



UNIVERSITÀ
DEGLI STUDI
FIRENZE

FLORE

Repository istituzionale dell'Università degli Studi di Firenze

Design, synthesis and biological evaluation of stereo- and regioisomers of amino aryl esters as multidrug resistance (MDR)

Questa è la versione Preprint (Submitted version) della seguente pubblicazione:

Original Citation:

Design, synthesis and biological evaluation of stereo- and regioisomers of amino aryl esters as multidrug resistance (MDR) reversers / Teodori E.; Contino M.; Riganti C.; Bartolucci G.; Braconi L.; Manetti D.; Romanelli M.N.; Trezza A.; Athanasios A.; Spiga O.; Perrone M.G.; Giampietro R.; Gazzano E.; Salerno M.; Colabufo N.A.; Dei S.. - In: EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY. - ISSN 0223-5234. - ELETTRONICO. - 182:(2019), pp. 1-25. [10.1016/j.ejmech.2019.111655]

Availability:

This version is available at: 2158/1175670 since: 2021-03-30T11:36:16Z

Published version:

DOI: 10.1016/j.ejmech.2019.111655

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

Publisher copyright claim:

(Article begins on next page)

Design, synthesis and biological evaluation of stereo- and regioisomers of amino aryl esters as multidrug resistance (MDR) reversers

Elisabetta Teodori,^{a,*} Marialessandra Contino,^b Chiara Riganti,^c Gianluca Bartolucci,^a Laura Braconi,^a Dina Manetti,^a Maria Novella Romanelli,^a Alfonso Trezza,^d Asimidis Athanasios,^d Ottavia Spiga,^d Maria Grazia Perrone,^b Roberta Giampietro,^b Elena Gazzano,^c Milena Salerno,^e Nicola Antonio Colabufo,^b Silvia Dei^a

^a Department of Neuroscience, Psychology, Drug Research and Child's Health - Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, via Ugo Schiff 6, 50019 Sesto Fiorentino (FI), Italy.

^b Department of Pharmacy-Drug Sciences, University of Bari "A. Moro", via Orabona 4, 70125, Bari, Italy.

^c Department of Oncology, University of Turin, Via Santena 5/bis, 10126 Torino, Italy.

^d Department of Biotechnology, Chemistry and Pharmacy, University of Siena, via Aldo Moro 2, 53100 Siena, Italy

^e Université Paris 13, Sorbonne Paris Cité, Laboratoire CSPBAT, CNRS (UMR 7244), UFR-SMBH, 74 rue Marcel Cachin, 93017 Bobigny, France.

* corresponding author:

Elisabetta Teodori, Department of Neuroscience, Psychology, Drug Research and Child's Health - Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, via Ugo Schiff 6, 50019 Sesto Fiorentino (FI), Italy. Tel +39 055 4573693. Email: elisabetta.teodori@unifi.it

Abstract

Stereo- and regioisomers of a series of *N,N*-bis(alkanol)amine aryl ester derivatives have been prepared and studied as multidrug resistance (MDR) modulators. The new compounds contain a 2-(methyl)propyl chain combined with a 3-, 5- or 7-methylenes long chain and carry different aromatic ester portions. Thus, these compounds have a methyl group on the 3-methylenes chain and represent branched homologues of previously studied derivatives. The introduction of the methyl group gives origin to a stereogenic center and consequently to (*R*) and (*S*) enantiomers. In the pirarubicin uptake assay on K562/DOX cell line these compounds showed good activity and efficacy and in many cases enantioselectivity was observed. Docking studies confirmed the influence of the stereocenter on the interaction in the P-gp pocket. The P-gp interaction mechanism and selectivity towards MRP1 and BCRP were also evaluated on MDCK transfected cells overexpressing the three transporters. Almost all these compounds inhibited both P-gp and BCRP, but only derivatives with specific structural characteristics showed MRP1 activity. Moreover, two compounds, (*S*)-**3** and (*R*)-**7**, showed the ability to induce collateral sensitivity (CS) against MDR cells. Therefore, these two CS-promoting agents could be considered interesting leads for the development of selective cytotoxic agents for drug-resistant cells.

Keyword

MDR reversers; P-gp modulators; enantiomers, molecular docking; K562 cells; MDCK cells; collateral sensitivity; human plasma stability.

