s, 6H, OCH_3); 2.82-2.54 (m, 4H, NCH_2); 2.53-2.38 (m, 2H, NCH_2); 2.13-1.98 (m, 2H, CH_2); 1.97-1.84 (m, 2H, CH_2); 1.76-1.66 (m, 2H, CH_2); 1.53-1.39 (m, 2H, CH_2); 1.38-1.23 (m, 6H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.60 (C); 166.23 (C); 162.22 (C); 161.15 (C); 155.81 (C); 152.92 (C); 143.35 (CH); 142.18 (C); 130.96 (C); 129.26 (CH); 128.69 (CH); 128.64 (CH); 128.35 (C); 128.04 (C); 126.92 (CH); 125.53 (C); 125.46 (CH); 124.94 (CH); 112.85 (CH); 112.69 (CH); 112.37 (C); 106.84 (CH); 101.33 (CH); 66.48 (OCH_2); 65.19 (OCH_2); 64.13 (OCH_2); 60.89 (OCH_3); 56.25 (OCH_3); 54.15 (NCH_2); 50.68 (NCH_2); 50.20 (NCH_2); 29.23 (CH_2); 28.70 (CH_2); 27.41 (CH_2); 27.19 (CH_2); 26.90 (CH_2); 26.78 (CH_2); 25.97 (CH_2) ppm.

Hydrochloride: pale yellow solid, mp 75-77 °C.

(*E*)-7-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)heptyl anthracene-9-carboxylate 53 (KIS 20)



Following method **B**, compound **53** (0.13 g, yield: 93.5 %) was synthesized as a pale-yellow oil, starting from anthracene-9-carboxylic acid (0.060 g, 0.27 mmol) and **232** (0.11 g, 0.18 mmol).

Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

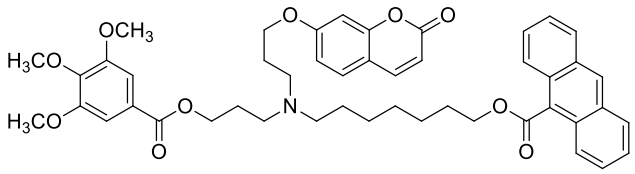
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.46 (s, 1H, CH arom.); 7.97 (t, *J* = 8.4 Hz, 4H, CH arom.); 7.54 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.51-7.41 (m, 5H, CH arom. and C*H*=CH); 7.21 (d, *J* = 9.6 Hz, 1H, CH arom.); 6.74-6.71 (m, 2H, CH arom.); 6.69 (s, 2H, CH arom.); 6.28 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.12 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.55 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.19 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.99 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.83 (s, 3H, OCH_3); 3.82 (s, 6H, OCH_3); 2.56-2.48 (m, 4H, NCH_2); 2.39-2.34 (m, 2H, NCH_2); 1.91-1.74 (m, 6H, CH_2); 1.47-1.21 (m, 8H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.69 (C); 166.91 (C); 162.22 (C); 161.18 (C); 155.82 (C); 153.41 (C); 144.73 (CH); 143.38 (CH); 140.10 (C); 130.96 (C); 129.86 (C); 129.21 (CH);

128.72 (CH); 128.62 (CH); 128.34 (C); 128.17 (C); 126.91 (CH); 125.46 (CH); 124.99 (CH); 117.24 (CH); 112.84 (CH); 112.64 (CH); 112.39 (C); 105.23 (CH); 101.39 (CH); 66.47 (OCH_2); 65.86 (OCH_2); 62.85 (OCH_2); 60.96 (OCH_3); 56.14 (OCH_3); 54.08 (NCH_2); 50.50 (NCH_2); 50.12 (NCH_2); 29.19 (CH_2); 28.73 (CH_2); 27.38 (CH_2); 27.05 (CH_2); 26.86 (CH_2); 26.58 (CH_2); 26.08 (CH_2) ppm.

Hydrochloride: pale yellow solid, mp 107-110 °C.

7-((3-((2-Oxo-2*H*-chromen-7-yl)oxy)propyl)(3-((3,4,5-trimethoxybenzoyl)oxy)propyl)amino)heptyl anthracene-9-carboxylate 54 (KIS 15)



Following method **B**, compound **54** (0.073 g, yield: 77.7 %) was synthesized as a pale-yellow oil, starting from anthracene-9-carboxylic acid (0.040 g, 0.18 mmol) and **231** (0.070 g, 0.12 mmol).

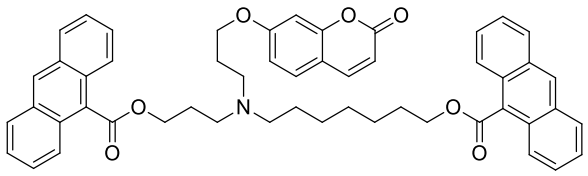
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.47 (s, 1H, CH arom.); 8.01 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.98 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.53-7.43 (m, 5H, CH arom. and C*H*=CH); 7.25 (s, 2H, CH arom.); 7.21 (d, *J* = 9.2 Hz, 1H, CH arom.); 6.74-6.71 (m, 2H, CH arom.); 6.15 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.58 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.31 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.99 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.88 (s, 3H, OCH_3); 3.86 (s, 6H, OCH_3); 2.58-2.52 (m, 4H, NCH_2); 2.40 (t, *J* = 6.4 Hz, 2H, NCH_2); 1.88-1.79 (m, 6H, CH_2); 1.48-1.37 (m, 4H, CH_2); 1.36-1.23 (m, 4H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.71 (C); 166.12 (C); 162.20 (C); 161.18 (C); 155.82 (C); 152.91 (C); 143.36 (CH); 142.18 (C); 130.98 (C); 129.22 (CH); 128.71 (CH); 128.63 (CH); 128.35 (C); 128.16 (C); 126.91 (CH); 125.46 (CH); 125.34 (C); 124.99 (CH); 112.89 (CH); 112.65 (CH); 112.40 (C); 106.75 (CH); 101.33 (CH); 66.42 (OCH_2); 65.86 (OCH_2); 63.40 (OCH_2); 60.92 (OCH_3); 56.23 (OCH_3); 54.10 (NCH_2); 50.40 (NCH_2); 50.11 (NCH_2); 29.20 (CH_2); 28.73 (CH_2); 27.39 (CH_2); 26.89 (CH_2); 26.57 (CH_2); 26.09 (CH_2) ppm.

Hydrochloride: yellow solid; mp 97-99 °C.

3-((7-((Anthracene-9-carbonyl)oxy)heptyl)(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)propyl anthracene-9-carboxylate 55 (LB122)



Following method **B**, compound **55** (0.11 g, yield: 91.1 %) was synthesized as a yellow oil, starting from anthracene-9-carboxylic acid (0.050 g, 0.23 mmol) and **233** (0.090 g, 0.15 mmol).

Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

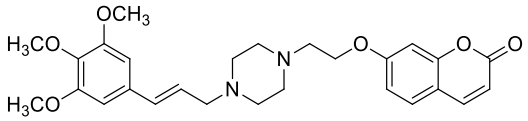
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.48 (s, 2H, CH arom.); 8.05-7.98 (m, 8H, CH arom.); 7.54-7.43 (m, 9H, CH arom. and C*H*=CH); 7.15 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.71-6.69 (m, 2H, CH arom.); 6.15 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.65 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.58 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.96 (t, *J* = 6.4 Hz, 2H, OCH_2); 2.70-2.49 (m, 4H, NCH_2); 2.42 (t, *J* = 6.4 Hz, 2H, NCH_2); 2.07-1.95 (m, 2H, CH_2); 1.91-1.78 (m, 4H, CH_2); 1.48-1.36 (m, 4H, CH_2); 1.34-1.22 (m, 4H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.75 (C); 169.66 (C); 162.17 (C); 161.26 (C); 155.77 (C); 143.39 (CH); 130.98 (C); 129.32 (CH); 129.25 (CH); 128.67 (CH); 128.37 (C); 128.18 (C); 128.01 (C); 126.97 (CH); 126.95 (CH); 125.49 (CH); 125.01 (CH); 124.95 (CH); 112.84 (CH); 112.65 (CH); 112.36 (C); 101.32 (CH); 66.41 (OCH_2); 65.90 (OCH_2); 64.11 (OCH_2); 54.10 (NCH_2); 50.64 (NCH_2); 50.16 (NCH_2); 29.18 (CH_2); 28.74 (CH_2); 27.38 (CH_2); 27.04 (CH_2); 26.78 (CH_2); 26.65 (CH_2); 26.08 (CH_2) ppm.
Hydrochloride: pale yellow solid; mp 117-119 °C.

7.1.1.1.3. Piperazine derivatives

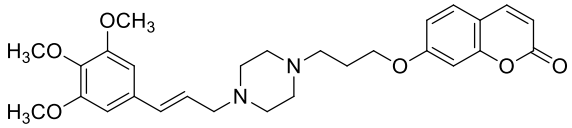
General procedures for the synthesis of piperazine derivatives 56-73.
To a solution of the proper intermediate **235-237**[81] (1 equiv.) in dry CH_3CN, K_2CO_3 (1.2 equiv.) and the adequate 7-(bromoalkoxy)-2*H*-chromen-2-one–**234** and **239-243** (1.2 equiv.) were added. The mixture was stirred at 60 °C overnight, then the solvent was removed under reduced pressure and the residue was treated with CH_2Cl_2. The organic layer was washed twice with 10 % NaOH solution, dried over Na_2SO_4 and concentrated under vacuum. Finally, the residue was purified by flash chromatography, using the proper eluting system, yielding the desired compounds as an oil.

(*E*)-7-(2-(4-(3-(3,4,5-Trimethoxyphenyl)allyl)piperazin-1-yl)ethoxy)-2*H*-chromen-2-one 56 (DAP7)



Following the general procedure, compound **56** (0.020 g, yield: 30.6 %) was synthesized as a pale-yellow oil, starting from **235**[81] (0.040 g, 0.14 mmol) and **239** (0.090 g, 0.15 mmol) in 2.5 mL of dry CH_3CN.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.62 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 7.36 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.85-6.80 (m, 2H, CH arom.); 6.60 (s, 2H, CH arom.); 6.46 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.29-6.15 (m, 2H, CH arom. and C*H*=CH); 4.15 (t, *J* = 5.6 Hz, 2H, OCH_2); 3.85 (s, 6H, OCH_3); 3.83 (s, 3H, OCH_3); 3.20 (d, *J* = 6.4 Hz, 2H, NCH_2Ar); 2.87 (t, *J* = 5.6 Hz, 2H, NCH_2); 2.80-2.45 (m, 8H, NCH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.96 (C); 161.15 (C); 155.85 (C); 153.31 (C); 143.36 (CH); 137.81 (C); 133.11 (CH); 132.55 (C); 128.73 (CH); 125.87 (CH); 113.18 (CH); 113.00 (CH) 112.63 (C); 103.37 (CH); 101.50 (CH); 66.56 (CH_2); 60.91 (OCH_3); 60.87 (CH_2); 56.84 (CH_2); 56.05 (OCH_3); 53.59 (CH_2); 53.09 (CH_2) ppm.
Hydrochloride: white solid.

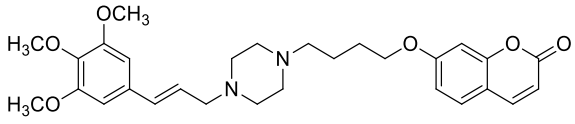
(*E*)-7-(3-(4-(3-(3,4,5-Trimethoxyphenyl)allyl)piperazin-1-yl)propoxy)-2*H*-chromen-2-one 57 (DAP3)



Following the general procedure, compound **57** (0.040 g, yield: 34.0 %) was synthesized as a pale-yellow oil, starting from **235**[81] (0.070 g, 0.24 mmol) and **234** (0.080 g, 0.29 mmol) in 4.0 mL of dry CH_3CN.

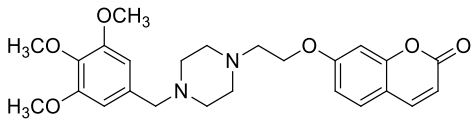
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 7.34 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.85-6.76 (m, 2H, CH arom.); 6.60 (s, 2H, CH arom.); 6.44 (d, *J* = 15.6 Hz, 1H, C*H*=CH); 6.25-6.13 (m, 2H, CH arom. and C*H*=CH); 4.07 (t, *J* = 5.6 Hz, 2H, OCH_2); 3.85 (s, 6H, OCH_3); 3.82 (s, 3H, OCH_3); 3.16 (d, *J* = 6.8 Hz, 2H, NCH_2Ar); 2.75-2.38 (m, 10H, NCH_2); 2.00 (t, *J* = 6.8 Hz, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 162.26 (C); 161.26 (C); 155.88 (C); 153.28 (C); 143.46 (CH); 137.67 (C); 132.99 (CH); 132.60 (C); 128.72 (CH); 126.06 (CH); 112.98 (CH); 112.45 (C); 103.26 (CH); 101.34 (CH); 66.84 (CH_2); 60.94 (CH_2); 60.87 (OCH_3); 56.03 (OCH_3); 54.88 (CH_2); 53.21 (CH_2); 26.84 (CH_2) ppm.
Hydrochloride: white solid; mp 240-243 °C.

(*E*)-7-(4-(4-(3-(3,4,5-Trimethoxyphenyl)allyl)piperazin-1-yl)butoxy)-2*H*-chromen-2-one 58 (DAP5)



Following the general procedure, compound **58** (0.010 g, yield: 23.0 %) was synthesized as a pale-yellow oil, starting from **235**[81] (0.025 g, 0.085 mmol) and **240** (0.030 g, 0.10 mmol) in 4.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.62 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 7.35 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.84-6.77 (m, 2H, CH arom.); 6.60 (s, 2H, CH arom.); 6.45 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.24-6.14 (m, 2H, CH arom. and C*H*=CH); 4.03 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.85 (s, 6H, OCH_3); 3.83 (s, 3H, OCH_3); 3.17 (d, *J* = 6.4 Hz, 2H, NCH_2Ar); 2.90-2.37 (m, 10H, NCH_2); 1.93-1.78 (m, 2H, CH_2); 1.92-1.64 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 162.28 (C); 161.23 (C); 155.92 (C); 153.31 (C); 143.41 (CH); 137.84 (C); 133.16 (CH); 132.55 (C); 128.71 (CH); 125.88 (CH); 112.99 (CH); 112.44 (C); 103.38 (CH); 101.31 (CH); 68.32 (CH_2); 60.92 (OCH_3); 60.88 (CH_2); 58.03 (CH_2); 56.06 (OCH_3); 53.09 (CH_2); 26.96 (CH_2); 23.26 (CH_2) ppm.
Hydrochloride: white solid.

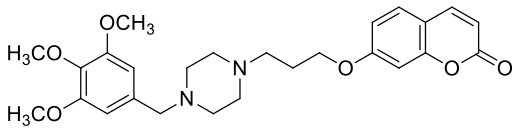
7-(2-(4-(3,4,5-Trimethoxybenzyl)piperazin-1-yl)ethoxy)-2*H*-chromen-2-one 59 (DAP 9)



Following the general procedure, compound **59** (0.12 g, yield: 78.0 %) was synthesized as a yellow oil, starting from **236**[81] (0.090 g, 0.34 mmol) and **239** (0.11 g, 0.40 mmol) in 5.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.58 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 7.31 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.81-6.75 (m, 2H, CH arom.); 6.52 (s, 2H, CH arom.); 6.18 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.11 (t, *J* = 5.6 Hz, 2H, OCH_2); 3.81 (s, 6H, OCH_3); 3.78 (s, 3H, OCH_3); 3.41 (NCH_2Ar); 2.81 (t, *J* = 5.6 Hz, 2H, NCH_2); 2.70-2.55 (m, 4H, NCH_2); 2.54-2.35 (m, 4H, NCH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.94 (C); 161.10 (C); 155.80 (C); 153.07 (C); 143.39 (CH); 136.97 (C); 133.63 (C); 128.76 (CH); 113.08 (CH); 112.94 (CH); 112.60 (C); 105.89 (CH) 101.48 (CH); 66.59 (CH_2); 63.11 (CH_2); 60.78 (OCH_3); 56.81 (CH_2); 56.11 (OCH_3); 53.57 (CH_2); 52.89 (CH_2) ppm.

Hydrochloride: white solid; mp 239-241 °C.

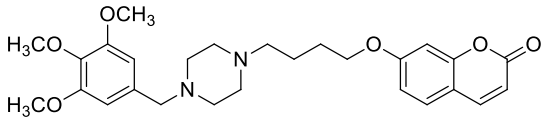
7-(3-(4-(3,4,5-Trimethoxybenzyl)piperazin-1-yl)propoxy)-2*H*-chromen-2-one 60 (DAP11)



Following the general procedure, compound **60** (0.11 g, yield: 89.9 %) was synthesized as a yellow oil, starting from **236**[81] (0.070 g, 0.26 mmol) and **234** (0.090 g, 0.32 mmol) in 5.0 mL of dry CH_3CN.

Free base: Chromatographic eluent: CH_2Cl_2/2-propanol/NH_4OH 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.60 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 7.32 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.83-6.77 (m, 2H, CH arom.); 6.53 (s, 2H, CH arom.); 6.20 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.05 (t, *J* = 5.6 Hz, 2H, OCH_2); 3.82 (s, 6H, OCH_3); 3.80 (s, 3H, OCH_3); 3.42 (s, 2H, NCH_2Ar); 2.66-2.30 (m, 10H, NCH_2); 2.00-1.94 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 162.28 (C); 161.19 (C); 155.88 (C); 153.06 (C); 143.43 (CH); 136.90 (C); 133.98 (C); 128.71 (CH); 112.94 (CH); 112.44 (C); 105.84 (CH); 101.38 (CH); 66.88 (CH_2); 63.21 (CH_2); 60.81 (OCH_3); 56.11 (OCH_3); 54.86 (CH_2); 53.25 (CH_2); 53.07 (CH_2); 26.51 (CH_2) ppm.
Hydrochloride: white solid; mp 220-223 °C.

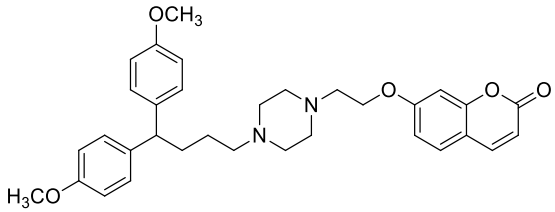
7-(4-(4-(3,4,5-Trimethoxybenzyl)piperazin-1-yl)butoxy)-2*H*-chromen-2-one 61 (DAP10)



Following the general procedure, compound **61** (0.12 g, yield: 82.7 %) was synthesized as a yellow oil, starting from **236**[81] (0.080 g, 0.30 mmol) and **240** (0.10 g, 0.36 mmol) in 5.0 mL of dry CH_3CN.

Free base: Chromatographic eluent: CH_2Cl_2/2-propanol/NH_4OH 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 7.33 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.82-6.76 (m, 2H, CH arom.); 6.54 (s, 2H, CH arom.); 6.21 (d, J=9.2 Hz , 1H, C*H*=CH); 4.01 (t, *J* = 5.6 Hz, 2H, OCH_2); 3.83 (s, 6H, OCH_3); 3.81 (s, 3H, OCH_3); 3.42 (s, 2H, NCH_2Ar); 2.76-2.27 (m, 10H, NCH_2); 1.86-1.77 (m, 2H, CH_2); 1.70-1.62 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 162.28 (C); 161.27 (C); 155.90 (C); 153.05 (C); 143.48 (CH); 136.82 (C); 133.98 (C); 128.73 (CH); 112.98 (CH); 112.94 (CH); 112.40 (C); 105.78 (CH); 101.27 (CH); 68.33 (CH_2); 63.23 (CH_2); 60.84 (OCH_3); 58.10 (CH_2); 56.11 (OCH_3); 53.22 (CH_2); 52.06 (CH_2); 26.99 (CH_2); 23.32 (CH_2) ppm.
Hydrochloride: white solid; mp 215-218 °C.

7-(2-(4-(4,4-bis(4-Methoxyphenyl)butyl)piperazin-1-yl)ethoxy)-2*H*-chromen-2-one 62 (DAP13)

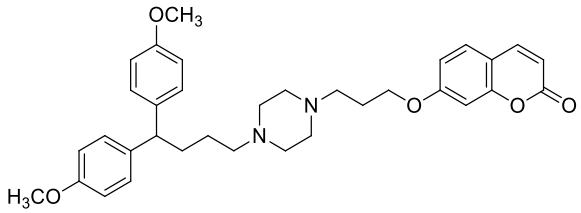


Following the general procedure, compound **62** (0.060 g, yield: 78.5 %) was synthesized as a pale-yellow oil, starting from **237**[81] (0.050 g, 0.14 mmol) and **239** (0.045 g, 0.17 mmol) in 5.0 mL of dry CH_3CN.

Free base: Chromatographic eluent: CH_2Cl_2/2-propanol/NH_4OH 96:4:0.6.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 7.34 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.11 (d, *J* = 8.8 Hz, 4H, CH arom.); 6.85-6.77 (m, 6H, CH arom.); 6.23 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.13 (t, *J* = 6.0 Hz, 2H, OCH_2); 3.78 (t, *J* = 8.0 Hz, 1H, CH); 3.75 (s, 6H, OCH_3); 2.82 (t, *J* = 6.0 Hz, 2H, NCH_2); 2.71-2.54 (m, 4H, NCH_2); 2.53-2.42 (m, 4H, NCH_2); 2.41-2.30 (m, 2H, NCH_2); 2.04-1.94 (m, 2H, CH_2); 1.50-1.42 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.97 (C); 161.20 (C); 157.81 (C); 155.85 (C); 143.40 (CH); 137.54 (C); 128.75 (CH); 128.60 (CH); 113.79 (CH); 113.16 (CH); 113.01 (CH); 112.62 (C); 101.50 (CH); 66.55 (CH_2); 58.47 (CH_2); 56.83 (CH_2); 55.22 (OCH_3); 53.49 (CH_2); 53.01 (CH_2);49.55 (CH); 33.89 (CH_2); 25.25 (CH_2) ppm.
Hydrochloride: white solid; mp 206-209 °C.

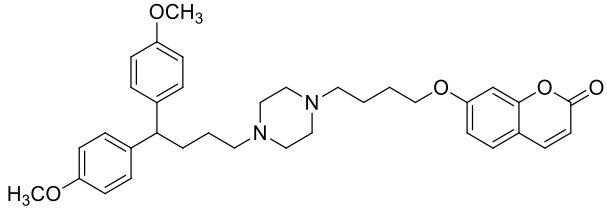
7-(3-(4-(4,4-bis(4-Methoxyphenyl)butyl)piperazin-1-yl)propoxy)-2*H*-chromen-2-one 63 (DAP 14)



Following the general procedure, compound **63** (0.040 g, yield: 57.4 %) was synthesized as a yellow oil, starting from **237**[81] (0.050 g, 0.14 mmol) and **234** (0.045 g, 0.17 mmol) in 5.0 mL of dry CH_3CN.

Free base: Chromatographic eluent: CH_2Cl_2/2-propanol/NH_4OH 96:4:0.6.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 7.34 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.11 (d, *J* = 8.8 Hz, 4H, CH arom.); 6.85-6.70 (m, 6H, CH arom.); 6.23 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.06 (t, *J* = 6.0 Hz, 2H, OCH_2); 3.79 (t, *J* = 8.0 Hz, 1H, CH); 3.76 (s, 6H, OCH_3); 2.63-2.26 (m, 12H, NCH_2); 2.05-1.88 (m, 4H, CH_2); 1.52-1.34 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 162.27 (C); 161.19 (C); 157.83 (C); 155.91 (C); 143.40 (CH); 137.53 (C); 128.71 (CH); 128.60 (CH); 113.80 (CH); 113.01 (CH); 112.94 (CH); 112.47 (C); 101.40 (CH); 66.83 (CH_2); 58.45 (CH_2); 55.22 (OCH_3); 54.82 (CH_2); 53.02 (CH_2); 49.54 (CH); 33.89 (CH_2); 26.43 (CH_2); 25.18 (CH_2) ppm.
Hydrochloride: white solid; mp 196-199 °C.

7-(4-(4-(4,4-bis(4-Methoxyphenyl)butyl)piperazin-1-yl)butoxy)-2*H*-chromen-2-one 64 (DAP 12)

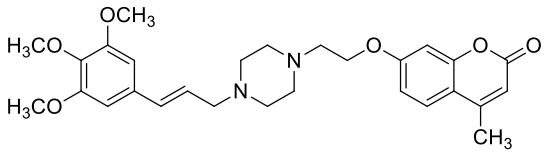


Following the general procedure, compound **64** (0.040 g, yield: 62.6 %) was synthesized as a pale-yellow oil, starting from **237**[81] (0.040 g, 0.11 mmol) and **240** (0.039 g, 0.13 mmol) in 5.0 mL of dry CH_3CN.

Free base: Chromatographic eluent: CH_2Cl_2/2-propanol/NH_4OH 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 7.34 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.11 (d, *J* = 8.8 Hz, 4H, CH arom.); 6.82-6.78 (m, 6H, CH arom.); 6.23 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.01 (t, *J* = 6.0 Hz, 2H, OCH_2); 3.78 (t, *J* = 8.0 Hz, 1H, CH); 3.75 (s, 6H, OCH_3); 2.66-2.33 (m, 12H, NCH_2); 2.02-1.94 (m, 2H, CH_2); 1.87-1.77 (m, 2H, CH_2); 1.71-1.62 (m, 2H, CH_2); 1.48-1.40 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 162.29 (C); 161.28 (C); 157.80 (C); 155.91 (C); 143.46 (CH); 137.56 (C); 128.73 (CH); 128.60 (CH); 113.78 (CH);112.97 (CH); 112.42 (C); 101.31 (CH);

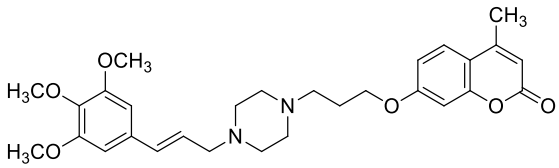
68.33 (CH_2); 58.53 (CH_2); 58.07 (CH_2); 55.23 (OCH_3); 53.06 (CH_2); 49.55 (CH); 33.93 (CH_2); 26.98 (CH_2); 25.30 (CH_2); 23.28 (CH_2) ppm.
Hydrochloride: white solid; mp 188-191 °C.

(*E*)-4-Methyl-7-(2-(4-(3-(3,4,5-trimethoxyphenyl)allyl)piperazin-1-yl)ethoxy)-2*H*-chromen-2-one 65 (FMU 4)



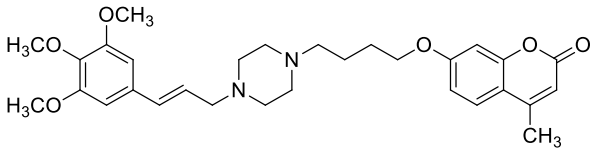
Following the general procedure, compound **65** (0.020 g, yield: 25.4 %) was synthesized as a pale-yellow oil, starting from **235**[81] (0.040 g, 0.14 mmol) and **241** (0.046 g, 0.16 mmol) in 5.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: EtOAc/CH_3OH/NH_4OH 99:1:0.1.
TLC: CH_2Cl_2/CH_3OH /NH_4OH 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.48 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.86 (dd, *J* = 8.8, 2.4 Hz, 1H, CH arom.); 6.81 (d, *J* = 2.4 Hz, 1H, CH arom.); 6.60 (s, 2H, CH arom.); 6.45 (d, *J* = 15.6, 1H, C*H*=CH); 6.18 (dt, *J* = 15.6, 6.8 Hz, 1H, C*H*=CH); 6.13 (s, 1H, CH); 4.16 (t, *J* = 5.6 Hz, 2H, OCH_2); 3.85 (s, 6H, OCH_3); 3.83 (s, 3H, OCH_3); 3.17 (d, *J* = 6.8 Hz, 2H, NCH_2); 2.86 (t, *J* = 5.6 Hz, 2H, NCH_2); 2.76-2.45 (m, 8H, CH_2); 2.39 (s, 3H, CH_3) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.77 (C); 161.25 (C); 155.24 (C); 153.32 (C); 152.49 (C); 135.56 (C); 133.18 (CH); 132.56 (C); 125.51 (CH); 113.70 (C); 112.71 (CH); 112.06 (CH); 103.37 (CH); 101.52 (CH); 66.51 (CH_2); 60.93 (OCH_3); 60.88 (CH_2); 56.87 (CH_2); 56.06 (OCH_3); 53.58 (CH_2); 53.09 (CH_2); 18.67 (CH_3) ppm.
Hydrochloride: white solid; mp 139-141 °C.

(*E*)-4-Methyl-7-(3-(4-(3-(3,4,5-trimethoxyphenyl)allyl)piperazin-1-yl)propoxy)-2*H*-chromen-2-one 66 (FMU 5)



Following the general procedure, compound **66** (0.030 g, yield: 57.3 %) was synthesized as a pale-yellow oil, starting from **235**[81] (0.030 g, 0.10 mmol) and **242** (0.036 g, 0.12 mmol) in 5.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: CH_2Cl_2/2-propanol/NH_4OH 95:5:0.5.
TLC: CH_2Cl_2/CH_3OH /NH_4OH 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.47 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.85-6.81 (m, 2H, CH arom.); 6.61 (s, 2H, CH arom); 6.45 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.20 (dt, *J* = 15.6, 6.8 Hz, 1H, C*H*=CH); 6.12 (s, 1H, CH); 4.07 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.85 (s, 6H, OCH_3); 3.83 (s, 3H, OCH_3); 3.16 (d, *J* = 6.8 Hz, 2H, NCH_2); 2.85-2.43 (m, 10H, NCH_2); 2.39 (s, 3H, CH_3); 2.04-1.96 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 162.08 (C); 161.37 (C); 155.29 (C); 153.30 (C); 152.59 (C); 135.56 (C); 133.06 (CH); 132.59 (C); 125.48 (CH); 113.51 (C); 112.70 (CH); 111.90 (CH); 103.29 (CH); 101.35 (CH); 66.80 (CH_2); 61.23 (CH_2); 60.88 (OCH_3); 56.04 (OCH_3); 54.90 (CH_2); 53.19 (CH_2); 26.50 (CH_2); 18.70 (CH_3) ppm.
Hydrochloride: grey solid; mp 70-73 °C.

(*E*)-4-Methyl-7-(4-(4-(3-(3,4,5-trimethoxyphenyl)allyl)piperazin-1-yl)butoxy)-2*H*-chromen-2-one 67 (FMU 10)



Following the general procedure, compound **67** (0.060 g, yield: 96.0 %) was synthesized as a pale-yellow oil, starting from **235**[81] (0.030 g, 0.10 mmol) and **243** (0.032 g, 0.12 mmol) in 5.0 mL of dry CH_3CN.

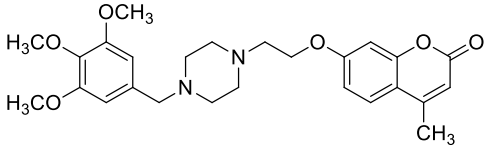
Free base: Chromatographic eluent: CH_2Cl_2/2-propanol/NH_4OH 95:5:0.5.

TLC: CH_2Cl_2/CH_3OH /NH_4OH 95:5:0.5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.47 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.85-6.80 (m, 2H, CH arom.); 6.60 (s, 2H, CH arom); 6.45 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.19 (dt, *J* = 15.6, 6.8 Hz, 1H, C*H*=CH); 6.12 (s, 1H, CH); 4.03 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.85 (s, 6H, OCH_3); 3.83 (s, 3H, OCH_3); 3.16 (d, *J* = 6.8 Hz, 2H, NCH_2); 2.85-2.43 (m, 10H, NCH_2); 2.38 (s, 3H, CH_3); 1.87-1.77 (m, 2H, CH_2); 1.73-1.63 (m, 2H, CH_2) ppm.

Hydrochloride: yellow solid; mp 180-183 °C.

4-Methyl-7-(2-(4-(3,4,5-trimethoxybenzyl)piperazin-1-yl)ethoxy)-2*H*-chromen-2-one 68 (DAP 21)



Following the general procedure, compound **68** (0.090 g, yield: 94.8 %) was synthesized as a pale-yellow oil, starting from **236**[81] (0.050 g, 0.20 mmol) and **241** (0.056 g, 0.24 mmol) in 5.0 mL of dry CH_3CN.

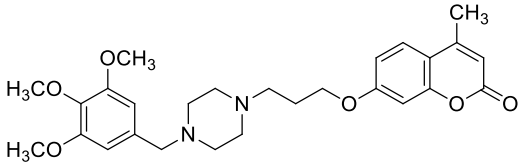
Free base: Chromatographic eluent: CH_2Cl_2/2-propanol/NH_4OH 95:5:0.5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.44 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.83-6.77 (m, 2H, CH arom.); 6.52 (s, 2H, CH arom.); 6.08 (s, 1H, C*H*=CH); 4.12 (t, *J* = 5.6 Hz, 2H, OCH_2); 3.82 (s, 6H, OCH_3); 3.79 (s, 3H, OCH_3); 3.42 (s, 2H, NCH_2); 2.82 (t, *J* = 5.6 Hz, 2H, NCH_2); 2.60-2.48 (m, 8H, NCH_2); 2.35 (s, 3H, CH_3) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.77 (C); 161.21 (C); 155.20 (C); 153.06 (C); 152.51 (C); 136.94 (C); 133.83 (C); 125.51 (CH); 113.63 (C); 112.65 (CH); 111.96 (CH); 105.85 (CH); 101.50 (CH); 66.55 (CH_2); 63.16 (CH_2); 60.80 (CH_3); 56.86 (CH_2); 56.11 (CH_3); 53.66 (CH2); 52.93 (CH_2); 18.62 (CH_3) ppm.

Hydrochloride: white solid; mp 231-234 °C.

4-Methyl-7-(3-(4-(3,4,5-trimethoxybenzyl)piperazin-1-yl)propoxy)-2*H*-chromen-2-one 69 (DAP 18)



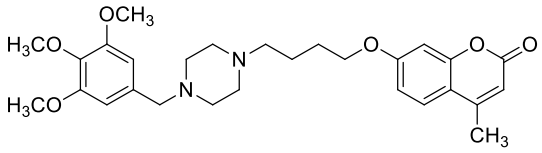
Following the general procedure, compound **69** (0.050 g, yield: 55.4 %) was synthesized as a pink oil, starting from **236**[81] (0.050 g, 0.19 mmol) and **242** (0.066 g, 0.22 mmol) in 5.0 mL of dry CH_3CN.

Free base: Chromatographic eluent: CH_2Cl_2/2-propanol/NH_4OH 95:5:0.5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.46 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.83-6.79 (m, 2H, CH arom.); 6.55 (s, 2H, CH arom.); 6.10 (s, 1H, C*H*=CH); 4.06 (t, *J* = 6.0 Hz, 2H, OCH_2); 3.84 (s,

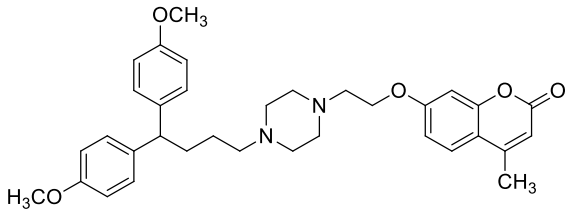
6H, OCH_3); 3.81 (s, 3H, OCH_3); 3.43 (s, 2H, NCH_2); 2.64-2.40 (m, 10H, NCH_2); 2.37 (s, 3H, CH_3); 2.03-1.94 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 162.09 (C); 161.29 (C); 155.28 (C); 153.07 (C); 152.54 (C); 136.94 (C); 133.91 (C); 125.47 (CH); 113.49 (C); 112.64 (CH); 111.87 (CH); 105.86 (CH); 101.40 (CH); 66.84 (CH_2); 63.20 (CH_2); 60.83 (OCH_3); 56.13 (OCH_3); 54.88 (CH_2); 53.22 (CH_2); 53.03 (CH_2); 26.50 (CH_2); 18.64 (CH_3) ppm.
Hydrochloride: white solid; mp 233-236 °C.

4-Methyl-7-(4-(4-(3,4,5-trimethoxybenzyl)piperazin-1-yl)butoxy)-2*H*-chromen-2-one 70 (DAP 19)



Following the general procedure, compound **70** (0.060 g, yield: 64.2 %) was synthesized as a pale-yellow oil, starting from **236**[81] (0.050 g, 0.19 mmol) and **243** (0.069 g, 0.22 mmol) in 5.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: CH_2Cl_2/2-propanol/NH_4OH 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.43 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.82-6.70 (m, 2H, CH arom.); 6.52 (s, 2H, CH arom.); 6.06 (s, 1H, CH arom.); 3.99 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.81 (s, 6H, OCH_3); 3.78 (s, 3H, OCH_3); 3.42 (s, 2H, NCH_2); 2.70-2.37 (m, 10H, NCH_2); 2.34 (s, 3H, CH_3); 1.85-1.78 (m, 2H, CH_2); 1.70-1.65 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 162.06 (C); 161.26 (C); 155.25 (C); 153.05 (C); 152.57 (C); 136.95 (C); 133.65 (C); 125.49 (CH); 113.43 (C); 112.60 (CH); 111.79 (CH); 105.92 (CH); 101.30 (CH); 68.22 (CH_2); 63.07 (CH_2); 60.79 (OCH_3); 57.92 (CH_2); 56.11 (OCH_3); 52.97 (CH_2); 52.76 (CH_2); 26.95 (CH_2); 23.09 (CH_2); 18.61 (CH_3) ppm.
Hydrochloride: white solid; mp 216-219 °C.

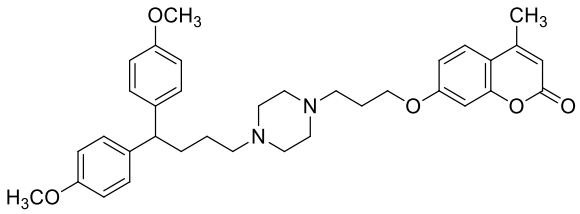
7-(2-(4-(4,4-bis(4-Methoxyphenyl)butyl)piperazin-1-yl)ethoxy)-4-methyl-2*H*-chromen-2-one 71 (FMU2)



Following the general procedure, compound **71** (0.070 g, yield: 74.0 %) was synthesized as a pale-yellow oil, starting from **237**[81] (0.060 g, 0.17 mmol) and **241** (0.056 g, 0.20 mmol) in 5.0 mL of dry CH_3CN.
Free base: Chromatographic eluent: CH_2Cl_2/2-propanol/NH_4OH 97:3:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.47 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.11 (d, *J* = 8.8Hz, 4H, CH arom.); 6.85 (dd, *J* = 8.8, 2.4 Hz, 1H, CH arom.); 6.82-6.73 (m, 5H, CH arom.); 6.12 (s, 1H, CH); 4.13 (t, *J* = 5.6 Hz, 2H, OCH_2); 3.79 (t, *J* = 7.6 Hz, 1H, CH); 3.75 (s, 6H, OCH_3); 2.82 (t, *J* = 5.6 Hz, 2H, NCH_2); 2.73-2.51 (m, 4H, NCH_2); 2.50-2.26 (m, 6H, CH_2); 2.38 (s, 3H, CH_3); 2.00-1.91 (m, 2H, CH_2); 1.50-1.36 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.83 (C); 161.21 (C); 157.83 (C); 155.26 (C); 152.44 (C); 137.62 (C); 128.60 (CH); 125.48 (CH); 114.55 (C); 113.79 (CH); 113.66 (C); 112.68 (CH); 112.02 (CH); 101.56 (CH); 66.56 (CH_2); 58.54 (CH_2); 56.90 (CH_2); 55.22 (OCH_3); 53.68 (CH_2); 53.11 (CH_2); 49.59 (CH); 33.95 (CH_2); 25.41 (CH_2); 18.61 (CH_3) ppm.

Hydrochloride: white solid; mp 147-150 °C.

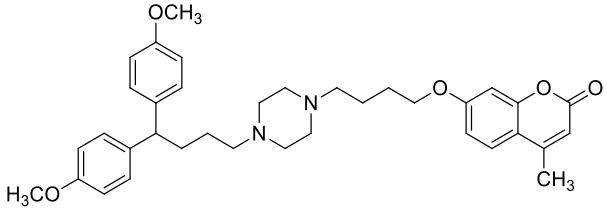
7-(3-(4-(4,4-bis(4-Methoxyphenyl)butyl)piperazin-1-yl)propoxy)-4-methyl-2*H*-chromen-2-one 72 (FMU1)



Following the general procedure, compound **72** (0.050 g, yield: 53.1 %) was synthesized as a pale-yellow oil, starting from **237**[81] (0.060 g, 0.17 mmol) and **242** (0.059 g, 0.20 mmol) in 5.0 mL of dry CH_3CN.

Free base: Chromatographic eluent: CH_2Cl_2/2-propanol/NH_4OH 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.46 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.11 (d, *J* = 8.8Hz, 4H, CH arom.); 6.84 (dd, *J* = 8.8, 2.4 Hz, 1H, CH arom.); 6.82-6.75 (m, 5H, CH arom.); 6.11 (s, 1H, CH); 4.05 (t, *J* = 5.6 Hz, 2H, OCH_2); 3.79 (t, *J* = 7.6 Hz, 1H, CH); 3.75 (s, 6H, OCH_3); 2.70-2.27 (m, 12H, NCH_2); 2.37 (s, 3H, CH_3); 2.00-1.91 (m, 4H, CH_2); 1.48-1.38 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 162.12 (C); 161.27 (C); 157.82 (C); 155.29 (C); 152.51 (C); 137.62 (C); 128.60 (CH); 125.46 (CH); 114.16 (C); 113.79 (CH); 113.49 (C); 112.63 (CH); 111.87 (CH); 101.43 (CH); 66.87 (CH_2); 58.56 (CH_2); 55.21 (OCH_3); 54.90 (CH_2); 53.20 (CH_2); 49.57 (CH); 33.96 (CH_2); 26.52 (CH_2); 25.38 (CH_2); 18.61 (CH_3) ppm.
Hydrochloride: white solid; mp 176-178 °C.

7-(4-(4-(4,4-bis(4-Methoxyphenyl)butyl)piperazin-1-yl)butoxy)-4-methyl-2*H*-chromen-2-one 73 (FMU3)



Following the general procedure, compound **73** (0.050 g, yield: 78.8 %) was synthesized as a pale-yellow oil, starting from **237**[81] (0.040 g, 0.11 mmol) and **243** (0.042 g, 0.14 mmol) in 5.0 mL of dry CH_3CN.

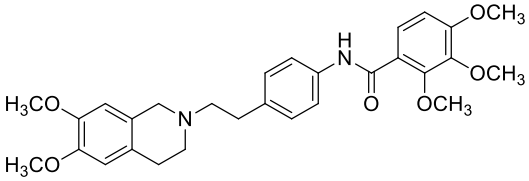
Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.46 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.11 (d, *J* = 8.8Hz, 4H, CH arom.); 6.87-6.75 (m, 6H, CH arom.); 6.11 (s, 1H, CH); 4.02 (t, *J* = 5.6 Hz, 2H, OCH_2); 3.78 (t, *J* = 7.6 Hz, 1H, CH); 3.75 (s, 6H, OCH_3); 2.66-2.24 (m, 10H, NCH_2); 2.38 (s, 3H, CH_3); 2.03-1.88 (m, 4H, CH_2); 1.86-1.76 (m, 2H, CH_2); 1.72-1.60 (m, 2H, CH_2); 1.50-1.36 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 162.12 (C); 161.35 (C); 157.80 (C); 155.30 (C); 152.57 (C); 137.61 (C); 128.60 (CH); 125.48 (CH); 113.77 (CH); 113.45 (C); 112.66 (CH); 111.85 (CH); 101.33 (CH); 68.32 (CH_2); 58.59 (CH_2); 58.14 (CH_2); 55.22 (OCH_3); 53.19 (CH_2); 49.57 (CH); 33.97 (CH_2); 27.03 (CH_2); 25.40 (CH_2); 23.35 (CH_2); 18.65 (CH_3) ppm.
Hydrochloride: white solid; mp 58-61 °C.

7.1.1.2. Tariquidar analogues

7.1.1.2.1. Amide and ester compounds

General procedure for the synthesis of amide (74-86) and ester (87-99) compounds
Compounds were synthesized using two different general procedures.
Method A: In an ice-bath, to a solution of the aniline **244**[91], for the amides, or the phenol **245**[91], for the esters, in the adequate amount of dry CH_2Cl_2 or CH_3CN, the proper carboxylic acid (1 equiv.), DMAP (0.8 equiv.) and EDC hydrochloride (1.8 equiv.) were added in this order. The reaction mixture was stirred at 0 °C for 1 h, then at rt for 48 h. Then, the mixture was treated with CH_2Cl_2, and the organic layer was washed twice with water and with a saturated solution of $NaHCO_3$, dried over Na_2SO_4 and concentrated under reduced pressure. Finally, the residue was purified by flash chromatography using the proper eluting system, to obtain the desired compound as an oil. Final compounds were transformed into the corresponding hydrochloride as solid. The salts were crystallized from abs. ethanol/petroleum ether.
Method B: The proper carboxylic acid (1 equiv.) was transformed into the corresponding acyl chloride by reaction with $SOCl_2$ (10 equiv.) in the adequate amount of $CHCl_3$ (free of ethanol) or dry CH_3CN at 60 °C for 6-8 h. Upon completion of the reaction, the mixture was cooled to rt, and the solvent was removed under reduced pressure. The residue was then treated twice with CHX, and the solvent was removed under reduce pressure. The obtained acyl chloride was dissolved in the proper amount of $CHCl_3$ (free of ethanol), and the aniline **244**[91], for the amides, or the phenol **245**[91], for the esters, was added. The mixture was kept at rt for 24 h, then the organic layer was washed twice with a saturated solution of $NaHCO_3$, dried over Na_2SO_4 and concentrated under reduced pressure. Finally, the residue was purified by flash chromatography using the proper eluting system, yielding the desired compound as an oil. Final compounds were transformed into the corresponding hydrochloride as solid. The salts were crystallized from abs. ethanol/petroleum ether.

_N_-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1_H_)-yl)ethyl)phenyl)-2,3,4-trimethoxybenzamide 74 (FF2)

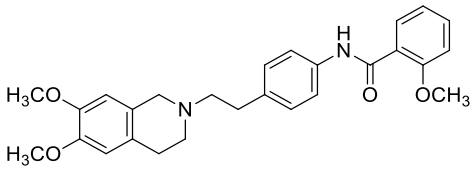


Following method **B** in $CHCl_3$ (free of ethanol), compound **74** (0.022 g, yield: 15.1 %) was synthesized as a yellow oil, starting from 2,3,4-trimethoxybenzoic acid (0.12 g, 0.58 mmol) and **244**[91] (0.090 g, 0.29 mmol).

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.92 (bs, 1H, NH); 7.98 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.60 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.23 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.82 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.60 (s, 1H, CH arom.); 6.53 (s, 1H, CH arom.); 4.05 (s, 3H, OCH_3); 3.92 (s, 3H, OCH_3); 3.90 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 3.69 (s, 2H, NCH_2Ar); 2.96-2.89 (m, 2H, CH_2); 2.88-2.75 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.71 (C); 156.78 (C); 152.14 (C); 147.66 (C); 147.32 (C); 136.69 (C); 135.84 (C); 129.26 (CH); 126.96 (CH); 125.97 (C); 125.91 (C); 120.33 (CH); 119.00 (C); 111.38 (CH); 109.49 (CH); 107.89 (CH); 61.97 (OCH_3); 61.05 (OCH_3); 59.93

(CH_2); 56.10 (OCH_3); 55.95 (OCH_3); 55.91 (OCH_3); 55.46 (CH_2); 50.91 (CH_2); 33.24 (CH_2); 28.35 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{29}H_{35}N_2O_6$= 507.2490, found 507.2492.
ESI-MS *m/z* (%): 507.1 (100%) [$M+H^+$].
Hydrochloride: yellow solid; mp 208-211°C.

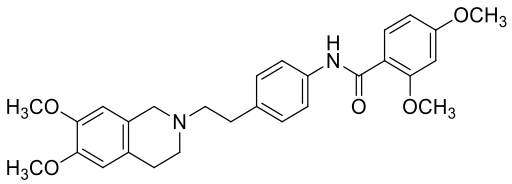
N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-2-methoxybenzamide 75 (LB70)



Following method **A**, compound **75** (0.15 g, yield: 88.9 %) was synthesized as a pale-yellow oil, starting from **244**[91] (0.11 g, 0.35 mmol) and 2-methoxybenzoic acid (0.054 g, 0.35 mmol) in 4.0 mL of dry CH_2Cl_2.

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.71 (bs, 1H, NH); 8.22 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.56 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.41 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.18 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.06 (t, *J* = 8.0 Hz, 1H, CH arom.); 6.95 (d, *J* = 8.0 Hz, 1H, CH arom.); 6.55 (s, 1H, CH arom.); 6.49 (s, 1H, CH arom.); 3.96 (s, 3H, OCH_3); 3.79 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.60 (s, 2H, NCH_2Ar); 2.90-2.67 (m, 8H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.13 (C); 157.19 (C); 147.59 (C); 147.27 (C); 136.50 (C); 136.09 (C); 133.16 (CH); 132.39 (CH); 129.17 (CH); 126.29 (C); 126.06 (C); 121.83 (C); 121.59 (CH); 120.58 (CH); 111.57 (CH); 111.44 (CH); 109.56 (CH); 60.03 (CH_2); 56.21 (OCH_3); 55.95 (OCH_3); 55.91 (OCH_3); 55.56 (CH_2); 50.96 (CH_2); 33.33 (CH_2); 28.53 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{27}H_{31}N_2O_4$= 447.2278, found 447.2279.
ESI-MS *m/z* (%): 447.1 (100%) [$M+H^+$].
Hydrochloride: yellow solid; mp 226-228°C.

N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-2,4-dimethoxybenzamide 76 (LB79)

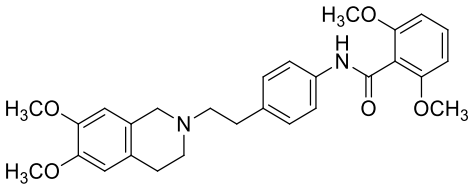


Following method **B** in $CHCl_3$ (free of ethanol), compound **76** (0.050 g, yield: 54.7 %) was synthesized as a yellow oil, starting from 2,4-dimethoxybenzoic acid (0.035 g, 0.19 mmol) and **244**[91] (0.060 g, 0.19 mmol).

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.69 (bs, 1H, NH); 8.26 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.60 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.23 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.66 (dd, *J* = 8.8 Hz, 2.2 Hz, 1H, CH arom.); 6.62 (s, 1H, CH arom.); 6.55 (s, 1H, CH arom.); 6.54 (d, *J* = 2.2 Hz, 1H, CH arom.); 4.03 (s, 3H, OCH_3); 3.88 (s, 3H, OCH_3); 3.86 (s, 3H, OCH_3); 3.85 (s, 3H, OCH_3); 3.73 (s, 2H, NCH_2Ar); 3.00-2.94 (m, 2H, CH_2); 2.93-2.77 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.70 (C); 163.04 (C); 158.51 (C); 147.66 (C); 147.31 (C); 136.78 (C); 135.43 (C); 134.16 (CH); 129.18 (CH); 125.71 (C); 120.57 (CH); 114.67 (C); 111.27 (CH); 109.38 (CH); 105.65 (CH); 98.74 (CH); 59.80 (CH_2); 56.20 (OCH_3); 55.93

(OCH_3); 55.89 (OCH_3); 55.59 (OCH_3); 55.30 (CH_2); 50.85 (CH_2); 33.11 (CH_2); 28.16 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{28}H_{33}N_2O_5$= 477.2384, found 477.2379.
Hydrochloride: yellow solid; mp 233-235°C.

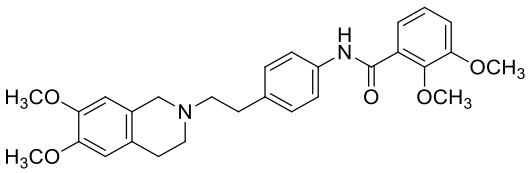
N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-2,6-dimethoxybenzamide 77 (LB72)



Following method **B** in $CHCl_3$ (free of ethanol), compound **77** (0.080 g, yield: 75.0 %) was synthesized as a yellow oil, starting from 2,6-dimethoxybenzoic acid (0.041 g, 0.22 mmol) and **244**[91] (0.070 g, 0.22 mmol).

Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH 95:5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.53 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.50 (bs, 1H, NH); 7.24 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.15 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.55 (s, 1H, CH arom.); 6.53 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.48 (s, 1H, CH arom.); 3.79 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.76 (s, 6H, OCH_3); 3.63 (s, 2H, NCH_2Ar); 2.90-2.70 (m, 8H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.65 (C); 157.55 (C); 147.64 (C); 147.31 (C); 136.58 (C); 135.85 (C); 131.03 (CH); 129.13 (CH); 125.91 (C); 119.82 (CH); 116.00 (C); 111.39 (CH); 109.52 (CH); 104.11 (CH); 59.92 (CH_2); 56.02 (OCH_3); 55.94 (OCH_3); 55.90 (OCH_3); 55.42 (CH_2); 50.90 (CH_2); 33.17 (CH_2); 28.34 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{28}H_{33}N_2O_5$= 477.2384, found 477.2384.
ESI-MS *m/z* (%): 477.1 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 212-214°C.

N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-2,3-dimethoxybenzamide 78 (LB74)

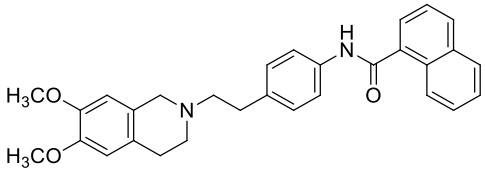


Following method **B** in $CHCl_3$ (free of ethanol), compound **78** (0.070 g, yield: 65.6 %) was synthesized as a yellow oil, starting from 2,3-dimethoxybenzoic acid (0.041 g, 0.22 mmol) and **244**[91] (0.070 g, 0.22 mmol).

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.93 (bs, 1H, NH); 7.73 (dd, *J* = 8.0, 1.6 Hz, 1H, CH arom.); 7.58 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.20 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.15 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.03 (dd, *J* = 8.0, 1.6 Hz, 1H, CH arom.); 6.56 (s, 1H, CH arom.); 6.50 (s, 1H, CH arom.); 3.93 (s, 3H, OCH_3); 3.87 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.79 (s, 3H, OCH_3); 3.62 (s, 2H, NCH_2Ar); 2.90-2.84 (m, 2H, CH_2); 2.83-2.69 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.90 (C); 152.61 (C); 147.57 (C); 147.25 (C); 147.20 (C); 136.51 (C); 136.28 (C); 129.29 (CH); 126.93 (C); 126.40 (C); 126.10 (C); 124.74 (CH); 122.93 (CH); 120.28 (CH); 115.70 (CH); 111.40 (CH); 109.52 (CH); 61.65 (OCH_3); 60.13 (CH_2); 56.14 (OCH_3); 55.94 (OCH_3); 55.91 (OCH_3); 55.64 (CH_2); 51.00 (CH_2); 33.40 (CH_2); 28.62 (CH_2) ppm.

ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{28}H_{33}N_2O_5$= 477.2384, found 477.2375.
ESI-MS *m/z* (%): 477.2 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 230-233°C.

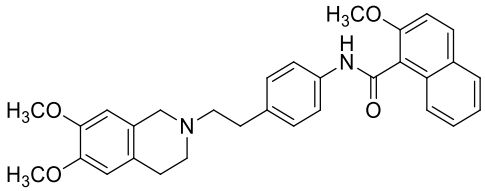
N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-1-naphthamide 79 (LB76)



Following method **B** in $CHCl_3$ (free of ethanol), compound **79** (0.040 g, yield: 53.6 %) was synthesized as a yellow oil, starting from 1-naphthoic acid (0.028 g, 0.16 mmol) and **244**[91] (0.050 g, 0.16 mmol).

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.29-8.27 (m, 1H, CH arom.); 7.95 (bs, 1H, NH); 7.87 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.84-7.81 (m, 1H, CH arom.); 7.62 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.56 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.51-7.46 (m, 2H, CH arom.); 7.38 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.19 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.56 (s, 1H, CH arom.); 6.49 (s, 1H, CH arom.); 3.79 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.63 (s, 2H, NCH_2Ar); 2.92-2.85 (m, 2H, CH_2); 2.84-2.68 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.58 (C); 147.60 (C); 147.28 (C); 136.67 (C); 136.24 (C); 134.49 (C); 133.72 (C); 130.91 (CH); 130.10 (C); 129.31 (CH); 128.40 (CH); 127.27 (CH); 126.53 (CH); 126.28 (C); 126.07 (C); 125.30 (CH); 125.10 (CH); 124.71 (CH); 120.29 (CH); 111.42 (CH); 109.54 (CH); 60.05 (CH_2); 55.94 (OCH_3); 55.90 (OCH_3); 55.61 (CH_2); 51.00 (CH_2); 33.36 (CH_2); 28.56 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{30}H_{31}N_2O_3$= 467.2329, found 467.2320.
Hydrochloride: yellow solid; mp 218-220°C.

N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-2-methoxy-1-naphthamide 80 (LB82)

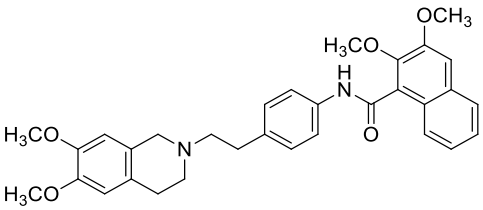


Following method **A**, compound **80** (0.030 g, yield: 30.5 %) was synthesized as a pale-yellow oil, starting from **246** (0.040 g, 0.19 mmol) and **244**[91] (0.062 g, 0.19 mmol) in 5.0 mL of dry CH_2Cl_2.

Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH 95:5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.99 (d, *J* = 8.0 Hz, 1H, CH arom); 7.84 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.81 (bs, 1H, NH); 7.75 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.60 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.44 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.33 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.22 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.21 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.56 (s, 1H, CH arom.); 6.50 (s, 1H, CH arom.); 3.91 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.79 (s, 3H, OCH_3); 3.63 (s, 2H, NCH_2Ar); 2.95-2.86 (m, 2H, CH_2); 2.85-2.69 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.45 (C); 153.71 (C); 147.60 (C); 147.28 (C); 136.45 (C); 131.57 (CH); 129.28 (CH); 128.85 (C); 128.03 (CH); 127.66 (CH); 126.38 (C); 126.11 (C); 124.34 (CH); 124.25 (CH); 120.51 (C); 120.07 (CH); 113.08 (CH); 111.44 (CH); 109.57 (CH);

60.12 (CH_2); 56.77 (OCH_3); 55.95 (OCH_3); 55.91 (OCH_3); 55.63 (CH_2); 51.01 (CH_2); 33.37 (CH_2); 28.60 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{31}H_{33}N_2O_4$= 497.2435, found 497.2425.
Hydrochloride: pale-yellow solid; mp 239-241°C.

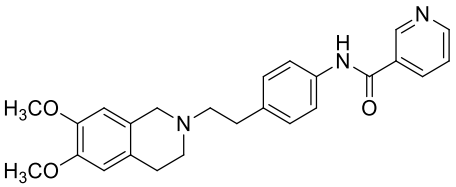
N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-2,3-dimethoxy-1-naphthamide 81 (LB87)



Following method **A**, compound **81** (0.030 g, yield: 24.1 %) was synthesized as a pale-yellow oil, starting from **247** (0.055 g, 0.24 mmol) and **244**[91] (0.074 g, 0.24 mmol) in 3.0 mL of dry CH_2Cl_2.

Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH 95:5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.98 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.90 (bs, 1H, NH), 7.67 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.61 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.42-7.33 (m, 2H, CH arom.); 7.24 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.10 (s, 1H, CH arom.); 6.57 (s, 1H, CH arom.); 6.51 (s, 1H, CH arom.); 3.93 (s, 3H, OCH_3); 3.88 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.66 (s, 2H, NCH_2Ar); 2.95-2.88 (m, 2H, CH_2); 2.87-2.74 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.75 (C); 151.32 (C); 147.63 (C); 147.30 (C); 146.22 (C); 136.57 (C); 136.27 (C); 131.39 (C); 129.38 (CH); 127.22 (C); 126.73 (CH); 126.03 (C); 125.96 (CH); 125.12 (CH); 124.71 (CH); 120.17 (CH); 111.37 (CH); 109.49 (CH); 108.85 (CH); 62.40 (OCH_3); 60.01 (CH_2); 55.95 (OCH_3); 55.91 (OCH_3); 55.76 (OCH_3); 55.55 (CH_2); 50.97 (CH_2); 33.32 (CH_2); 28.47 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{32}H_{35}N_2O_5$= 527.2541, found 527.2534.
Hydrochloride: pale-yellow solid; mp 245-248°C.

N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)phenyl)nicotinamide 82 (FF15)

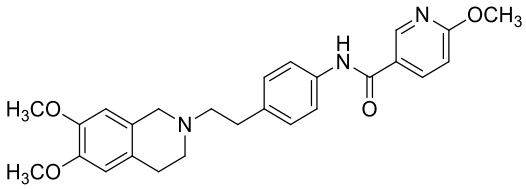


Following method **B** in dry CH_3CN, compound **82** (0.047 g, yield: 40.5 %) was synthesized as a yellow oil, starting from nicotinic acid (0.069 g, 0.56 mmol) and **244**[91] (0.087 g, 0.28 mmol).

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.3.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.02 (bs, 1H, NH); 8.70 (s, 1H, CH arom.); 8.62 (d, *J* = 4.4 Hz, 1H, CH arom.); 8.13 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.52 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.30 (dd, *J* = 8.0, 4.4 Hz, 1H, CH arom.); 7.16 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.54 (s, 1H, CH arom.); 6.48 (s, 1H, CH arom.); 3.77 (s, 3H, OCH_3); 3.76 (s, 3H, OCH_3); 3.60 (s, 2H, NCH_2Ar); 2.87-2.67 (m, 8H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.18 (C); 152.07 (CH); 148.30 (CH); 147.52 (C); 147.19 (C); 136.87 (C); 135.95 (C); 135.48 (CH); 130.80 (C); 129.21 (CH); 126.26 (C); 126.04 (C); 123.49 (CH); 120.97 (CH); 111.36 (CH); 109.48 (CH); 59.96 (CH_2); 55.88 (OCH_3); 55.84 (OCH_3); 55.57 (CH_2); 50.96 (CH_2); 33.30 (CH_2); 28.53 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{25}H_{28}N_3O_3$= 418.2125, found 418.2128.

ESI-MS *m/z* (%): 418.1 (100%) [M+H$^+$].
Hydrochloride: yellow solid; mp 151-154°C.

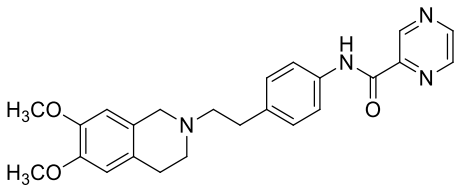
N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-6-methoxynicotinamide 83 (FF22)



Following method **B** in dry CH_3CN, compound **83** (0.032 g, yield: 29.3 %) was synthesized as a yellow oil, starting from 6-methoxynicotinic acid (0.076 g, 0.49 mmol) and **244**[91] (0.076 g, 0.25 mmol).

Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 97:3:0.3.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.65 (s, 1H, CH arom.); 8.03 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.89 (bs, 1H, NH); 7.50 (d, *J* = 6.8 Hz, 2H, CH arom.); 7.18 (d, *J* = 6.8 Hz, 2H, CH arom.); 6.75 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.56 (s, 1H, CH arom.); 6.50 (s, 1H, CH arom.); 3.95 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.79 (s, 3H, OCH_3); 3.63 (s, 2H, NCH_2Ar); 2.91-2.70 (m, 8H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.07 (C); 164.08 (C); 147.67 (C); 147.33 (C); 146.84 (CH); 137.93 (CH); 136.31 (C); 136.15 (C); 129.20 (CH); 125.83 (C); 124.04 (C); 120.79 (CH); 111.38 (CH); 110.78 (CH); 109.50 (CH); 59.69 (CH_2); 55.92 (OCH_3); 55.89 (OCH_3); 55.34 (CH_2); 53.94 (OCH_3); 50.82 (CH_2); 33.08 (CH_2); 28.25 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for [M+H]$^+$ ion species $C_{26}H_{30}N_3O_4$= 448.2231, found 448.2236.
ESI-MS *m/z* (%): 448.1 (100%) [M+H$^+$].
Hydrochloride: yellow solid; mp 123-126°C.

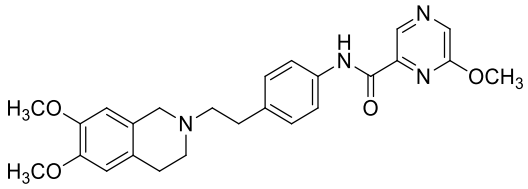
N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)pyrazine-2 carboxamide 84 (FF13)



Following method **A**, compound **84** (0.070 g, yield: 63.8 %) was synthesized as a yellow solid, starting from **244**[91] (0.082 g, 0.26 mmol) and pyrazine-2-carboxylic acid (0.049 g, 0.39 mmol) in 5.0 mL of dry CH_2Cl_2.

Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 97:3:0.3.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.59 (bs, 1H, NH); 9.45 (d, *J* = 1.2 Hz, 1H, CH arom.); 8.73 (d, *J* = 2.4 Hz, 1H, CH arom.); 8.51 (dd, *J* = 2.4, 1.2 Hz, 1H, CH arom.); 7.64 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.22 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.55 (s, 1H, CH arom.); 6.48 (s, 1H, CH arom.); 3.79 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.61 (s, 2H, NCH_2Ar); 2.90-2.83 (m, 2H, CH_2); 2.82-2.69 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 160.55 (C); 147.60 (C); 147.47 (CH); 147.28 (C); 144.62 (CH); 144.44 (C); 142.35 (CH); 136.95 (C); 135.35 (C); 129.40 (CH); 126.32 (C); 126.07 (C); 119.96 (CH); 111.42 (CH); 109.53 (CH); 59.98 (CH_2); 55.94 (OCH_3); 55.90 (OCH_3); 55.62 (CH_2); 50.99 (CH_2); 33.41 (CH_2); 28.57 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for [M+H]$^+$ ion species $C_{24}H_{27}N_4O_3$= 419.2078, found 419.2079.
ESI-MS *m/z* (%): 419.1 (100%) [M+H$^+$].
Hydrochloride: yellow solid; mp 246-249°C.

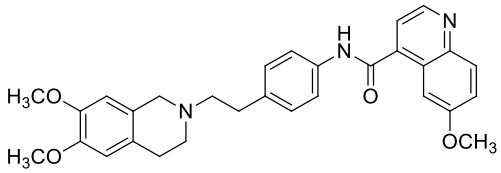
N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-6-methoxypyrazine-2-carboxamide 85 (FF19)



Following method **B** in dry CH_3CN, compound **85** (0.097 g, yield: 89.5 %) was synthesized as a yellow oil, starting from 6-methoxypyrazine-2-carboxylic acid (0.074 g, 0.48 mmol) and **244**[91] (0.075 g, 0.24 mmol).

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.3.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.32 (bs, 1H, NH); 9.02 (s, 1H, CH arom.); 8.41 (s, 1H, CH arom.); 7.62 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.24 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.57 (s, 1H, CH arom.); 6.50 (s, 1H, CH arom.); 4.06 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.65 (s, 2H, NCH_2Ar); 2.95-2.87 (m, 2H, CH_2); 2.86-2.73 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 160.76 (C); 140.43 (C); 139.35 (CH); 138.65 (C); 136.53 (C); 136.14 (CH); 135.37 (C); 132.67 (C); 132.13 (C); 129.45 (CH); 125.67 (C); 125.45 (C); 120.13 (CH); 111.31 (CH); 109.42 (CH); 59.58 (CH_2); 55.95 (OCH_3); 55.91 (OCH_3); 55.29 (CH_2); 53.89 (OCH_3); 50.82 (CH_2); 33.10 (CH_2); 28.04 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{25}H_{29}N_4O_4$= 449.2183, found 449.2187.
ESI-MS *m/z* (%): 449.1 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 224-226°C.

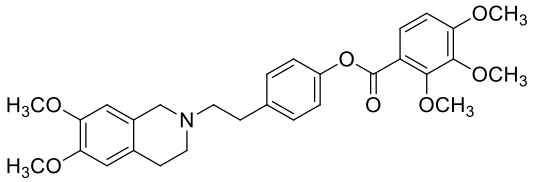
N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-6-methoxyquinoline-4-carboxamide 86 (FF7)



Following method **B** in $CHCl_3$ (free of ethanol), compound **86** (0.12 g, yield: 100.0 %) was synthesized as a yellow oil, starting from **248**[97] (0.082 g, 0.40 mmol) and **244**[91] (0.063 g, 0.20 mmol).

Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.24 (bs, 1H, NH); 8.35 (d, *J* = 4.0 Hz, 1H, CH arom.); 7.78 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.68 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.30 (d, *J* = 2.0 Hz, 1H, CH arom.); 7.24-7.20 (m, 3H, CH arom.); 7.13 (d, *J* = 4.0 Hz, 1H, CH arom.); 6.55 (s, 1H, CH arom.); 6.49 (s, 1H, CH arom.); 3.78 (s, 6H, OCH_3); 3.76 (s, 3H, OCH_3); 3.63 (s, 2H, NCH_2Ar); 2.93-2.87 (m, 2H, CH_2); 2.85-2.73 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.86 (C); 158.41 (C); 147.50 (C); 147.16 (C); 146.62 (CH); 144.34 (C); 140.14 (C); 136.87 (C); 136.27 (C); 130.51 (CH); 129.32 (CH); 126.09 (C); 125.95 (C); 125.45 (C); 122.94 (CH); 120.57 (CH); 118.92 (CH); 111.31 (CH); 109.43 (CH); 102.57 (CH); 59.92 (CH_2); 55.87 (OCH_3); 55.83 (OCH_3); 55.52 (OCH_3); 55.48 (CH_2); 50.88 (CH_2); 33.21 (CH_2); 28.42 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{30}H_{32}N_3O_4$= 498.2387, found 498.2395.
ESI-MS *m/z* (%): 498.1 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 228-230°C.

4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 2,3,4-trimethoxybenzoate 87 (FF1)



Following method **B** in $CHCl_3$ (free of ethanol), compound **87** (0.030 g, yield: 17.2 %) was synthesized as a yellow oil, starting from 2,3,4-trimethoxybenzoic acid (0.072 g, 0.34 mmol) and **245**[91] (0.11 g, 0.34 mmol).

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.28 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.13 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.75 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.60 (s, 1H, CH arom.); 6.53 (s, 1H, CH arom.); 3.97 (s, 3H, OCH_3); 3.93 (s, 3H, OCH_3); 3.89 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 3.68 (s, 2H, NCH_2Ar); 2.97-2.91 (m, 2H, CH_2); 2.90-2.76 (m, 6H, CH_2) ppm.

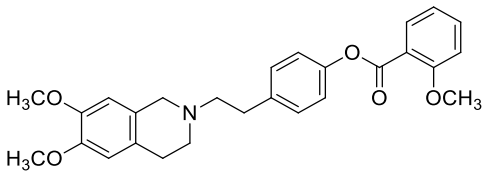
^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.97 (C); 157.79 (C); 155.34 (C); 149.32 (C); 147.66 (C); 147.33 (C); 143.19 (C); 137.61 (C); 129.67 (CH); 127.50 (CH); 126.03 (C); 125.94 (C); 121.76 (CH); 117.10 (C); 111.39 (CH); 109.51 (CH); 107.03 (CH); 61.90 (OCH_3); 61.06 (OCH_3); 59.88 (CH_2); 56.15 (OCH_3); 55.95 (OCH_3); 55.91 (OCH_3); 55.50 (CH_2); 50.96 (CH_2); 33.25 (CH_2); 28.40 (CH_2) ppm.

ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{29}H_{34}NO_7$= 508.2330, found 508.2326.

ESI-MS *m/z* (%): 508.1 (100%) $[M+H^+]$.

Hydrochloride: yellow solid; mp 207-210°C.

4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 2-methoxybenzoate 88 (LB71)



Following method **B** in $CHCl_3$ (free of ethanol), compound **88** (0.070 g, yield: 44.6 %) was synthesized as a yellow oil, starting from 2-methoxybenzoic acid (0.054 g, 0.35 mmol) and **245**[91] (0.11 g, 0.35 mmol).

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.95 (dd, *J* = 8.0, 1.8 Hz, 1H, CH arom.); 7.48 (td, *J* = 8.0, 1.8 Hz, 1H, CH arom.); 7.23 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.11 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.02-6.96 (m, 2H, CH arom.); 6.56 (s, 1H, CH arom.); 6.50 (s, 1H, CH arom.); 3.87 (s, 3H, OCH_3); 3.79 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.61 (s, 2H, NCH_2Ar); 2.93-2.85 (m, 2H, CH_2); 2.84-2.70 (m, 6H, CH_2) ppm.

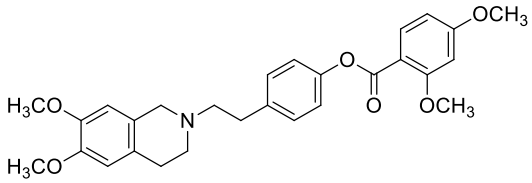
^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.53 (C); 159.79 (C); 149.31 (C); 147.62 (C); 147.30 (C); 137.70 (C); 134.22 (CH); 132.09 (CH); 129.59 (CH); 126.31 (C); 126.07 (C); 121.72 (CH); 120.21 (CH); 119.28 (C); 112.27 (CH); 111.46 (CH); 109.58 (CH); 59.97 (CH_2); 56.05 (OCH_3); 55.95 (OCH_3); 55.92 (OCH_3); 55.59 (CH_2); 51.00 (CH_2); 33.34 (CH_2); 28.55 (CH_2) ppm.

ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{27}H_{30}NO_5$= 448.2119, found 448.2123.

ESI-MS *m/z* (%): 448.1 (100%) $[M+H^+]$.

Hydrochloride: yellow solid; mp 220-222°C.

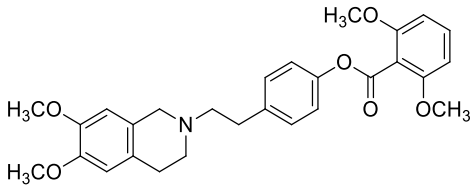
4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 2,4-dimethoxybenzoate 89 (LB78)



Following method **B** in $CHCl_3$ (free of ethanol), compound **89** (0.020 g, yield: 18.7 %) was synthesized as a yellow oil, starting from 2,4-dimethoxybenzoic acid (0.041 g, 0.22 mmol) and **245**[91] (0.070 g, 0.22 mmol).

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.02 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.23 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.09 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.57 (s, 1H, CH arom.); 6.52 (dd, *J* = 8.8, 2.2 Hz, 1H, CH arom.); 6.51 (s, 1H, CH arom.); 6.49 (d, *J* = 2.2 Hz, 1H, CH arom.); 3.87 (s, 3H, OCH_3); 3.85 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.66 (s, 2H, NCH_2Ar); 2.96-2.89 (m, 2H, CH_2); 2.88-2.74 (m, 6H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.90 (C); 163.83 (C); 162.15 (C); 149.52 (C); 147.80 (C); 147.44 (C); 136.84 (C); 134.43 (CH); 129.55 (CH); 125.51 (C); 121.97 (CH); 111.33 (CH); 109.44 (CH); 104.79 (CH); 99.04 (CH); 59.45 (CH_2); 56.03 (OCH_3); 55.96 (OCH_3); 55.92 (OCH_3); 55.57 (OCH_3); 55.11 (CH_2); 50.77 (CH_2); 32.91 (CH_2); 27.87 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{28}H_{32}NO_6$= 478.2224, found 478.2230.
Hydrochloride: pale-yellow solid; mp 221-223°C.

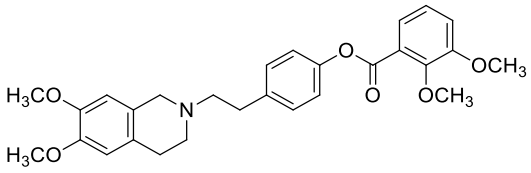
4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 2,6-dimethoxybenzoate 90 (LB73)



Following method **B** in $CHCl_3$ (free of ethanol), compound **90** (0.040 g, yield: 37.4 %) was synthesized as a yellow oil, starting from 2,6-dimethoxybenzoic acid (0.046 g, 0.26 mmol) and **245**[91] (0.080 g, 0.26 mmol).

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.29 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.24 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.14 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.56 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.56 (s, 1H, CH arom.); 6.50 (s, 1H, CH arom.); 3.83 (s, 6H, OCH_3); 3.80 (s, 3H, OCH_3); 3.79 (s, 3H, OCH_3); 3.65 (s, 2H, NCH_2Ar); 2.94-2.87 (m, 2H, CH_2); 2.86-2.70 (m, 6H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 165.12 (C); 157.63 (C); 149.47 (C); 147.71 (C); 147.37 (C); 137.59 (C); 131.58 (CH); 129.62 (CH); 125.87 (C); 121.75 (CH); 112.52 (C); 111.42 (CH); 109.55 (CH); 104.04 (CH); 59.73 (CH_2); 56.13 (OCH_3); 55.96 (OCH_3); 55.92 (OCH_3); 55.39 (CH_2); 50.90 (CH_2); 33.19 (CH_2); 28.28 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{28}H_{32}NO_6$= 478.2224, found 478.2224.
ESI-MS *m/z* (%): 478.1 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 213-215°C.

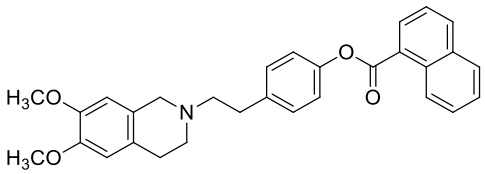
4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 2,3-dimethoxybenzoate 91 (LB75)



Following method **A**, compound **91** (0.060 g, yield: 65.5 %) was synthesized as a pale-yellow oil, starting from **245**[91] (0.060 g, 0.19 mmol) and 2,3-dimethoxybenzoic acid (0.035 g, 0.19 mmol) in 5.0 mL of dry CH_2Cl_2.

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.47 (dd, *J* = 7.2, 2.2 Hz, 1H, CH arom.); 7.26 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.15-7.07 (m, 4H, CH arom.); 6.57 (s, 1H, CH arom.); 6.51 (s, 1H, CH arom.); 3.92 (s, 3H, OCH_3); 3.87 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.66 (s, 2H, NCH_2Ar); 2.96-2.89 (m, 2H, CH_2); 2.88-2.68 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.74 (C); 153.76 (C); 149.71 (C); 149.27 (C); 147.66 (C); 147.33 (C); 137.78 (C); 129.72 (CH); 125.93 (C); 125.43 (C); 123.95 (CH); 122.54 (CH); 121.68 (CH); 116.44 (CH); 111.38 (CH); 109.50 (CH); 61.60 (OCH_3); 59.88 (CH_2); 56.14 (OCH_3); 55.95 (OCH_3); 55.92 (OCH_3); 55.51 (CH_2); 50.97 (CH_2); 33.27 (CH_2); 28.42 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{28}H_{32}NO_6$= 478.2224, found 478.2221.
ESI-MS *m/z* (%): 478.1 (100%) $[M+H^+]$.
Hydrochloride: pale-yellow solid; mp 237-239°C.

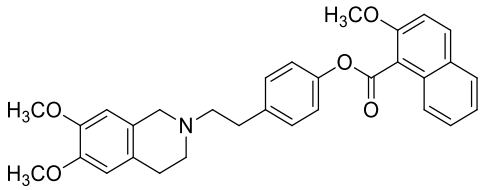
4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 1-naphthoate 92 (LB77)



Following method **A**, compound **92** (0.080 g, yield: 77.5 %) was synthesized as a pale-yellow oil, starting from **245**[91] (0.069 g, 0.22 mmol) and 1-naphthoic acid (0.038 g, 0.22 mmol) in 4.0 mL of dry CH_2Cl_2.

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.00 (d, *J* = 8.0 Hz, 1H, CH arom.); 8.44 (d, *J* = 7.6 Hz, 1H, CH arom.); 8.07 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.89 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.61 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.54 (t, *J* = 8.0 Hz, 2H, CH arom.); 7.31 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.19 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.59 (s, 1H, CH arom.); 6.53 (s, 1H, CH arom.); 3.82 (s, 6H, OCH_3); 3.67 (s, 2H, NCH_2Ar); 2.99-2.91 (m, 2H, CH_2); 2.90-2.76 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.97 (C); 149.32 (C); 147.67 (C); 147.35 (C); 137.93 (C); 134.30 (CH); 133.95 (C); 131.70 (C); 131.19 (CH); 129.82 (CH); 128.71 (CH); 128.17 (CH); 126.42 (CH); 126.14 (C); 126.00 (C); 125.76 (CH); 124.54 (CH); 121.81 (CH); 111.42 (CH); 109.54 (CH); 59.94 (CH_2); 55.97 (OCH_3); 55.94 (OCH_3); 55.59 (CH_2); 51.01 (CH_2); 33.35 (CH_2); 28.50 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{30}H_{30}NO_4$= 468.2169, found 468.2174.
Hydrochloride: pale-yellow solid; mp 227-230°C.

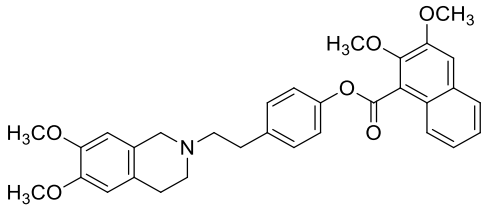
4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 2-methoxy-1-naphthoate 93 (LB81)



Following method **A**, compound **93** (0.080 g, yield: 73.3 %) was synthesized as a yellow oil, starting from **245**[91] (0.077 g, 0.25 mmol) and **246** (0.050 g, 0.25 mmol) in 8.0 mL of dry CH_2Cl_2.

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.92 (d, *J* = 8.8 Hz, 2H, CH arom); 7.80 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.52 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.38 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.34-7.27 (m, 3H, CH arom.); 7.25 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.58 (s, 1H, CH arom.); 6.52 (s, 1H, CH arom.); 4.00 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 3.65 (s, 2H, NCH_2Ar); 3.00-2.90 (m, 2H, CH_2); 2.89-2.75 (m, 6H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 166.67 (C); 155.02 (C); 149.38 (C); 147.67 (C); 147.34 (C); 137.85 (C); 132.26 (CH); 131.02 (C); 129.79 (CH); 128.55 (C); 128.24 (CH); 127.92 (CH); 125.86 (C); 124.29 (CH); 123.58 (CH); 121.71 (CH); 116.70 (C); 113.17 (CH); 111.36 (CH); 109.49 (CH); 59.78 (CH_2); 56.92 (OCH_3); 55.95 (OCH_3); 55.92 (OCH_3); 55.43 (CH_2); 50.92 (CH_2); 33.24 (CH_2); 28.32 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{31}H_{32}NO_5$= 498.2275, found 498.2272.
Hydrochloride: yellow solid; mp 208-210°C.

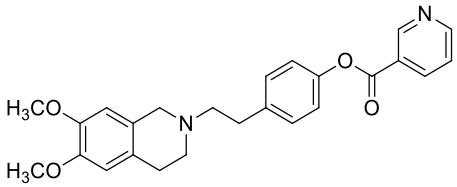
4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 2,3-dimethoxy-1-naphthoate 94 (LB86)



Following method **A**, compound **94** (0.030 g, yield: 26.5 %) was synthesized as a yellow oil, starting from **245**[91] (0.067 g, 0.22 mmol) and **247** (0.050 g, 0.22 mmol) in 5.0 mL of dry CH_2Cl_2.

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.89-7.83 (m 1H, CH arom.); 7.75-7.69 (m, 1H, CH arom.); 7.44-7.38 (m, 2H, CH arom.); 7.32 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.26 (s, 1H, CH arom.); 7.25 (d, *J* = 8.0 Hz, 2H, CH arom.); 6.58 (s, 1H, CH arom.); 6.52 (s, 1H, CH arom.); 4.02 (s, 3H, OCH_3); 3.98 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.65 (s, 2H, NCH_2Ar); 2.98-2.90 (m, 2H, CH_2); 2.89-2.74 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.08 (C); 151.66 (C); 149.22 (C); 147.66 (C); 147.48 (C); 147.33 (C); 138.08 (C); 131.14 (C); 129.90 (CH); 126.90 (CH); 125.90 (CH); 125.47 (C); 125.31 (CH); 123.95 (CH); 123.59 (C); 121.66 (CH); 111.36 (CH); 109.61 (CH); 109.48 (CH); 61.94 (OCH_3); 59.85 (CH_2); 55.95 (OCH_3); 55.91 (OCH_3); 55.87 (OCH_3); 55.49 (CH_2); 50.95 (CH_2); 33.27 (CH_2); 28.39 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{32}H_{34}NO_6$= 528.2381, found 528.2384.
Hydrochloride: yellow solid; mp 183-185°C.

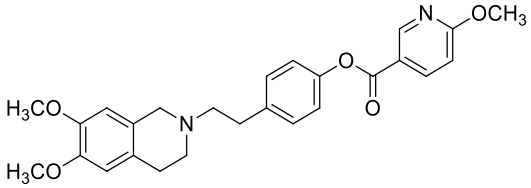
4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl nicotinate 95 (FF16)



Following method **A**, compound **95** (0.018 g, yield: 16.7 %) was synthesized as a yellow solid, starting from **245**[91] (0.080 g, 0.26 mmol) and nicotinic acid (0.063 g, 0.51 mmol) in 6.0 mL of dry CH_3CN.