List of Abbreviations

P-gp, P-glycoprotein; MRP1, multidrug-resistance-associated protein-1; BCRP, breast cancer resistance protein; ROS, reactive oxygen species; CS, collateral sensitivity; K562/DOX, doxorubicin-resistant erythroleukemia K562 cells; DOX, Doxorubicin; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; DMAP, 4-dimethylaminopyridine; PDB, Protein Data Bank; MDCK, Madin-Darby Canine Kidney; P_{app} , apparent permeability; BA, basolateral to apical; AB,

apical to basolateral; Calcein-AM; calcein acetoxymethyl ester; SR, selectivity ratio; NAC, N-acetylcysteine; RLU, relative luminescence units; PBS, phosphate buffer solution; KEE, ketoprofen ethylester; TR, retention time.

1. Introduction

The anticancer chemotherapy is often impaired by the resistance that cancer cells develop to cytotoxic drugs after an exposure period. This resistance is called multidrug resistance (MDR) when it is towards a multiplicity of structurally unrelated chemotherapeutic drugs [1]. This cross-resistance is often due to an enhanced energy dependent drug efflux, caused by the overexpression of some transmembrane ATP-Binding Cassette (ABC) transporter proteins. They are able to transport substrates through the membrane against the concentration gradient [2,3] and are constitutively expressed in many tissues playing important protective roles through the regulation of the permeability of substances through biological membranes. [4]. The overexpression of these proteins in cancer cells causes the reduction of the intracellular concentration of antineoplastic agents. The transport proteins P-glycoprotein (P-gp, ABCB1), Multidrug-Resistance-associated Protein-1 (MRP1, ABCC1), and Breast Cancer Resistance Protein (BCRP, ABCG2) have mainly been associated with MDR as they were found overexpressed in malignant tissues [5-7]. P-gp was the first ABC transporter to be discovered playing a role in cancer cells drug resistance [8,9]. In fact, it is overexpressed in several tumor cells as a result of anticancer treatment and is responsible of an acquired resistance to a number of structurally and mechanistically unrelated chemotherapeutic drugs [10,11]. MRP1 is overexpressed in cancer cells that are not sensitive to several chemotherapeutic drugs such as doxorubicin, methotrexate, cisplatin, etoposide and vincristine [12]. The most recently discovered ABC efflux protein involved in multidrug-resistance is BCRP. Its overexpression in many solid tumors and leukemia, together with P-gp, impairs the efficacy of many chemotherapeutic agents [13]. BCRP was also found expressed in cancer stem cells causing their insensitivity to anticancer drugs and consequently the long-lasting ineffectiveness of many chemotherapy treatments [14,15]. P-gp and BCRP are the two prevalent ABC efflux proteins placed at the blood-brain barrier (BBB) and are the cause of the reduced BBB penetration of many drugs, including antineoplastic agents, that are actively extruded being substrate of these two efflux pumps [16,17].

A number of approaches to reverse MDR have been extensively studied and the identification of ABC transporter protein inhibitors has been considered an appropriate strategy. For this reason, many modulators of these proteins have been synthesized over the past few decades. These compounds are defined chemosensitizers, in fact, when co-administered with anticancer agents which are substrates of ABC transporters, they are able to restore their effectiveness in resistant tumor cells [18,19].

In the last years several ABC transporter modulators have been discovered; they are classified as first-, second- or third-generation, according to their chronology and characteristics [20-23]. Several of them have reached clinical trials [24-27], however no substantial benefits have been established. The observed problems are mainly due to their low potency and toxicity and also to cytochrome isoform inhibitory effects [28]; even though the more recently discovered MDR reversing agents show lower negative properties.

Consequently, the search for more potent and efficacious MDR reversers is still ongoing with the aim of overcoming some of the obstacles concerning the use of chemosensitizers.

Compounds able to interact with ABC transporters present different chemical structures; however, some general features for the binding with these proteins have been identified, for instance the presence of aromatic moieties and one or more protonable nitrogen atoms, the ability of establishing hydrogen bond interactions and high lipophilicity [29]. These features are in agreement with the information collected on the structure of ABC transporters suggesting that they are characterized, in particular for P-gp, by large and polymorphous recognition sites where different compounds can be accommodated in a plurality of binding modes establishing hydrophobic interactions, hydrogen and π - π , π -ion bonds [30,31].