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.3.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.36 (s, 1H, CH arom.); 8.82 (d, *J* = 4.8, 1H, CH arom.); 8.41 (d, *J* = 8.0, 1H, CH arom.); 7.43 (dd, *J* = 8.0, 4.8 Hz, 1H, CH arom.); 7.28 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.12 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.57 (s, 1H, CH arom.); 6.51 (s, 1H, CH arom.); 3.81 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.65 (s, 2H, NCH_2Ar); 2.97-2.90 (m, 2H, CH_2); 2.88-2.74 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.00 (C); 153.97 (CH); 151.37 (CH); 148.87 (C); 147.73 (C); 147.39 (C); 138.14 (C); 137.57 (CH); 129.85 (CH); 125.84 (C); 125.65 (C); 123.45 (CH); 121.46 (CH); 111.40 (CH); 109.52 (CH); 59.64 (CH_2); 55.96 (OCH_3); 55.92 (OCH_3); 55.40 (CH_2); 50.88 (CH_2); 33.16 (CH_2); 28.26 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{25}H_{27}N_2O_4$= 419.1965, found 419.1958.
ESI-MS *m/z* (%): 419.1 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 98-100°C.

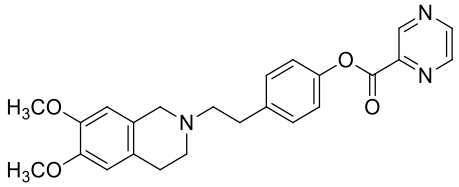
4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 6-methoxynicotinate 96 (FF21)



Following method **B** in dry CH_3CN, compound **96** (0.023 g, yield: 21.0 %) was synthesized as a yellow oil, starting from 6-methoxynicotinic acid (0.075 g, 0.49 mmol) and **245**[91] (0.067 g, 0.21 mmol).

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.97 (d, *J* = 2.0 Hz, 1H, CH arom.); 8.25 (dd, *J* = 8.8, 2.0 Hz, 1H, CH arom.); 7.27 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.10 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.80 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.58 (s, 1H, CH arom.); 6.51 (s, 1H, CH arom.); 4.00 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.69 (s, 2H, NCH_2Ar); 3.00-2.92 (m, 2H, CH_2); 2.91-2.77 (m, 6H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 166.35 (C); 152.99 (C); 150.79 (CH); 149.13 (C); 147.52 (C); 139.90 (CH); 137.39 (C); 129.77 (CH); 125.87 (C); 125.60 (C); 121.69 (CH); 119.06 (C); 111.38 (CH); 110.90 (CH); 109.49 (CH); 59.37 (CH_2); 55.97 (OCH_3); 55.93 (OCH_3); 55.07 (CH_2); 54.14 (OCH_3); 50.74 (CH_2); 32.87 (CH_2); 27.94 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{26}H_{29}N_2O_5$= 449.2071, found 449.2063.
Hydrochloride: yellow solid; mp 254-256°C.

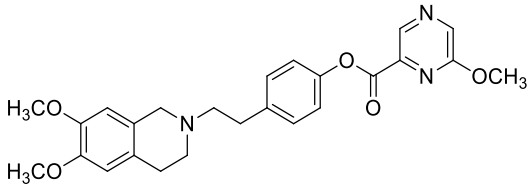
4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl pyrazine-2-carboxylate 97 (FF14)



Following method **B** in $CHCl_3$ (free of ethanol), compound **97** (0.036 g, yield: 21.8 %) was synthesized as a yellow oil, starting from pyrazine-2-carboxylic acid (0.097 g, 0.78 mmol) and **245**[91] (0.12 g, 0.39 mmol).

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.40 (d, *J* = 1.2 Hz, 1H, CH arom.); 8.78 (d, *J* = 2.4 Hz, 1H, CH arom.); 8.75 (dd, *J* = 2.4, 1.2 Hz, 1H, CH arom.); 7.26 (d, *J* = 8.8 Hz, 2H, CH arom.); 7.14 (d, *J* = 8.8 Hz, 2H, CH arom.); 6.55 (s, 1H, CH arom.); 6.49 (s, 1H, CH arom.); 3.79 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.61 (s, 2H, NCH_2Ar); 2.92-2.85 (m, 2H, CH_2); 2.83-2.72 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.66 (C); 148.80 (C); 148.16 (CH); 147.71 (C); 147.36 (C); 146.77 (CH); 144.67 (CH); 142.96 (C); 138.18 (C); 129.93 (CH); 125.62 (C); 125.36 (C); 121.40 (CH); 111.29 (CH); 109.40 (CH); 59.38 (CH_2); 55.93 (OCH_3); 55.89 (OCH_3); 55.17 (CH_2); 50.73 (CH_2); 32.97 (CH_2); 27.97 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{24}H_{26}N_3O_4$= 420.1918, found 420.1911.
Hydrochloride: yellow solid; mp 96-99°C.

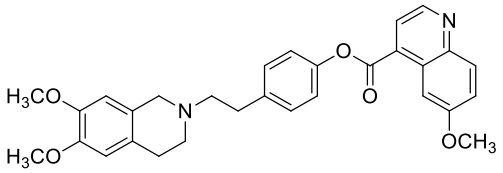
4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 6-methoxypyrazine-2-carboxylate 98 (FF20)



Following method **B** in dry CH_3CN, compound **98** (0.076 g, yield: 75.6 %) was synthesized as a dark yellow oil, starting from 6-methoxypyrazine-2-carboxylic acid (0.063 g, 0.41 mmol) and **245**[91] (0.064 g, 0.20 mmol).

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.3.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.95 (s, 1H, CH arom.); 8.41 (s, 1H, CH arom.); 7.28 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.14 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.57 (s, 1H, CH arom.); 6.50 (s, 1H, CH arom.); 4.05 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.63 (s, 2H, NCH_2Ar); 2.95-2.90 (m, 2H, CH_2); 2.89-2.73 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.79 (C); 159.99 (C); 148.90 (C); 147.62 (C); 147.29 (C); 140.08 (CH); 139.08 (C); 138.38 (CH); 138.33 (C); 129.82 (CH); 126.09 (C); 125.96 (C); 121.34 (CH); 111.37 (CH); 109.49 (CH); 59.80 (CH_2); 55.93 (OCH_3); 55.90 (OCH_3); 55.52 (CH_2); 54.13 (OCH_3); 50.94 (CH_2); 33.27 (CH_2); 28.44 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{25}H_{28}N_3O_5$= 450.2024, found 450.2029.
ESI-MS *m/z* (%): 450.1 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 214-216°C.

4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 6-methoxyquinoline-4-carboxylate 99 (FF8)



Following method **B** in $CHCl_3$ (free of ethanol), compound **99** (0.030 g, yield: 32.5 %) was synthesized as a yellow oil, starting from **248**[97] (0.074 g, 0.36 mmol) and **245**[91] (0.057 g, 0.18 mmol).

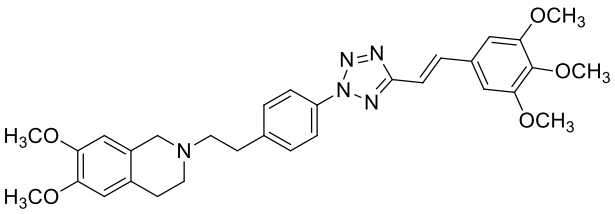
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3. Yield: %.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.93 (d, *J* = 4.8 Hz, 1H, CH arom.); 8.33 (d, *J* = 2.4 Hz, 1H, CH arom.); 8.19 (d, *J* = 4.8 Hz, 1H, CH arom.); 8.09 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.44 (dd, *J* = 9.2, 2.4 Hz, 1H, CH arom.); 7.36 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.20 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.61 (s, 1H, CH arom.); 6.55 (s, 1H, CH arom.); 3.93 (s, 3H, OCH_3); 3.85 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.71 (s, 2H, NCH_2Ar); 3.02-2.97 (m, 2H, CH_2); 2.92-2.80 (m, 6H, CH_2) ppm.
^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.03 (C); 159.57 (C); 148.91 (C); 147.79 (C); 147.42 (C); 146.93 (CH); 145.80 (C); 137.98 (C); 131.57 (CH); 131.20 (C); 130.04 (CH); 127.04 (C); 125.50 (C); 123.37 (CH); 123.15 (CH); 121.68 (CH); 111.26 (CH); 109.37 (CH); 102.97 (CH); 59.36 (CH_2); 55.95 (OCH_3); 55.91 (OCH_3); 55.65 (OCH_3); 55.12 (CH_2); 50.74 (CH_2); 32.91 (CH_2); 27.90 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{30}H_{31}N_2O_5$= 499.2228, found 499.2226.
ESI-MS *m/z* (%): 499.1 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 203-206°C.

7.1.1.2.2. Bioisosteric heterocycles: tetrazole and oxadiazole derivatives

7.1.1.2.2.1. 2,5-disubstituted-2*H*-tetrazoles

General procedure for the synthesis of 2,5-substituted-2*H*-tetrazoles 100-105.
Final compounds were synthesized following the procedure described by Köhler et al.[101] with slight modifications. In an ice-bath, to a solution of the aniline **244**[91] (1 equiv.) and concentrated HCl (8 equiv.) in 2.0 mL of an 1:1 mixture of ethanol/water, a cooled solution of $NaNO_2$ (3 equiv.) in 2.0 mL of water was added. The obtained solution was stirred at rt for 1 h, then added dropwise, between -10 °C and -15 °C, to a solution of the proper benzenesulfonohydrazide (**253-258**, 1 equiv.) in the adequate amount of pyridine. The reaction was stirred until reaching rt, then extracted with CH_2Cl_2. The organic phase was collected, dried over Na_2SO_4, and concentrated under reduced pressure. Finally, the residue was purified by flash chromatography, using $CH_2Cl_2/CH_3OH/NH_4OH$ 99:1:0.1 as the proper eluting system, obtaining the desired compound as an oil. Final compounds were transformed into the corresponding hydrochloride as solid. The salts were crystallized from abs. ethanol/petroleum ether.

(*E*)-6,7-Dimethoxy-2-(4-(5-(3,4,5-trimethoxystyryl)-2*H*-tetrazol-2-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline 100 (LB ANL29)



Following the general procedure, compound **100** (0.080 g, yield: 54.0 %) was synthesized as a pale-yellow oil, starting from the aniline **244**[91] (0.083 g, 0.27 mmol) and the benzenesulfonohydrazide **253** (0.10 g, 0.27 mmol).

Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

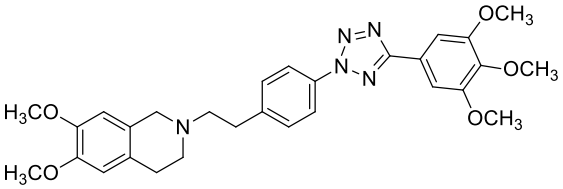
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.00 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.71 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.38 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.09 (d, *J* = 16.0 Hz, 1H, CH=C*H*); 6.78 (s, 2H, CH arom.); 6.56 (s, 1H, CH arom.); 6.49 (s, 1H, CH arom.); 3.87 (s, 6H, OCH_3); 3.84 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.79 (s, 3H, OCH_3); 3.61 (s, 2H, NCH_2Ar); 2.97-2.91 (m, 2H, CH_2); 2.82-2.74 (m, 6H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.17 (C); 153.50 (C); 147.61 (C); 147.28 (C); 142.42 (C); 139.16 (C); 136.79 (CH); 135.07 (C); 131.31 (C); 129.95 (CH); 126.30 (C); 126.07 (C); 119.76 (CH); 112.68 (CH); 111.38 (CH); 109.47 (CH); 104.32 (CH); 60.99 (OCH_3); 59.64 (CH_2); 56.15 (OCH_3); 55.95 (OCH_3); 55.91 (OCH_3); 55.70 (CH_2); 51.04 (CH_2); 33.65 (CH_2); 28.65 (CH_2) ppm.

ESI-MS *m/z* (%): 558.2 (100%) [$M+H^+$].

Hydrochloride: pale-yellow solid; mp 226-228 °C.

6,7-Dimethoxy-2-(4-(5-(3,4,5-trimethoxyphenyl)-2*H*-tetrazol-2-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline 101 (ANL 2)



Following the general procedure, compound **101** (0.13 g, yield: 42.8 %) was synthesized as a yellow oil, starting from the aniline **244**[91] (0.18 g, 0.57 mmol) and the benzenesulfonohydrazide **254** (0.20 g, 0.57 mmol).

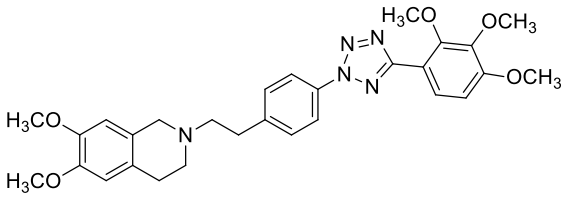
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.7.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.08 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.46 (s, 2H, CH arom.); 7.42 (d, *J* = 8.0 Hz, 2H, CH arom.); 6.58 (s, 1H, CH arom.); 6.51 (s, 1H, CH arom.); 3.95 (s, 6H, OCH_3); 3.89 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.69 (s, 2H, NCH_2Ar); 3.07-2.96 (m, 2H, CH_2); 2.91-2.78 (m, 6H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.98 (C); 153.71 (C); 147.62 (C); 147.30 (C); 142.55 (C); 135.12 (C); 129.91 (CH); 126.39 (C); 126.11 (C); 122.48 (C); 119.94 (CH); 111.43 (CH); 109.52 (CH); 104.17 (CH); 60.96 (OCH_3); 59.64 (CH_2); 56.33 (OCH_3); 55.92 (OCH_3); 55.70 (CH_2); 51.03 (CH_2); 33.66 (CH_2); 28.68 (CH_2) ppm.

Hydrochloride: orange solid; mp 207-210 °C.

6,7-Dimethoxy-2-(4-(5-(2,3,4-trimethoxyphenyl)-2*H*-tetrazol-2-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline 102 (ANL 14)



Following the general procedure, compound **102** (0.020 g, yield: 7.8 %) was synthesized as an orange oil, starting from the aniline **244**[91] (0.15 g, 0.48 mmol) and the benzenesulfonohydrazide **255** (0.17 g, 0.48 mmol).

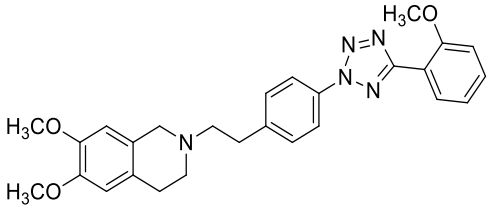
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.08 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.78 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.40 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.80 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.58 (s, 1H, CH arom.); 6.51 (s, 1H, CH arom.); 3.99 (s, 3H, OCH_3); 3.92 (s, 3H, OCH_3); 3.91 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.67 (s, 2H, NCH_2Ar); 3.04-2.94 (m, 2H, CH_2); 2.88-2.77 (m, 6H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 162.97 (C); 155.60 (C); 147.86 (C); 147.50 (C); 141.62 (C); 135.39 (C); 129.90 (CH); 125.57 (C); 124.97 (CH); 119.98 (CH); 114.66 (C); 111.39 (CH); 109.48 (CH); 107.87 (CH); 61.60 (OCH_3); 61.07 (OCH_3); 59.05 (CH_2); 56.12 (OCH_3); 55.97 (OCH_3); 55.93 (OCH_3); 55.18 (CH_2); 50.77 (CH_2); 33.17 (CH_2); 27.96 (CH_2) ppm.

Hydrochloride: yellow solid; mp 202-204 °C.

6,7-Dimethoxy-2-(4-(5-(2-methoxyphenyl)-2*H*-tetrazol-2-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline 103 (LB99)



Following the general procedure, compound **103** (0.11 g, yield: 73.0 %) was synthesized as a yellow oil, starting from the aniline **244**[91] (0.10 g, 0.32 mmol) and the benzenesulfonohydrazide **256** (0.093 g, 0.32 mmol).

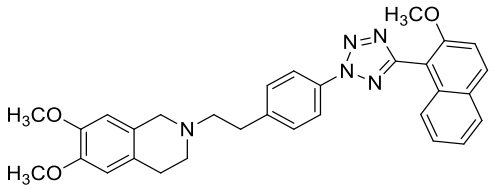
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.07 (d, *J* = 8.4 Hz, 2H, CH arom.); 8.00 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.43 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.39 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.08-7.03 (m, 2H, CH arom.); 6.57 (s, 1H, CH arom.); 6.51 (s, 1H, CH arom.); 3.92 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.63 (s, 2H, NCH_2Ar); 2.98-2.93 (m, 2H, CH_2); 2.82-2.75 (m, 6H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 163.36 (C); 157.67 (C); 147.61 (C); 147.29 (C); 142.23 (C); 135.25 (C); 131.77 (CH); 130.85 (CH); 129.85 (CH); 126.24 (C); 126.03 (C); 120.79 (CH); 120.03 (CH); 116.34 (C); 111.96 (CH); 111.40 (CH); 109.50 (CH); 59.57 (CH_2); 56.04 (OCH_3); 55.93 (OCH_3); 55.61 (CH_2); 50.98 (CH_2); 33.57 (CH_2); 28.57 (CH_2) ppm.

Hydrochloride: yellow solid; mp 206-209 °C.

6,7-Dimethoxy-2-(4-(5-(2-methoxynaphthalen-1-yl)-2*H*-tetrazol-2-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline 104 (LB ANL24)



Following the general procedure, compound **104** (0.080 g, yield: 48.0 %) was synthesized as a yellow oil, starting from the aniline **244**[91] (0.10 g, 0.32 mmol) and the benzenesulfonohydrazide **257** (0.11 g, 0.32 mmol).

Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

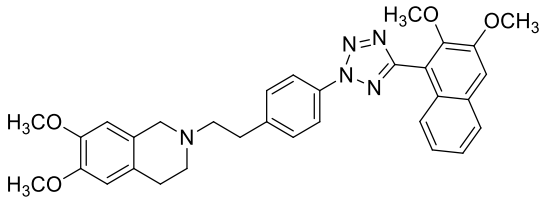
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.14 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.98 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.81 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.56 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.45-7.31 (m, 5H, CH arom.); 6.58 (s, 1H, CH arom.); 6.51 (s, 1H, CH arom.); 3.89 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.63 (s, 2H, NCH_2Ar); 3.04-2.93 (m, 2H, CH_2); 2.85-2.75 (m, 6H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.48 (C); 156.53 (C); 147.58 (C); 147.25 (C); 142.37 (C); 135.32 (C); 133.56 (C); 132.38 (CH); 129.95 (CH); 128.75 (C); 128.15 (CH); 127.58 (CH); 126.27 (C); 126.04 (C); 124.14 (CH); 124.07 (CH); 120.01 (CH); 113.18 (CH); 111.33 (CH); 109.42 (CH); 59.71 (CH_2); 56.82 (OCH_3); 55.95 (OCH_3); 55.91 (OCH_3); 55.70 (CH_2); 51.05 (CH_2); 33.67 (CH_2); 28.65 (CH_2) ppm.

ESI-MS *m/z* (%): 522.3 (100%) [$M+H^+$].

Hydrochloride: yellow solid; mp 186-188 °C.

2-(4-(5-(2,3-Dimethoxynaphthalen-1-yl)-2*H*-tetrazol-2-yl)phenethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline 105 (LB ANL28)



Following the general procedure, compound **105** (0.080 g, yield: 45.4 %) was synthesized as a yellow oil, starting from the aniline **244**[91] (0.10 g, 0.32 mmol) and the benzenesulfonohydrazide **258** (0.12 g, 0.32 mmol).

Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.14 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.74 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.59 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.42 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.39 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.34-7.26 (m, 2H, CH arom.); 6.58 (s, 1H, CH arom.); 6.52 (s, 1H, CH arom.); 4.00 (s, 3H, OCH_3); 3.92 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.64 (s, 2H, NCH_2Ar); 3.01-2.95 (m, 2H, CH_2); 2.83-2.76 (m, 6H, CH_2) ppm.

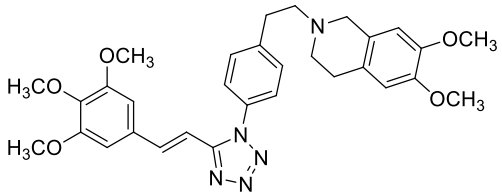
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.27 (C); 151.96 (C); 149.44 (C); 147.66 (C); 147.34 (C); 142.55 (C); 135.29 (C); 131.19 (C); 129.97 (CH); 128.02 (C); 126.81 (CH); 126.45 (C); 126.16 (C); 125.71 (CH); 124.97 (CH); 124.75 (CH); 119.95 (CH.); 117.84 (C); 111.50 (CH); 109.80 (CH); 109.60 (CH); 62.26 (OCH_3); 59.65 (CH_2); 55.98 (OCH_3); 55.94 (OCH_3); 55.83 (OCH_3); 55.72 (CH_2); 51.04 (CH_2); 33.67 (CH_2); 28.69 (CH_2) ppm.

Hydrochloride: yellow solid; mp 203-205 °C.

7.1.1.2.2.2. 1,5-disubstituted-1*H*-tetrazoles

General procedure for the synthesis of 1,5-substituted-1*H*-tetrazoles 106-111.
The proper intermediate **276-280** and **283** (1 equiv.) was dissolved in dry CH_3CN, then Et_3N (6 equiv.) and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (1.2 equiv.) were added. The reaction was refluxed overnight, then cooled to rt and the solvent was removed under reduced pressure. The mixture was treated with CH_2Cl_2, and the organic layer was washed twice with water, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography using the proper eluting system, yielding the desired compound as an oil. Final compounds were transformed into the corresponding hydrochloride as solid. The salts were crystallized from abs. ethanol/petroleum ether.

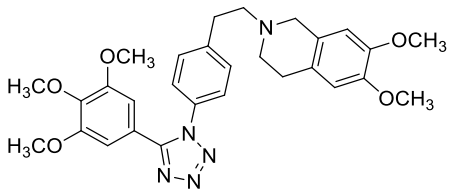
(*E*)-6,7-Dimethoxy-2-(4-(5-(3,4,5-trimethoxystyryl)-1*H*-tetrazol-1-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline 106 (LB116)



Following the general procedure, compound **106** (0.0065 g, yield: 18.1 %) was synthesized as a pale-yellow oil, starting from **283** (0.034 g, 0.064 mmol) and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (0.018 g, 0.077 mmol) in 4.0 mL of dry CH_3CN.

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.90 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.52 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.46 (d, *J* = 8.0 Hz, 2H, CH arom.); 6.71 (s, 2H, CH arom.); 6.67 (d, *J* = 16.0 Hz, 1H, CH=C*H*); 6.62 (s, 1H, CH arom.); 6.54 (s, 1H, CH arom.); 3.87 (s, 9H, OCH_3); 3.86 (s, 2H, NCH_2Ar); 3.85 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.24-3.12 (m, 2H, CH_2); 3.09-2.89 (m, 6H, CH_2) ppm.
Hydrochloride: pale yellow solid.

6,7-Dimethoxy-2-(4-(5-(3,4,5-trimethoxyphenyl)-1*H*-tetrazol-1-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline 107 (ANL 22)

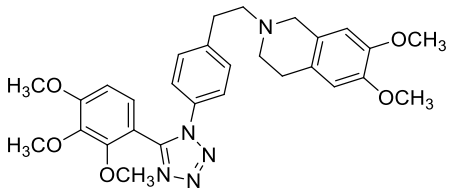


Following the general procedure, compound **107** (0.050 g, yield: 60.0 %) was synthesized as a pale-yellow oil, starting from **276** (0.080 g, 0.16 mmol) and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (0.043 g, 0.19 mmol) in 5.0 mL of dry CH_3CN.

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.40 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.31 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.73 (s, 2H, CH arom.); 6.56 (s, 1H, CH arom.); 6.49 (s, 1H, CH arom.); 3.83 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.63 (s, 2H, NCH_2Ar); 3.60 (s, 6H, OCH_3); 3.02-2.94 (m, 2H, CH_2); 2.84-2.72 (m, 6H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 153.42 (C); 153.37 (C); 147.70 (C); 147.36 (C); 143.41 (C); 132.84 (C); 130.19 (CH); 125.85 (C); 125.75 (CH); 118.36 (C); 111.34 (CH); 109.39 (CH);

106.17 (CH); 60.97 (OCH_3); 59.37 (CH_2); 55.98 (OCH_3); 55.95 (OCH_3); 55.91 (OCH_3); 55.55 (CH_2); 50.90 (CH_2); 33.41 (CH_2); 28.46 (CH_2) ppm.
ESI-MS *m/z* (%): 532.3 (100%) [$M+H^+$].
Hydrochloride: pale yellow solid; mp 180-183 °C.

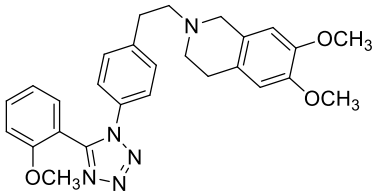
6,7-Dimethoxy-2-(4-(5-(2,3,4-trimethoxyphenyl)-1*H*-tetrazol-1-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline 108 (ANL 27)



- Follow the procedure described by Al-Hourani et al.[106], a solution of **74** (0.10 g, 0.20 mmol) in 4.0 mL of $SOCl_2$ was refluxed for 3.5 h, then it was concentrated under reduced pressure and the residue was treated twice with CHX and the solvent was removed under vacuum, to eliminate the excess of $SOCl_2$. The mixture was dissolved in 4.0 mL of dry DMF and NaN_3 (0.026 g, 0.40 mmol) was added. The suspension was stirred at rt for 48 h, then it was extracted with EtOAc. The organic layer was washed twice with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. Unfortunately, the ^{1}H-NMR and the ESI-MS spectra of the mixture did not reveal the signals of the desired compound.
- Following the procedure reported in ref. [107], NaN_3 (0.26 g, 3.96 mmol) was suspended in 3.0 mL of dry CH_3CN, and Cl_4Si (0.092 mL, 0.79 mmol) was added: the mixture was refluxed for 2 h, then it was cooled to rt and a solution of **74** (0.10 g, 0.20 mmol) in 6.0 mL of dry CH_3CN was added. The reaction was stirred at 90 °C for 48 h, then the solvent was removed under vacuum. Unfortunately, the ^{1}H-NMR and the ESI-MS spectra of the mixture did not reveal the signals of the desired compound.
- Following the general procedure, compound **108** (0.080 g, yield: 40.5 %) was synthesized as a pale-yellow oil, starting from **277** (0.19 g, 0.37 mmol) and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (0.10 g, 0.44 mmol) in 8.0 mL of dry CH_3CN.

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 96:4:0.4.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.25 (d, *J* = 8.8 Hz, 2H, CH arom.); 7.22 (d, *J* = 8.8 Hz, 2H, CH arom.); 7.18 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.71 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.55 (s, 1H, CH arom.); 6.47 (s, 1H, CH arom.); 3.87 (s, 3H, OCH_3); 3.79 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.67 (s, 3H, OCH_3); 3.57 (s, 2H, NCH_2Ar); 3.36 (s, 3H, OCH_3); 2.93-2.84 (m, 2H, CH_2); 2.80-2.64 (m, 6H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 156.66 (C); 151.72 (C); 151.52 (C); 147.59 (C); 147.25 (C); 142.30 (C); 133.24 (C); 129.71 (CH); 126.21 (C); 125.99 (CH); 123.38 (CH); 111.33 (CH); 110.73 (C); 109.40 (CH); 107.25 (CH); 60.87 (OCH_3); 60.63 (OCH_3); 59.56 (CH_2); 56.12 (OCH_3); 55.94 (OCH_3); 55.90 (OCH_3); 55.63 (CH_2); 50.99 (CH_2); 33.53 (CH_2); 28.61 (CH_2) ppm.
ESI-MS *m/z* (%): 532.2 (100%) [$M+H^+$].
Hydrochloride: pale-yellow solid; mp 217-219 °C.

6,7-Dimethoxy-2-(4-(5-(2-methoxyphenyl)-1*H*-tetrazol-1-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline 109 (LB 94)



Following the general procedure, compound **109** (0.030 g, yield: 47.9 %) was synthesized as a pale-yellow oil, starting from **278** (0.060 g, 0.13 mmol) and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (0.037 g, 0.16 mmol) in 5.0 mL of dry CH_3CN

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 96:4:0.4.

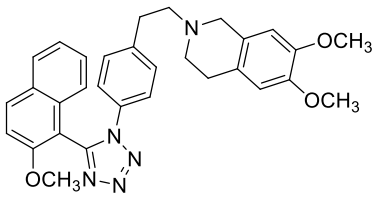
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.57 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.45 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.24 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.19 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.06 (t, *J* = 8.0 Hz, 1H, CH arom.); 6.76 (d, *J* = 8.0 Hz, 1H, CH arom.); 6.56 (s, 1H, CH arom.); 6.48 (s, 1H, CH arom.); 3.81 (s, 3H, OCH_3); 3.79 (s, 3H, OCH_3); 3.58 (s, 2H, NCH_2Ar); 3.25 (s, 3H, OCH_3); 2.93-2.85 (m, 2H, CH_2); 2.80-2.68 (m, 6H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 156.66 (C); 152.04 (C); 147.67 (C); 147.33 (C); 142.19 (C); 133.63 (C); 132.96 (CH); 131.60 (CH); 129.49 (CH); 126.38 (C); 126.11 (C); 123.16 (CH); 121.14 (CH); 113.60 (C); 111.44 (CH); 109.53 (CH); 59.51 (CH_2); 55.98 (OCH_3); 55.94 (OCH_3); 55.67 (CH_2); 54.85 (OCH_3); 50.96 (CH_2); 33.54 (CH_2); 28.67 (CH_2) ppm.

ESI-MS *m/z* (%): 472.2 (100%) [$M+H^+$].

Hydrochloride: pale yellow solid; mp 168-170 °C.

6,7-Dimethoxy-2-(4-(5-(2-methoxynaphthalen-1-yl)-1*H*-tetrazol-1-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline 110 (LB106)



Following the general procedure, compound **110** (0.030 g, yield: 41.1 %) was synthesized as a yellow oil, starting from **279** (0.070 g, 0.14 mmol) and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (0.039 g, 0.17 mmol) in 5.0 mL of dry CH_3CN

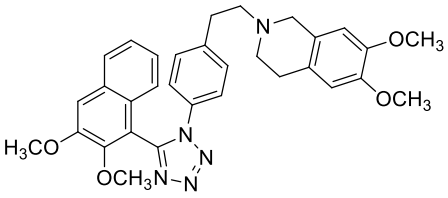
Free base: Chromatographic eluent: EtOAc 100.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.00 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.84 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.53 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.48 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.41 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.21-7.13 (m, 5H, CH arom.); 6.58 (s, 1H, CH arom.); 6.49 (s, 1H, CH arom.); 3.83 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 3.64 (s, 2H, NCH_2Ar); 3.53 (s, 3H, OCH_3); 2.93-2.88 (m, 2H, CH_2); 2.86-2.70 (m, 6H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 155.65 (C); 150.53 (C); 147.59 (C); 147.25 (C); 142.30 (C); 133.50 (CH); 132.98 (C); 132.86 (C); 129.51 (CH); 128.69 (C); 128.39 (CH); 128.32 (CH); 126.31 (C); 126.05 (C); 124.56 (CH); 123.58 (CH); 123.20 (CH); 112.47 (CH); 111.36 (CH); 109.44 (CH); 106.57 (C); 59.40 (CH_2); 55.93 (OCH_3); 55.63 (CH_2); 50.92 (CH_2); 33.49 (CH_2); 28.62 (CH_2) ppm.

Hydrochloride: pale yellow solid.

2-(4-(5-(2,3-Dimethoxynaphthalen-1-yl)-1*H*-tetrazol-1-yl)phenethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline 111 (LB113)



Following the general procedure, compound **111** (0.013 g, yield: 40.8 %) was synthesized as a yellow oil, starting from **280** (0.032 g, 0.060 mmol) and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (0.016 g, 0.072 mmol) in 3.0 mL of dry CH_3CN.

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.75 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.42 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.36-7.28 (m, 2H, CH arom.); 7.24 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.19 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.16 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.57 (s, 1H, CH arom.); 6.49 (s, 1H, CH arom.); 3.97 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.66 (s, 3H, OCH_3); 3.60 (s, 2H, NCH_2Ar); 2.92-2.65 (m, 8H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 151.35 (C); 150.19 (C); 148.79 (C); 147.82 (C); 147.44 (C); 141.87 (C); 132.49 (C); 130.97 (C); 129.79 (CH); 127.57 (C); 126.99 (CH); 126.16 (CH); 125.69 (CH); 125.51 (C); 123.82 (CH); 123.50 (CH); 114.30 (C); 111.32 (CH); 110.62 (CH); 109.39 (CH); 61.64 (OCH_3); 58.84 (CH_2); 55.95 (OCH_3); 55.91 (OCH_3); 55.79 (OCH_3); 55.12 (CH_2); 50.72 (CH_2); 33.04 (CH_2); 27.90 (CH_2) ppm.

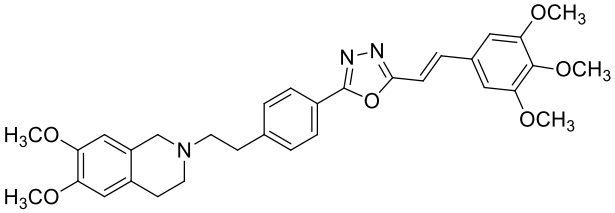
Hydrochloride: pale yellow solid.

7.1.1.2.2.3. 2,5-disubstituted-1,3,4-oxadiazoles

General procedure for the synthesis of 2,5-substituted-1,3,4-oxadiazole compounds 112-117.

Final compounds were synthesized following the procedure described by Stabile et al.[112] with slight modifications. The hydrazide **288** (1 equiv.) and the proper carboxylic acid (1 equiv.) were dissolved in dry CH_3CN, then at 0 °C DIPEA (3 equiv.) and HATU (1.45 equiv.) were added in this order. The mixture was stirred 10 minutes at 0 °C, then at rt for 4 h. When all the acid is consumed, DIPEA (2 equiv.) and p-toluenesulfonyl chloride (3 equiv.) were added and the reaction is maintained at rt for 16 h. Then the solvent is removed under reduced pressure, and the mixture was treated with CH_2Cl_2. The organic layer was washed twice with water and with a saturated solution of $NaHCO_3$, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by flash chromatography using the proper eluting system, then triturated with Et_2O, obtaining the desired compounds as oils. Final compounds were transformed into the corresponding hydrochloride as solid. The salts were crystallized from abs. ethanol/petroleum ether.

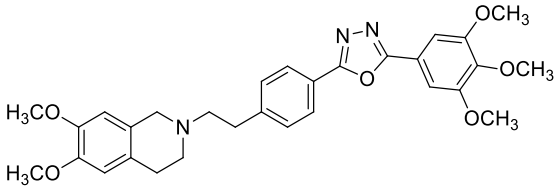
(*E*)-2-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-5-(3,4,5-trimethoxystyryl)-1,3,4-oxadiazole 112 (MES6)



Following the general procedure, compound **112** (0.020 g, yield 18.2 %) was synthesized as a yellow oil, starting from the hydrazide **288** (0.070 g, 0.20 mmol) and

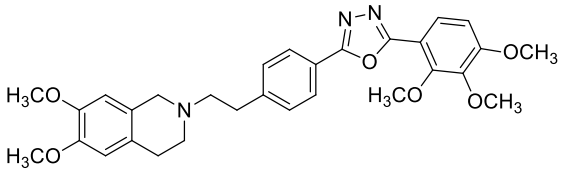
(*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (0.045 g, 0.20 mmol) in 2.5 mL of dry CH_3CN.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.04 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.54 (d, *J* = 16.4 Hz, 1H, C*H*=CH); 7.41 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.02 (d, *J* = 16.4 Hz, 1H, C*H*=CH); 6.81 (s, 2H, CH arom.); 6.61 (s, 1H, CH arom.); 6.53 (s, 1H, CH arom.); 3.92 (s, 6H, OCH_3); 3.90 (s, 3H, OCH_3); 3.87 (s, 2H, NCH_2); 3.85 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.12-3.06 (m, 2H, CH_2); 3.04-2.90 (m, 6H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.18 (C); 163.95 (C); 153.60 (C); 148.11 (C); 147.70 (C); 143.54 (C); 138.79 (CH); 130.35 (C); 129.56 (CH); 127.20 (CH); 125.00 (C); 122.08 (C); 111.34 (CH); 109.45 (CH); 109.32 (CH); 104.72 (CH); 61.02 (OCH_3); 57.95 (CH_2); 56.22 (OCH_3); 55.97 (OCH_3); 54.31 (CH_2); 50.12 (CH_2); 32.87 (CH_2); 26.95 (CH_2) ppm.
ESI-MS *m/z* (%): 558.1 (100%) [$M+H^+$].
Hydrochloride: yellow solid; mp 222-224 °C.

2-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole 113 (MES 5)



Following the general procedure, compound **113** (0.025 g, yield 23.8 %) was synthesized as an orange oil, starting from the hydrazide **288** (0.070 g, 0.20 mmol) and 3,4,5-trimethoxybenzoic acid (0.042 g, 0.20 mmol) in 2.5 mL of dry CH_3CN.
Free base: Chromatographic eluent: EtOAc/CH_3OH 99:1. TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.06 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.41 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.35 (s, 2H, CH arom.); 6.60 (s, 1H, CH arom.); 6.53 (s, 1H, CH arom.); 3.97 (s, 6H, OCH_3); 3.93 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 3.71 (s, 2H, NCH_2Ar); 3.08-3.00 (m, 2H, CH_2); 2.91-2.81 (m, 6H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.55 (C); 164.38 (C); 153.72 (C); 147.76 (C); 147.41 (C); 144.40 (C); 141.20 (C); 129.49 (CH); 127.09 (CH); 125.80 (C); 121.89 (C); 119.08 (C); 111.40 (CH); 109.48 (CH); 104.25 (CH); 61.04 (OCH_3); 59.21 (CH_2); 56.44 (OCH_3); 55.97 (OCH_3); 55.93 (OCH_3); 55.41 (CH_2); 50.89 (CH_2); 33.80 (CH_2); 28.29 (CH_2) ppm.
ESI-MS *m/z* (%): 532.2 (100%) [$M+H^+$].
Hydrochloride: pale-yellow solid; mp 245-248 °C.

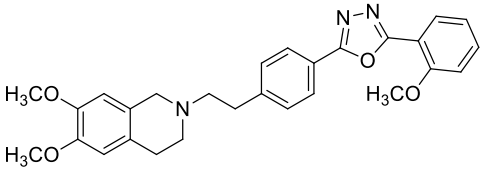
2-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-5-(2,3,4-trimethoxyphenyl)-1,3,4-oxadiazole 114 (MES 8)



Following the general procedure, compound **114** (0.030 g, yield 29.0 %) was synthesized as a yellow oil, starting from the hydrazide **288** (0.070 g, 0.20 mmol) and 2,3,4-trimethoxybenzoic acid (0.042 g, 0.20 mmol) in 2.5 mL of dry CH_3CN.
Free base: Chromatographic eluent: EtOAc/CH_3OH 90:10. TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 90:10:1.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.04 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.75 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.39 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.81 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.60 (s, 1H, CH arom.); 6.53 (s, 1H, CH arom.); 4.04 (s, 3H, OCH_3); 3.94 (s, 3H, OCH_3); 3.93 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 3.68 (s, 2H, NCH_2Ar); 3.03-2.97 (m, 2H, CH_2); 2.88-2.79 (m, 6H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.32 (C); 162.87 (C); 156.64 (C); 152.83 (C); 147.68 (C); 147.35 (C); 144.26 (C); 143.12 (C); 129.46 (CH); 126.96 (CH); 125.92 (C); 124.97 (CH); 122.11 (C); 111.49 (C); 111.41 (CH); 109.50 (CH); 107.90 (CH); 61.68 (OCH_3); 61.06 (OCH_3); 59.36 (CH_2); 56.18 (OCH_3); 55.95 (OCH_3); 55.92 (OCH_3); 55.50 (CH_2); 50.93 (CH_2); 33.86 (CH_2); 28.41 (CH_2) ppm.
ESI-MS *m/z* (%): 532.2 (100%) [$M+H^+$].
Hydrochloride: yellow solid; mp 204-206 °C.

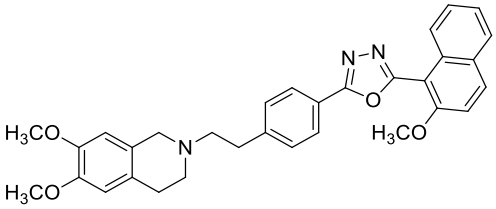
2-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-5-(2-methoxyphenyl)-1,3,4-oxadiazole 115 (MES 7)



Following the general procedure, compound **115** (0.050 g, yield 47.1 %) was synthesized as a yellow oil, starting from the hydrazide **288** (0.080 g, 0.22 mmol) and 2-methoxybenzoic acid (0.034 g, 0.22 mmol) in 2.5 mL of dry CH_3CN.

Free base: Chromatographic eluent: EtOAc/CH_3OH 90:10.
TLC: CH_2Cl_2/CH_3OH/NH_4OH 90:10:1.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.06 (d, *J* = 8.0 Hz, 2H, CH arom.); 8.00 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.51 (t, 1H, *J* = 7.6 Hz, 1H, CH arom.); 7.40 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.11-7.05 (m, 2H, CH arom.); 6.60 (s, 1H, CH arom.); 6.53 (s, 1H, CH arom.); 3.99 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 3.70 (s, 2H, NCH_2Ar); 3.05-2.99 (m, 2H, CH_2); 2.91-2.82 (m, 6H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 163.22 (C); 157.91 (C); 147.80 (C); 147.44 (C); 144.02 (C); 133.02 (CH); 130.43 (CH); 129.42 (CH); 127.09 (CH); 125.73 (C); 122.18 (C); 120.76 (CH); 112.04 (CH); 111.43 (CH); 109.54 (CH); 59.05 (CH_2); 56.04 (OCH_3) 55.97 (OCH_3); 55.93 (OCH_3); 55.24 (CH_2); 50.76 (CH_2); 33.63 (CH_2); 28.09 (CH_2) ppm.
ESI-MS *m/z* (%): 472.2 (100%) [$M+H^+$].
Hydrochloride: yellow solid; mp 197-199 °C.

2-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-5-(2-methoxynaphthalen-1-yl)-1,3,4-oxadiazole 116 (MES 9)

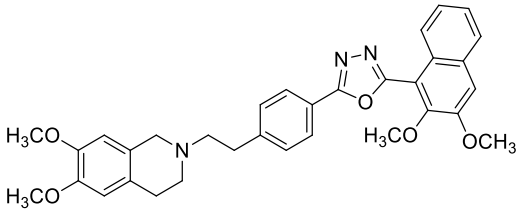


Following the general procedure, compound **116** (0.030 g, yield 20.9 %) was synthesized as a yellow oil, starting from the hydrazide **288** (0.065 g, 0.18 mmol) and **246** (0.036 g, 0.18 mmol) in 3.0 mL of dry CH_3CN.

Free base: Chromatographic eluent: EtOAc/CH_3OH 93:7.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.08 (d, *J* = 8.0 Hz, 2H, CH arom.); 8.05 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.91 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.84 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.50 (t, *J*

= 8.4 Hz, 1H, CH arom.); 7.42-7.35 (m, 4H, CH arom.); 6.60 (s, 1H, CH arom.); 6.53 (s, 1H, CH arom.); 3.97 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 3.71 (s, 2H, NCH_2Ar); 3.05-3.00 (m, 2H, CH_2); 2.91-2.82 (m, 6H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 165.35 (C); 161.50 (C); 157.37 (C); 147.93 (C); 147.55 (C); 133.65 (CH); 133.08 (C); 129.48 (CH); 128.63 (C); 128.23 (CH); 127.23 (CH); 124.42 (CH); 124.10 (CH); 122.35 (C); 112.88 (CH); 111.41 (CH); 109.50 (CH); 58.88 (CH_2); 56.77 (OCH_3); 55.98 (OCH_3); 55.94 (OCH_3); 55.09 (CH_2); 50.72 (CH_2); 33.47 (CH_2); 27.84 (CH_2) ppm.
ESI-MS *m/z* (%): 522.2 (100%) [M+H$^+$].
Hydrochloride: yellow solid; mp 163-165 °C.

2-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-5-(2,3-dimethoxynaphthalen-1-yl)-1,3,4-oxadiazole 117 (MES 10)



Following the general procedure, compound **117** (0.020 g, yield 20.1 %) was synthesized as a yellow oil, starting from the hydrazide **288** (0.065 g, 0.18 mmol) and **247** (0.042 g, 0.18 mmol) in 3.0 mL of dry CH_3CN.

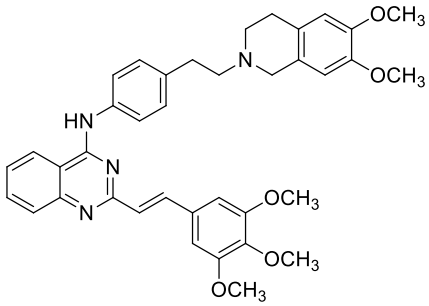
Free base: Chromatographic eluent: EtOAc/CH_3OH 90:10.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.08 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.92 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.77 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.48-7.35 (m, 5H, CH arom.); 6.60 (s, 1H, CH arom.); 6.54 (s, 1H, CH arom.); 4.04 (s, 3H, OCH_3); 3.99 (s, 3H, OCH_3); 3.84 (s, 6H, OCH_3); 3.69 (s, 2H, NCH_2Ar); 3.05-2.96 (m, 2H, CH_2); 2.90-2.78 (m, 6H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 165.43 (C); 161.03 (C); 151.62 (C); 150.11 (C); 147.63 (C); 147.31 (C); 144.62 (C); 131.06 (C); 129.55 (CH); 127.32 (C); 127.15 (CH); 126.88 (CH); 126.08 (CH); 125.95 (C); 125.60 (CH); 124.63 (CH); 121.97 (C); 111.35 (CH); 110.93 (CH); 109.46 (CH); 62.24 (OCH_3); 59.49 (CH_2); 55.91 (OCH_3); 55.61 (CH_2); 51.01 (CH_2); 33.95 (CH_2); 28.52 (CH_2) ppm.
ESI-MS *m/z* (%): 552.2 (100%) [M+H$^+$].
Hydrochloride: yellow solid; mp 157-160 °C.

7.1.1.3. Quinazoline derivatives

General procedure for the synthesis of final compounds 118-152.
Final compounds were synthesized using two different general procedures.
Method A:[115] To a solution of the proper 4-chloroquinazoline (1 equiv.) in the adequate amount of abs. ethanol, the suitable amine (1 equiv.) and methanesulfonic acid (5.0 µL) were added. The reaction mixture was refluxed for 4 h, then it was cooled to rt and the solvent was removed under reduced pressure. The residue was suspended into a 1 N NaOH solution and stirred for 1 h, then it was treated with CH_2Cl_2. The organic layer was washed twice with water and brine, dried over Na_2SO_4 and concentrated under vacuum. The desired derivatives were obtained as pure solids, or they were purified by flash chromatography using the proper eluting system. Final compounds were transformed into the corresponding hydrochloride as solid. The salts were crystallized from abs. ethanol/petroleum ether.

Method B: To a solution of the proper 4-chloroquinazolines (1 equiv.) in the adequate amount of dry DMF, the suitable amine (1 equiv.) and K_2CO_3 (1 equiv.) were added. The mixture was heated at 60 °C for 5 h, then was cooled to rt. A proper amount of cold water was added: if a solid precipitated, it was filtrated and dried under vacuum. Otherwise, the mixture was extracted with CH_2Cl_2, and the organic phase was washed twice with brine, dried over Na_2SO_4 and concentrated under vacuum. The desired derivatives were obtained as pure solids, or they were purified by flash chromatography using the proper eluting system. Final compounds (except for **129**) were transformed into the corresponding hydrochloride as solid. The salts were crystallized from abs. ethanol/petroleum ether.

(*E*)-*N*-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-2-(3,4,5-trimethoxystyryl)quinazolin-4-amine 118 (LB16)



Following the method **A**, compound **118** (0.067 g, yield: 49.2 %) was synthesized as a yellow solid, starting from the 4-chloroquinazoline **297** (0.076 g, 0.21 mmol) and **244**[91] (0.067 g, 0.21 mmol) in 4.0 mL of abs. ethanol.

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 98:2:0.2.

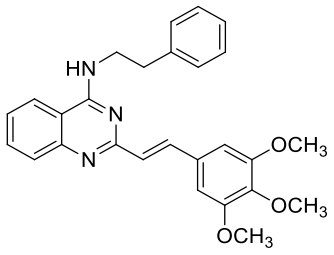
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.88-7.82 (m, 3H, CH arom. and C*H*=CH); 7.77 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.72 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.56 (bs, 1H, NH); 7.43 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.29 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.13 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.82 (s, 2H, CH arom.); 6.57 (s, 1H, CH arom.); 6.51 (s, 1H, CH arom.); 3.87 (s, 6H, OCH_3); 3.85 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.64 (s, 2H, CH_2); 2.95-2.90 (m, 2H, CH_2); 2.87-2.73 (m, 6H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 160.59 (C); 157.03 (C); 153.36 (C); 150.80 (C); 147.60 (C); 147.28 (C); 138.92 (C); 137.60 (CH); 136.82 (C); 136.14 (C); 132.94 (CH); 132.16 (C); 129.13 (CH); 128.54 (CH); 128.40 (CH); 126.41 (C); 126.12 (C); 125.91 (CH); 121.53 (CH); 120.77 (CH); 114.06 (C); 111.43 (CH); 109.54 (CH); 104.76 (CH); 60.97 (OCH_3); 60.20 (CH_2); 56.17 (OCH_3); 55.94 (OCH_3); 55.90 (OCH_3); 55.73 (CH_2); 51.07 (CH_2); 33.50 (CH_2); 28.67 (CH_2) ppm.

ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{38}H_{41}N_4O_5$= 633.3072, found 633.3073.

Hydrochloride: orange solid; mp 244-246 (dec) °C.

(*E*)-*N*-Phenethyl-2-(3,4,5-trimethoxystyryl)quinazolin-4-amine 119 (LB15)



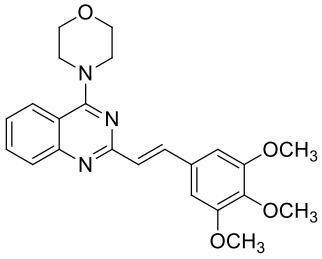
Following the method **A**, compound **119** (0.044 g, yield: 34.8 %) was synthesized as a pale yellow solid, starting from the 4-chloroquinazoline **297** (0.10 g, 0.29 mmol) and 2-phenylethanamine (0.040 mL, 0.29 mmol) in 5.0 mL of abs. ethanol.

Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH 99:1.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.95 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.78 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.66 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.52 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.34-7.24 (m, 6H, CH arom.); 7.14 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.86 (s, 2H, CH arom.); 5.90-5.78 (m,

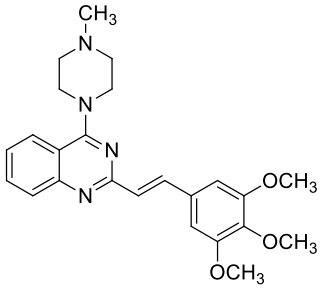
1H, NH); 4.01 (q, *J* = 6.8 Hz, 2H, CH_2); 3.88 (s, 6H, OCH_3); 3.86 (s, 3H, OCH_3); 3.07 (t, *J* = 6.8 Hz, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 160.67 (C); 158.99 (C); 153.37 (C); 150.04 (C); 139.15 (C); 137.24 (CH); 132.66 (CH); 132.24 (C); 128.90 (CH); 128.74 (CH); 128.42 (CH); 128.08 (CH); 126.64 (CH); 125.45 (CH); 120.66 (CH); 113.91 (C); 104.59 (CH); 60.98 (OCH_3); 56.10 (OCH_3); 42.47 (CH_2); 35.37 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{27}H_{28}N_3O_3$= 442.2125, found 442.2123.
Hydrochloride: yellow solid; mp 125-128 °C.

(*E*)-4-(2-(3,4,5-Trimethoxystyryl)quinazolin-4-yl)morpholine 120 (LB14)



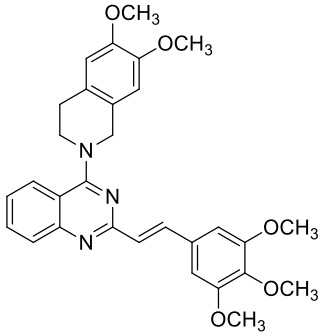
Following the method **A**, compound **120** (0.032 g, yield: 27.3 %) was synthesized as a pale yellow solid, starting from the 4-chloroquinazoline **297** (0.10 g, 0.29 mmol) and morpholine (0.025 mL, 0.29 mmol) in 5.0 mL of abs. ethanol.
Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH 99:1.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.89-7.81 (m, 3H, CH arom. and C*H*=CH); 7.67 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.36 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.14 (d, *J* = 15.6 Hz, 1H, C*H*=CH); 6.84 (s, 2H, CH arom.); 3.92-3.90 (m, 4H, CH_2); 3.87 (s, 6H, OCH_3); 3.85 (s, 3H, OCH_3); 3.78-3.76 (m, 4H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.64 (C); 159.76 (C); 153.39 (C); 152.54 (C); 138.87 (C); 137.35 (CH); 132.66 (CH); 132.07 (C); 128.59 (CH); 128.12 (CH); 125.08 (CH); 124.76 (CH); 115.48 (C); 104.60 (CH); 66.80 (CH_2); 60.97 (OCH_3); 56.13 (OCH_3); 50.39 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{23}H_{26}N_3O_4$= 408.1918, found 408.1916.
Hydrochloride: yellow solid; mp 244-246 (dec) °C.

(*E*)-4-(4-Methylpiperazin-1-yl)-2-(3,4,5-trimethoxystyryl)quinazoline 121 (LB13)



Following the method **A**, compound **121** (0.080 g, yield: 100.0 %) was synthesized as a pale yellow solid, starting from the 4-chloroquinazoline **297** (0.068 g, 0.19 mmol) and 1-methylpiperazine (0.021 mL, 0.19 mmol) in 5.0 mL of abs. ethanol.
Free base: TLC: CH_2Cl_2/CH_3OH 96:4.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.83 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.78 (d, *J* = 8.8 Hz, 2H, CH arom.); 7.61 (t, *J* = 8.8 Hz, 1H, CH arom.); 7.30 (t, *J* = 8.8 Hz, 1H, CH arom.); 7.10 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.81 (s, 2H, CH arom.); 3.83 (s, 6H, OCH_3); 3.81 (s, 3H, OCH_3); 3.77 (t, *J* = 4.8 Hz, 4H, CH_2); 2.58 (t, *J* = 4.8 Hz, 4H, CH_2); 2.31 (s, 3H, NCH_3) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.38 (C); 159.68 (C); 153.33 (C); 152.52 (C); 138.78 (C); 137.11 (CH); 132.44 (CH); 132.14 (C); 128.40 (CH); 128.27 (CH); 124.97 (CH); 124.79 (CH); 115.47 (C); 104.58 (CH); 60.91 (OCH_3); 56.09 (OCH_3); 54.96 (CH_2); 49.65 (CH_2); 46.15 (NCH_3) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{24}H_{29}N_4O_3$= 421.2234, found 421.2237.
Hydrochloride: yellow solid; mp 203-205 (dec) °C.

(*E*)-4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-(3,4,5-trimethoxystyryl)quinazoline 122 (NAS 9)



Following the method **B**, compound **122** (0.18 g, yield: 96.1 %) was synthesized as a yellow solid, starting from the 4-chloroquinazoline **297** (0.13 g, 0.36 mmol), 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (0.071 g, 0.36 mmol) and K_2CO_3 (0.050 g, 0.36 mmol) in 4.0 mL of dry DMF.

Free base: TLC: CH_2Cl_2/CH_3OH 95:5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.98-7.94 (m, 2H, CH arom. and C*H*=CH); 7.90 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.72 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.43 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.21 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.91 (s, 2H, CH arom.); 6.72 (s, 2H, CH arom.); 4.95 (s, 2H, NCH_2Ar); 4.08 (t, *J* = 5.4 Hz, 2H, CH_2); 3.93 (s, 6H, OCH_3); 3.89 (s, 9H, OCH_3); 3.14 (t, *J* = 5.4 Hz, 2H, CH_2) ppm.

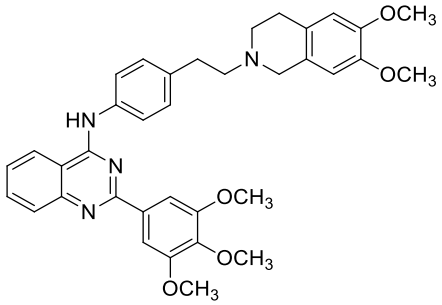
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 163.99 (C); 159.58 (C); 153.37 (C); 152.32 (C); 147.90 (C); 147.74 (C); 138.84 (C); 137.16 (CH); 132.50 (CH); 132.18 (C); 128.21 (CH); 126.54 (C); 125.72 (C); 124.97 (CH); 124.77 (CH); 115.47 (C); 111.60 (CH); 109.42 (CH); 104.64 (CH); 60.95 (OCH_3); 56.14 (OCH_3); 56.04 (OCH_3); 55.99 (OCH_3); 51.00 (CH_2); 48.32 (CH_2); 28.51 (CH_2) ppm.

ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{30}H_{32}N_3O_5$= 514.2337, found 514.2333.

ESI-MS *m/z* (%): 514.2 (100%) $[M+H^+]$.

Hydrochloride: yellow solid; mp 228-231 (dec) °C.

N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-2-(3,4,5-trimethoxyphenyl) quinazolin-4-amine 123 (LB 4)[115]



The compound was already described in ref. [115]. Following the method **A**, compound **123** (0.13 g, yield: 80.9 %) was synthesized as a yellow solid, starting from the 4-chloroquinazoline **298**[115] (0.086 g, 0.26 mmol) and **244**[91] (0.081 g, 0.26 mmol) in 3.0 mL of abs. ethanol.

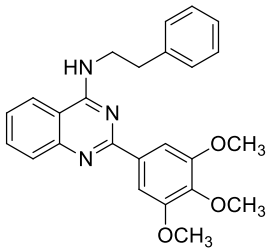
Free base: TLC: CH_2Cl_2/CH_3OH 95:5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.91 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.86 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.85 (s, 2H, CH arom.); 7.77 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.73 (bs, 1H, NH); 7.70 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.38 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.22 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.57 (s, 1H, CH arom.); 6.51 (s, 1H, CH arom.); 3.93 (s, 6H, OCH_3); 3.89 (s, 3H, OCH_3); 3.79 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.62 (s, 2H, CH_2); 2.91-2.71 (m, 8H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 159.62 (C); 157.33 (C); 153.06 (C); 151.11 (C); 147.66 (C); 147.35 (C); 136.89 (C); 136.27 (C); 134.09 (C); 132.81 (CH); 129.05 (CH); 128.87 (CH); 126.41 (C); 126.13 (C); 125.86 (CH); 121.96 (CH); 120.67 (CH); 113.77 (C); 111.51 (CH); 109.61 (CH); 105.61 (CH); 60.92 (OCH_3); 60.23 (CH_2); 56.02 (OCH_3); 55.97 (OCH_3); 55.93 (OCH_3); 55.72 (CH_2); 51.05 (CH_2); 33.46 (CH_2); 28.66 (CH_2) ppm.

Hydrochloride: orange solid; mp 216-219 (dec) °C.

N-Phenethyl-2-(3,4,5-trimethoxyphenyl)quinazolin-4-amine 124 (LB 5)



Following the method **A**, compound **124** (0.077 g, yield: 47.7 %) was synthesized as a pale yellow solid, starting from the 4-chloroquinazoline **298**[115] (0.13 g, 0.39 mmol) and 2-phenylethanamine (0.049 mL, 0.39 mmol) in 6.0 mL of abs. ethanol.

Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH 99:1.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.90 (s, 2H, CH arom.); 7.89 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.68 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.56 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.35 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.32-7.21 (m, 5H, CH arom.); 5.85 (bs, 1H, NH); 4.04-4.00 (m, 2H, CH_2); 3.98 (s, 6H, OCH_3); 3.91 (s, 3H, OCH_3); 3.08 (t, *J* = 7.2 Hz, 2H, CH_2) ppm.

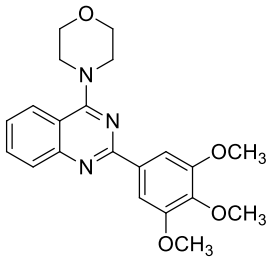
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 160.03 (C); 159.40 (C); 153.09 (C); 150.52 (C); 140.08 (C); 139.10 (C); 134.58 (C); 132.53 (CH); 128.80 (CH); 128.74 (CH); 126.64 (CH); 125.37 (CH); 120.41 (CH); 113.64 (C); 105.64 (CH); 60.95 (OCH_3); 56.18 (OCH_3); 42.49 (CH_2); 35.38 (CH_2) ppm.

ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{25}H_{26}N_3O_3$= 416.1969, found 416.1966.

ESI-MS m/z (%): 416.4 (100%) $[M+H^+]$

Hydrochloride: pale yellow solid; mp 241-243 (dec) °C.

4-(2-(3,4,5-Trimethoxyphenyl)quinazolin-4-yl)morpholine 125 (LB 6)



Following the method **A**, compound **125** (0.064 g, yield: 43.9 %) was synthesized as a pale yellow solid, starting from the 4-chloroquinazoline **298**[115] (0.12 g, 0.38 mmol) and morpholine (0.035 mL, 0.38 mmol) in 6.0 mL of abs. ethanol.

Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH 99:1.

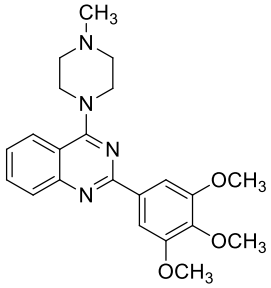
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.95 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.85 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.83 (s, 2H, CH arom.); 7.71 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.39 (t, *J* = 8.0 Hz, 1H, CH arom.); 3.98 (s, 6H, OCH_3); 3.94-3.91 (m, 4H, CH_2); 3.89 (s, 3H, OCH_3); 3.81-3.79 (m, 4H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.99 (C); 159.00 (C); 153.16 (C); 152.79 (C); 140.30 (C); 133.99 (C); 132.57 (CH); 129.10 (CH); 125.08 (CH); 124.63 (CH); 115.29 (C); 105.70 (CH); 66.76 (CH_2); 60.94 (OCH_3); 56.22 (OCH_3); 50.42 (CH_2) ppm.

ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{21}H_{24}N_3O_4$= 382.1761, found 382.1763.

Hydrochloride: pale yellow solid; mp 239-241 (dec) °C.

4-(4-Methylpiperazin-1-yl)-2-(3,4,5-trimethoxyphenyl)quinazoline 126 (LB 7)



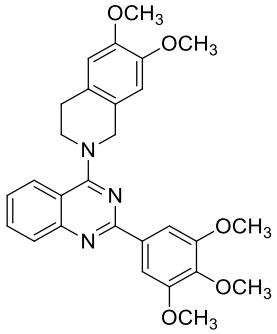
Following the method **A**, compound **126** (0.11 g, yield: 93.9 %) was synthesized as a pale yellow solid, starting from the 4-chloroquinazoline **298**[115] (0.10 g, 0.30 mmol) and 1-methylpiperazine (0.035 mL, 0.30 mmol) in 10.0 mL of abs. ethanol.

Free base: TLC: CH_2Cl_2/CH_3OH 96:4.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.88 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.81 (s, 2H, CH arom.); 7.79 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.62 (t, *J* =

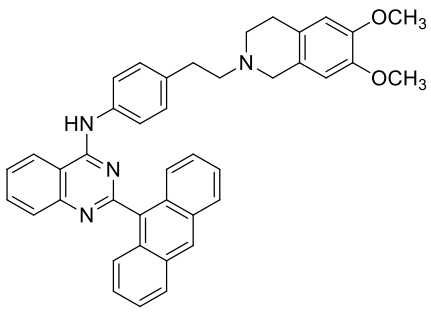
8.0 Hz, 1H, CH arom.); 7.31 (t, *J* = 8.0 Hz, 1H, CH arom.); 3.93 (s, 6H, OCH_3); 3.85 (s, 3H, OCH_3); 3.82-3.73 (m, 4H, CH_2); 2.60-2.51 (s, 4H, CH_2); 2.30 (s, 3H, NCH_3) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.76 (C); 158.86 (C); 153.08 (C); 152.75 (C); 140.17 (C); 134.14 (C); 132.35 (CH); 128.89 (CH); 124.84 (CH); 124.80 (CH); 115.29 (C); 105.68 (CH); 60.88 (OCH_3); 56.16 (OCH_3); 54.88 (CH_2); 49.71 (CH_2); 46.15 (NCH_3) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{22}H_{27}N_4O_3$= 395.2078, found 395.2080.
Hydrochloride: yellow solid; mp 250-252 (dec) °C.

4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-(3,4,5-trimethoxyphenyl)quinazoline 127 (NAS 3)



Following the method **A**, compound **127** (0.090 g, yield: 71.1 %) was synthesized as a pale yellow oil, starting from the 4-chloroquinazoline **298**[115] (0.10 g, 0.32 mmol) and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (0.062 g, 0.32 mmol) in 4.0 mL of abs. ethanol.
Free base: Chromatographic eluent: CHX/EtOAc 50:50. TLC: CH_2Cl_2/CH_3OH 98:2.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.98 (d, *J* = 7.6 Hz, 2H, CH arom.); 7.88 (s, 2H, CH arom.); 7.72 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.42 (t, *J* = 7.6 Hz, 1H, CH arom.); 6.69 (s, 1H, CH arom.); 6.66 (s, 1H, CH arom.); 4.97 (s, 2H, NCH_2Ar); 4.11 (t, *J* = 5.4 Hz, 2H, CH_2); 4.02 (s, 6H, OCH_3); 3.92 (s, 3H, OCH_3); 3.87 (s, 3H, OCH_3); 3.86 (s, 3H, OCH_3); 3.11 (t, *J* = 5.4 Hz, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.20 (C); 153.15 (C); 148.01 (C); 147.95 (C); 126.53 (C); 124.95 (CH); 111.62 (CH); 109.28 (CH); 106.05 (CH); 60.95 (OCH_3); 56.37 (OCH_3); 56.02 (OCH_3); 51.26 (CH_2); 48.05 (CH_2); 28.17 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{28}H_{30}N_3O_5$= 488.2180, found 488.2179.
ESI-MS *m/z* (%): 488.1 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 155-158 (dec) °C.

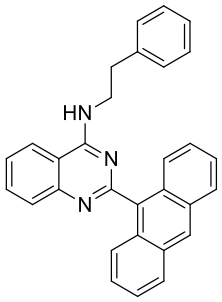
2-(Anthracen-9-yl)-*N*-(4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)quinazolin-4-amine 128 (LB 131)



Following the method **A**, compound **128** (0.040 g, yield: 24.6 %) was synthesized as a pale yellow solid, starting from the 4-chloroquinazoline **299** (0.090 g, 0.26 mmol) and **244**[91] (0.082 g, 0.26 mmol) in 1.5 mL of abs. ethanol.
Free base: Chromatographic eluent: CH_2Cl_2/2-propanol/NH_4OH 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.47 (s, 1H, CH arom.); 8.05-7.99 (m, 4H, CH arom.); 7.97 (bs, 1H, NH); 7.86 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.80 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.60 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.46 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.41 (t, *J* = 8.0 Hz, 2H, CH arom.); 7.33 (t, *J* = 8.0 Hz, 2H, CH arom.); 6.94 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.54 (s, 1H, CH arom.); 6.45 (s, 1H, CH arom.); 3.81 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.53 (s, 2H, CH_2); 2.79-2.64 (m, 6H, CH_2); 2.61-2.53 (m, 2H, CH_2) ppm.

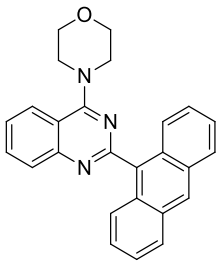
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 162.71 (C); 157.46 (C); 150.56 (C); 147.61 (C); 147.27 (C); 136.62 (C); 135.66 (C); 134.89 (C); 133.08 (CH); 131.52 (C); 129.72 (C); 129.04 (CH); 128.42 (CH); 127.55 (CH); 126.66 (CH); 126.31 (CH); 125.90 (C); 125.75 (CH); 125.03 (CH); 121.24 (CH); 120.95 (CH); 113.63 (C); 111.37 (CH); 109.48 (CH); 59.78 (CH_2); 55.91 (OCH_3); 55.39 (CH_2); 50.83 (CH_2); 33.05 (CH_2); 28.34 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{41}H_{37}N_4O_2$= 617.2911, found 617.2905.
Hydrochloride: yellow solid; mp 285-288 (dec) °C.

2-(Anthracen-9-yl)-*N*-phenethylquinazolin-4-amine 129 (NAS 28)



Following the method **B**, compound **129** (0.070 g, yield: 66.3 %) was synthesized as a white solid, starting from the 4-chloroquinazoline **299** (0.085 g, 0.25 mmol), 2-phenylethanamine (0.031 mL, 0.25 mmol) and K_2CO_3 (0.034 g, 0.25 mmol) in 2.0 mL of dry DMF.
Free base: mp 236-238 (dec) °C. Chromatographic eluent: CH_2Cl_2/CH_3OH 99.5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.53 (s, 1H, CH arom.); 8.04 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.97 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.84 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.80 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.70 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.53 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.44 (t, *J* = 7.6 Hz, 2H, CH arom.); 7.36 (t, *J* = 7.6 Hz, 2H, CH arom.); 7.32-7.23 (m, 3H, CH arom.); 7.18 (d, *J* = 6.8 Hz, 2H, CH arom.); 5.95 (bs, 1H, NH); 3.89 (q, *J* = 6.8 Hz, 2H, CH_2); 2.98 (t, *J* = 6.8 Hz, 2H, CH_2) ppm.
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.70 (bs, 1H, NH); 8.68 (s, 1H, CH arom.); 8.39 (d, *J* = 8.0 Hz, 1H, CH arom.); 8.13 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.83 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.74 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.70 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.61 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.49 (t, *J* = 8.4 Hz, 2H, CH arom.); 7.40 (t, *J* = 8.4 Hz, 2H, CH arom.); 7.16-7.06 (m, 5H, CH, arom.); 3.67 (q, *J* = 6.8 Hz, 2H, CH_2); 2.93 (t, *J* = 6.8 Hz, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, DMSO-d_6) δ: 162.45 (C); 160.16 (C); 149.61 (C); 139.86 (C); 135.90 (C); 133.46 (CH); 131.34 (C); 129.21 (C); 129.05 (CH); 128.76 (CH); 128.69 (CH); 127.23 (CH); 126.54 (CH); 126.48 (CH); 126.30 (CH); 125.77 (CH); 123.34 (CH); 114.04 (C); 42.57 (CH_2); 34.87 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{30}H_{24}N_3$= 426.1965, found 426.1963.
ESI-MS *m/z* (%): 426.3 (100%) $[M+H^+]$.

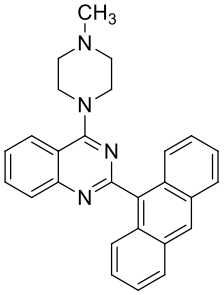
4-(2-(Anthracen-9-yl)quinazolin-4-yl)morpholine 130 (NAS 29)



Following the method **B**, compound **130** (0.060 g, yield: 100.0 %) was synthesized as a yellow solid, starting from the 4-chloroquinazoline **299** (0.052 g, 0.15 mmol), morpholine (0.013 mL, 0.15 mmol) and K_2CO_3 (0.021 g, 0.15 mmol) in 2.5 mL of dry DMF.
Free base: Chromatographic eluent: CH_2Cl_2. TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 98:2:0.2.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.55 (s, 1H, CH arom.); 8.17-8.08 (m, 1H, CH arom.); 8.05 (d, *J* = 8.4 Hz, 3H, CH arom.); 7.84 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.76 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.59 (t, 7.6 Hz, 1H, CH arom.); 7.45 (t, *J* = 8.4 Hz, 2H, CH arom.); 7.37 (t, *J* = 8.4 Hz, 2H, CH arom.) 3.88 (s, 8H, CH_2) ppm.

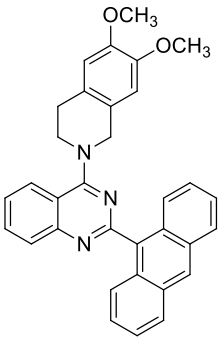
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.23 (C); 161.13 (C); 133.49 (CH); 131.43 (C); 130.12 (C); 129.77 (C); 128.59 (CH); 128.33 (CH); 126.14 (CH); 125.79 (CH); 125.15 (CH); 125.07 (CH); 124.85 (CH); 114.14 (C); 66.87 (CH_2); 50.29 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{26}H_{22}N_3O$= 392.1757, found 392.1758.
ESI-MS *m/z* (%): 392.3 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 198-201 (dec) °C.

2-(Anthracen-9-yl)-4-(4-methylpiperazin-1-yl)quinazoline 131 (LB130)



Following the method **B**, compound **131** (0.060 g, yield: 84.3 %) was synthesized as a pale-yellow solid, starting from the 4-chloroquinazoline **299** (0.060 g, 0.18 mmol), 1-methylpiperazine (0.019 mL, 0.18 mmol) and K_2CO_3 (0.024 g, 0.18 mmol) in 3.0 mL of dry DMF.
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.52 (s, 1H, CH arom.); 8.07-8.02 (m, 4H, CH arom.); 7.83-7.78 (m, 3H, CH arom.); 7.54 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.44 (t, *J* = 8.0 Hz, 2H, CH arom.); 7.37 (t, *J* = 8.0 Hz, 2H, CH arom.); 3-90-3.80 (m, 4H, CH_2); 2.58 (t, *J* = 4.0 Hz, 4H, CH_2); 2.33 (s, 3H, CH_3) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.61 (C); 161.91 (C); 152.58 (C); 134.88 (C); 132.73 (CH); 131.60 (C); 129.79 (C); 129.10 (CH); 128.47 (CH); 127.61 (CH); 126.30 (CH); 125.76 (CH); 125.57 (CH); 125.05 (CH); 125.01 (CH); 114.94 (C); 55.00 (CH_2); 49.66 (CH_2); 46.11 (NCH_3) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{27}H_{25}N_4$= 405.2074, found 405.2074.
Hydrochloride: orange solid; mp 278-280 (dec) °C.

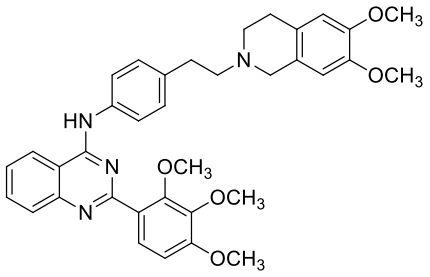
2-(Anthracen-9-yl)-4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)quinazoline 132 (NAS 27)



Following the method **B**, compound **132** (0.050 g, yield: 40.2 %) was synthesized as a pale-yellow oil, starting from the 4-chloroquinazoline **299** (0.085 g, 0.25 mmol), 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (0.048 g, 0.25 mmol) and K_2CO_3 (0.034 g, 0.25 mmol) in 2.0 mL of dry DMF.
Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH 99.5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.53 (s, 1H, CH arom.); 8.15 (d, *J* = 8.4 Hz, 1H, CH arom.); 8.08 (d, *J* = 8.4 Hz, 1H, CH arom.); 8.04 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.84-7.99 (m, 3H, CH arom.); 7.58 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.44 (t, *J* = 7.2 Hz, 2H, CH arom.); 7.35 (t, *J* = 7.2 Hz, 2H, CH arom.); 6.66 (s, 1H, CH arom.); 6.57 (s, 1H, CH arom.); 4.94 (s, 2H, NCH_2Ar); 4.11 (t, *J* = 5.6 Hz, 2H, CH_2); 3.86 (s, 3H, OCH_3); 3.77 (s, 3H, OCH_3); 3.04 (t, *J* = 5.6 Hz, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 163.82 (C); 161.77 (C); 152.27 (C); 147.88 (C); 147.76 (C); 134.80 (C); 132.74 (CH); 131.57 (C); 129.77 (C); 128.76 (CH); 128.47 (CH); 127.61 (CH); 126.45 (C); 126.30 (CH); 125.78 (CH); 125.60 (C); 125.41 (CH); 125.06 (CH); 114.73 (C); 111.53 (CH); 109.31 (CH); 56.03 (OCH_3); 55.95 (OCH_3); 51.12 (CH_2); 48.21 (CH_2); 28.55 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{33}H_{28}N_3O_2$= 498.2176, found 498.2176.
ESI-MS *m/z* (%): 498.4 (100%) $[M+H^+]$.

Hydrochloride: yellow solid; mp 210-213 (dec) °C.

N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-2-(2,3,4-trimethoxyphenyl)quinazolin-4-amine 133 (NAS 26)



Following the method **A**, compound **133** (0.070 g, yield: 47.5 %) was synthesized as an orange solid, starting from the 4-chloroquinazoline **300** (0.080 g, 0.24 mmol) and **244**[91] (0.076 g, 0.24 mmol) in 2.0 mL of abs. ethanol. **133** was recrystallized from ethanol.

Free base: TLC: CH_2Cl_2/CH_3OH/NH_4OH 95:5:0.5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.97-7.88 (m, 2H, CH arom.); 7.79-7.54 (m, 4H, CH arom.); 7.46-7.34 (m, 1H, CH arom.); 7.25-7.12 (m, 2H, CH arom.); 6.75 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.58 (s, 1H, CH arom.); 6.52 (s, 1H, CH arom.); 3.87 (s, 6H, OCH_3); 3.82 (s, 6H, OCH_3); 3.81 (s, 3H, OCH_3); 3.63 (s, 2H, CH_2); 3.90-2.70 (m, 8H, CH_2) ppm.

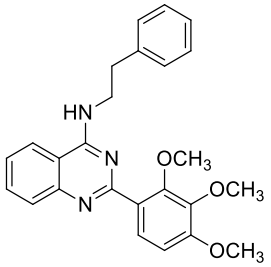
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.08 (C); 157.30 (C); 154.65 (C); 153.16 (C); 150.86 (C); 147.54 (C); 147.22 (C); 142.70 (C); 136.90 (C); 135.99 (C); 132.67 (CH); 130.34 (CH); 129.10 (CH); 129.00 (CH); 127.48 (C); 126.45 (C); 126.33 (CH); 126.11 (C); 125.99 (CH); 121.70 (CH); 120.58 (CH); 113.43 (C); 111.38 (CH); 109.49 (CH); 107.20 (CH); 61.69 (OCH_3); 60.99 (OCH_3); 60.23 (CH_2); 56.07 (OCH_3); 55.89 (OCH_3); 55.70 (CH_2); 51.06 (CH_2); 50.59 (OCH_3); 33.40 (CH_2); 28.64 (CH_2) ppm.

ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{36}H_{39}N_4O_5$= 607.2915, found 607.2915.

ESI-MS *m/z* (%): 607.3 (100%) $[M+H^+]$.

Hydrochloride: orange solid; mp 110-113 (dec) °C.

N-Phenethyl-2-(2,3,4-trimethoxyphenyl)quinazolin-4-amine 134 (NAS 7)



- Following the method **A**, starting from the 4-chloroquinazoline **300** (0.10 g, 0.30 mmol) and 2-phenylethanamine (0.038 mL, 0.30 mmol) in 4.0 mL of abs. ethanol, we obtained a reaction mixture, that was purified by flash chromatography, using CHX/EtOAc 80:20 as the proper eluting system, but we did not obtain the desired product.
- Following the method **B**, compound **134** (0.030 g, yield: 21.7 %) was synthesized as a pale-yellow oil, starting from the 4-chloroquinazoline **300** (0.11 g, 0.33 mmol), 2-phenylethanamine (0.042 mL, 0.33 mmol) and K_2CO_3 (0.046 g, 0.33 mmol) in 4.0 mL of dry DMF.

Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH 97:3.

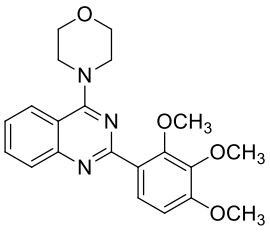
TLC: CH_2Cl_2/CH_3OH/NH_4OH 98:2:0.2.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.85 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.73-7.67 (m, 3H, CH arom.); 7.39 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.33-7.23 (m, 5H, CH arom.); 6.79 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.35 (bs, 1H, NH); 4.02-3.95 (m, 5H, CH_2 and OCH_3); 3.92 (s, 6H, OCH_3); 3.06 (t, *J* = 6.8 Hz, 2H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.03 (C); 159.32 (C); 154.89 (C); 153.04 (C); 142.71 (C); 139.14 (C); 132.58 (CH); 128.90 (CH); 128.70 (CH); 127.91 (CH); 126.55 (CH); 126.27 (CH);

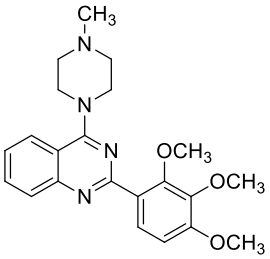
125.69 (CH); 120.94 (CH); 113.21 (C); 107.36 (CH); 61.78 (OCH_3); 61.01 (OCH_3); 56.14 (OCH_3); 42.49 (CH_2); 35.38 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{25}H_{26}N_3O_3$= 416.1969, found 416.1967.
ESI-MS *m/z* (%): 416.3 (100%) $[M+H^+]$.
Hydrochloride: white solid; mp 165-168 (dec) °C.

4-(2-(2,3,4-Trimethoxyphenyl)quinazolin-4-yl)morpholine 135 (NAS 22)



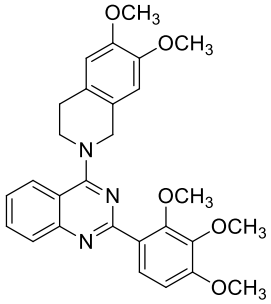
Following the method **B**, compound **135** (0.080 g, yield: 77.2 %) was synthesized as a yellow oil, starting from the 4-chloroquinazoline **300** (0.090 g, 0.27 mmol), morpholine (0.024 mL, 0.27 mmol) and K_2CO_3 (0.038 g, 0.27 mmol) in 4.0 mL of dry DMF.
Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH 99:1.
TLC: CH_2Cl_2/CH_3OH/NH_4OH 98:2:0.2
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.02 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.88 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.72 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.65 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.43 (t, *J* = 8.0 Hz, 1H, CH arom.); 6.78 (d, *J* = 8.8 Hz, 1H, CH arom.); 3.98 (s, 3H, OCH_3); 3.94-3.88 (m, 10H, CH_2 and OCH_3); 3.83-3.79 (m, 4H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.65 (C); 160.03 (C); 154.94 (C); 153.05 (C); 152.11 (C); 142.81 (C); 132.59 (CH); 128.72 (CH); 126.37 (CH); 125.24 (CH); 124.55 (CH); 114.65 (C); 107.35 (CH); 66.85 (CH_2); 61.75 (OCH_3); 60.98 (OCH_3); 56.10 (OCH_3); 50.42 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{21}H_{24}N_3O_4$= 382.1761, found 382.1761.
ESI-MS *m/z* (%): 382.1 (100%) $[M+H^+]$.
Hydrochloride: brown solid; mp 176-178 °C.

4-(4-Methylpiperazin-1-yl)-2-(2,3,4-trimethoxyphenyl)quinazoline 136 (NAS 6)



Following the method **A**, compound **136** (0.027 g, yield: 37.7 %) was synthesized as a yellow oil, starting from the 4-chloroquinazoline **300** (0.060 g, 0.18 mmol) and 1-methylpiperazine (0.020 mL, 0.18 mmol) in 4.0 mL of abs. ethanol.
Free base: Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.95 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.87 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.71 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.64 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.42 (t, *J* = 8.0 Hz, 1H, CH arom.); 6.78 (d, *J* = 8.8 Hz, 1H, CH arom.); 3.96 (s, 3H, OCH_3); 3.95-3.91 (m, 4H, CH_2); 3.90 (s, 6H, OCH_3); 2.84-2.71 (m, 4H, CH_2); 2.46 (s, 3H, CH_3) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.43 (C); 160.13 (C); 154.81 (C); 153.04 (C); 152.56 (C); 142.84 (C); 132.48 (CH); 128.98 (CH); 127.11 (C); 126.29 (CH); 125.20 (CH); 124.57 (CH); 114.80 (C); 107.36 (CH); 61.74 (OCH_3); 60.97 (OCH_3); 56.10 (OCH_3); 54.49 (CH_2); 49.02 (CH_2); 45.60 (NCH_3) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{22}H_{27}N_4O_3$= 395.2078, found 395.2075.
ESI-MS *m/z* (%): 395.2 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 247-249 °C.

4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-(2,3,4-trimethoxyphenyl)quinazoline 137 (NAS 23)



Following the method **B**, compound **137** (0.11 g, yield: 83.1 %) was synthesized as a yellow solid, starting from the 4-chloroquinazoline **300** (0.090 g, 0.27 mmol), 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (0.053 g, 0.27 mmol) and K_2CO_3 (0.038 g, 0.27 mmol) in 4.0 mL of dry DMF.

Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.05-7.94 (m, 2H, CH arom.); 7.73 (t, *J* = 7.2 Hz, 1H, CH arom.); 7.67 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.46 (t, *J* = 7.2 Hz, 1H, CH arom.); 6.80 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.70 (s, 1H, CH arom.); 6.65 (s, 1H, CH arom.); 4.93 (s, 2H, NCH_2Ar); 4.12-4.02 (m, 2H, CH_2); 3.99 (s, 3H, OCH_3); 3.92 (s, 6H, OCH_3); 3.88 (s, 3H, OCH_3); 3.86 (s, 3H, OCH_3); 3.17-3.07 (m, 2H, CH_2) ppm.

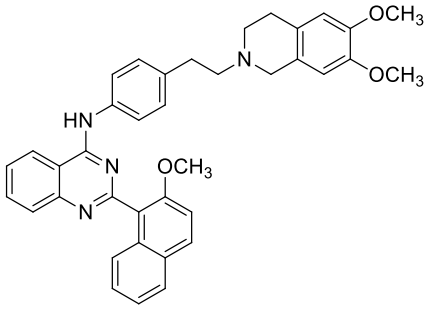
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.11 (C); 160.18 (C); 154.67 (C); 153.02 (C); 152.57 (C); 147.82 (C); 147.70 (C); 142.79 (C); 132.23 (CH); 128.80 (CH); 127.46 (C); 126.51 (C); 126.29 (CH); 125.80 (C); 124.80 (CH); 124.72 (CH); 114.83 (C); 111.56 (CH); 109.33 (CH); 107.32 (CH); 61.78 (OCH_3); 60.99 (OCH_3); 56.10 (OCH_3); 55.99 (OCH_3); 51.06 (CH_2); 48.40 (CH_2); 28.58 (CH_2) ppm.

ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{28}H_{30}N_3O_5$= 488.2180, found 488.2184.

ESI-MS *m/z* (%): 488.2 (100%) $[M+H^+]$.

Hydrochloride: orange solid; mp 94-96 °C.

N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-2-(2-methoxynaphthalen-1-yl)quinazolin-4-amine 138 (NAS 13)



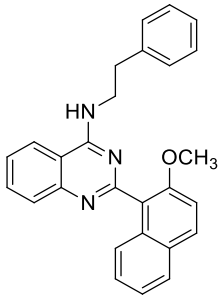
- Following the method **B**, starting from the 4-chloroquinazoline **301** (0.070 g, 0.22 mmol), **244**[91] (0.068 g, 0.22 mmol) and K_2CO_3 (0.030 g, 0.22 mmol) in 3.5 mL of dry DMF, we did not obtain the desired product.
- A solution of **301** (0.090 g, 0.28 mmol), **244**[91] (0.088 g, 0.28 mmol) and dry Et_3N (0.050 mL, 0.22 mmol) in 4.0 mL of $CHCl_3$ (free of ethanol) was refluxed for 24 h, then it was cooled to rt and the solvent was removed under reduced pressure. Unfortunately, the ^{1}H-NMR spectrum of the reaction mixture did not present the specific signals of the desired compound.
- Following the method **A**, compound **138** (0.080 g, yield: 47.7 %) was synthesized as a yellow oil, starting from the 4-chloroquinazoline **301** (0.090 g, 0.28 mmol) and **244**[91] (0.088 g, 0.28 mmol) in 3.0 mL of abs. ethanol.

Free base: Chromatographic eluent: $CH_2Cl_2/CH_3COCH_3/NH_4OH$ 80:20:0.2.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.99-7.91 (m, 3H, NH and CH arom.); 7.86 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.79 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.72 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.60-7.56 (m, 3H, CH arom.); 7.38-7.26 (m, 4H, CH arom.); 6.95 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.55 (s, 1H, CH arom.); 6.47 (s, 1H, CH arom.); 3.82 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.79 (s, 3H,

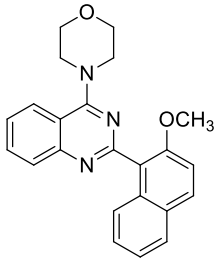
OCH_3); 3.55 (s, 2H, NCH_2Ar); 2.82-2.76 (m, 2H, CH_2); 2.75-2.66 (m, 4H, CH_2); 2.62-2.52 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.08 (C); 157.67 (C); 154.41 (C); 150.66 (C); 147.47 (C); 147.15 (C); 136.77 (C); 135.68 (C); 132.98 (C); 132.71 (CH); 129.99 (CH); 129.05 (C); 128.90 (CH); 127.87 (CH); 126.61 (CH); 126.48 (C); 126.22 (CH); 126.10 (C); 124.85 (CH); 124.29 (C); 123.52 (CH); 121.23 (CH); 121.06 (CH); 113.84 (C); 113.77 (CH); 111.31 (CH); 109.45 (CH); 60.12 (CH_2); 56.62 (OCH_3); 55.89 (OCH_3); 55.64 (CH_2); 51.00 (CH_2); 33.30 (CH_2); 28.63 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{38}H_{37}N_4O_3$= 597.2860, found 597.2856.
ESI-MS *m/z* (%): 597.3 (100%) $[M+H^+]$.
Hydrochloride: orange solid; mp 215-218 (dec) °C.

2-(2-Methoxynaphthalen-1-yl)-*N*-phenethylquinazolin-4-amine 139 (NAS 11)



Following the method **B**, compound **139** (0.11 g, yield: 86.9 %) was synthesized as a yellow solid, starting from the 4-chloroquinazoline **301** (0.10 g, 0.31 mmol), 2-phenylethanamine (0.040 mL, 0.31 mmol) and K_2CO_3 (0.043 g, 0.31 mmol) in 4.0 mL of dry DMF.
Free base: TLC: CH_2Cl_2/CH_3OH/NH_4OH 98:2:0.2.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.94 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.88 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.82-7.77 (m, 1H, CH arom.); 7.76-7.64 (m, 2H, CH arom.); 7.58-7.50 (m, 1H, CH arom.); 7.43 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.39 – 7.24 (m, 5H, CH arom.); 7.22 (d, *J* = 6.8 Hz, 1H, CH arom.); 7.17 (d, *J* = 7.2 Hz, 2H, CH arom.); 6.14 (bs, 1H, NH); 3.93-3.81 (m, 5H, CH_2 and OCH_3); 2.99 (t, *J* = 6.8 Hz, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.56 (C); 159.74 (C); 154.36 (C); 149.91 (C); 139.21 (C); 132.98 (C); 132.47 (CH); 129.91 (CH); 129.15 (C); 128.89 (CH); 128.61 (CH); 128.49 (CH); 127.88 (CH); 126.53 (CH); 126.44 (CH); 125.80 (CH); 124.93 (CH); 124.75 (C); 123.52 (CH); 120.75 (CH); 114.13 (CH); 113.52 (C); 56.95 (OCH_3); 42.43 (CH_2); 35.12 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{27}H_{24}N_3O$= 406.1914, found 406.1917.
ESI-MS *m/z* (%): 406.2 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 232-234 (dec) °C.

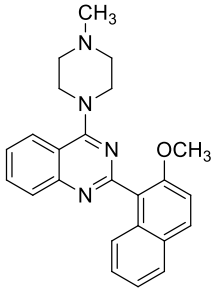
4-(2-(2-Methoxynaphthalen-1-yl)quinazolin-4-yl)morpholine 140 (NAS 14)



Following the method **B**, compound **140** (0.090 g, yield: 97.3 %) was synthesized as a yellow solid, starting from the 4-chloroquinazoline **301** (0.080 g, 0.25 mmol), morpholine (0.022 mL, 0.25 mmol) and K_2CO_3 (0.034 g, 0.25 mmol) in 3.5 mL of dry DMF.
Free base: TLC: CH_2Cl_2/CH_3OH 95:5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.06 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.98 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.91 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.82-7.75 (m, 2H, CH arom.); 7.51 (t, *J* = 7.2 Hz, 1H, CH arom.); 7.47-7.43 (m, 1H, CH arom.); 7.38 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.35-7.28 (m, 2H, CH arom.); 3.92-3.85 (m, 7H, CH_2 and OCH_3); 3.84-3.77 (m, 4H, CH_2) ppm.

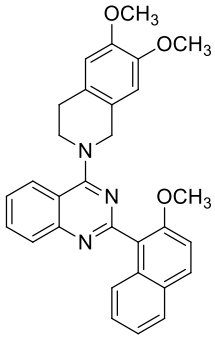
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.87 (C); 160.28 (C); 154.61 (C); 132.92 (C); 132.60 (CH); 130.19 (CH); 129.14 (C); 128.99 (CH); 127.94 (CH); 126.65 (CH); 125.52 (CH); 124.66 (CH); 123.59 (CH); 114.96 (C); 114.24 (CH); 66.87 (CH_2); 57.06 (OCH_3); 50.43 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{23}H_{22}N_3O_2$= 372.1707, found 372.1709.
ESI-MS *m/z* (%): 372.2 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 244-246 (dec) °C.

2-(2-Methoxynaphthalen-1-yl)-4-(4-methylpiperazin-1-yl)quinazoline 141 (NAS 12)



Following the method **B**, compound **141** (0.12 g, yield: 95.4 %) was synthesized as a yellow oil, starting from the 4-chloroquinazoline **301** (0.11 g, 0.33 mmol), 1-methylpiperazine (0.036 mL, 0.33 mmol) and K_2CO_3 (0.045 g, 0.33 mmol) in 4.0 mL of dry DMF.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 98:2:0.2.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.99 (t, *J* = 8.4 Hz, 2H, CH arom.); 7.90 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.83-7.78 (m, 1H, CH arom.); 7.75 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.53-7.43 (m, 2H, CH arom.); 7.38 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.35-7.27 (m, 2H, CH arom.); 3.88 (s, 3H, OCH_3); 3.85 (t, *J* = 4.8 Hz, 4H, CH_2); 2.62 (t, *J* = 4.8 Hz, 4H, CH_2); 2.37 (s, 3H, CH_3) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.87 (C); 160.37 (C); 154.56 (C); 152.58 (C); 132.99 (C); 132.36 (CH); 130.00 (CH); 129.19 (C); 129.03 (CH); 127.88 (CH); 126.54 (CH); 125.24 (CH); 124.88 (CH); 124.83 (CH); 124.60 (C); 123.56 (CH); 115.12 (C); 114.43 (CH); 57.14 (OCH_3); 55.01 (CH_2); 49.73 (CH_2); 46.17 (NCH_3) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{24}H_{25}N_4O$= 385.2023, found 385.2025.
ESI-MS *m/z* (%): 385.2 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 135-138 °C.

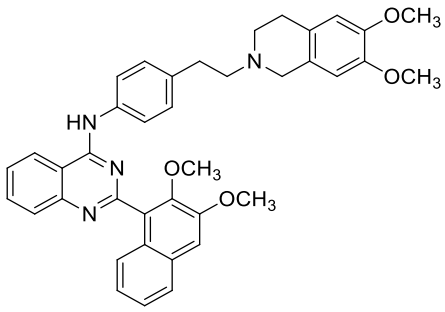
4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-(2-methoxynaphthalen-1-yl)quinazoline 142 (NAS 15)



Following the method **B**, compound **142** (0.090 g, yield: 73.6 %) was synthesized as a yellow solid, starting from the 4-chloroquinazoline **301** (0.083 g, 0.26 mmol), 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (0.050 g, 0.26 mmol) and K_2CO_3 (0.036 g, 0.26 mmol) in 3.0 mL of dry DMF.
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.05 (d, *J* = 8.4 Hz, 1H, CH arom.); 8.02 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.89 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.83-7.76 (m, 1H, CH arom.); 7.75 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.50 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.47-7.42 (m, 1H, CH arom.); 7.37 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.36-7.27 (m, 2H, CH arom.); 6.66 (s, 1H, CH arom.); 6.59 (s, 1H, CH arom.); 4.89 (s, 2H, NCH_2Ar); 4.05 (t, *J* = 5.6 Hz, 2H, CH_2); 3.87 (s, 3H, OCH_3); 3.85 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.06 (t, *J* = 5.6 Hz, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.08 (C); 160.18 (C); 154.57 (C); 152.38 (C); 147.86 (C); 147.75 (C); 133.01 (C); 132.34 (CH); 130.02 (CH); 129.17 (C); 128.74 (CH); 127.86 (CH); 126.53 (CH); 125.79 (C); 125.05 (CH); 124.85 (CH); 123.55 (CH); 114.88 (C); 114.38 (CH);

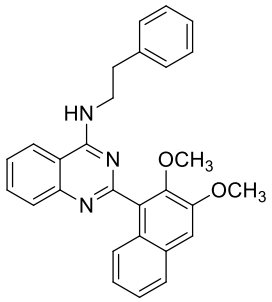
111.58 (CH); 109.39 (CH); 57.11 (OCH_3); 56.02 (OCH_3); 55.96 (OCH_3); 51.07 (CH_2); 48.32 (CH_2); 28.61 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{30}H_{28}N_3O_3$= 478.2125, found 478.2126.
ESI-MS *m/z* (%): 478.1 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 184-186 (dec) °C.

N-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-2-(2,3-dimethoxynaphthalen-1-yl)quinazolin-4-amine 143 (LB 112)



Following the method **A**, compound **143** (0.030 g, yield: 21.0 %) was synthesized as a yellow oil, starting from the 4-chloroquinazoline **302** (0.080 g, 0.23 mmol) and **244**[91] (0.071 g, 0.23 mmol) in 3.0 mL of abs. ethanol.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3COCH_3/NH_4OH$ 80:20:0.2.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.04 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.97 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.87 (bs, 1H, NH); 7.77 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.72 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.66 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.53-7.47 (m, 2H, CH arom.); 7.34 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.23-7.19 (m, 2H, CH arom.); 7.02 (d, *J* = 8.0 Hz, 2H, CH arom.); 6.56 (s, 1H, CH arom.); 6.48 (s, 1H, CH arom.); 3.98 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.59 (s, 2H, NCH_2Ar); 2.82-2.70 (m, 6H, CH_2); 2.67-2.61 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 160.72 (C); 157.49 (C); 152.17 (C); 150.47 (C); 147.61 (C); 147.27 (C); 147.07 (C); 136.78 (C); 135.54 (C); 132.82 (CH); 131.42 (C); 131.13 (C); 129.00 (CH); 127.83 (C); 126.53 (CH); 126.46 (CH); 125.88 (C); 125.30 (CH); 125.18 (CH); 124.19 (CH); 121.41 (CH); 120.87 (CH); 113.80 (C); 111.33 (CH); 109.45 (CH); 107.64 (CH); 61.72 (OCH_3); 59.84 (CH_2); 55.91 (OCH_3); 55.73 (OCH_3); 55.38 (CH_2); 50.86 (CH_2); 33.07 (CH_2); 28.34 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{39}H_{39}N_4O_4$= 627.2966, found 627.2971.
ESI-MS *m/z* (%): 627.4 (100%) $[M+H^+]$.
Hydrochloride: yellow solid; mp 256-259 (dec) °C.

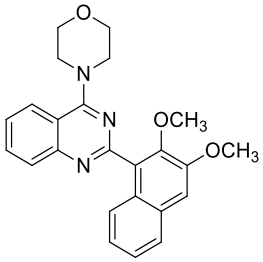
2-(2,3-Dimethoxynaphthalen-1-yl)-*N*-phenethylquinazolin-4-amine 144 (NAS 19)



Following the method **B**, compound **144** (0.060 g, yield: 69.0 %) was synthesized as a white solid, starting from the 4-chloroquinazoline **302** (0.070 g, 0.20 mmol), 2-phenylethanamine (0.025 mL, 0.20 mmol) and K_2CO_3 (0.028 g, 0.20 mmol) in 3.0 mL of dry DMF.
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 98:2:0.2.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.93 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.76-7.71 (m, 2H, CH arom.); 7.66 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.50-7.41 (m, 2H, CH arom.); 7.35 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.31-7.15 (m, 7H, CH arom.); 6.02 (bs, 1H, NH); 4.01 (s, 3H, OCH_3); 3.94-3.88 (m, 5H, CH_2 and OCH_3); 2.98 (t, *J* = 6.8 Hz, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.12 (C); 159.58 (C); 152.20 (C); 149.55 (C); 146.94 (C); 139.04 (C); 132.60 (CH); 131.48 (C); 131.16 (C); 128.85 (CH); 128.65 (CH); 128.43 (CH);

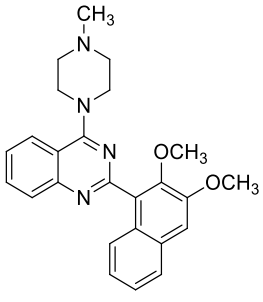
127.84 (C); 126.56 (CH); 126.50 (CH); 125.97 (CH); 125.29 (CH); 125.14 (CH); 124.13 (CH); 120.69 (CH); 113.50 (C); 107.73 (CH); 61.70 (OCH_3); 55.77 (OCH_3); 42.32 (CH_2); 35.26 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{28}H_{26}N_3O_2$= 436.2020, found 436.2016.
ESI-MS *m/z* (%): 436.1 (100%) $[M+H^+]$.
Hydrochloride: white solid; mp 256-259 (dec) °C.

4-(2-(2,3-Dimethoxynaphthalen-1-yl)quinazolin-4-yl)morpholine 145 (NAS 18)



Following the method **B**, compound **145** (0.070 g, yield: 87.2 %) was synthesized as a white solid, starting from the 4-chloroquinazoline **302** (0.070 g, 0.20 mmol), morpholine (0.017 mL, 0.20 mmol) and K_2CO_3 (0.028 g, 0.20 mmol) in 3.0 mL of dry DMF.
Free base: TLC: CH_2Cl_2/CH_3OH 95:5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.03 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.98 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.77 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.73 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.51 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.42 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.35 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.26 (s, 1H, CH arom.); 7.22 (t, *J* = 8.0 Hz, 1H, CH arom.); 4.01 (s, 3H, OCH_3); 3.90 (s, 3H, OCH_3); 3.89-3.84 (m, 4H, CH_2); 3.83-3.78 (m, 4H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.75 (C); 159.93 (C); 152.21 (C); 147.16 (C); 132.67 (CH); 131.48 (C); 130.70 (C); 129.00 (CH); 127.78 (C); 126.65 (CH); 125.61 (CH); 125.20 (CH); 125.08 (CH); 124.70 (CH); 124.23 (CH); 115.02 (C); 107.89 (CH); 66.85 (CH_2); 61.73 (OCH_3); 55.79 (OCH_3); 50.40 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{24}H_{24}N_3O_3$= 402.1812, found 402.1812.
ESI-MS *m/z* (%): 402.1 (100%) $[M+H^+]$.
Hydrochloride: white solid; mp 219-221 (dec) °C.

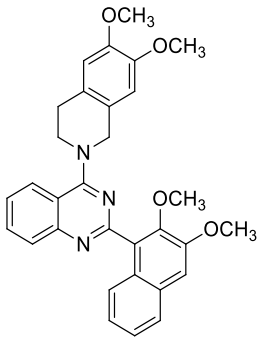
2-(2,3-Dimethoxynaphthalen-1-yl)-4-(4-methylpiperazin-1-yl)quinazoline 146 (NAS 21)



Following the method **B**, compound **146** (0.050 g, yield: 60.3 %) was synthesized as a white solid, starting from the 4-chloroquinazoline **302** (0.070 g, 0.20 mmol), 1-methylpiperazine (0.022 mL, 0.20 mmol) and K_2CO_3 (0.028 g, 0.20 mmol) in 3.0 mL of dry DMF.
Free base: Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 98:2:0.2.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.99 (d, *J* = 9.2 Hz, 2H, CH arom.); 7.79-7.68 (m, 2H, CH arom.); 7.49 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.43 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.34 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.25 (s, 1H, CH arom.); 7.21 (t, *J* = 8.0 Hz, 1H, CH arom.); 4.01 (s, 3H, OCH_3); 3.89 (s, 3H, OCH_3); 3.86 (t, *J* = 4.4 Hz, 4H, CH_2); 2.62 (t, *J* = 4.4 Hz, 4H, CH_2); 2.36 (s, 3H, NCH_3) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.73 (C); 159.98 (C); 152.41 (C); 152.25 (C); 147.14 (C); 132.44 (CH); 131.48 (C); 131.01 (C); 129.04 (CH); 127.86 (C); 126.58 (CH); 125.34 (CH); 125.20 (CH); 125.13 (CH); 124.88 (CH); 124.14 (CH); 115.17 (C); 107.78 (CH); 61.71 (OCH_3); 55.78 (OCH_3); 54.96 (CH_2); 49.65 (CH_2); 46.06 (NCH_3) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{25}H_{27}N_4O_2$= 415.2129, found 415.2132.
ESI-MS *m/z* (%): 415.1 (100%) $[M+H^+]$.

Hydrochloride: yellow solid; mp 181-183 (dec) °C.

4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-(2,3-dimethoxynaphthalen-1-yl)quinazoline 147 (NAS 20)



Following the method **B**, compound **147** (0.090 g, yield: 95.8 %) was synthesized as a white solid, starting from the 4-chloroquinazoline **302** (0.065 g, 0.19 mmol), 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (0.036 g, 0.19 mmol) and K_2CO_3 (0.026 g, 0.19 mmol) in 3.0 mL of dry DMF.

Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 98:2:0.2.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.07 (d, *J* = 8.0 Hz, 1H, CH arom.); 8.04-7.96 (m, 1H, CH arom.); 7.79-7.71 (m, 2H, CH arom.); 7.52 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.42 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.35 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.26 (s, 1H, CH arom.); 7.20 (t, *J* = 7.6 Hz, 1H, CH arom.); 6.67 (s, 1H, CH arom.); 6.60 (s, 1H, CH arom.); 4.90 (s, 2H, NCH_2Ar); 4.08 (t, *J* = 5.2 Hz, 2H, CH_2); 4.02 (s, 3H, OCH_3); 3.87 (s, 3H, OCH_3); 3.86 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.07 (t, *J* = 5.2 Hz, 2H, CH_2) ppm.

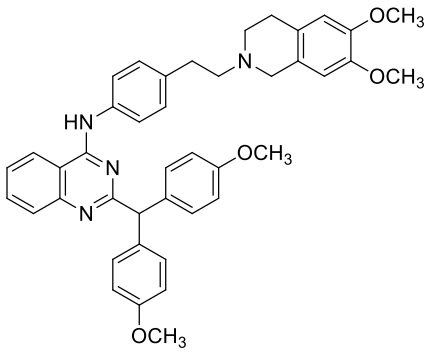
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 163.93 (C); 152.21 (C); 147.87 (C); 147.77 (C); 147.10 (C); 132.48 (CH); 131.45 (C); 127.85 (C); 126.58 (CH); 126.48 (C); 125.67 (C); 125.20 (CH); 125.16 (CH); 124.91 (CH); 124.16 (CH); 114.92 (C); 111.56 (CH); 109.36 (CH); 107.78 (CH); 61.72 (OCH_3); 56.02 (OCH_3); 55.96 (OCH_3); 55.78 (OCH_3); 51.05 (CH_2); 48.36 (CH_2); 28.62 (CH_2) ppm.

ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{31}H_{30}N_3O_4$= 508.2231, found 508.2231.

ESI-MS *m/z* (%): 508.1 (100%) $[M+H^+]$.

Hydrochloride: white solid; mp 190-193 (dec) °C.

2-(bis(4-Methoxyphenyl)methyl)-*N*-(4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)quinazolin-4-amine 148 (LB 126)



Following the method **A**, compound **148** (0.040 g, yield: 29.3 %) was synthesized as a pale yellow solid, starting from the 4-chloroquinazoline **306** (0.080 g, 0.20 mmol) and **244**[91] (0.064 g, 0.20 mmol) in 1.5 mL of abs. ethanol.

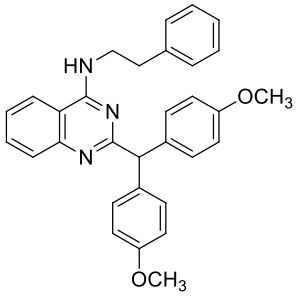
Free base: Chromatographic eluent: CH_2Cl_2/CH_3COCH_3 70:30.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.85 (t, *J* = 8.8 Hz, 2H, CH arom.); 7.71 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.63 (bs, 1H, NH); 7.52 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.43 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.30 (d, *J* = 8.8 Hz, 4H, CH arom.); 7.13 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.81 (d, *J* = 8.8 Hz, 4H, CH arom.); 6.60 (s, 1H, CH arom.); 6.54 (s, 1H, CH arom.); 5.63 (s, 1H, CH); 3.84 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 3.75 (s, 6H, OCH_3); 3.69 (s, 2H, CH_2); 2.93-2.77 (m, 8H, CH_2) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.57 (C); 158.08 (C); 157.34 (C); 150.57 (C); 147.63 (C); 147.30 (C); 136.88 (C); 135.50 (C); 135.07 (C); 132.71 (CH); 130.40 (CH); 128.91 (CH); 128.60 (CH); 126.13 (C); 126.02 (CH); 121.17 (CH); 120.48 (CH); 113.47 (CH); 111.39 (CH);

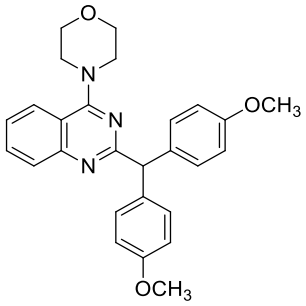
109.50 (CH); 60.07 (CH_2); 59.22 (CH); 55.94 (OCH_3); 55.91 (OCH_3); 55.61 (CH_2); 55.24 (OCH_3); 51.03 (CH_2); 33.31 (CH_2); 28.50 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{42}H_{43}N_4O_4$= 667.3279, found 667.3282.
Hydrochloride: yellow solid; mp 258-260 (dec) °C.

2-(bis(4-Methoxyphenyl)methyl)-*N*-phenethylquinazolin-4-amine 149 (LB 124)



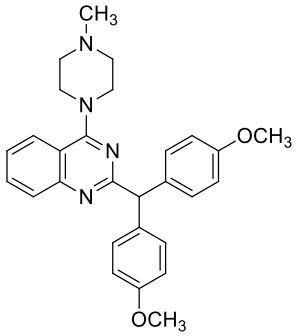
Following the method **B**, compound **149** (0.070 g, yield: 95.7 %) was synthesized as a pale-yellow solid, starting from the 4-chloroquinazoline **306** (0.060 g, 0.15 mmol), 2-phenylethanamine (0.019 mL, 0.15 mmol) and K_2CO_3 (0.021 g, 0.15 mmol) in 3.0 mL of dry DMF.
Free base: TLC: CH_2Cl_2/CH_3OH/NH_4OH 97:3:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.80 (bs, 1H, NH); 7.75 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.63 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.48-7.37 (m, 5H, CH arom.); 7.34-7.20 (m, 3H, CH arom.); 7.15-7.01 (m, 3H, CH arom.); 6.84 (d, *J* = 8.4 Hz, 4H, CH arom.); 5.64 (s, 1H, CH); 3.86-3.76 (m, 2H, CH_2); 3.75 (s, 6H, OCH_3); 2.90 (t, *J* = 7.2 Hz, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.32 (C); 159.82 (C); 158.23 (C); 148.51 (C); 139.15 (C); 134.94 (C); 132.72 (CH); 130.36 (CH); 128.84 (CH); 128.61 (CH); 126.76 (CH); 126.45 (CH); 125.71 (CH); 121.24 (CH); 113.54 (CH); 113.27 (C); 58.49 (CH); 55.24 (OCH_3); 42.83 (CH_2); 35.36 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{31}H_{30}N_3O_2$= 476.2333, found 476.2333.
Hydrochloride: white solid; mp 155-158 (dec) °C.

4-(2-(bis(4-Methoxyphenyl)methyl)quinazolin-4-yl)morpholine 150 (LB 118)



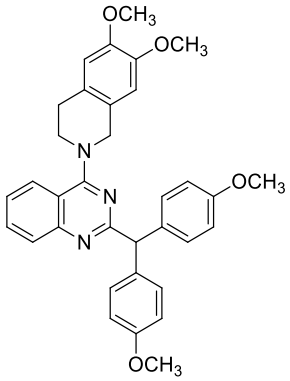
Following the method **B**, compound **150** (0.040 g, yield: 70.9 %) was synthesized as a white solid, starting from the 4-chloroquinazoline **306** (0.050 g, 0.13 mmol), morpholine (0.011 mL, 0.13 mmol) and K_2CO_3 (0.018 g, 0.13 mmol) in 3.0 mL of dry DMF.
Free base: TLC: CH_2Cl_2/CH_3OH/NH_4OH 97:3:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.93 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.81 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.69 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.41-7.34 (m, 5H, CH arom.); 6.82 (d, *J* = 8.4 Hz, 4H, CH arom.); 5.61 (s, 1H, CH); 3.84-3.78 (m, 4H, CH_2); 3.77-3.72 (m, 10H, OCH_3 and CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 165.99 (C); 164.63 (C); 158.15 (C); 151.99 (C); 135.07 (C); 132.54 (CH); 130.23 (CH); 128.44 (CH); 125.13 (CH); 124.48 (CH); 114.61 (C); 113.45 (CH); 66.75 (CH_2); 58.83 (CH); 55.21 (OCH_3); 50.25 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{27}H_{28}N_3O_3$= 442.2125, found 442.2127.
Hydrochloride: white solid; mp 236-238 (dec) °C.

2-(bis(4-Methoxyphenyl)methyl)-4-(4-methylpiperazin-1-yl)quinazoline 151 (LB 123)



Following the method **B**, compound **151** (0.030 g, yield: 42.9 %) was synthesized as a white solid, starting from the 4-chloroquinazoline **306** (0.060 g, 0.15 mmol), 1-methylpiperazine (0.017 mL, 0.15 mmol) and K_2CO_3 (0.021 g, 0.15 mmol) in 3.0 mL of dry DMF.
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.89 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.81 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.68 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.40-7.33 (m, 5H, CH arom.); 6.81 (d, *J* = 8.4 Hz, 4H, CH arom.); 5.58 (s, 1H, CH); 3.88-3.78 (m, 4H, CH_2); 3.75 (s, 6H, OCH_3); 2.69-2.51 (m, 4H, CH_2); 2.36 (s, 3H, CH_3) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 166.07 (C); 164.64 (C); 158.11 (C); 152.26 (C); 135.18 (C); 132.47 (CH); 130.22 (CH); 128.54 (CH); 125.10 (CH); 124.56 (CH); 114.79 (C); 113.43 (CH); 58.89 (CH); 55.22 (OCH_3); 54.27 (CH_2); 48.99 (CH_2); 45.56 (NCH_3) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{28}H_{31}N_4O_2$= 455.2442, found 455.2442.
Hydrochloride: white solid; mp 233-235 (dec) °C.

2-(bis(4-Methoxyphenyl)methyl)-4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)quinazoline 152 (LB 125)



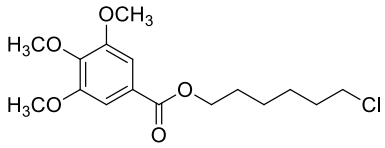
Following the method **B**, compound **152** (0.070 g, yield: 82.5 %) was synthesized as a pale-yellow solid, starting from the 4-chloroquinazoline **306** (0.061 g, 0.16 mmol), 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (0.030 g, 0.16 mmol) and K_2CO_3 (0.022 g, 0.16 mmol) in 3.0 mL of dry DMF.
Free base: TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.93 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.88 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.67 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.41-7.35 (m, 5H, CH arom.); 6.82 (d, *J* = 8.4 Hz, 4H, CH arom.); 6.66 (s, 1H, CH arom.); 6.63 (s, 1H, CH arom.); 5.59 (s, 1H, CH); 4.86 (s, 2H, CH_2); 4.02-3.98 (m, 2H, CH_2); 3.88 (s, 3H, OCH_3); 3.86 (s, 3H, OCH_3); 3.76 (s, 6H, OCH_3); 2.96-2.90 (m, 2H, CH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 166.01 (C); 163.98 (C); 158.07 (C); 152.48 (C); 147.84 (C); 147.71 (C); 135.44 (C); 132.22 (CH); 130.29 (CH); 128.42 (CH); 126.64 (C); 125.74 (C); 124.80 (CH); 124.64 (CH); 114.77 (C); 113.40 (CH); 111.56 (CH); 109.27 (CH); 59.07 (CH); 56.06 (OCH_3); 56.01 (OCH_3); 55.22 (OCH_3); 51.17 (CH_2); 47.78 (CH_2); 28.14 (CH_2) ppm.
ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{34}H_{34}N_3O_4$= 548.2544, found 548.2548.
Hydrochloride: yellow solid; mp 186-189 (dec) °C.

7.1.2. Intermediates

7.1.2.1. P-gp/hCAXII inhibitors

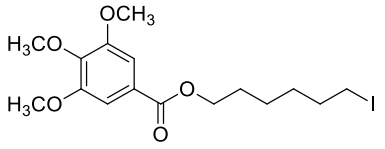
7.1.2.1.1. Coumarins and sulfamoyl benzoate diester compounds

6-Chlorohexyl 3,4,5-trimethoxybenzoate 193 (LB42)



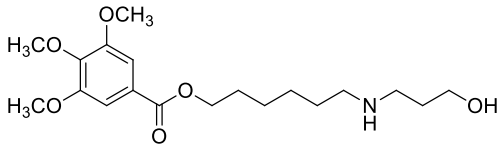
A solution of 3,4,5-trimethoxybenzoic acid (0.91 g 4.28 mmol) in 25.0 mL of dry CH_2Cl_2 was cooled at 0 °C and 6-chlorohexan-1-ol (0.33 mL, 2.86 mmol), DMAP (0.28 g, 2.28 mmol) and EDC hydrochloride (0.98 g, 5.14 mmol) were added in this order. The reaction mixture was stirred at 0 °C for 1 h and at rt for 48 h, then treated with CH_2Cl_2. The organic layer was washed three times with water and twice with a saturated solution of $NaHCO_3$, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was then purified by flash chromatography using CH_2Cl_2/CH_3OH 98:2 as eluent, obtaining **193** (0.94 g, yield 100.0 %) as an oil.
TLC: CH_2Cl_2/CH_3OH 95:5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.22 (s, 2H, CH arom.); 4.24 (t, *J* = 6.8 Hz, 2H, OCH_2); 3.84 (s, 6H, OCH_3); 3.83 (s, 3H, OCH_3); 3.47 (t, *J* = 6.4 Hz, 2H, CH_2Cl); 1.75-1.69 (m, 4H, CH_2); 1.49-1.39 (m, 4H, CH_2) ppm.

6-Iodohexyl 3,4,5-trimethoxybenzoate 194 (LB44)



To a solution of **193** (1.07 g, 3.24 mmol) in 15.0 mL of acetone, NaI (2.90 g, 19.4 mmol) was added, and the resulting mixture was refluxed in the dark for 19 h. The reaction was cooled to rt, and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and the organic layer was washed with water, dried over Na_2SO_4, and the solvent was removed under reduced pressure. **194** (1.27 g, yield 93.0 %) was obtained as an oil which was used as such for the next reaction.
TLC: CH_2Cl_2/CH_3OH 95:5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.21 (s, 2H, CH arom.); 4.24 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.83 (s, 6H, OCH_3); 3.82 (s, 3H, OCH_3); 3.11 (t, *J* = 6.8 Hz, 2H, CH_2I); 1.77-1.69 (m, 4H, CH_2); 1.40-1.35 (m, 4H, CH_2) ppm.

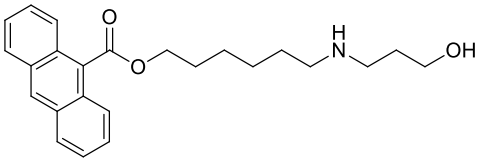
6-((3-Hydroxypropyl)amino)hexyl 3,4,5-trimethoxybenzoate 195 (LB45)



To a solution of **194** (1.27 g, 3.00 mmol) in 20.0 mL of dry CH_3CN, K_2CO_3 (0.42 g, 3.00 mmol) and 3-aminopropan-1-ol (0.70 mL, 9.00 mmol) were added. The mixture was heated at 80 °C for 18 h. Then the solvent was removed under reduced pressure, and the residue was treated with CH_2Cl_2. The organic layer was washed with 10% NaOH solution, dried over Na_2SO_4, and the solvent was

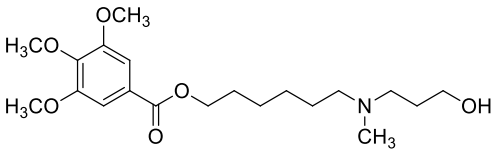
removed under reduced pressure. The residue was purified by flash chromatography using $CH_2Cl_2/CH_3OH/NH_4OH$ 90:10:1 as eluent, obtaining **195** (0.950 g, yield 86%) as an oil.
TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.22 (s, 2H, CH arom.); 4.24 (t, *J* = 6.8 Hz, 2H, OCH_2); 3.85 (s, 6H, OCH_3); 3.83 (s, 3H, OCH_3); 3.73 (t, *J* = 5.2 Hz, 2H, CH_2OH); 3.45 (bs, 2H, NH and OH); 2.82 (t, *J* = 5.6 Hz, 2H, NCH_2); 2.56 (t, *J* = 7.2 Hz, 2H, NCH_2); 1.72-1.63 (m, 4H, CH_2); 1.49-1.33 (m, 6H, CH_2) ppm.

6-((3-Hydroxypropyl)amino)hexyl anthracene-9-carboxylate 196 (LB41)



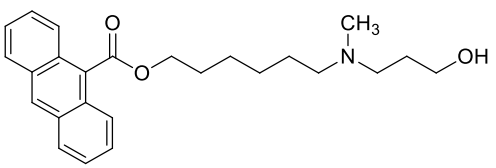
Following the same procedure described for compound **195**, starting from 6-iodohexyl anthracene-9-carboxylate[80] (1.44 g, 3.34 mmol), compound **196** (0.77 g, yield 60.0 %) was obtained as an oil.
TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.44 (s, 1H, CH arom.); 7.98-7.93 (m, 4H, CH arom.); 7.50-7.40 (m, 4H, CH arom.); 4.55 (t, *J* = 6.8 Hz, 2H, OCH_2); 3.67 (t, *J* = 5.2 Hz, 2H, CH_2OH); 2.70 (t, *J* = 5.6 Hz, 2H, NCH_2); 2.49 (t, *J* = 6.8 Hz, 2H, NCH_2); 1.83-1.77 (m, 2H, CH_2); 1.63-1.57 (m, 2H, CH_2); 1.42-1.31 (m, 6H, CH_2) ppm.

6-((3-Hydroxypropyl)(methyl)amino)hexyl 3,4,5-trimethoxybenzoate 197 (LB47)



Compound **195** (0.24 g, 0.65 mmol) was dissolved in 5.0 mL of abs. ethanol, then HCOOH (0.40 mL, 10.10 mmol) and 37% HCHO solution (0.09 mL, 3.23 mmol) were added. The mixture was refluxed for 4-5 h and concentrated in vacuo. The residue was dissolved in CH_2Cl_2, and the organic layer was washed with 10% NaOH solution, dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography using $CH_2Cl_2/CH_3OH/NH_4OH$ 90:10:1 as eluent, obtaining **197** (0.169 g, yield 68.0 %) as an oil.
TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 90:10:1.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.24 (s, 2H, CH arom.); 4.25 (t, *J* = 6.8 Hz, 2H, OCH_2); 3.86 (s, 6H, OCH_3); 3.85 (s, 3H, OCH_3); 3.74 (t, *J* = 5.2 Hz, 2H, CH_2OH); 2.55 (t, *J* = 5.6 Hz, 2H, NCH_2); 2.33 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.20 (s, 3H, NCH_3); 1.74-1.63 (m, 4H, CH_2); 1.51-1.30 (m, 6H, CH_2) ppm.

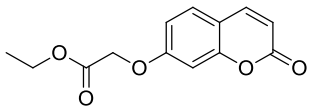
6-((3-Hydroxypropyl)(methyl)amino)hexyl anthracene-9-carboxylate 198 (LB43)



Following the same procedure described for compound **197**, starting from **196** (0.22 g, 0.59 mmol), compound **198** (0. 23 g, yield 98.0 %) was obtained as an oil.
TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 90:10:1.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.39 (s, 1H, CH arom.); 8.01 (d, *J* =8.8 Hz, 2H, CH arom.); 7.90 (d, *J* = 8.8 Hz, 2H, CH arom.); 7.50-7.38 (m, 4H, CH arom.); 4.56 (t, *J* = 6.8 Hz, 2H, OCH_2); 3.71 (t, *J* = 5.2 Hz, 2H, CH_2OH); 2.43 (t, *J* = 5.6 Hz, 2H, NCH_2); 2.23 (t, *J* = 7.2 Hz,

2H, NCH_2); 2.12 (s, 3H, NCH_3); 1.85-1.78 (m, 2H, CH_2); 1.61-1.50 (m, 2H, CH_2); 1.49-1.28 (m, 6H, CH_2) ppm.

Ethyl 2-((2-oxo-2H-chromen-7-yl)oxy)acetate 211[82]

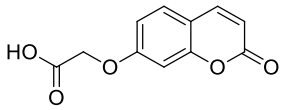


To a solution of 7-hydroxy-2*H*-chromen-2-one (0.24 g, 0.97 mmol) in 8.0 mL of acetone, K_2CO_3 (0.23 g, 1.63 mmol) was added. The mixture was stirred at rt for 15 min, then ethyl bromoacetate (0.18 mL, 1.63 mmol) dissolved in 6.0 mL of acetone was added dropwise. The reaction was refluxed for 6 h and, after cooling, water (10.0 mL) was added. The suspension was filtered under vacuum and the obtained solid was dried under vacuum, obtaining **211** (yield 89.0 %) as a white solid.

TLC: CH_2Cl_2/CH_3OH 95:5. Mp: 107-110 °C.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.64 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 7.40 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.89 (dd, *J* = 8.4, 2.2 Hz, 1H, CH arom.); 6.78 (d, *J* = 2.2 Hz, 1H, CH arom.); 6.28 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.68 (s, 2H, OCH_2); 4.30 (q, *J* = 7.2 Hz, 2H, OCH_2); 1.32 (t, *J* = 7.2 Hz, 3H, CH_3) ppm.

2-((2-Oxo-2H-chromen-7-yl)oxy)acetic acid 212[82]



211 (0.23 g, 0.95 mmol) was suspended in 10.0 mL of 10% NaOH solution. The mixture was stirred at 100 °C for 3 h. After cooling, the solution was acidified with 2N HCl and the solid was filtered under vacuum and then dried, obtaining **212** (yield 100.0 %) as a white solid.

TLC: CH_2Cl_2/CH_3OH 95:5. Mp 220-223 °C.

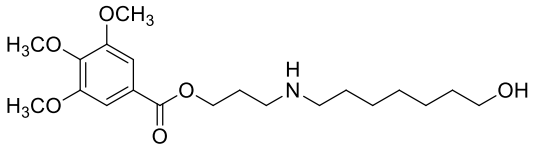
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.98 (d, *J* = 9.6, Hz 1H, C*H*=CH); 7.62 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.94 (s, 1H, CH arom.); 6.93 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.28 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.81 (s, 2H, OCH_2) ppm.

7.1.2.1.2. (*N*-Alkylcoumarin)aminoaryl diester compounds

General procedure for the synthesis of (hydroxyalkyl)aminoesters 221-223.

To a solution of the proper bromoester **213-215**[84,86] (1 equiv.) in the adequate amount of dry CH_3CN, K_2CO_3 (1 equiv.) and 7-aminoheptan-1-ol[89] (2 equiv.) were added. The mixture was stirred at 60 °C overnight, then the solvent was removed under reduced pressure and the residue was treated with CH_2Cl_2. The organic layer was washed twice with 10% NaOH solution, dried over Na_2SO_4 and concentrated under reduced pressure. Finally, the residue was purified by flash chromatography, using $CH_2Cl_2/CH_3OH/NH_4OH$ 90:10:1 as eluent, yielding the desired (hydroxyalkyl)aminoester as a pale-yellow oil.

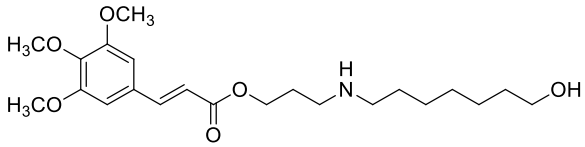
3-((7-Hydroxyheptyl)amino)propyl 3,4,5-trimethoxybenzoate 221 (KIS12)



Following the general procedure, compound **221** (0.10 g, yield: 70.6 %) was synthesized from **213**[84] (0.12 g, 0.37 mmol) and 7-aminoheptan-1-ol[89] (0.10 g, 0.74 mmol) in 5.0 mL of dry CH_3CN.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.25 (s, 2H, CH arom.); 4.35 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.87 (s, 9H, OCH_3); 3.57 (t, *J* = 6.8 Hz, 2H, CH_2OH); 2.74 (t, *J* = 6.8 Hz, 2H, NCH_2); 2.59 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.22 (bs, 2H, NH and OH); 1.99-1.93 (m, 2H, CH_2); 1.50-1.47 (m, 4H, CH_2); 1.28 (s, 6H, CH_2) ppm.

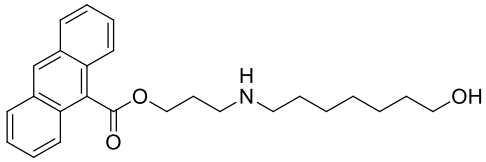
(*E*)-3-((7-Hydroxyheptyl)amino)propyl 3-(3,4,5-trimethoxyphenyl)acrylate 222 (KIS18)



Following the general procedure, compound **222** (0.17 g, yield: 71.0 %) was synthesized from **214**[86] (0.21 g, 0.58 mmol) and 7-aminoheptan-1-ol[89] (0.15 g, 1.17 mmol) in 6.0 mL of dry CH_3CN.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.51 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.68 (s, 2H, CH arom.); 6.27 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 4.20 (t, *J* = 6.0 Hz, 2H, OCH_2); 3.81 (s, 6H, OCH_3); 3.80 (s, 3H, OCH_3); 3.52 (t, *J* = 6.4 Hz, 2H, CH_2OH); 2.66 (t, *J* = 6.8 Hz, 2H, NCH_2); 2.53 (t, *J* = 6.8 Hz, 2H, NCH_2); 2.06 (bs, 2H, NH and OH); 1.85-1.82 (m, 2H, CH_2); 1.52-1.39 (m, 4H, CH_2); 1.30-1.19 (m, 6H, CH_2) ppm.

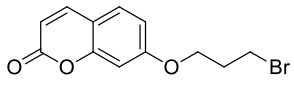
3-((7-Hydroxyheptyl)amino)propyl anthracene-9-carboxylate 223 (LB119)



Following the general procedure, compound **223** (0.33 g, yield: 72.4 %) was synthesized from **215**[84] (0.40 g, 1.17 mmol) and 7-aminoheptan-1-ol[89] (0.31 g, 2.33 mmol) in 15.0 mL of dry CH_3CN.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.49 (s, 1H, CH arom.); 7.99 (t, *J* = 9.2 Hz, 4H, CH arom.); 7.53-7.43 (m, 4H, CH arom.); 4.66 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.62 (bs, 2H, NH and OH); 3.53 (t, *J* = 6.4 Hz, 2H, CH_2OH); 2.82 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.59 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.14-2.07 (m, 2H, CH_2); 1.51-1.37 (m, 4H, CH_2); 1.29-1.16 (m, 6H, CH_2) ppm.

7-(3-Bromopropoxy)-2*H*-chromen-2-one 234 (LB50)



To a solution of 7-hydroxy-2*H*-chromen-2-one (0.40 g, 2.46 mmol) in 30.0 mL of acetone, K_2CO_3 (1.02 g, 7.39 mmol) and 1,3-dibromopropane (1.25 mL, 12.31 mmol) were added. The reaction was refluxed overnight, then it was cooled to rt and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed twice with water, then the organic phase was dried over Na_2SO_4 and concentrated under vacuum. **234** (0.64 g, yield 91.5%) was obtained as a pure white solid.

TLC: CH_2Cl_2/CH_3OH 95:5.

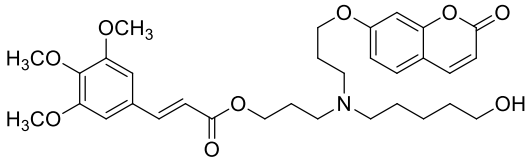
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 7.35 (d, *J* = 8.0 Hz, 1H, CH arom.); 6.83-6.80 (m, 2H, CH arom.); 6.23 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.14 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.58 (t, *J* = 6.4 Hz, 2H, CH_2Br); 2.36-2.29 (m, 2H, CH_2) ppm.

General procedure for the synthesis of ((hydroxyalkyl)alkylcoumarin)aminoester 224-233

The suitable (hydroxyalkyl)aminoester (1 or 1.2 equiv.) was dissolved in the adequate amount of dry CH_3CN, then K_2CO_3 (3 equiv.) and **234** (1 or 1.2 equiv.) were added. The mixture was

stirred at 60 °C for 20 h, then it was cooled to rt and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2, then the organic layer was washed twice with 10% NaOH solution, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography using the proper eluting system, yielding the desired compound as a pale-yellow oil.

(*E*)-3-((5-Hydroxypentyl)(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)propyl 3-(3,4,5-trimethoxyphenyl)acrylate 224 (KIS 6)

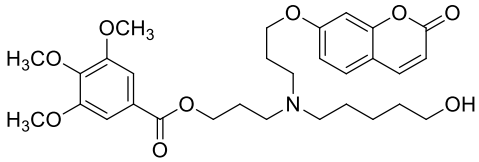


Following the general procedure, compound **224** (0.30 g, yield: 57.7 %) was synthesized from **216**[86] (0.34 g, 0.89 mmol) and **234** (0.37 g, 1.07 mmol) in 13.0 mL of dry CH_3CN.

Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.54 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 7.51 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.27 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.77-6.75 (m, 2H, CH arom.); 6.68 (s, 2H, CH arom.); 6.25 (d, *J* =16.0 Hz, 1H, C*H*=CH); 6.14 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.17 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.02 (t, *J* = 6.0 Hz, 2H, OCH_2); 3.82 (s, 6H, OCH_3); 3.81 (s, 3H, OCH_3); 3.57 (t, *J* = 6.4 Hz, 2H, CH_2OH); 2.56 (t, *J* = 6.4 Hz, 2H, NCH_2); 2.50 (t, *J* = 6.4 Hz, 2H, NCH_2); 2.39 (t, *J* = 6.4 Hz, 2H, NCH_2); 2.00-1.83 (m, 3H, CH_2 and OH); 1.82-1.74 (m, 2H, CH_2); 1.52-1.37 (m, 4H, CH_2); 1.35-1.26 (m, 2H, CH_2) ppm.

3-((5-Hydroxypentyl)(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)propyl 3,4,5-trimethoxybenzoate 225 (LB62)

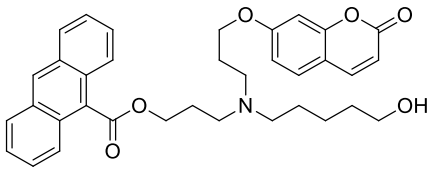


Following the general procedure, compound **225** (0.16 g, yield: 53.0 %) was synthesized from **217**[86] (0.23 g, 0.64 mmol) and **234** (0.15 g, 0.54 mmol) in 20.0 mL of dry CH_3CN.

Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.53 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 7.26 (d, *J* =8.4 Hz, 1H, CH arom.); 7.21 (s, 2H, CH arom.); 6.75-6.73 (m, 2H, CH arom.); 6.14 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.26 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.00 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.83 (s, 3H, OCH_3); 3.82 (s, 6H, OCH_3); 3.53 (t, *J* = 6.4 Hz, 2H, CH_2OH); 2.56-2.49 (m, 4H, NCH_2); 2.37 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.14 (bs, 1H, OH); 1.90-1.80 (m, 4H, CH_2); 1.49-1.36 (m, 4H, CH_2); 1.35-1.26 (m, 2H, CH_2) ppm.

3-((5-Hydroxypentyl)(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)propyl anthracene-9-carboxylate 226 (LB57)

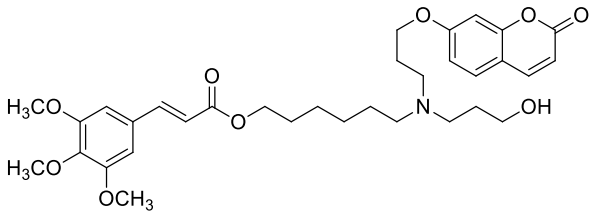


Following the general procedure, compound **226** (0.15 g, yield: 46.8 %) was synthesized from **218**[86] (0.25 g, 0.69 mmol) and **234** (0.16 g, 0.58 mmol) in 20.0 mL of dry CH_3CN.

Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.46 (s, 1H, CH arom.); 7.97 (t, *J* = 8.0 Hz, 4H, CH arom.); 7.50-7.42 (m, 5H, CH arom. and C*H*=CH); 7.19 (d, *J* = 9.2 Hz, 1H, CH arom.); 6.73-6.70 (m, 2H, CH arom.); 6.15 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.61 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.97 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.52 (t, *J* = 6.4 Hz, 2H, CH_2OH); 2.60-2.54 (m, 4H, NCH_2); 2.40 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.00-1.92 (m, 2H, CH_2); 1.88-1.82 (m, 2H, CH_2); 1.47-1.39 (m, 4H, CH_2); 1.33-1.26 (m, 2H, CH_2) ppm.

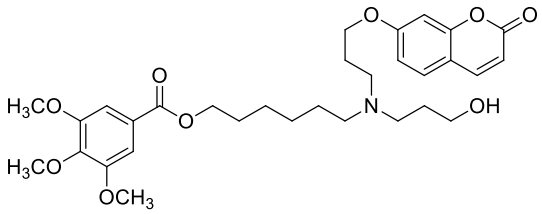
(*E*)-6-((3-Hydroxypropyl)(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)hexyl 3-(3,4,5-trimethoxyphenyl)acrylate 227 (KIS 1)



Following the general procedure, compound **227** (0.27 g, yield: 59.6 %) was synthesized from **219**[80] (0.36 g, 0.91 mmol) and **234** (0.21 g, 0.76 mmol) in 27.0 mL of dry CH_3CN.

Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.59 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 7.56 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.33 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.82-6.77 (m, 2H, CH arom.); 6.73 (s, 2H, CH arom.); 6.32 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.21 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.14 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.03 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.86 (s, 6H, OCH_3); 3.85 (s, 3H, OCH_3); 3.77 (t, *J* = 6.4 Hz, 2H, CH_2OH); 2.66-2.59 (m, 4H, NCH_2); 2.44 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.00-1.94 (m, 2H, CH_2); 1.72-1.62 (m, 4H, CH_2); 1.51-1.44 (m, 2H, CH_2); 1.41-1.28 (m, 4H, CH_2) ppm.

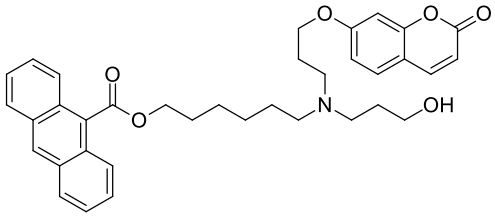
6-((3-Hydroxypropyl)(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)hexyl 3,4,5-trimethoxybenzoate 228 (LB51)



Following the general procedure, compound **228** (0.15 g, yield: 68.7 %) was synthesized from **195** (0.17 g, 0.47 mmol) and **234** (0.11 g, 0.39 mmol) in 10.0 mL of dry CH_3CN.

Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.7.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.50 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 7.23 (d, *J* =8.4 Hz, 1H, CH arom.); 7.18 (s, 2H, CH arom.); 6.70 (dd, *J* = 8.4, 2.2 Hz, 1H, CH arom.); 6.66 (d, *J* = 2.2 Hz, 1H, CH arom.); 6.09 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.16 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.94 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.78 (s, 9H, OCH_3); 3.65 (t, *J* = 6.4 Hz, 2H, CH_2OH); 2.60-2.44 (m, 4H, NCH_2); 2.35 (t, *J* = 7.2 Hz, 2H, NCH_2); 1.93-1.80 (m, 2H, CH_2); 1.69-1.52 (m, 4H, CH_2); 1.45-1.34 (m, 2H, CH_2); 1.33-1.18 (m, 4H, CH_2) ppm.

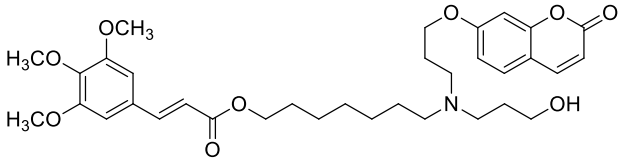
6-((3-Hydroxypropyl)(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)hexyl anthracene-9-carboxylate 229 (LB53)



Following the general procedure, compound **229** (0.13 g, yield: 60.4 %) was synthesized from **196** (0.16 g, 0.43 mmol) and **234** (0.10 g, 0.36 mmol) in 10.0 mL of dry CH_3CN.
Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.39 (s, 1H, CH arom.); 7.97 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.91 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.48-7.37 (m, 5H, CH arom. and C*H*=CH); 7.13 (d, *J* = 9.2 Hz, 1H, CH arom.); 6.66-6.64 (m, 2H, CH arom.); 6.08 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.54 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.87 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.69 (t, *J* = 6.4 Hz, 2H, CH_2OH); 2.54-2.47 (m, 4H, NCH_2); 2.34 (t, *J* = 7.2 Hz, 2H, NCH_2); 1.85-1.72 (m, 4H, CH_2); 1.65-1.53 (m, 2H, CH_2); 1.49-1.35 (m, 4H, CH_2); 1.34-1.23 (m, 2H, CH_2) ppm.

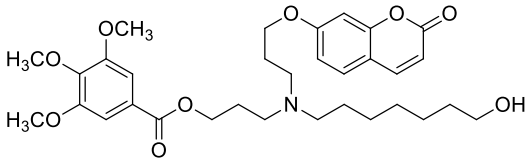
(*E*)-7-((3-Hydroxypropyl)(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)heptyl 3-(3,4,5-trimethoxyphenyl)acrylate 230 (KIS 10)



Following the general procedure, compound **230** (0.16 g, yield: 63.1 %) was synthesized from **220**[80] (0.17 g, 0.42 mmol) and **234** (0.14 g, 0.50 mmol) in 7.0 mL of dry CH_3CN.
Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 90:10:1.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.57 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 7.53 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.30 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.79-6.74 (m, 2H, CH arom.); 6.70 (s, 2H, CH arom.); 6.29 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.17 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.12 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.01 (t, *J* = 6.0 Hz, 2H, OCH_2); 3.82 (s, 6H, OCH_3); 3.81 (s, 3H, OCH_3); 3.73 (t, *J* = 5.2 Hz, 2H, CH_2OH); 2.66-2.61 (m, 4H, NCH_2); 2.44 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.00-1.93 (m, 2H, CH_2); 1.70-1.59 (m, 4H, CH_2); 1.51-1.41 (m, 2H, CH_2); 1.37-1.19 (m, 7H, CH_2 and OH) ppm.

3-((7-Hydroxyheptyl)(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)propyl 3,4,5-trimethoxybenzoate 231 (KIS 14)

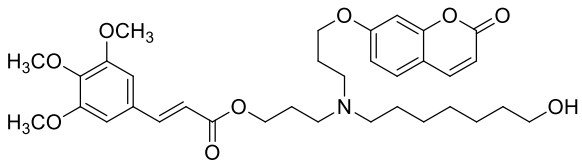


Following the general procedure, compound **231** (0.16 g, yield: 55.1 %) was synthesized from **221** (0.18 g, 0.50 mmol) and **234** (0.16 g, 0.56 mmol) in 7.0 mL of dry CH_3CN.
Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.58 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 7.31 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.24 (s, 2H, CH arom.); 6.79-6.77 (m, 2H, CH arom.); 6.20 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.31 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.05 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.88 (s, 3H, OCH_3); 3.81 (s, 6H, OCH_3); 3.58 (t, *J* = 6.4 Hz, 2H, CH_2OH); 2.61-2.54 (m, 4H, NCH_2); 2.40 (t, *J* =

7.2 Hz, 2H, NCH_2); 1.93-1.87 (m, 4H, CH_2); 1.69 (bs, 1H, OH); 1.53-1.45 (m, 2H, CH_2); 1.44-1.35 (m, 2H, CH_2); 1.33-1.20 (m, 6H, CH_2) ppm.

(*E*)-3-((7-Hydroxyheptyl)(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)propyl 3-(3,4,5-trimethoxyphenyl)acrylate 232 (KIS 19)

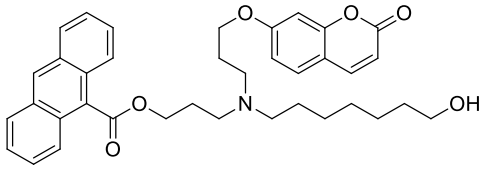


Following the general procedure, compound **232** (0.15 g, yield: 59.1 %) was synthesized from **222** (0.17 g, 0.42 mmol) and **234** (0.14 g, 0.50 mmol) in 7.0 mL of dry CH_3CN.

Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.53 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 7.50 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.27 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.77-6.73 (m, 2H, CH arom.); 6.67 (s, 2H, CH arom.); 6.24 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.13 (d, *J* = 9.6 Hz, 1H, C*H*=CH); 4.16 (t, *J* = 6.4 Hz, 2H, OCH_2); 4.01 (t, *J* = 6.0 Hz, 2H, OCH_2); 3.81 (s, 6H, OCH_3); 3.80 (s, 3H, OCH_3); 3.53 (t, *J* = 6.4 Hz, 2H, CH_2OH); 2.53 (t, *J* = 6.4 Hz, 2H, NCH_2); 2.47 (t, *J* = 6.8 Hz, 2H, NCH_2); 2.34 (t, *J* = 6.8 Hz, 2H, NCH_2); 1.94 (bs, 1H, OH); 1.90-1.82 (m, 2H, CH_2); 1.80-1.72 (m, 2H, CH_2); 1.49-1.40 (m, 2H, CH_2); 1.39-1.30 (m, 2H, CH_2); 1.29-1.16 (m, 6H, CH_2) ppm.

3-((7-Hydroxyheptyl)(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)propyl anthracene-9-carboxylate 233 (LB120)



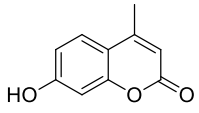
Following the general procedure, compound **233** (0.32 g, yield: 64.0 %) was synthesized from **223** (0.33 g, 0.84 mmol) and **234** (0.28 g, 1.01 mmol) in 15.0 mL of dry CH_3CN.

Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97:3:0.3.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.48 (s, 1H, CH arom.); 8.00 (t, *J* = 9.6 Hz, 4H, CH arom.); 7.51-7.41 (m, 5H, CH arom. and C*H*=CH); 7.21 (d, *J* = 9.2 Hz, 1H, CH arom.); 6.74-6.72 (m, 2H, CH arom.); 6.17 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.64 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.99 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.56 (t, *J* = 6.4 Hz, 2H, CH_2OH); 2.62-2.56 (m, 4H, NCH_2); 2.41 (t, *J* = 7.2 Hz, 2H, NCH_2); 2.04-1.96 (m, 2H, CH_2); 1.94-1.83 (m, 2H, CH_2); 1.54-1.33 (m, 4H, CH_2); 1.32-1.16 (m, 6H, CH_2) ppm.

7.1.2.1.3. Piperazine derivatives

7-Hydroxy-4-methyl-2H-chromen-2-one 238 (DAP15)



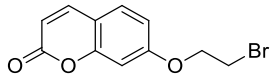
A solution of resorcinol (0.40 g, 3.63 mmol) in ethyl acetoacetate (0.5 mL, 3.99 mmol) was added dropwise at 0 °C in 4.0 mL of concentrated H_2SO_4. The mixture was stirred at rt for 15 minutes, then cold water was added. A solid precipitated, which was filtrated under reduced pressure and dried, obtaining **238** (0.41 g, yield 64.2 %) as a pale-yellow solid.

TLC: CH_2Cl_2/CH_3OH 95:5.

^{1}H-NMR (400 MHz, DMSO) δ: 7.53 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.75 (d, *J* =8.8 Hz, 1H, CH arom.); 6.64 (s, 1H, CH arom.); 6.05 (s, 1H, CH arom.); 2.29 (s, 3H, CH_3) ppm

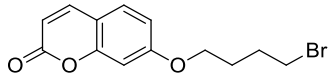
General procedure for the synthesis of 7-**(bromoalkoxy)-2*H*-chromen-2-ones 239-243.**
To a solution of 7-hydroxy-2*H*-chromen-2-one (1 equiv.) or 7-hydroxy-4-methyl-2*H*-chromen-2-one **238** (1 equiv.) in acetone, K_2CO_3 (3 equiv.) and the proper dibromoalkane (5 equiv.) were added. The reaction was refluxed overnight, then it was cooled to rt and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed twice with water, then the organic phase was dried over Na_2SO_4 and concentrated under vacuum, yielding the desired compounds as solids.

7-(2-Bromoethoxy)-2*H*-chromen-2-one 239 (DAP6)



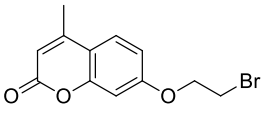
Following the general procedure, compound **239** (0.30 g, yield: 90.6 %) was synthesized as a white solid, from 7-hydroxy-2*H*-chromen-2-one (0.20 g, 1.23 mmol) and 1,2-dibromoethane (0.61 mL, 6.50 mmol) in 15.0 mL of acetone.
TLC: CH_2Cl_2/CH_3OH 90:10.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.63 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 7.38 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.87-6.80 (m, 2H, CH arom.); 6.26 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.34 (t, *J* = 6.4 Hz, 2H, OCH_2); 3.66 (t, *J* = 6.4 Hz, 2H, CH_2Br) ppm.

7-(4-Bromobutoxy)-2*H*-chromen-2-one 240 (DAP4)



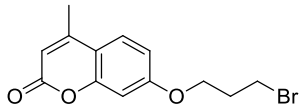
Following the general procedure, compound **240** (0.33 g, yield: 85.0 %) was synthesized as a white solid, from 7-hydroxy-2*H*-chromen-2-one (0.21 g, 1.30 mmol) and 1,4-dibromobutane (0.77 mL, 6.50 mmol) in 15.0 mL of acetone.
TLC: CH_2Cl_2/CH_3OH 90:10.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.62 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 7.35 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.83-6.78 (m, 2H, CH arom.); 6.23 (d, *J* = 9.2 Hz, 1H, C*H*=CH); 4.04 (t, *J* = 6.0 Hz, 2H, OCH_2); 3.48 (t, *J* = 6.0 Hz, 2H, CH_2Br); 2.10-2.03 (m, 2H, CH_2); 2.01-1.96 (m, 2H, CH_2) ppm.

7-(2-Bromoethoxy)-4-methyl-2*H*-chromen-2-one 241 (DAP20)



Following the general procedure, compound **241** (0.13 g, yield: 62.4 %) was synthesized as a white solid, from **238** (0.13 g, 0.74 mmol) and 1,3-dibromoethane (0.51 mL, 5.95 mmol) in 10.0 mL of acetone.
TLC: CH_2Cl_2/CH_3OH 90:10.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.51 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.90-6.80 (m, 2H, CH arom.); 6.13 (s, 1H, CH arom.); 4.35 (t, *J* = 6.0 Hz, 2H, OCH_2); 3.61 (t, *J* = 6.0 Hz, 2H, CH_2Br); 2.39 (s, 3H, CH_3) ppm.

7-(3-Bromopropoxy)-4-methyl-2*H*-chromen-2-one 242 (DAP17)

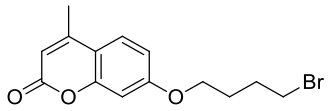


Following the general procedure, compound **242** (0.19 g, yield: 74.8 %) was synthesized as a white solid, from **238** (0.15 g, 0.85 mmol) and 1,3-dibromopropane (0.43 mL, 4.26 mmol) in 11.0 mL of acetone.

TLC: CH_2Cl_2/CH_3OH 90:10.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.50 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.86-6.81 (m, 2H, CH arom.); 6.13 (s, 1H, CH arom.); 4.16 (t, *J* = 6.0 Hz, 2H, OCH_2); 3.61 (t, *J* = 6.0 Hz, 2H, CH_2Br); 2.39 (s, 3H, CH_3); 2.36-2.32 (m, 2H, CH_2) ppm.

7-(4-Bromobutoxy)-4-methyl-2*H*-chromen-2-one 243 (DAP16)



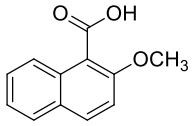
Following the general procedure, compound **243** (0.16 g, yield: 76.3 %) was synthesized as a white solid, from **238** (0.12 g, 0.68 mmol) and 1,4-dibromobutane (0.40 mL, 3.40 mmol) in 9.0 mL of acetone.

TLC: CH_2Cl_2/CH_3OH 90:10.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.48 (d, *J* = 8.4 Hz, 1H, CH arom.); 6.85-6.79 (m, 2H, CH arom.); 6.13 (s, 1H, CH arom.); 4.05 (t, *J* = 6.0 Hz, 2H, OCH_2); 3.49 (t, *J* = 6.0 Hz, 2H, CH_2Br); 2.39 (s, 3H, CH_3); 2.08-2.06 (m, 2H, CH_2); 2.00-1.96 (m, 2H, CH_2) ppm.

7.1.2.2. Tariquidar analogues

2-Methoxy-1-naphthoic acid 246 (LB80)

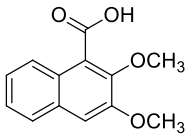


- Following the procedure described in ref [96], 2-methoxy-1-naphthaldehyde (0.20 g, 1.10 mmol) was dissolved in 5.0 mL of acetone, and a solution of Na_2CO_3 (0.18 g, 1.65 mmol) in 4.0 mL of water was added. Then $KMnO_4$ (0.26 g, 1.65 mmol) was added portion wise. The suspension was stirred at rt for 5 h, then filtered and the filtrate was concentrated under vacuum to eliminate acetone. The aqueous solution was washed with EtOAc, then acidified to pH 1 with 1 N HCl, and extracted with new EtOAc to collect the desired acid. The second organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure, obtaining **246** (0.18 g, yield 81.1 %) as a pale-yellow solid.
- Following the procedure described in ref [95], to a stirred solution of $CuBr_2$ (0.010 g, 0.040 mmol) and 2-methoxy-1-naphthaldehyde (0.15 g, 0.80 mmol) in 3.0 mL of dry CH_3CN, *t*-BuOOH (70% solution in water, 0.15 mL, 1.61 mmol) was added: the solution was stirred at rt for 4 days. When the aldehyde had been consumed, the solvent was removed under reduced pressure. The residue was treated with a saturated solution of $NaHCO_3$, and the aqueous phase was washed with EtOAc, then acidified with 2 M HCl and extracted with new EtOAc to collect the desired acid. The second organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure, obtaining **246** (0.11 g, yield 67.6 %) as a pale-yellow solid.

TLC: CHX/EtOAc 20:80. Mp: 175-177 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.38 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.95 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.78 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.55 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.39 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.30 (d, *J* = 9.2 Hz, 1H, CH arom.); 4.05 (s, 3H, OCH_3).
ESI-MS *m/z* (%): 200.8 (100%) $[M-H]^-$.

2,3-Dimethoxy-1-naphthoic acid 247 (LB85)[96]

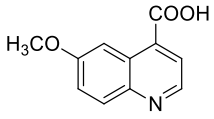


- Following the procedure described in ref [95], 2,3-dimethoxy-1-naphthaldehyde (0.20 g, 0.93 mmol) was dissolved in 3.0 mL of dry CH_3CN, then $CuBr_2$ (0.021 g, 0.93 mmol) and *t*-BuOOH (70% solution in water, 0.35 mL, 3.70 mmol) were added: the solution was stirred at rt for 48 h. When the aldehyde had been consumed, the solvent was removed under reduced pressure. The residue was treated with a saturated solution of $NaHCO_3$ and the aqueous phase was washed with EtOAc, then acidified with 2 M HCl and extracted with new EtOAc to collect the desired acid. The second organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure: unfortunately, the ^{1}H-NMR spectrum of the mixture did not reveal the signals of the desired compound.
- Following the procedure described in ref [96], 2,3-dimethoxy-1-naphthaldehyde (0.050 g, 0.23 mmol) was dissolved in 3.0 mL of acetone, and a solution of Na_2CO_3 (0.025 g, 0.23 mmol) in 1.0 mL of water was added. Then $KMnO_4$ (0.037 g, 0.23 mmol) was added portion wise. The suspension was stirred at rt overnight, then filtered and the filtrate was concentrated under vacuum to eliminate acetone. The aqueous solution was washed with EtOAc, then acidified to pH 1 with 1 N HCl, and extracted with new EtOAc to collect the desired acid. The second organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure, obtaining **247** (0.050 g, yield 93.3 %) as a pale-yellow solid.

TLC: CH_2Cl_2/CH_3OH/CH_3COOH 90:10:1. Mp: 155-156 °C.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.40 (bs, 1H, OH); 7.89 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.63 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.37-7.27 (m, 3H, CH arom.); 7.14 (s, 1H, CH arom.); 3.92 (s, 3H, OCH_3); 3.89 (s, 3H, OCH_3) ppm.

7.1.2.2.1. Amide and ester compounds

6-Methoxyquinoline-4-carboxylic acid 248 (CTS14)[97]



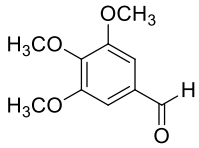
To a solution of quinine sulphate (0.70 g, 0.89 mmol) in 12.0 mL of 10% H_2SO_4, MnO_2 (0.15 g, 1.70 mmol) was added. The reaction was heated to the boiling point, then a solution of CrO_3 (1.43 g, 14.30 mmol) in 3.0 mL of water was added dropwise during 1 h. The mixture was refluxed for 3 h, then 126.0 mL of water and 28.0 mL of 15 N ammonia were added. The reaction was stirred at 100 °C for 18 h, then the suspension was filtered with Celite: the residue was washed several times with hot 15 N ammonia solution. The combined filtrates were concentrated under reduced pressure, then acidified with acetic acid and filtered, obtaining **248** (0.16 g, yield: 89.4 %) as pure yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.83 (d, *J* = 2.0 Hz, 1H, CH arom.); 8.16 (s, 1H, CH arom.); 7.99 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.88 (d, *J* = 2.0 Hz, 1H, CH arom.); 7.46 (d, *J* = 9.2 Hz, 1H, CH arom.); 3.88 (s, 3H, OCH_3).

7.1.2.2.2. Bioisosteric heterocycles: tetrazole and oxadiazole derivatives

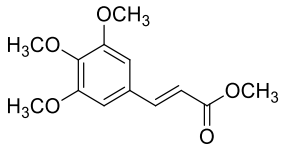
7.1.2.2.2.1. 2,5-disubstituted 2*H*-tetrazoles

3,4,5-Trimethoxybenzaldehyde 249 (RNZ22)



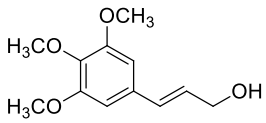
To a suspension of pyridinium chlorochromate (0.49 g, 2.27 mmol) and Celite (0.34 g) in 8.0 mL of dry CH_2Cl_2, (3,4,5-trimethoxyphenyl)methanol (0.30 g, 1.51 mmol) was added. The mixture was stirred at rt for 5 h, then it was cooled to rt, filtered under vacuum, and concentrated under reduced pressure. The residue was purified by flash chromatography, using CH_2Cl_2/CH_3OH 99:1 as the proper eluting system, and **249** (0.23 g, yield 77.6 %) was synthesized as a white solid.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.84 (s, 1H, CHO); 7.10 (s, 2H, CH arom.); 3.92 (s, 6H, OCH_3); 3.91 (s, 3H, OCH_3) ppm.

(*E*)-Methyl 3-(3,4,5-trimethoxyphenyl)acrylate 250 (MC218)



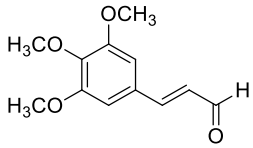
(*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (0.30 g, 1.26 mmol) was dissolved in 6.0 mL of dry methanol, and $SOCl_2$ (0.090 mL, 1.26 mmol) was added. The mixture was refluxed for 4 h, then the solvent was removed under vacuum. The residue was treated twice with CHX, and the solvent was removed under reduce pressure, obtaining **250** (0.33 g, yield: 97.6 %) as a white solid.
TLC: CH_2Cl_2/CH_3OH 93:7. Mp: 138-139 °C.
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.58 (d, *J* = 16.0 Hz, 1H, C*H*=CH), 6.72 (s, 2H, CH arom.), 6.31 (d, *J* = 16.0 Hz, 1H, C*H*=CH), 3.85 (s, 6H, OCH_3), 3.84 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3) ppm.

(*E*)-3-(3,4,5-Trimethoxyphenyl)prop-2-en-1-ol 251 (MC142)[104]



A solution of **250** (0.45 g, 1.78 mmol) in 10.0 mL of dry CH_2Cl_2 was cooled to -78 °C, then DIBAL-H (1.5 M in toluene, 6.44 mL, 6.42 mmol) was added dropwise in two steps. The reaction was stirred at -78 °C for 3 h, then was allowed to reach rt and a saturated aqueous potassium sodium tartrate solution was added. The solution was stirred for 1.5 h, then the mixture was extracted with EtOAc. The organic layer was washed dried over Na_2SO_4, and concentrated under vacuum. Finally, the residue was purified by flash chromatography, using CH_2Cl_2/CH_3OH 97:3 as eluent, obtaining **251** (0.30 g, yield 75.3 %) as a pale-yellow oil.
TLC: CH_2Cl_2/CH_3OH 95:5
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.59 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.52 (s, 2H, CH arom.); 6.32-6.25 (dt, *J* = 5.6, 16.0 Hz 1H, C*H*=CH,); 4.32 (d, *J* = 6.0 Hz, 2H, CH_2); 3.87 (s, 6H, OCH_3); 3.83 (s, 3H, OCH_3); 2.49 (bs, 1H, OH) ppm.

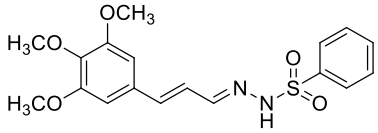
(*E*)-3-(3,4,5-Trimethoxyphenyl)acrylaldehyde 252 (GL21)[91]



To a suspension of pyridinium chlorochromate (0.23 g, 1.07 mmol) and Celite (0.18 g) in 4.0 mL of dry CH_2Cl_2, **251** (0.16 g, 0.71 mmol) was added. The mixture was stirred at rt for 5 h, then it was cooled to rt, filtered under vacuum, and concentrated under reduced pressure. The residue was purified by flash chromatography, using CH_2Cl_2/CH_3OH 99:1 as the proper eluting system, and compound **252**[91] (0.090 g, yield 56.8 %) was synthesized as a pale-yellow solid,
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.66 (d, *J* = 7.6 Hz, 1H, COH), 7.38 (d, *J* = 15.6 Hz, 1H, C*H*=CH), 6.78 (s, 2H, CH arom.), 6.62 (dd, *J* = 15.6, 7.6 Hz, 1H, C*H*=CH), 3.88 (s, 9H, OCH_3) ppm.

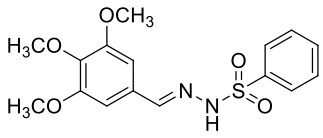
General procedure for the synthesis of benzenesulfonohydrazides 253-258.
Following the procedure described by Gujarati et al.[105] with slight modifications, the proper aldehyde (1 equiv.) and benzenesulfonyl hydrazide (1 equiv.) were suspended in the adequate amount of ethanol and the mixture was stirred at rt for 3 h. Upon completion of the reaction, if a solid precipitated, it is filtered and dried under vacuum, yielding the benzenesulfonohydrazides as solids. Otherwise, the solvent was removed under reduced pressure and the desired compounds were obtained as pure solids, or they were purified by flash chromatography using the proper eluting system.

(*E*)-*N*'-((*E*)-3-(3,4,5-Trimethoxyphenyl)allylidene)benzenesulfonohydrazide 253 (ANL12)



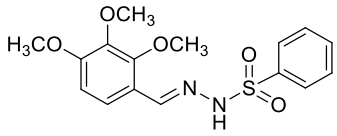
Following the general procedure, compound **253** (0.10 g, yield 65.7 %) was synthesized as a yellow oil, starting from **252**[91] (0.090 g, 0.40 mmol) and benzenesulfonohydrazide (0.084 g, 0.49 mmol) in 3.0 mL of ethanol.
Chromatographic eluent: CH_2Cl_2/CH_3OH 99:1. TLC: CH_2Cl_2/CH_3OH/NH_4OH 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.94 (d, *J* = 7.6 Hz, 2H, CH arom.); 7.63-7.46 (m, 5H, CH arom., N=CH and C*H*=CH); 6.72 (d, *J* = 8.4 Hz, 1H, C*H*=CH); 6.69 (bs, 1H, NH); 6.60 (s, 2H, CH arom.); 3.81 (s, 9H, OCH_3) ppm.

(*E*)-*N*'-(3,4,5-Trimethoxybenzylidene)benzenesulfonohydrazide 254 (ANL4)



Following the general procedure, compound **254** (0.34 g, yield 83.0 %) precipitated as a pure white solid, starting from **249** (0.23 g, 1.17 mmol) and benzenesulfonohydrazide (0.20 g, 1.17 mmol) in 8.0 mL of ethanol.
TLC: CH_2Cl_2/CH_3OH/NH_4OH 93:7:0.7. Mp: 181-183 °C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.95 (d, *J* = 7.2 Hz, 2H, CH arom.); 7.86 (bs, 1H, NH); 7.67 (s, 1H, N=CH); 7.57 (t, *J* = 7.2 Hz, 1H, CH arom.); 7.49 (t, *J* = 7.2 Hz, 2H, CH arom.); 6.78 (s, 2H, CH arom.); 3.83 (s, 6H, OCH_3); 3.82 (s, 3H, OCH_3) ppm.

(*E*)-*N*'-(2,3,4-Trimethoxybenzylidene)benzenesulfonohydrazide 255 (ANL13)

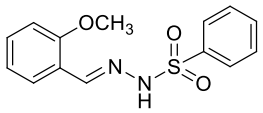


Following the general procedure, compound **255** (0.45 g, yield 86.9 %) precipitated as a pure yellow solid, starting from 2,3,4-trimethoxy-1-benzaldehyde (0.29 g, 1.48 mmol) and benzenesulfonohydrazide (0.25 g, 1.48 mmol) in 8.0 mL of ethanol.

TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 93:7:0.7.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.04-7.92 (m, 3H, CH arom. and N=CH); 7.88 (bs, 1H, NH); 7.62-7.42 (m, 4H, CH arom.); 6.64 (d, *J* = 8.4 Hz, 1H, CH arom.); 3.83 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.79 (s, 3H, OCH_3) ppm.

(*E*)-*N*'-(2-Methoxybenzylidene)benzenesulfonohydrazide 256 (LB98)

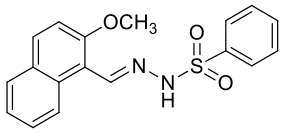


Following the general procedure, **256** (0.17 g, yield 79.8 %) was synthesized as a white solid, starting from 2-methoxybenzaldehyde (0.09 mL, 0.73 mmol) and benzenesulfonohydrazide (0.13 g, 0.73 mmol) in 6.0 mL of ethanol.

Chromatographic eluent: CH_2Cl_2/CH_3OH 99:1. Mp: 184-187 °C.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.19 (s, 1H, N=CH); 7.99 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.82 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.59-7.48 (m, 3H, CH arom.); 7.33 (t, *J* = 8.4 Hz, 1H, CH arom.); 6.93 (t, *J* = 8.4 Hz, 1H, CH arom.); 6.85 (d, *J* = 8.4 Hz, 1H, CH arom.); 3.80 (s, 3H, OCH_3) ppm.

(*E*)-*N*'-((2-Methoxynaphthalen-1-yl)methylene)benzenesulfonohydrazide 257 (ANL15)



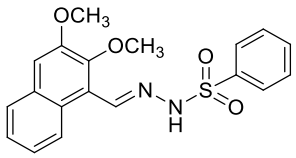
Following the general procedure, **257** (0.36 g, yield 98.8 %) precipitated as a pale-yellow solid, starting from 2-methoxy-1-naphthaldehyde (0.20 g, 1.07 mmol) and benzenesulfonohydrazide (0.18 g, 1.07 mmol) in 5.0 mL of ethanol.

TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.51 (d, *J* = 7.6 Hz, 1H, CH arom.); 8.50 (s, 1H, N=CH); 7.95 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.93-7.87 (m, 2H, CH arom.); 7.81 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.67-7.58 (m, 3H, CH arom.); 7.40 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.36 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.31 (t, *J* = 7.6 Hz, 1H, CH arom.); 3.88 (s, 3H, OCH_3) ppm.

ESI-MS m/z (%): 341.1 (100%) [$M+H^+$].

(*E*)-*N*'-((2,3-Dimethoxynaphthalen-1-yl)methylene)benzenesulfonohydrazide 258 (ANL16)



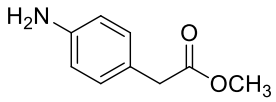
Following the general procedure, **258** (0.34 g, yield 99.2 %) was synthesized as a white solid, starting from 2,3-dimethoxy-1-naphthaldehyde (0.20 g, 0.92 mmol) and benzenesulfonohydrazide (0.16 g, 0.92 mmol) in 5.0 mL of ethanol.

Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 99:1:0.1. Mp: 142-144 °C.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.44 (s, 1H, N=CH); 8.43 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.93-7.86 (m, 2H, CH arom.); 7.77 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.67-7.59 (m, 3H, CH arom.); 7.44 (s, 1H, CH arom.); 7.36 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.27 (t, *J* = 7.6 Hz, 1H, CH arom.);

7.1.2.2.2.2. 1,5-disubstituted 1*H*-tetrazoles

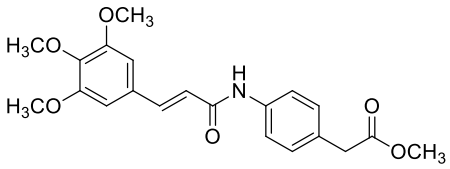
Methyl 2-(4-aminophenyl)acetate 259 (LB 88)



To a solution of 2-(4-aminophenyl)acetic acid (1.00 g, 6.61 mmol) in 20.0 mL of dry methanol, $SOCl_2$ (0.48 mL, 6.61 mmol) was added. The reaction mixture was refluxed for 3 h, then cooled to rt and the solvent was removed under reduced pressure. The residue was treated twice with cyclohexane and the solvent was removed under vacuum, to eliminate the excess of $SOCl_2$. The mixture was dissolved in CH_2Cl_2 and the organic phase was washed twice with Na_2CO_3, dried over Na_2SO_4 and concentrated under reduced pressure, obtaining **259** (1.08 g, yield: 99.0 %) as a brown oil.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.03 (d, *J* = 7.6 Hz, 2H, CH arom.); 6.60 (d, *J* = 7.6 Hz, 2H, CH arom.); 3.64 (s, 3H, OCH_3); 3.55 (bs, 2H, NH_2); 3.48 (s, 2H, CH_2) ppm.

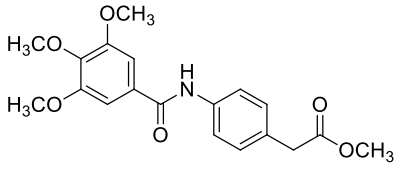
General procedure for the synthesis of amides 260-264.
To a solution of the proper carboxylic acid (1 equiv.) in the adequate amount of $CHCl_3$ (free of ethanol), $SOCl_2$ (10 equiv.) was added. The mixture was refluxed for 5 h, then it was cooled to rt, and the solvent was removed under reduced pressure. The residue was treated twice with cyclohexane and the solvent was removed under vacuum, to eliminate the excess of $SOCl_2$. The obtained acyl chloride was dissolved in the adequate amount of $CHCl_3$ (free of ethanol) and **259** (1 equiv.) was added: the reaction was stirred at rt overnight, then treated with CH_2Cl_2. The organic layer was washed with 1 N HCl, then four times with a saturated solution of $NaHCO_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The desired compounds were obtained as pure oils/solids, or the residue was purified by flash chromatography using the proper eluting system.

(*E*)-Methyl 2-(4-(3-(3,4,5-trimethoxyphenyl)acrylamido)phenyl)acetate 260 (LB95)



Following the general procedure, starting from (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (0.58 g, 2.43 mmol) and **259** (0.40 g, 2.43 mmol), **260** (0.76 g, yield 84.3 %) was obtained as a pure yellow oil.
TLC: CHX/EtOAc 50:50.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.13 (bs, 1H, NH); 7.62 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.58 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.20 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.68 (s, 2H, CH arom.); 6.54 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 3.85 (s, 3H, OCH_3); 3.78 (s, 6H, OCH_3); 3.67 (s, 3H, OCH_3); 3.57 (s, 2H, CH_2) ppm.

Methyl 2-(4-(3,4,5-trimethoxybenzamido)phenyl)acetate 261 (ANL 17)

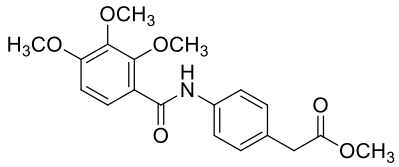


Following the general procedure, starting from 3,4,5-trimethoxybenzoic acid (0.26 g, 1.21 mmol) and **259** (0.20 g, 1.21 mmol), **261** (0.41 g, yield 94.3 %) was obtained as a yellow solid.

Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 98:2:0.2. Mp: 132-134 °C.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.24 (bs, 1H, NH); 7.54 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.18 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.01 (s, 2H, CH arom.); 3.82 (s, 3H, OCH_3); 3.78 (s, 6H, OCH_3); 3.64 (s, 3H, OCH_3); 3.55 (s, 2H, CH_2) ppm.

Methyl 2-(4-(2,3,4-trimethoxybenzamido)phenyl)acetate 262 (ANL 18)

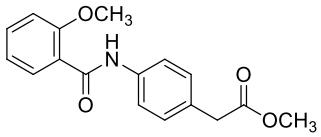


Following the general procedure, starting from 2,3,4-trimethoxybenzoic acid (0.26 g, 1.21 mmol) and **259** (0.20 g, 1.21 mmol), **262** (0.29 g, yield 66.7 %) was obtained as a yellow solid.

Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 98:2:0.2.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.92 (bs, 1H, NH); 7.93 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.60 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.23 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.78 (d, *J* = 9.2 Hz, 1H, CH arom.); 4.01 (s, 3H, OCH_3); 3.88 (s, 3H, OCH_3); 3.86 (s, 3H, OCH_3); 3.64 (s, 3H, OCH_3); 3.57 (s, 2H, CH_2) ppm.

Methyl 2-(4-(2-methoxybenzamido)phenyl)acetate 263 (LB 89)

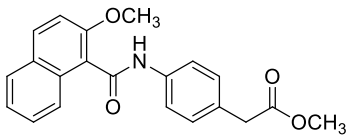


Following the general procedure, starting from 2-methoxybenzoic acid (0.16 g, 1.05 mmol) and **259** (0.17 g, 1.05 mmol), **263** (0.23 g, yield 73.3 %) was obtained as a pale-yellow solid.

Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 97:3:0.3.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.75 (bs, 1H, NH); 8.23 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.60 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.44 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.23 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.07 (t, *J* = 7.6 Hz, 1H, CH arom.); 6.97 (d, *J* = 7.6 Hz, 1H, CH arom.); 3.98 (s, 3H, OCH_3); 3.65 (s, 3H, OCH_3); 3.57 (s, 2H, CH_2) ppm.

Methyl 2-(4-(2-methoxy-1-naphthamido)phenyl)acetate 264 (LB97)

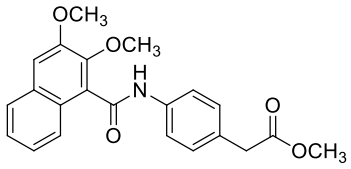


Following the general procedure, starting from **246** (0.40 g, 1.98 mmol) and **259** (0.33 g, 1.98 mmol), **264** (0.38 g, yield 55.0 %) was obtained as a yellow solid.

Chromatographic eluent: CH_2Cl_2/CH_3OH/NH_4OH 98:2:0.2.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.00 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.91-7.83 (m, 2H, CH arom. and NH); 7.78 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.66 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.47 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.37 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.28-7.24 (m, 3H, CH arom.); 3.94 (s, 3H, OCH_3); 3.68 (s, 3H, OCH_3); 3.60 (s, 2H, CH_2) ppm.

Methyl 2-(4-(2,3-dimethoxy-1-naphthamido)phenyl)acetate 265 (LB108)



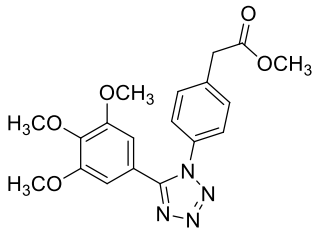
In an ice-bath, to a solution of **247** (0.40 g, 1.98 mmol) and **259** (0.33 g, 1.98 mmol) in 15.0 mL of dry CH_2Cl_2, DMAP (0.16 g, 1.27 mmol) and EDC hydrochloride (0.55 g, 2.87 mmol) were added in this order. The reaction mixture was stirred at 0 °C for 1 h, then at rt for 48 h. The mixture was treated with CH_2Cl_2, and the organic layer was washed twice with water and 1 N HCl, then four times with a saturated solution of $NaHCO_3$, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography, using hexane/EtOAc 60:40 as the proper eluting system, and **265** (0.11 g, yield 18.3 %) was obtained as a pale-yellow solid.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.12 (bs, 1H, NH); 7.97 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.73-7.65 (m, 3H, CH arom.); 7.44-7.36 (m, 2H, CH arom.); 7.30 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.02 (s, 1H, CH arom.); 3.93 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.69 (s, 3H, OCH_3); 3.62 (s, 2H, CH_2) ppm.

General procedure for the synthesis of methyl 2-(4-(5-(aryl)-1*H*-tetrazol-1-yl)phenyl)acetate 266-270.

Following the procedure reported by Jedhe et al.[108], in an ice bath to a solution of the proper amide **261-265** (1 equiv.) in the adequate amount of dry CH_2Cl_2, dry pyridine (5 equiv.) and oxalyl chloride (3 equiv.) were added dropwise in this order. The suspension was stirred at rt for 24 h, then the solvent was removed under reduced pressure. The obtained benzimidoyl chloride was dissolved in dry DMF and added dropwise, over a period of 10 min, to a stirring suspension of NaN_3 (7 equiv.) in dry DMF. The mixture was maintained at 60 °C overnight, then cooled to rt and treated with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography using CHX/EtOAc 60:40 as the proper eluting system, yielding the desired tetrazole derivatives as oils or solids.

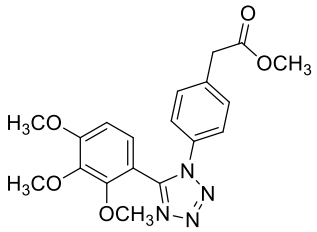
Methyl 2-(4-(5-(3,4,5-trimethoxyphenyl)-1*H*-tetrazol-1-yl)phenyl)acetate 266 (ANL 19)



Following the general procedure, starting from **261** (0.25 g, 0.70 mmol) and NaN_3 (0.32 g, 4.87 mmol), **266** (0.14 g, yield 52.3 %) was obtained as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.43 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.36 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.73 (s, 2H, CH arom.); 3.83 (s, 3H, OCH_3); 3.70 (s, 2H, CH_2); 3.68 (s, 3H, OCH_3); 3.63 (s, 6H, OCH_3) ppm.

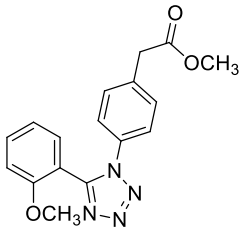
Methyl 2-(4-(5-(2,3,4-trimethoxyphenyl)-1*H*-tetrazol-1-yl)phenyl)acetate 267 (ANL 23)



Following the general procedure, starting from **262** (0.30 g, 0.83 mmol) and NaN_3 (0.38 g, 5.84 mmol), **267** (0.24 g, yield 74.8 %) was obtained as a pale-yellow solid.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.30 (d, *J* = 8.8 Hz, 2H, CH arom.); 7.27 (d, *J* = 8.8 Hz, 2H, CH arom.); 7.18 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.72 (d, *J* = 8.8 Hz, 1H, CH arom.); 3.87 (s, 3H, OCH_3); 3.68 (s, 3H, OCH_3); 3.63 (s, 3H, OCH_3); 3.61 (s, 2H, CH_2); 3.40 (s, 3H, OCH_3) ppm.

Methyl 2-(4-(5-(2-methoxyphenyl)-1*H*-tetrazol-1-yl)phenyl)acetate 268 (LB 90)



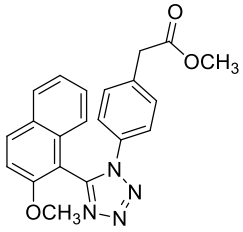
- Following the procedure reported by Al-Hourani et al.[106], compound **263** (0.060 g, 0.23 mmol) was dissolved in 1.5 mL of $SOCl_2$, and the reaction was refluxed for 5 h, then maintained at rt overnight. The solvent was removed under reduced pressure and the residue was treated twice with CHX and concentrated under vacuum, to eliminate the excess of $SOCl_2$. But the ^{1}H-NMR spectrum of the mixture did not reveal the signals of the desired imidoyl chloride, but those of **263**.
- Following the procedure reported by Kennedy et al.[109], to a solution of **263** (0.070 g, 0.23 mmol) and dry pyridine (0.11 mL, 1.40 mmol) in 3.0 mL of dry CH_2Cl_2, PCl_5 (0.15 g, 0.70 mmol) was added: the mixture was refluxed overnight, then azidotrimethylsilane (0.12 mL, 0.94 mmol) was added and the reaction was stirred at rt for 24 h. The mixture was treated with 0.2 mL of a saturated solution of $NaHCO_3$ and stirred for 15 minutes: the organic layer was collected, dried over Na_2SO_4, and concentrated under vacuum. Finally, the residue was purified by flash chromatography, using CHX/EtOAc 70:30 as eluent, obtaining **268** (0.030 g, yield 39.6 %) as a white solid.
- Following the general procedure, starting from **263** (0.15 g, 0.50 mmol) and NaN_3 (0.23 g, 3.51 mmol), **268** (0.090 g, yield 61.5 %) was obtained as a white solid.

ESI-MS *m/z* (%): 325.1 (100%) [$M+H^+$].

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.56 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.43 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.26 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.21 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.04 (t, *J* = 7.6 Hz, 1H, CH arom.); 6.79 (d, *J* = 7.6 Hz, 1H, CH arom.); 3.65 (s, 3H, OCH_3); 3.63 (s, 2H, CH_2); 3.28 (s, 3H, OCH_3) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 171.29 (C); 156.56 (C); 152.10 (C); 135.54 (C); 134.60 (C); 133.15 (CH); 131.57 (CH); 130.24 (CH); 123.23 (CH); 121.20 (CH); 113.32 (C); 111.52 (CH); 54.84 (OCH_3); 52.19 (OCH_3); 40.52 (CH_2) ppm.

Methyl 2-(4-(5-(2-methoxynaphthalen-1-yl)-1*H*-tetrazol-1-yl)phenyl)acetate 269 (LB101)

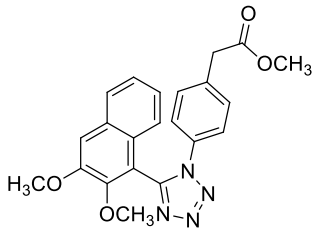


Following the general procedure, starting from **264** (0.28 g, 0.80 mmol) and NaN_3 (0.36 g, 5.62 mmol), **269** (0.070 g, yield 23.3 %) was obtained as a pale-yellow oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.01 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.85 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.56 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.49

(t, *J* = 8.0 Hz, 1H, CH arom.); 7.42 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.26-7.17 (m, 5H, CH arom.); 3.66 (s, 3H, OCH_3); 3.59 (s, 2H, CH_2); 3.54 (s, 3H, OCH_3) ppm.

Methyl 2-(4-(5-(2,3-dimethoxynaphthalen-1-yl)-1*H*-tetrazol-1-yl)phenyl)acetate 270 (LB109)



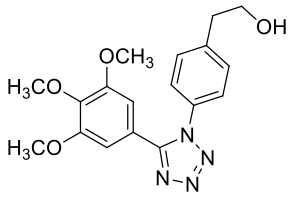
Following the general procedure, starting from **265** (0.10 g, 0.26 mmol) and NaN_3 (0.12 g, 1.82 mmol), **270** (0.035 g, yield 32.7 %) was obtained as a yellow oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.75 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.42 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.34-7.27 (m, 4H, CH arom.); 7.22-7.17 (m, 3H, CH arom.); 3.97 (s, 3H, OCH_3); 3.66 (s, 3H, OCH_3); 3.63 (s, 3H, OCH_3); 3.55 (s, 2H, CH_2) ppm.

General procedure for the synthesis of intermediates 271-275.

In an ice bath, to a solution of the proper methyl 2-(4-(5-(aryl)-1*H*-tetrazol-1-yl)phenyl)acetate **266-270** (1 equiv.) in the adequate amount of dry THF, $LiAlH_4$ (3 equiv.) was added portion wise. The reaction mixture was kept at rt for 4 h, then was quenched with ice, and 10% NaOH solution was added. The obtained suspension was filtered under vacuum and the filtrate was concentrated under reduced pressure, to eliminate THF. The mixture was extracted with CH_2Cl_2, then the organic layer was dried over Na_2SO_4, and the solvent was removed under vacuum. Finally, the residue was purified by flash chromatography using the proper eluting system, obtaining the desired compound as oils.

2-(4-(5-(3,4,5-Trimethoxyphenyl)-1*H*-tetrazol-1-yl)phenyl)ethanol 271 (ANL 20)

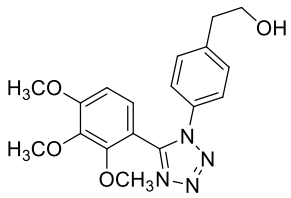


Following the general procedure, starting from **266** (0.14 g, 0.36 mmol) and $LiAlH_4$ (0.041 g, 1.09 mmol) in 3.0 mL of dry THF, **271** (0.080 g, yield 62.3 %) was obtained as a pale-yellow oil.

Chromatographic eluent: CH_2Cl_2/CH_3OH 95:5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.37 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.30 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.71 (s, 2H, CH arom.); 3.94 (t, *J* = 6.4 Hz, 2H, CH_2OH); 3.80 (s, 3H, OCH_3); 3.60 (s, 6H, OCH_3); 2.89 (t, *J* = 6.4 Hz, 2H, CH_2) ppm.

2-(4-(5-(2,3,4-Trimethoxyphenyl)-1*H*-tetrazol-1-yl)phenyl)ethanol 272 (ANL 25)

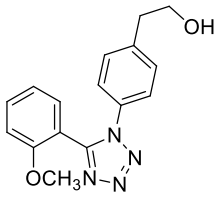


Following the general procedure, starting from **267** (0.24 g, 0.62 mmol) and $LiAlH_4$ (0.071 g, 1.87 mmol) in 6.0 mL of dry THF, **272** (0.11 g, yield 49.8 %) was obtained as a pale-yellow oil.

Chromatographic eluent: CH_2Cl_2/CH_3OH 95:5.

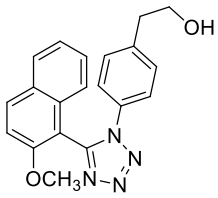
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.20 (d, *J* = 8.8 Hz, 2H, CH arom.); 7.17 (d, *J* = 8.8 Hz, 2H, CH arom.); 7.11 (d, *J* = 8.8 Hz, 1H, CH arom.); 6.68 (d, *J* = 8.8 Hz, 1H, CH arom.); 3.82 (s, 3H, OCH_3); 3.74 (t, *J* = 6.8 Hz, 2H, CH_2OH); 3.64 (s, 3H, OCH_3); 3.34 (s, 3H, OCH_3); 2.79 (t, *J* = 6.8 Hz, 2H, CH_2); 2.62 (bs, 1H, OH) ppm.

2-(4-(5-(2-Methoxyphenyl)-1*H*-tetrazol-1-yl)phenyl)ethanol 273 (LB 92)



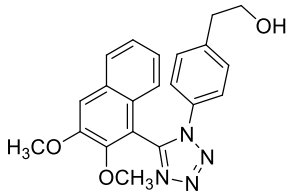
Following the general procedure, starting from **268** (0.070 g, 0.22 mmol) and $LiAlH_4$ (0.025 g, 0.65 mmol) in 5.0 mL of dry THF, **273** (0.050 g, yield 78.2 %) was obtained as a pale-yellow oil.
Chromatographic eluent: CH_2Cl_2/CH_3OH 95:5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.52 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.43 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.21 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.15 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.03 (t, *J* = 7.6 Hz, 1H, CH arom.); 6.78 (d, *J* = 7.6 Hz, 1H, CH arom.); 3.79 (t, *J* = 6.4 Hz, 2H, CH_2OH); 3.25 (s, 3H, OCH_3); 2.83 (t, *J* = 6.4 Hz, 2H, CH_2); 2.48 (bs, 1H, OH) ppm.

2-(4-(5-(2-Methoxynaphthalen-1-yl)-1*H*-tetrazol-1-yl)phenyl)ethanol 274 (LB102)



Following the general procedure, starting from **269** (0.070 g, 0.19 mmol) and $LiAlH_4$ (0.021 g, 0.56 mmol) in 4.0 mL of dry THF, **274** (0.050 g, yield 77.3 %) was obtained as a pale-yellow oil.
Chromatographic eluent: EtOAc 100.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.99 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.83 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.51-7.45 (m, 2H, CH arom.); 7.40 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.20-7.12 (m, 5H, CH arom.); 3.78 (t, *J* = 6.4 Hz, 2H, CH_2OH); 3.55 (s, 3H, OCH_3); 2.80 (t, *J* = 6.4 Hz, 2H, CH_2); 1.82 (bs, 1H, OH) ppm.

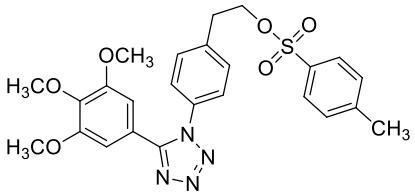
2-(4-(5-(2,3-Dimethoxynaphthalen-1-yl)-1*H*-tetrazol-1-yl)phenyl)ethanol 275 (LB110)



Following the general procedure, starting from **270** (0.035 g, 0.086 mmol) and $LiAlH_4$ (0.010 g, 0.26 mmol) in 3.0 mL of dry THF, **275** (0.030 g, yield 94.2 %) was obtained as a pure yellow oil.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.74 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.41 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.34-7.27 (m, 2H, CH arom.); 7.24 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.17 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.12 (d, *J* = 8.4 Hz, 2H, CH arom.); 3.96 (s, 3H, OCH_3); 3.74 (t, *J* = 6.4 Hz, 2H, CH_2OH); 3.65 (s, 3H, OCH_3); 2.76 (t, *J* = 6.4 Hz, 2H, CH_2); 1.83 (bs, 1H, OH) ppm.

General procedure for the synthesis of compounds 276-280.
To a solution of intermediates **271-275** (1 equiv.) and Et_3N (6 equiv.) in dry CH_2Cl_2, *p*-toluenesulfonyl chloride (2.5 equiv.) was added at 0 °C. The solution was stirred at rt overnight, then it was treated with water, and the mixture was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. Finally, the residue was purified by flash chromatography using the proper eluting system, obtaining the desired compound as oils.

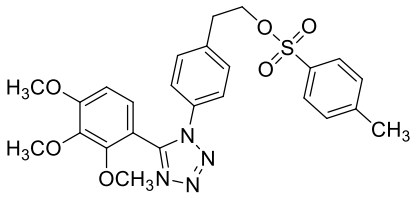
4-(5-(3,4,5-Trimethoxyphenyl)-1*H*-tetrazol-1-yl)phenethyl 4-methylbenzenesulfonate 276 (ANL21)



Following the general procedure, starting from **271** (0.15 g, 0.42 mmol) and *p*-toluenesulfonyl chloride (0.20 g, 1.05 mmol) in 6.0 mL of dry CH_2Cl_2, **276** (0.080 g, yield 37.2 %) was obtained as a pale-yellow oil.
Chromatographic eluent: CH_2Cl_2/CH_3OH 99:1.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.63 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.32-7.22 (m, 6H, CH arom.); 6.71 (s, 2H, CH arom.); 4.21 (t, *J* = 6.0 Hz, 2H, CH_2OS); 3.82 (s, 3H, OCH_3); 3.61 (s, 6H, OCH_3); 3.00 (t, *J* = 6.0 Hz, 2H, CH_2); 2.38 (s, 3H, CH_3) ppm.

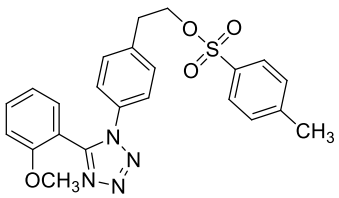
4-(5-(2,3,4-Trimethoxyphenyl)-1*H*-tetrazol-1-yl)phenethyl 4-methylbenzenesulfonate 277 (ANL 26)



Following the general procedure, starting from **272** (0.11 g, 0.31 mmol) and *p*-toluenesulfonyl chloride (0.15 g, 0.77 mmol) in 5.0 mL of dry CH_2Cl_2, **277** (0.16 g, yield 100.0 %) was obtained as a pale-yellow oil.
Chromatographic eluent: CH_2Cl_2/CH_3OH 98:2.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.56 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.20 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.16 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.13 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.10 (d, *J* = 8.4 Hz, 2H, CH arom.); 6.69 (d, *J* = 8.8 Hz, 1H, CH arom.); 4.12 (t, *J* = 6.4 Hz, 2H, CH_2OS); 3.82 (s, 3H, OCH_3); 3.64 (s, 3H, OCH_3); 3.34 (s, 3H, OCH_3); 2.89 (t, *J* = 6.4 Hz, 2H, CH_2); 2.33 (s, 3H, CH_3) ppm.

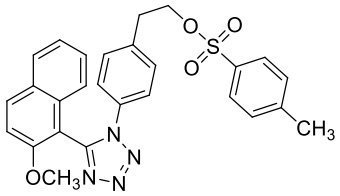
4-(5-(2-Methoxyphenyl)-1*H*-tetrazol-1-yl)phenethyl 4-methylbenzenesulfonate 278 (LB 93)



Following the general procedure, starting from **273** (0.050 g, 0.17 mmol) and *p*-toluenesulfonyl chloride (0.050 g, 0.25 mmol) in 4.0 mL of dry CH_2Cl_2, **278** (0.060 g, yield 78.9 %) was obtained as a pale-yellow oil.
Chromatographic eluent: CH_2Cl_2/CH_3OH 98:2.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.60 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.56 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.44 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.24 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.15 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.12 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.05 (t, *J* = 7.6 Hz, 1H, CH arom.); 6.79 (d, *J* = 7.6 Hz, 1H, CH arom.); 4.17 (t, *J* = 6.4 Hz, 2H, CH_2OS); 3.24 (s, 3H, OCH_3); 2.92 (t, *J* = 6.4 Hz, 2H, CH_2); 2.37 (s, 3H, CH_3) ppm.

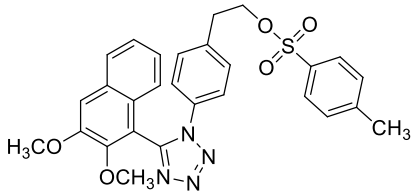
4-(5-(2-Methoxynaphthalen-1-yl)-1*H*-tetrazol-1-yl)phenethyl 4-methylbenzenesulfonate 279 (LB103)



Following the general procedure, starting from **274** (0.063 g, 0.18 mmol) and *p*-toluenesulfonyl chloride (0.087 g, 0.45 mmol) in 4.0 mL of dry CH_2Cl_2, **279** (0.070 g, yield 77.8 %) was obtained as a pale-yellow oil.
Chromatographic eluent: hexane/EtOAc 50:50.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.99 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.83 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.60 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.54 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.48 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.40 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.23 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.18-7.12 (m, 3H, CH arom.); 7.03 (d, *J* = 8.4 Hz, 2H, CH arom.); 4.14 (t, *J* = 6.4 Hz, 2H, CH_2OS); 3.51 (s, 3H, OCH_3); 2.88 (t, *J* = 6.4 Hz, 2H, CH_2); 2.38 (s, 3H, CH_3) ppm.

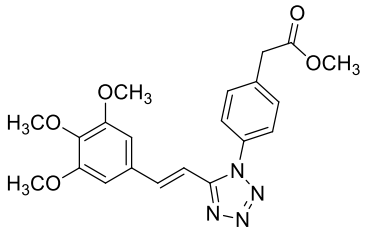
4-(5-(2,3-Dimethoxynaphthalen-1-yl)-1*H*-tetrazol-1-yl)phenethyl 4-methylbenzenesulfonate 280 (LB111)



Following the general procedure, starting from **275** (0.031 g, 0.081 mmol) and p-toluenesulfonyl chloride (0.039 g, 0.20 mmol) in 2.0 mL of dry CH_2Cl_2, **280** (0.032 g, yield 74.0 %) was obtained as a yellow oil.
Chromatographic eluent: hexane/EtOAc 50:50.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.76 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.59 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.43 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.37-7.30 (m, 2H, CH arom.); 7.24-7.15 (m, 5H, CH arom.); 7.01 (d, *J* = 8.0 Hz, 2H, CH arom.); 4.12 (t, *J* = 6.4 Hz, 2H, CH_2OS); 3.97 (s, 3H, OCH_3); 3.66 (s, 3H, OCH_3); 2.86 (t, *J* = 6.4 Hz, 2H, CH_2); 2.39 (s, 3H, CH_3) ppm.

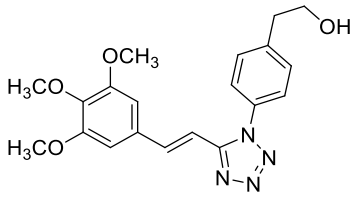
(*E*)-Methyl 2-(4-(5-(3,4,5-trimethoxystyryl)-1*H*-tetrazol-1-yl)phenyl)acetate 281 (LB96)



- Following the general procedure described for **266-270**, starting from **260** (0.13 g, 0.35 mmol) and NaN_3 (0.16 g, 2.45 mmol), the ^{1}H-NMR spectrum of the mixture did not reveal the signals of the desired compound.
- Following the procedure reported by Li et al.[121] with slight modifications, **260** (0.090 g, 0.24 mmol) and dry pyridine (0.12 mL, 1.45 mmol) were dissolved in 3.6 mL of dry CH_2Cl_2, then PCl_5 (0.15 g, 0.73 mmol) was added. The suspension was refluxed for 3 h, then the solvent was removed under reduced pressure. In an ice bath, the solution of the obtained benzimidoyl chloride in 4.0 mL dry DMF was added dropwise to a stirring suspension of NaN_3 (0.11 g, 1.70 mmol) in 1.0 mL of dry DMF. The reaction was maintained at rt overnight, then cooled to 0 °C and treated with 6.0 mL of water. The mixture was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated under vacuum. Finally, the residue was purified by flash chromatography using CH_2Cl_2/CH_3OH 97:3 as eluent, obtaining **281** (0.060 g, yield 60.5 %) as a pale-yellow solid.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.85 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.51 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.47 (d, *J* = 8.0 Hz, 2H, CH arom.); 6.70 (s, 2H, CH arom.); 6.66 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 3.85 (s, 6H, OCH_3); 3.84 (s, 3H, OCH_3); 3.75 (s, 2H, CH_2); 3.72 (s, 3H, OCH_3) ppm.

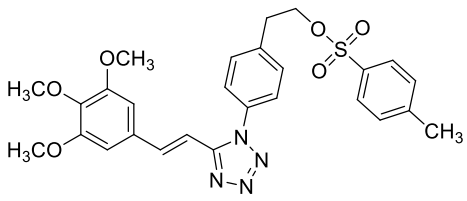
(*E*)-2-(4-(5-(3,4,5-Trimethoxystyryl)-1*H*-tetrazol-1-yl)phenyl)ethanol 282 (LB107)



- Following the general procedure described for **271-275**, starting from **281** (0.25 g, 0.61 mmol) and $LiAlH_4$ (0.069 g, 1.83 mmol) in 25.0 mL of dry THF, the ^{1}H-NMR spectrum of the mixture did not reveal the signals of the desired compound.
- A solution of **281** (0.060 g, 0.15 mmol) in 5.0 mL of dry CH_2Cl_2 was cooled to -78 °C, then DIBAL-H (1 M in toluene, 0.18 mL, 0.18 mmol) was added dropwise. The reaction was maintained at -78 °C for 1 h. Since **281** is always present in the mixture, DIBAL-H (1 M in toluene, 0.18 mL, 0.18 mmol) was added dropwise and the solution was stirred at -78 °C for 1 h. Upon completion of the reaction, 1.2 mL of 10 % NaOH solution were added. The mixture was allowed to reach rt and it was stirred for 15 minutes, then it was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, and concentrated under vacuum, but the ^{1}H-NMR spectrum of the mixture revealed the presence of the corresponding aldehyde instead of the desired compound.
- A solution of **281** (0.090 g, 0.22 mmol) in 3.0 mL of dry CH_2Cl_2 was cooled to -30 °C, then DIBAL-H (1 M in toluene, 0.26 mL, 0.26 mmol) was added dropwise. The reaction was stirred at -30 °C for 1 h. Since **281** is always present in the mixture, DIBAL-H (1 M in toluene, 0.26 mL, 0.26 mmol) was added dropwise and the solution was stirred at -15 °C for 1 h. Upon completion of the reaction, 2.7 mL of 10 % NaOH solution were added. The mixture was allowed to reach rt and it was stirred for 15 minutes, then it was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under vacuum. Finally, the residue was purified by flash chromatography, using CH_2Cl_2/CH_3COCH_3 80:20 as eluent, obtaining **282** (0.015 g, yield 21.5 %) as a pale-yellow oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.88 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.49 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.45 (d, *J* = 8.0 Hz, 2H, CH arom.); 6.71 (s, 2H, CH arom.); 6.66 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 3.97 (t, *J* = 6.4 Hz, 2H, CH_2OH); 3.86 (s, 9H, OCH_3); 3.00 (t, *J* = 6.4 Hz, 2H, CH_2); 1.90 (bs, 1H, OH) ppm.

(*E*)-4-(5-(3,4,5-Trimethoxystyryl)-1*H*-tetrazol-1-yl)phenethyl 4-methylbenzenesulfonate 283 (LB114)



Following the general procedure described for **276-280**, starting from **282** (0.055 g, 0.14 mmol) and *p*-toluenesulfonyl chloride (0.069 g, 0.36 mmol) in 4.0 mL of dry CH_2Cl_2, **283** (0.035 g, yield 45.3 %) was obtained as a pale-yellow oil.

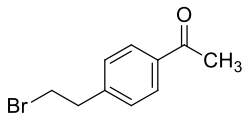
Chromatographic eluent: hexane/EtOAc 70:30.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.88 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.67 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.42 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.38 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.29 (d, *J*

= 8.0 Hz, 2H, CH arom.); 6.70 (s, 2H, CH arom.); 6.65 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 4.29 (t, *J* = 6.4 Hz, 2H, CH_2OS); 3.85 (s, 3H, OCH_3); 3.84 (s, 6H, OCH_3); 3.07 (t, *J* = 6.4 Hz, 2H, CH_2); 2.41 (s, 3H, CH_3) ppm.

8.1.2.2.2.3. 2,5-disubstituted-1,3,4-oxadiazoles

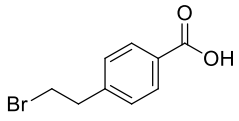
1-(4-(2-Bromoethyl)phenyl)ethenone 284 (LB127)



- Following the procedure described in ref.[122], $AlCl_3$ (0.13 g, 0.97 mmol) and acetyl chloride (0.080 mL, 1.08 mmol) were dissolved in 0.5 mL of CS_2, then at 0 °C a solution of (2-bromoethyl)benzene (0.15 mL, 1.08 mmol) in acetyl chloride (0.15 mL, 2.16 mmol) was added dropwise. The reaction was stirred at 0 °C for 3 h, and at rt overnight; then, it was treated with 0.3 mL of concentrated HCl and 3.0 g of ice. The mixture was extracted with CH_2Cl_2, and the organic layer was washed twice with 10 % NaOH solution and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography using hexane/EtOAc 80:20 as eluent, obtaining **284** as a yellow oil.
- Following the procedure described in ref. [110], in an ice bath, to a suspension of $AlCl_3$ (1.73 g, 13.00 mmol) in 5.0 mL of CH_2Cl_2, acetyl chloride (2.30 mL, 32.30 mmol) and (2-bromoethyl)benzene (1.46 mL, 10.80 mmol) were added in this order. The reaction was stirred at rt overnight, then quenched with ice and 1.0 mL of cold water. The mixture was stirred 15 minutes and extracted with CH_2Cl_2. The organic layer was washed twice with water, a 10 % NaOH solution and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography using hexane/EtOAc 80:20 as eluent, obtaining **284** (0.52 g, yield: 21.2 %) as a yellow oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.92 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.31 (d, *J* = 8.0 Hz, 2H, CH arom.); 3.59 (t, *J* = 8.0 Hz, 2H, CH_2Br); 3.22 (t, *J* = 8.0 Hz, 2H, CH_2); 2.59 (s, 3H, CH_3) ppm.

4-(2-Bromoethyl)benzoic acid 285 (LB128)

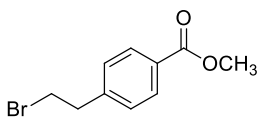


Following the procedure described in ref. [111], NaOH (1.97 g, 49.30 mmol) was dissolved in 8.0 mL of water, then Br_2 (1.04 mL, 20.20 mmol) and at 0 °C a solution of **284** (0.43 g, 1.89 mmol) in 2.0 mL of dioxane were added dropwise: the mixture was stirred at rt for 1.5 h. Upon completion of the reaction, the solution was acidified with concentrated HCl: **285** (0.40 g, yield: 92.5 %) precipitated as a pure white solid, that was filtered and dried under vacuum.

TLC: CH_2Cl_2/CH_3OH/CH_3COOH 99:1:0.1. Mp: 200-202 °C.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.07 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.33 (d, *J* = 8.0 Hz, 2H, CH arom.); 3.60 (t, *J* = 7.6 Hz, 2H, CH_2Br); 3.25 (t, *J* = 7.6 Hz, 2H, CH_2) ppm.

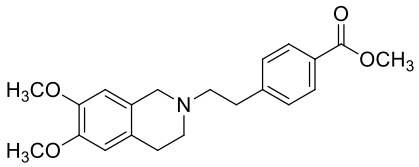
Methyl 4-(2-bromoethyl)benzoate 286 (MES1)



To a solution of **285** (0.42 g, 1.83 mmol) in 8.0 mL of methanol, $SOCl_2$ (0.20 mL, 2.75 mmol) was added, and the mixture was refluxed for 3 h. Upon completion of the reaction, the solution was concentrated under

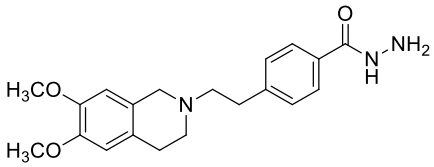
reduced pressure, then the residue was treated twice with CHX, and the solvent was removed under vacuum, to eliminate the excess of $SOCl_2$. Finally, **286** (0.44 g, yield: 100.0 %) was obtained as a brown oil.
TLC: CH_2Cl_2/CH_3OH 99:1.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.99 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.28 (d, *J* = 8.0 Hz, 2H, CH arom.); 3.91 (s, 3H, OCH_3); 3.58 (t, *J* = 7.6 Hz, 2H, CH_2Br); 3.22 (t, *J* = 7.6 Hz, 2H, CH_2) ppm.

Methyl 4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)benzoate 287 (MES2)



A solution of **286** (0.21 g, 0.86 mmol), 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (0.18 g, 0.95 mmol) and K_2CO_3 (0.094 g, 0.95 mmol) in 4.0 mL of dry CH_3CN was refluxed for 18 h. Upon completion of the reaction, the solvent was removed under reduced pressure. The mixture was treated with CH_2Cl_2, then the organic layer was washed twice with water and a saturated solution of $NaHCO_3$, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography using $CH_2Cl_2/CH_3OH/NH_4OH$ 98:2:0.2 as eluent, obtaining **287** (0.12 g, yield: 68.7 %) as a yellow oil.
TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.97 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.31 (d, *J* = 8.0 Hz, 2H, CH arom.); 6.60 (s, 1H, CH arom.); 6.53 (s, 1H, CH arom.); 3.90 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 3.65 (s, 2H, NCH_2Ar); 3.00-2.94 (m, 2H, CH_2); 2.88-2.74 (m, 6H, CH_2) ppm.

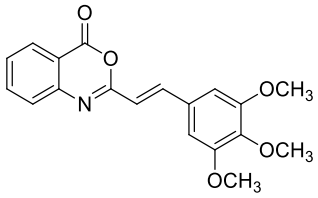
4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)benzohydrazide 288 (MES 3)



To a solution of **287** (0.41 g, 1.15 mmol) in 3.0 mL of ethanol, hydrazine hydrate (0.56 mL, 11.50 mmol) was added, and the reaction was refluxed for 24 h. Upon completion of the reaction, the solvent was removed under reduced pressure, obtaining **288** (0.41 g, yield: 100.0 %) as a yellow oil.
TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 96:4:0.4.
ESI-MS *m/z* (%): 356.3 (100%) $[M+H^+]$.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.67 (d, *J* = 8.0 Hz, 2H, CH arom.); 7.36 (bs, 1H, NH); 7.31 (d, *J* = 8.0 Hz, 2H, CH arom.); 6.60 (s, 1H, CH arom.); 6.52 (s, 1H, CH arom.); 4.10 (bs, 2H, NH_2); 3.84 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 3.66 (s, 2H, NCH_2Ar); 3.00-2.92 (m, 2H, CH_2); 2.88-2.70 (m, 6H, CH_2) ppm.

7.1.2.3. Quinazoline derivatives

(*E*)-2-(3,4,5-Trimethoxystyryl)-4*H*-benzo[*d*][1,3]oxazin-4-one 289 (LB 10)

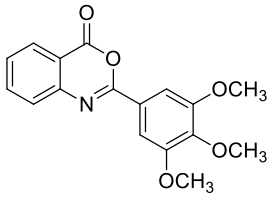


Following the procedure described in ref. [115], to a solution of (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (1.00 g, 4.21 mmol) in 14.0 mL of $CHCl_3$ (free of ethanol), $SOCl_2$ (1.53 mL, 21.00 mmol) was added. The reaction was refluxed for 6 h, then it was cooled to rt and the solvent was removed under reduced pressure. The residue was treated twice with CHX and the solvent was removed under reduced pressure, to eliminate the excess of $SOCl_2$. The obtained (*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl chloride was solubilized in 2.0 mL of dry pyridine and cooled to 0 °C, and a solution of 2-aminobenzoic acid (0.87 g, 6.33 mmol) in 5.0 mL of dry pyridine was added dropwise. The mixture was stirred at 0 °C for 30 minutes, then at rt for 12 h. After the completion of the reaction, 47.2 mL of water were added, and the suspension was stirred for 20 minutes, yielding **289** (0.32 g, yield: 22.1 %) as a yellow solid that was filtered, and dried under vacuum.

TLC: CH_2Cl_2/CH_3OH/CH_3COOH 99:1:1.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.12 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.23 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.69 (d, *J* = 16.4 Hz, 1H, C*H*=CH); 7.51 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.42 (t, *J* = 7.6 Hz, 1H, CH arom.); 6.75 (s, 2H, CH arom.); 6.63 (d, *J* = 16.4 Hz, 1H, C*H*=CH); 3.84 (s, 9H, OCH_3) ppm.

2-(3,4,5-Trimethoxyphenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one 290 (LB 1)[115]

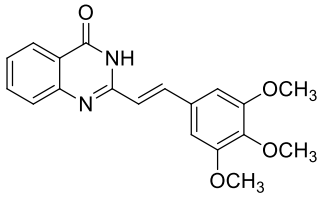


The compound was already described in ref. [115]. Following the procedure described for **289**, compound **290** (0.49 g, yield: 33.4 %) was synthesized as a white solid, starting from 3,4,5-trimethoxybenzoic acid (1.00 g, 4.72 mmol) and 2-aminobenzoic acid (0.87 g, 6.33 mmol) in 5.0 mL of dry pyridine.

TLC: CH_2Cl_2/CH_3OH/CH_3COOH 99:1:1.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.21 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.81 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.67 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.54 (s, 2H, CH arom.); 7.49 (t, *J* = 7.6 Hz, 1H, CH arom.); 3.96 (s, 6H, OCH_3); 3.92 (s, 3H, OCH_3) ppm.

(*E*)-2-(3,4,5-Trimethoxystyryl)quinazolin-4(3*H*)-one 291 (LB 11)

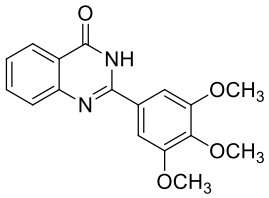


Following the procedure described in ref. [115], a solution of **289** (0.46 g, 1.36 mmol) and 33.0 % ammonia water (3.0 mL) in 7.0 mL of abs. ethanol was refluxed under pressure for 20 h. Then, the mixture was cooled to rt, obtaining compound **291** (0.35 g, yield: 77.0 %) as a yellow solid that was filtered, and dried under vacuum.

TLC: CH_2Cl_2/CH_3OH 96:4.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 11.45 (bs, 1H, NH); 8.29 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.84 (d, *J* = 16.4 Hz, 1H, C*H*=CH); 7.79-7.71 (m, 2H, CH arom.); 7.42 (t, *J* = 8.0 Hz, 1H, CH arom.); 6.87 (s, 2H, CH arom.); 6.86 (d, *J* = 16.4 Hz, 1H, C*H*=CH); 3.92 (s, 6H, OCH_3); 3.89 (s, 3H, OCH_3) ppm.

2-(3,4,5-Trimethoxyphenyl)quinazolin-4(3*H*)-one 292 (LB 2)[115]

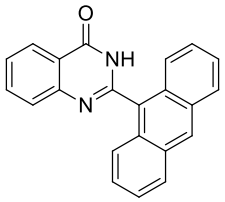


The compound was already described in ref. [115]. Following the procedure described for **291**, compound **292** (0.51 g, yield: 71.7 %) was synthesized as a white solid, starting from **290**[115] (0.73 g, 2.32 mmol) and 33.0 % ammonia water (5.0 mL) in 10.0 mL of abs. ethanol.
TLC: CH_2Cl_2/CH_3OH 96:4.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.76 (bs, 1H, NH); 8.24 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.82-7.76 (m, 2H, CH arom.); 7.47 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.37 (s, 2H, CH arom.); 4.00 (s, 6H, OCH_3); 3.92 (s, 3H, OCH_3) ppm.

General procedure for the synthesis of quinazolin-4(3*H*)-ones 293-296.
Following the procedure described in ref. [117], to a solution of anthranilamide (1 equiv.) and the proper aldehyde (1 equiv.) in the adequate amount of ethanol, $CuCl_2$ (2 equiv.) was added. The reaction mixture was refluxed for 16 h, then was cooled to rt. A proper amount of water was added, yielding a green solid that was filtered, dried under vacuum, and purified by flash chromatography. Finally, quinazolin-4(3*H*)-ones **293-296** were obtained as pure solids.

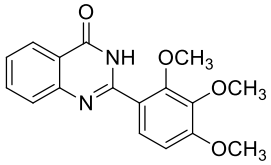
2-(Anthracen-9-yl)quinazolin-4(3*H*)-one 293 (LB NAS24)



Following the general procedure, compound **293** (0.23 g, yield: 100.0 %) was synthesized as a green solid, starting from anthranilamide (0.099 g, 0.73 mmol) and anthracene-9-carbaldehyde (0.15 g, 0.73 mmol) in 3.6 mL of ethanol.

Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 99:1:0.1. Mp: 301-303 °C (dec).
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.69 (bs, 1H, NH); 8.54 (s, 1H, CH arom.); 8.24 (d, *J* = 8.0 Hz, 1H, CH arom.); 8.02-7.98 (m, 2H, CH arom.); 7.89-7.85 (m, 2H, CH arom); 7.84-7.80 (m, 2H, CH arom.); 7.57 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.48-7.43 (m, 4H, CH arom.) ppm.

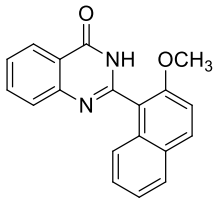
2-(2,3,4-Trimethoxyphenyl)quinazolin-4(3*H*)-one 294 (NAS 4)



- Following the general procedure, compound **294** (0.80 g, yield: 100.0 %) was synthesized as a white solid, starting from anthranilamide (0.35 g, 2.55 mmol) and 2,3,4-trimethoxybenzaldehyde (0.50 g, 2.55 mmol) in 14.0 mL of ethanol.
- Compound **294** was synthesized also following the procedure described in ref. [123]: to a solution of anthranilamide (0.55 g, 4.03 mmol) and 2,3,4-trimethoxybenzaldehyde (0.79 g, 4.03 mmol) in 3.3 mL of dry DMF, K_2CO_3 (0.56 g, 4.03 mmol) and I_2 (1.28 g, 5.03 mmol) were added. The black suspension was stirred at 80 °C for 5 h, then crushed ice was added and the solution pH was adjusted to 7 with concentrated HCl: the obtained black solid was filtered, dried under vacuum, and treated with a 20% $Na_2S_2O_3$ solution. The solid became yellow, then was filtered, dried under vacuum, and purified by flash chromatography, using CHX/EtOAc 50:50 as the proper eluting system. Compound **294** (0.40 g, yield: 31.8 %) was obtained as a white solid.

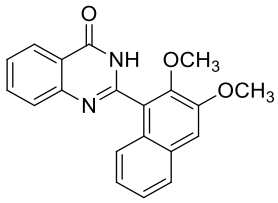
Chromatographic eluent: CH_2Cl_2/CH_3OH 95:5. Mp: 169-171 °C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 11.03 (bs, 1H, NH); 8.28 (d, *J* = 8.0 Hz, 1H, CH arom.); 8.23 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.82-7.70 (m, 2H, CH arom.); 7.44 (t, *J* = 8.0 Hz, 1H, CH arom.); 6.86 (d, *J* = 9.2 Hz, 1H, CH arom.); 4.04 (s, 3H, OCH_3); 3.94 (s, 3H, OCH_3); 3.91 (s, 3H, OCH_3) ppm.

2-(2-Methoxynaphthalen-1-yl)quinazolin-4(3*H*)-one 295 (NAS 8)



Following the general procedure, compound **295** (0.87 g, yield: 89.3 %) was synthesized as a white solid, starting from anthranilamide (0.44 g, 3.22 mmol) and 2-methoxy-1-naphthaldehyde (0.60 g, 3.22 mmol) in 24.0 mL of ethanol.
Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 98:2:0.2. TLC: CH_2Cl_2/CH_3OH 95:5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.94 (bs, 1H, NH); 8.30 (d, *J* = 8.0 Hz, 1H, CH arom.); 8.00 (d, *J* = 9.2 Hz, 1H, CH arom.); 7.95-7.77 (m, 4H, CH arom.); 7.54 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.46 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.38 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.34 (d, *J* = 9.2 Hz, 1H, CH arom.); 3.91 (s, 3H, OCH_3) ppm.

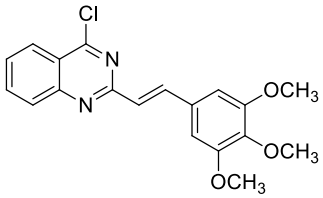
2-(2,3-Dimethoxynaphthalen-1-yl)quinazolin-4(3*H*)-one 296 (NAS 16)



Following the general procedure, compound **296** (0.46 g, yield: 85.5 %) was synthesized as a yellow solid, starting from anthranilamide (0.22 g, 1.62 mmol) and 2,3-dimethoxy-1-naphthaldehyde (0.35 g, 1.62 mmol) in 14.0 mL of ethanol.
Chromatographic eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 98:2:0.2. TLC: CH_2Cl_2/CH_3OH 95:5. Mp 244-246 °C (dec).
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.94 (bs, 1H, NH); 8.29 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.84-7.74 (m, 2H, CH arom.); 7.72 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.61 (d, *J* = 7.6 Hz, 1H, CH arom); 7.49 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.38 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.31 (t, *J* = 7.6 Hz, 1H, CH arom.); 6.62 (s, 1H, CH arom.); 3.84 (s, 3H, OCH_3); 3.15 (s, 3H, OCH_3) ppm.

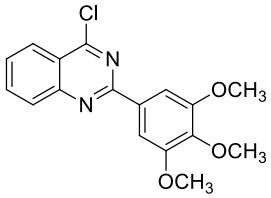
General procedure for the synthesis of 4-chloroquinazolines 297-299 and 301, 302.
The procedure described in ref. [115] was followed with slight modifications: to a solution of the quinazolin-4(3*H*)-one **291-293** and **295**, **296** (1 equiv.) in the adequate amount of $CHCl_3$ (free of ethanol), $SOCl_2$ (10 or 15 equiv.) and 3 drops of dry DMF were added. The reaction mixture was heated to 50 °C for 6 h and it was monitored by TLC, after a microextraction NaOH/EtOAc. After the completion of the reaction, the mixture cooled to rt and the solvent was removed under vacuum. The residue was treated twice with CHX and the solvent was removed under reduced pressure, to eliminate the excess of $SOCl_2$. The obtained red solid was suspended into a 1 N NaOH solution, stirred for 10 minutes, and then treated with CH_2Cl_2. The organic layer was washed twice with water and brine, dried over Na_2SO_4, and concentrated under vacuum, to afford the proper 4-chloroquinazoline as a solid.

(*E*)-4-Chloro-2-(3,4,5-trimethoxystyryl)quinazoline 297 (LB 12)



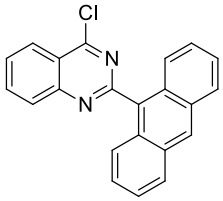
Following the general procedure, compound **297** (0.23 g, yield: 91.8 %) was synthesized as a yellow solid, starting from **291** (0.24 g, 0.71 mmol) and $SOCl_2$ (0.50 mL, 7.10 mmol) in 10.0 mL of $CHCl_3$ (free of ethanol).
TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 98:2:0.2.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.04 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.90 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 7.83 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.77 (t, 1H, *J* = 7.2 Hz, CH arom.); 7.48 (t, *J* = 7.2 Hz, 1H, CH arom.); 7.08 (d, *J* = 16.0 Hz, 1H, C*H*=CH); 6.77 (s, 2H, CH arom.); 3.81 (s, 9H, OCH_3) ppm.

4-Chloro-2-(3,4,5-trimethoxyphenyl)quinazoline 298 (LB 3)[115]



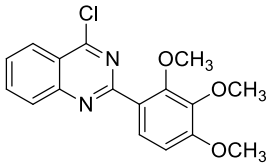
The compound was already described in ref. [115]. Following the general procedure, compound **298** (0.34 g, yield: 96.1 %) was synthesized as a white solid, starting from **290**[115] (0.33 g, 1.07 mmol) in 2.0 mL of $SOCl_2$.
TLC: CH_2Cl_2/CH_3OH 95:5.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.09 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.94 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.79 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.77 (s, 2H, CH arom.); 7.51 (t, *J* = 8.0 Hz, 1H, CH arom.); 3.95 (s, 6H, OCH_3); 3.89 (s, 3H, OCH_3) ppm.

2-(Anthracen-9-yl)-4-chloroquinazoline 299 (LB NAS25)



Following the general procedure, compound **299** (0.23 g, yield: 90.6 %) was synthesized as an orange solid, starting from **293** (0.24 g, 0.74 mmol) and $SOCl_2$ (0.81 mL, 11.10 mmol) in 8.0 mL of $CHCl_3$ (free of ethanol).
TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 98:2:0.2. Mp 237-240 °C (dec).
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.61 (s, 1H, CH arom.); 8.46 (d, *J* = 8.0 Hz, 1H, CH arom.); 8.23 (d, *J* = 8.0 Hz, 1H, CH arom.); 8.09-8.06 (m, 3H, CH arom.); 7.86 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.64 (d, *J* = 8.4 Hz, 2H, CH arom.); 7.47 (t, *J* = 8.4 Hz, 2H, CH arom.) 7.41 (t, *J* = 8.4 Hz, 2H, CH arom.) ppm.

4-Chloro-2-(2,3,4-trimethoxyphenyl)quinazoline 300 (NAS 5)



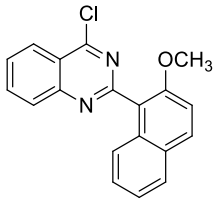
- Following the general procedure, a solution of **294** (0.80 g, 2.56 mmol) in 3 mL of $SOCl_2$ and 3 drops of dry DMF was heated to 50 °C for 24 h. The reaction was monitored by TLC after a microextraction NaOH/EtOAc, then the mixture was cooled to rt and the solvent was removed under vacuum. The residue was treated twice with CHX, and the solvent was removed under reduced pressure, to eliminate the excess of $SOCl_2$. The obtained orange solid was suspended into a 1 N NaOH solution, stirred for 10 minutes, and then treated with CH_2Cl_2. The organic layer was washed twice with water and brine, dried over Na_2SO_4, and concentrated under vacuum. The obtained orange solid was purified by flash chromatography using CHX/EtOAc 75:25 as the proper eluting system, affording a yellow solid which was not the

desired compound.

- Following the procedure described in ref. [116], the quinazolin-4(3*H*)-one **294** (0.73 g, 2.34 mmol) was treated with $POCl_3$ (6.80 mL, 75.79 mmol). The mixture was stirred at rt for 10 minutes, then it was refluxed for 12 h. The reaction was monitored by TLC after a microextraction NaOH/EtOAc, then the mixture was cooled to rt, and it was concentrated under vacuum. The residue was treated with 20.0 mL of ice water and the solution pH was adjusted to 7 with a $NaHCO_3$ solution: then 20.0 mL of CH_2Cl_2 were added and the mixture was stirred for 10 minutes. The organic layer was washed twice with brine, dried over Na_2SO_4, and concentrated under vacuum, obtaining compound **300** (0.58 g, yield: 75.1 %) as a yellow solid. TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 98:2:0.2. Mp 110-112 °C.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.26 (d, *J* = 8.0 Hz, 1H, CH arom.); 8.12 (d, *J* = 8.0 Hz, 1H, CH arom.); 7.94 (t, *J* = 8.0 Hz, 1H, CH arom.); 7.74 (d, *J* = 8.8 Hz, 1H, CH arom.); 7.68 (t, *J* = 8.0 Hz, 1H, CH arom.); 6.82 (d, *J* = 8.8 Hz, 1H, CH arom.); 4.04 (s, 3H, OCH_3); 3.92 (s, 6H, OCH_3) ppm.

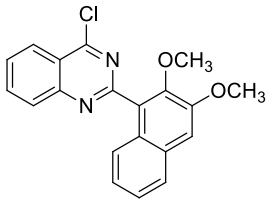
4-Chloro-2-(2-methoxynaphthalen-1-yl)quinazoline 301 (NAS 10)



Following the general procedure, compound **301** (0.81 g, yield: 88.8 %) was synthesized as a pale-yellow solid, starting from **295** (0.87 g, 2.88 mmol) and $SOCl_2$ (3.13 mL, 43.20 mmol) in 16.0 mL of $CHCl_3$ (free of ethanol).
TLC: $CH_2Cl_2/CH_3OH/NH_4OH$ 98:2:0.2. Mp 173-175 °C (dec).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.37 (d, *J* = 8.0 Hz, 1H, CH arom.); 8.18 (d, *J* = 8.4 Hz, 1H, CH arom.); 8.06-7.93 (m, 2H, CH arom.); 7.88-7.81 (m, 1H, CH arom.); 7.78 (t, *J* = 8.4 Hz, 1H, CH arom.); 7.43-7.29 (m, 4H, CH arom.); 3.89 (s, 3H, OCH_3) ppm.

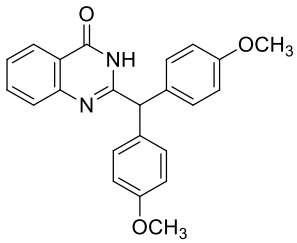
4-Chloro-2-(2,3-dimethoxynaphthalen-1-yl)quinazoline 302 (NAS 17)



Following the general procedure, compound **302** (0.36 g, yield: 74.1 %) was synthesized as a pale-yellow solid, starting from **296** (0.46 g, 1.38 mmol) and $SOCl_2$ (1.00 mL, 13.84 mmol) in 12.0 mL of $CHCl_3$ (free of ethanol).
TLC: CH_2Cl_2/CH_3OH 98:2.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.38 (d, *J* = 8.4 Hz, 1H, CH arom.); 8.18 (d, *J* = 8.4 Hz, 1H, CH arom.); 8.02 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.80 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.76 (d, *J* = 8.4 Hz, 1H, CH arom.); 7.43-7.34 (m, 2H, CH arom.); 7.31 (s, 1H, CH arom.); 7.26 (t, J = 8.4 Hz, 1H, CH arom.); 4.03 (s, 3H, OCH_3); 3.92 (s, 3H, OCH_3) ppm.

2-(bis(4-Methoxyphenyl)methyl)quinazolin-4(3*H*)-one 305 (LB 115)



• Following the procedure described in ref. [117], to a solution of anthranilamide (0.090 mg, 0.66 mmol) and **303**[91] (0.17 g, 0.66 mmol) in 5.0 mL of ethanol, $CuCl_2$ (0.23 g, 1.33 mmol) was added. The reaction mixture was refluxed for 16 h, then was cooled to rt. After the completion of the reaction, the mixture became red: it was cooled to rt, then 10.0 mL of water were added, yielding a red solid that was

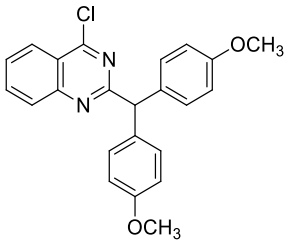
filtered, dried under vacuum, and purified by flash chromatography, by using CH_2Cl_2/CH_3OH 99:1 as the proper eluting system. Unfortunately, the ^{1}H-NMR spectrum did not reveal the signals of the desired compound, particularly that of the aliphatic CH.

- To a solution of **304**[85] (0.15 g, 0.55 mmol) in 3.0 mL of $CHCl_3$ (free of ethanol), $SOCl_2$ (0.20 mL, 2.76 mmol) was added. The mixture was refluxed for 3 h, then the mixture was cooled to rt and the solvent was removed under vacuum. The residue was treated twice with CHX, and the solvent was removed under reduced pressure, to eliminate the excess of $SOCl_2$. Then, the solution of the obtained 2,2-bis(4-methoxyphenyl)acetyl chloride in 5.0 mL of dry CH_3CN was added dropwise to a suspension of anthranilamide (0.075 mg, 0.55 mmol) and K_2CO_3 (0.15 g, 1.10 mmol) in 1.0 mL of dry CH_3CN. The reaction mixture was refluxed overnight, then it was cooled to rt and 20.0 mL of water were added, yielding a white solid that was filtered and dried under vacuum. The solid was suspended in 15.0 mL of EtOH, then 0.22 mL of 40 % (10 M) NaOH solution were added: the suspension was stirred at rt for 1.5 h, then it was cooled to 0 °C and acidified with concentrated HCl: compound **305** (0.11 g, yield 53.7 %) precipitated as a white solid which was filtered and dried under vacuum.
- Following the procedure described in ref. [118], to a solution of **304**[85] (0.50 g, 1.84 mmol) and HATU (0.84 g, 2.21 mmol) in 13.0 mL of dry CH_2Cl_2, DIPEA (0.64 mL, 3.68 mmol) was added. The mixture was stirred at rt for 1 h, then anthranilamide (0.25 mg, 1.84 mmol) was added and the solution was maintained at 50 °C for 20 h. After the completion of the reaction, the mixture was cooled to rt and 20.0 mL of 1 N HCl solution were added. The residue was extracted with CH_2Cl_2, then the organic layer was dried over Na_2SO_4, and concentrated under vacuum, obtaining an orange oil that was treated with 20.0 mL of EtOH, and 0.74 mL of 40 % (10 M) NaOH solution. The suspension was stirred at rt for 2 h, then it was acidified with concentrated HCl: compound **305** (0.60 g, yield: 87.6 %) precipitated as a white solid which was filtered and dried under vacuum.

TLC: CH_2Cl_2/CH_3OH 95:5.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.50 (bs, 1H, NH); 8.23 (d, *J* = 7.6 Hz, 1H, CH arom.); 7.78-7.71 (m, 2H, CH arom.); 7.47 (t, *J* = 7.6 Hz, 1H, CH arom.); 7.17 (d, *J* = 8.8 Hz, 4H, CH arom.); 6.86 (d, *J* = 8.8 Hz, 4H, CH arom.); 5.51 (s, 1H, CH); 3.77 (s, 6H, OCH_3) ppm.

2-(bis(4-Methoxyphenyl)methyl)-4-chloroquinazoline 306 (LB117)



- Following the general procedure, a solution of **305** (0.11 g, 0.30 mmol), $SOCl_2$ (0.21 mL, 2.89 mmol) and 3 drops of dry DMF in 3.0 mL of $CHCl_3$ (free of ethanol) was heated to 50 °C for 5 h. The reaction was monitored by TLC after a microextraction NaOH/EtOAc, then the mixture was cooled to rt and the solvent was removed under vacuum. The residue was treated twice with CHX, and the solvent was removed under reduced pressure, to eliminate the excess of $SOCl_2$. The obtained orange solid was suspended into a 1 N NaOH solution, stirred for 10 minutes, and then treated with CH_2Cl_2. The organic layer was washed twice with water and brine, dried over Na_2SO_4, and concentrated under vacuum. Unfortunately, the ^{1}H-NMR spectrum did not reveal the signals of the desired compound, particularly that of the aliphatic CH.
- Following the procedure described in ref. [116], the quinazolin-4(3*H*)-one **305** (0.050 g, 0.13 mmol) was treated with $POCl_3$ (0.40 mL, 4.30 mmol). The mixture was stirred at rt for 10

minutes, then it was refluxed for 5 h. The reaction was monitored by TLC after a microextraction NaOH/EtOAc, then the mixture was cooled to rt, and it was concentrated under vacuum. The residue was treated with 4.0 mL of ice water and the solution pH was adjusted to 7 with a $NaHCO_3$ solution: then 4.0 mL of CH_2Cl_2 were added and the mixture was stirred for 30 minutes. The organic layer was washed twice with brine, dried over Na_2SO_4, and concentrated under vacuum, obtaining compound **306** (0.050 g, yield: 95.6 %) was synthesized as a white solid.

Chromatographic eluent: CH_2Cl_2, then $CH_2Cl_2/CH_3OH/NH_4OH$ 99:1:0.1. Mp 143-146 °C (dec).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.20 (d, *J* = 8.4 Hz, 1H, CH arom.), 8.02 (d, *J* = 8.4 Hz, 1H, CH arom.), 7.89 (t, *J* = 8.4 Hz, 1H, CH arom.), 7.64 (t, *J* = 8.4 Hz, 1H, CH arom.), 7.37 (d, *J* = 8.8 Hz, 4H, CH arom.), 6.86 (d, *J* = 8.8 Hz, 4H, CH arom.), 5.77 (s, 1H, CH), 3.77 (s, 6H, OCH_3) ppm.

7. Experimental section

7.2. Drug analyses

7.2.1. Stability test

Stock solutions of analytes and Verapamil hydrochloride (ISTD) were prepared in acetonitrile at 1.0 mg mL^{-1} and stored at 4 °C. Working solutions of each analyte were freshly prepared by diluting stock solutions up to a concentration of 10 μM and 1 μM (working solution 1 and 2 respectively) in mQ water: acetonitrile 80:20 (v/v) solution. The ISTD working solution was prepared in acetonitrile at 60 ng mL^{-1} (ISTD solution).
A six levels calibration curve was prepared by adding proper volumes of working solution of each analyte to 300 μL of ISTD solution. The obtained solutions were dried under a gentle nitrogen stream and dissolved in 1.0 mL of 10 mM of formic acid in mQ water: acetonitrile 70:30 (v/v) solution. Final concentrations of calibration levels were: 0, 0.05, 0.10, 0.20, 0.50, 0.75 and 1.00 μM of analyte in the sample. All calibration levels were analysed six times by the appropriate LC-MS/MS method. Calibration curves of analytes were obtained by plotting the peak area ratios (PAR), between quantitation ions of analyte and ISTD, versus the nominal concentration of the calibration solution. A linear regression analysis was applied to obtain the best fitting function between the calibration points.

Phosphate buffer solution (PBS) was prepared by adding 8.01 g L^{-1} of NaCl, 0.2 g L^{-1} of KCl, 1.78 g L^{-1} of Na_2HPO_4 $2H_2O$ and 0.27 g L^{-1} of KH_2PO_4. Human plasma was collected from healthy male volunteer and kept at -80 °C until use.
Each sample was prepared adding 10 μL of working solution 1 to 100 μL of tested matrix (PBS or human plasma) in micro centrifuge tubes. The obtained solutions correspond to 1 μM of analyte. Each set of samples was incubated in triplicate at four different times, 0, 30, 60 and 120 min at 37 °C. Therefore, the degradation profile of each analyte was represented by a batch of 12 samples (4 incubation times x 3 replicates). After the incubation, the samples were added with 300 μL of ISTD solution and centrifuged (rt for 3 minutes at 10 000 rpm). The supernatants were transferred in auto sampler vials and dried under a gentle stream of nitrogen. The dried samples were dissolved in 1.0 mL of 10 mM of formic acid in mQ water: acetonitrile 80:20 solution. The obtained sample solutions were analysed by LC-MS/MS methods.
At the same time, plasma samples with ketoprofene ethylester were prepared with the same procedure (at time 0 and 2 h), to control the enzymatic activity of the used plasma samples. This molecule was chosen since, in previously conducted drug plasma stability tests, it had shown a half-life of 2 h.

The LC-MS/MS analysis was carried out using a Varian 1200L triple quadrupole system (Palo Alto, CA, USA) equipped by two Prostar 210 pumps, a Prostar 410 autosampler and an Elettrospray Source (ESI) operating in positive ions mode. Raw data were collected and processed by Varian Workstation Vers. 6.8 software. G-Therm 015 thermostatic oven was used to keep the samples at 37 °C during the degradation tests. Eppendorf microcentrifuge 5415D was employed to centrifuge plasma samples.

The chromatographic parameters employed to analyse the samples were tuned to minimize the run time and were reported as follows:
- column, Pursuit C18 length = 30 mm; internal diameter = 2mm; particle size = 3 μm purchased from Agilent Technologies (Palo Alto, CA, USA)
- acidic mobile phase, composed by 5 mM of ammonium formate and 10mM of formic acid in mQ water: acetonitrile 90:10 (v/v) solution (solvent A), 5 mM of ammonium formate and 10mM of formic acid in mQ water: acetonitrile 10:90 (v/v) solution (solvent B).
- flow rate and the injection volume were 0.25 mL min^{-1} and 5 μL respectively.
The elution gradient is shown in Table 7.1.

Table 7.1: Elution gradient of mobile phase used for LC-MS/MS analyses.

Time (min)	A (%)
0.00	90
4.00	10
7.00	10
7.01	90
10.00	90

7.2.2. Enantiomeric excess (ee) of (*R*) and (*S*) enantiomers evaluation

The separation of racemic mixture of the studied compounds was carried out by Agilent 1200 liquid chromatography system composed by autosampler, binary pumps, column oven and diode-array detector (LC-DAD) operating in UV range (210-400 nm). The analyses were performed by using a Phenomenex Lux Cellulose-3 column 250 mm length, 4.6 mm internal diameter and 5 μm particle size, in isocratic elution. The sample injection volume was 20 μL. The elution conditions employed to carry out the resolution of racemic mixtures of compounds **181-183**, **185**, **186** and **188-191** are reported in Table 7.2.

Table 7.2: Elution conditions employed to carry out the resolution of racemic mixtures of compounds **181-183**, **185**, **186** and **188-191**.

Racemic mixture	Mobile phase[a]	Flow (mL min^{-1})	T (°C)	UV (nm)
(R)-**181**/*(S)*-**181**, *(R)*-**183**/*(S)*-**183**, *(R)*-**185**/*(S)*-**185**, *(R)*-**189**/*(S)*-**189**, *(R)*-**191**/*(S)*-**191**	A	0.5	20	300
(R)-**182**/*(S)*-**182**	B	0.8	10	250
(R)-**186**/*(S)*-**186**, *(R)*-**190**/*(S)*-**190**	C	0.8	20	250
(R)-**188**/*(S)*-**188**	D	0.5	20	250

[a] A) CH_3OH:isopropanol 60:40 (v/v), with 0.1% of DEA; B) CH_3OH:CH_3CN 95:5 (v/v), with 0.1% of DEA; C) CH_3OH:CH_3CN 90:10 (v/v), with 0.1% of DEA; D) CH_3OH:CH_3CN 98:2 (v/v), with 0.1% of DEA.

7. Experimental section

7.3. Biological assays

7.3.1. CA Inhibition Assay

The CA inhibitory efficacy of all the tested compounds was evaluated on four human CA isoforms, the two cytosolic hCA I and II and the transmembrane tumor-associate hCA IX and XII isoforms. An SX.18MV-R Applied Photophysics (Oxford, UK) stopped-flow instrument was used to assay the catalytic/inhibition of various CA isozymes[87]. Phenol Red (at a concentration of 0.2 mM) was used as an indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.4) as a buffer and, 0.1 M Na_2SO_4 or $NaClO_4$ (for maintaining constant the ionic strength; these anions are not inhibitory in the used concentration), following the CA-catalyzed CO_2 hydration reaction for a period of 5-10 s. Saturated CO_2 solutions in water at 25 °C were used as a substrate. Stock solutions of inhibitors were prepared at a concentration of 10 μM (in DMSO-water 1:1, *v*/*v*) and dilutions up to 0.01 nM done with the assay buffer mentioned above. At least 7 different inhibitor concentrations have been used for measuring the inhibition constant. Inhibitor and enzyme solutions were preincubated together for 10 min at rt prior to the assay, in order to allow the formation of the E-I complex. Triplicate experiments were done for each inhibitor concentration, and the values reported throughout this thesis were the mean of such results. The inhibition constants were obtained by non-linear least-squares methods using the Cheng–Prusoff equation and represent the mean from at least three different determinations. All CA isozymes used here were recombinant proteins.

7.3.2. Cell lines and cultures

The K562 cell line is a highly undifferentiated erythroleukemia originally derived from a patient with chronic myelogenous leukemia[124]. These cells were cultured in RPMI 1640 medium with GlutaMAX I (GIBCO) medium supplemented with 10% fetal calf serum (FCS; GIBCO) at 37 °C in a humidified incubator with 5% CO_2. To maintain the resistance, every month, resistant cells were cultured for three days with 400 nM Doxorubicin. K562/DOX cells overexpress almost exclusively the membrane glycoprotein P-gp[88]. Human colon adenocarcinoma cell line (LoVo) was isolated from a metastatic nodule, and its MDR variant, LoVo/DOX, were obtained from LoVo (parental line) by exposure to increasing concentrations of doxorubicin and maintained in vitro in Ham's F12 medium supplemented with 10% fetal bovine serum and vitamins (Life Technology, Monza, Italy) at 37 °C in a 5% CO_2 atmosphere[125].
The Doxorubicin-resistant sublines, HT29/DOX and A549/DOX cells, were generated by stepwise selection in medium with increasing concentrations of Doxorubicin[126] and maintained in culture medium with 100 nM and 50 nM Doxorubicin, respectively. MDCK and Caco-2 cells were grown in DMEM high glucose, HT29 and HT29/DOX in RPMI-1640, A549 and A549/DX in HAM-F12 medium, all supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL Penicillin, 100 μg/mL Streptomycin, in a humidified incubator at 37 °C with a 5 % CO_2 atmosphere.
Caco-2 cells were seeded onto a Millicell® assay system (Millipore), where a cell monolayer is set in between a filter cell and a receiver plate, at a density of 10 000 cells/well. The culture

medium was replaced every 48 h and the cells kept for 21 days in culture. The Trans Epithelial Electrical Resistance (TEER) of the monolayers was measured daily, before and after the experiment, using an epithelial volt-ohmmeter (Millicell® -ERS). Generally, TEER values greater than 1000 Ω for a 21 days culture, are considered optimal.

7.3.3. Rhodamine-123 (Rhd 123) Uptake

The inhibition of P-gp activity was evaluated by measuring the uptake of the P-gp substrate Rhodamine-123 (Rhd 123) in K562/DOX and LoVo/DOX cells, in the absence or in the presence of compounds by a flow cytometric test.
Briefly, K562/DOX cells were sedimented and diluted to obtain a cell suspension at 5×10^5 cells/mL in complete RPMI 1640 medium; LoVo/DOX cells suspension was obtained by incubating monolayer cell cultures with EDTA and trypsin, then cells were diluted to obtain a cell suspension at 5×10^5 cells/mL in complete Ham's F12. Cells were loaded with Rhd 123, 5.0 µM, for 30 min at 37 °C in a humidified atmosphere with 5% CO_2 in the presence of the tested compounds at 3.0, 10 and 30 µM concentrations, or of the reference compound verapamil at 3.0 µM concentration. An aliquot of cells (control) was incubated with the fluorochrome in the absence of inhibitors. All compounds were added 15 min before Rhd 123. At the end of the uptake, cells were sedimented, washed twice in ice-cold PBS, placed in PBS on ice, and kept in the dark until flow cytometric analysis.
Samples were analyzed on a FACScantoflow cytometer (Becton Dickinson, San Jose CA, USA) equipped with two lasers at 488/633 nm and FACSDIVA software. The green fluorescence of Rhd 123 was collected by a 530-nm band pass filter and at least 10,000 events were acquired. Results were expressed as the FR (fluorescence ratio), which is the ratio between the average fluorescence intensity of rhodamine in the presence and in the absence of modulators. Value 1 was attributed to the average fluorescence intensity of the samples exposed only to rhodamine. The histograms were generated by program GraphPad Prism 5 (GraphPad Prism software, Inc. CA); the data were obtained from the ratio between the average fluorescence intensity of the samples preincubated with the compounds under study and subsequently exposed to rhodamine, and the fluorescence of the sample exposed only to the Rhodamine.

7.3.4. Enhancement of Doxorubicin cytotoxicity assay

K562/DOX and LoVo/DOX cell lines
Doxorubicin was used at the concentration corresponding to the IC_{20} of the resistant lines, which were 0.5 µM and 0.3 µM for K562/DOX and LoVo/DOX cell lines, respectively.
To evaluate the enhancement of Doxorubicin toxicity in the presence of the tested compounds, the cells, in exponential growth phase, were seeded at 10^4 cells/well and solutions of either compounds or Doxorubicin, or a solution of Doxorubicin in combination with the compounds, were added to the wells repeated in quadruplicate. Then the plates were incubated at 37 °C for 72 h in a humidified atmosphere with 5 % CO_2/95 % air. The MTT working solution was then added and plates were further incubated for 3 h. Following incubation cells and formazan crystals were inspected microscopically. The supernatant was then carefully removed by slow aspiration and the formazan crystals were dissolved in 150 mL of acidified isopropanol solution.

The absorbance of the solution was then read on an automated plate reader at a wavelength of 570 nm. The increase in the toxicity of Doxorubicin was quantified by the ratio between the growth of the cell sample treated with IC_{20} of Doxorubicin and that of the cell sample treated with IC_{20} of Doxorubicin in combination with the various concentrations of the tested compounds.

MDCK-MDR1 and HT29/DOX cells

The co-administration assay with Doxorubicin was performed in MDCK-MDR1, HT29 and HT29/DOX cells at 48 h as reported with minor modifications[127]. On day 1, 10000 cells/well were seeded into 96-well plates in a volume of 100 μL of fresh medium. On day 2, the tested drug was added alone to the cells at different concentrations (10 nM, 100 nM, 500 nM, 1 μM, 10 μM). On day 3, the medium was removed and the drug at the same concentrations was added alone and in co-administration with 10 μM Doxorubicin to the cells. After the established incubation time with the tested drug, MTT (0.5 mg/mL) was added to each well, and after 3-4 h incubation at 37 °C, the supernatant was removed. The formazan crystals were solubilized using 100 μL of DMSO/EtOH (1:1), and the absorbance values at 570 and 630 nm were determined on the microplate reader Victor 3 from PerkinElmer Life Sciences.

7.3.5. Characterization of P-gp interacting profile and ABC transporters selectivity

Calcein-AM experiments

Each cell line (30 000 cells per well) was seeded into black CulturePlate 96/wells plate with 100 μL medium and allowed to become confluent overnight. 100 μL of test compounds were solubilized in culture medium and added to monolayers, with final concentrations ranging from 1 nM to 100 μM. 96/Wells plate was incubated for 30 min in a humidified atmosphere 5% CO_2 at 37 °C. Calcein-AM was added in 100 μL of Phosphate Buffered Saline (PBS) to yield a final concentration of 2.5 μM and plate was incubated for 30 min in a humidified atmosphere 5% CO_2 at 37 °C. Each well was washed 3 times with ice cold PBS. Saline buffer was added to each well and the plate was read with Victor3 (PerkinElmer) at excitation and emission wavelengths of 485 nm and 535 nm, respectively. In these experimental conditions Calcein cell accumulation in the absence and in the presence of tested compounds was evaluated and fluorescence basal level was estimated with untreated cells. In treated wells the increase of fluorescence with respect to basal level was measured. EC_{50} values were determined by fitting the fluorescence increase percentage versus log[dose].

Hoechst 33342 experiment

Each cell line (30 000 cells per well) was seeded into black CulturePlate 96/wells plate with 100 μL medium and allowed to become confluent overnight. 100 μL of test compounds were solubilized in culture medium and added to monolayers, with final concentrations ranging from 1 nM to 100 μM. 96/Wells plate was incubated for 30 min in a humidified atmosphere 5% CO_2 at 37 °C. Hoechst 33342 was added in 100 μL of Phosphate Buffered Saline (PBS) to yield a final concentration of 8 μM and plate was incubated for 30 min in a humidified atmosphere 5% CO_2 at 37 °C. The supernatants were drained, and the cells were fixed for 20 min under light protection using 100 μL per well of a 4% PFA solution. Each well was washed 3 times with ice cold PBS. Saline buffer was added to each well and the plate was read with Victor3 (PerkinElmer) at excitation and emission wavelengths of 340/35 nm and 485/20 nm,

respectively. In these experimental conditions, Hoechst 33342 accumulation in the absence and in the presence of tested compounds was evaluated and fluorescence basal level was estimated with untreated cells. In treated wells the increase of fluorescence with respect to basal level was measured. EC_{50} values were determined by fitting the fluorescence increase percentage versus log[dose].

ATPlite assay

The MDCK-MDR1 cells were seeded into 96-well microplate in 100 μL of complete medium at a density 20 000 cells per well. The plate was incubated overnight (O/N) in a humidified atmosphere 5% CO_2 at 37 °C. The medium was removed and 100 μL of complete medium either alone or containing different concentrations (ranging from 1 nM to 100 μM) of test compounds was added. The plate was incubated for 2h in a humidified 5% CO_2 atmosphere at 37 °C. 50 μL of mammalian cell lysis solution was added to all wells and the plate shacked for five minutes in an orbital shaker. 50 μL of substrate solution was added to all wells and the plate shacked for five minutes in an orbital shaker. The plate was dark adapted for ten minutes and the luminescence was measured.

Permeability Experiments

After 21 days of Caco-2 cell growth, the medium was removed from filter wells and from the receiver plate, which were filled with fresh HBSS buffer (Invitrogen). This procedure was repeated twice, and the plates were incubated at 37 °C for 30 min. After incubation time, the HBSS buffer was removed and drug solutions and reference compounds were added to the filter well at the concentration of 100 μM, while fresh HBSS was added to the receiver plate. The plates were incubated at 37 °C for 120 min. Afterwards, samples were removed from the apical (filter well) and basolateral (receiver plate) side of the monolayer to measure the permeability. The apparent permeability (*Papp*), in units of nm/second, was calculated using the following equation:

$$P_{app} = \left(\frac{V_A}{\text{Area} \times \text{time}} \right) \times \left(\frac{[\text{drug}]_{acceptor}}{[\text{drug}]_{initial}} \right)$$

VA = the volume (in mL) in the acceptor well;
Area = the surface area of the membrane (0.11 cm^2 of the well);
time = the total transport time in seconds (7200 sec);
[drug]acceptor = the concentration of the drug measured by U.V. spectroscopy;
[drug]initial = the initial drug concentration (1×10^{-4} M) in the apical or basolateral wells.

7.3.6. Intracellular doxorubicin accumulation and kinetic parameters

Doxorubicin content was measured after incubating 10000 HT29 and HT29/DOX cells, seeded into 96-well plates in a volume of 100 μL of fresh medium, for 24 h with 5 μM doxorubicin, in the absence or presence of increasing concentration of compound **5**. Cells were collected and the intracellular drug content was measured fluorimetrically as detailed previously[128], using a Synregy HTX 96-well plate reader (Bio-Tek Instruments, Winooski, VT). The results were

expressed as nmol doxorubicin/mg cell proteins, according to a titration curve previously set. For the calculation of the kinetic parameters of doxorubicin efflux (Km and maximal velocity, Vmax), cells were incubated for 20 min with increasing (0-100 μmol/L) concentrations of doxorubicin, alone or with compound **5** at 10 μM, then washed and analysed for the intracellular concentration of doxorubicin. A second series of dishes, after the incubation with doxorubicin formulations under the same experimental conditions, were left for further 10 min at 37°C, then washed and tested for the intracellular drug content. The difference of doxorubicin concentration between the two series, expressed as nmol doxorubicin extruded/min/mg cell protein was plotted versus the initial drugs' concentration. Values were fitted to Michaelis-Menten equation to calculate Vmax and Km, using the Enzfitter software (Biosoft Corporation, Cambridge, United Kingdom)[128].

7. Experimental section

7.4. Molecular Modeling studies

In order to give a sensible explanation of the activity profile of target compounds towards P-gp, a molecular docking study was performed using the crystal structure of P-gp in its inward conformation (PDB code 4XWK)[119].

Initial structure of murine P-gp (4XWK[119] was retrieved from Protein Data Bank (www.rcsb.org).[129]) Inner missing regions were modeled using Modeller as implemented in UCSF Chimera 1.11.2[130]. The structure was then minimized with Amber force field ff14SB[131]. Molecular docking was carried out with Gold software v. 2020.2.0[132] using default settings. PyMOL was used for analysis and picture rendering (The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC.).

The simulation was carried out using Gold software v. 2020.2.0[132]. The internal surface of the transmembrane region of P-gp was set as interaction site. After a first run of rigid docking, for each compound a second run was carried out, setting flexibility for relevant residues in the binding region: the best poses of this second computation were selected for analysis.

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