In the last years, high-throughput screening of pharmacological libraries progressively identified also specific compounds which were unexpectedly more effective as cytotoxic agents in drug-resistant than in drug-sensitive cells [32-34]. Compounds increasing reactive oxygen species (ROS), interfering with the energy metabolism and the synthesis of ATP and altering the membrane fluidity exert selective toxicity in P-gp- and MRP1-overexpressing cells. However, the molecular bases of this paradoxical hypersensitivity, known as “collateral sensitivity” (CS), as well as the development of rationally-designed new sensitizer compounds are still under intensive investigation [35,36].

In previous papers we described the design and synthesis, and *in vitro* studies of many series of MDR modulators characterized by the presence of a basic nitrogen atom connected to two different aromatic ester portions by two linkers with different length and flexibility, such as polymethylenic chains or cyclohexane rings [37,38]. In particular, several *N,N*-bis(alkanol)amine aryl esters were synthesized; these compounds were characterized by different combinations of aromatic ester residues connected to the *N*-methylated basic portion by two polymethylenic chains of variable length. Most of the synthesized compounds showed to be potent and efficacious P-gp-dependent MDR reversers [37-42]. Some combinations of aromatic moieties and spacers seem to be very positive; in fact, the (*E*)-3-(3,4,5-trimethoxyphenyl)vinyl moiety with the 3,4,5-trimethoxyphenyl or the anthracene residues, and spacers of a total length of 6, 8 or 10 methylenes, gave interesting results (compounds **I-X**, structure A, Chart 1).

In the present study we explored the consequence of the modification of a three methylenes chain by the introduction of a methyl group on the position 2. The steric hindrance introduced in the chain could influence the binding mode of these derivatives and also prevent the enzymatic hydrolysis of the corresponding ester function. As a matter of fact, in a previous study [42] it was observed that in a series of derivatives with two chains of different length, the hydrolysis occurs, in particular, when the ester group was carried by a 3-methylenes *N*-alkyl chain. Moreover, this modification gives origin to a stereogenic center and the two possible enantiomers (*R*) and (*S*) could show different biological activity. For this purpose, we synthesized a new series of compounds with a 2-(methyl)propyl chain combined with a 3-, 5- or 7-methylenes long chain, in order to connect the two aromatic moieties with spacers of 6, 8 or 10 methylenes total length. All the possible stereo- and regioisomers were obtained (compounds **1-12**, structure B, Chart 1).

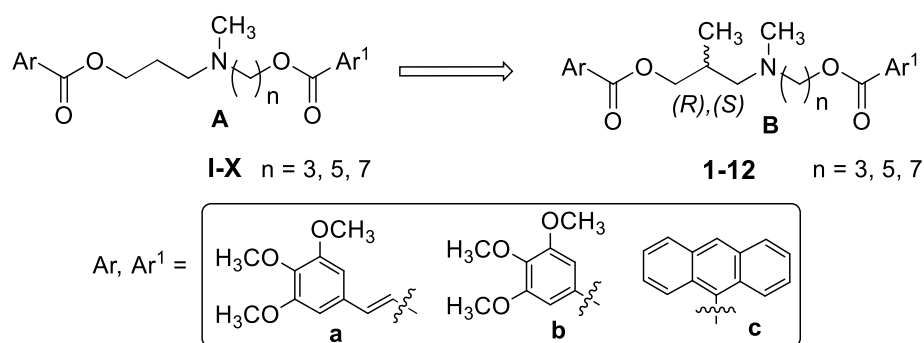


Chart 1. General structure of lead and newly designed compounds.

The new derivatives were studied on the doxorubicin-resistant erythroleukemia K562 cell line (K562/DOX) to evaluate their reversal activity by the pirarubicin uptake assay. Molecular docking simulation studies were performed in order to identify the binding mode of these compounds in the P-gp binding pocket.

The compounds were also studied to evaluate their P-gp interaction profile and selectivity towards two other ABC transporters, MRP1 and BCRP. For these studies Madin-Darby Canine Kidney (MDCK) transfected cells were used (MDCK-MDR1, MDCK-MRP1 and MDCK-BCRP cells overexpressing P-gp, MRP1 and BCRP, respectively). Among the compounds displaying the best P-gp activity and selectivity profile in these tests, the enantiomers (*R*)-**3** and (*R*)-**7** were selected for the

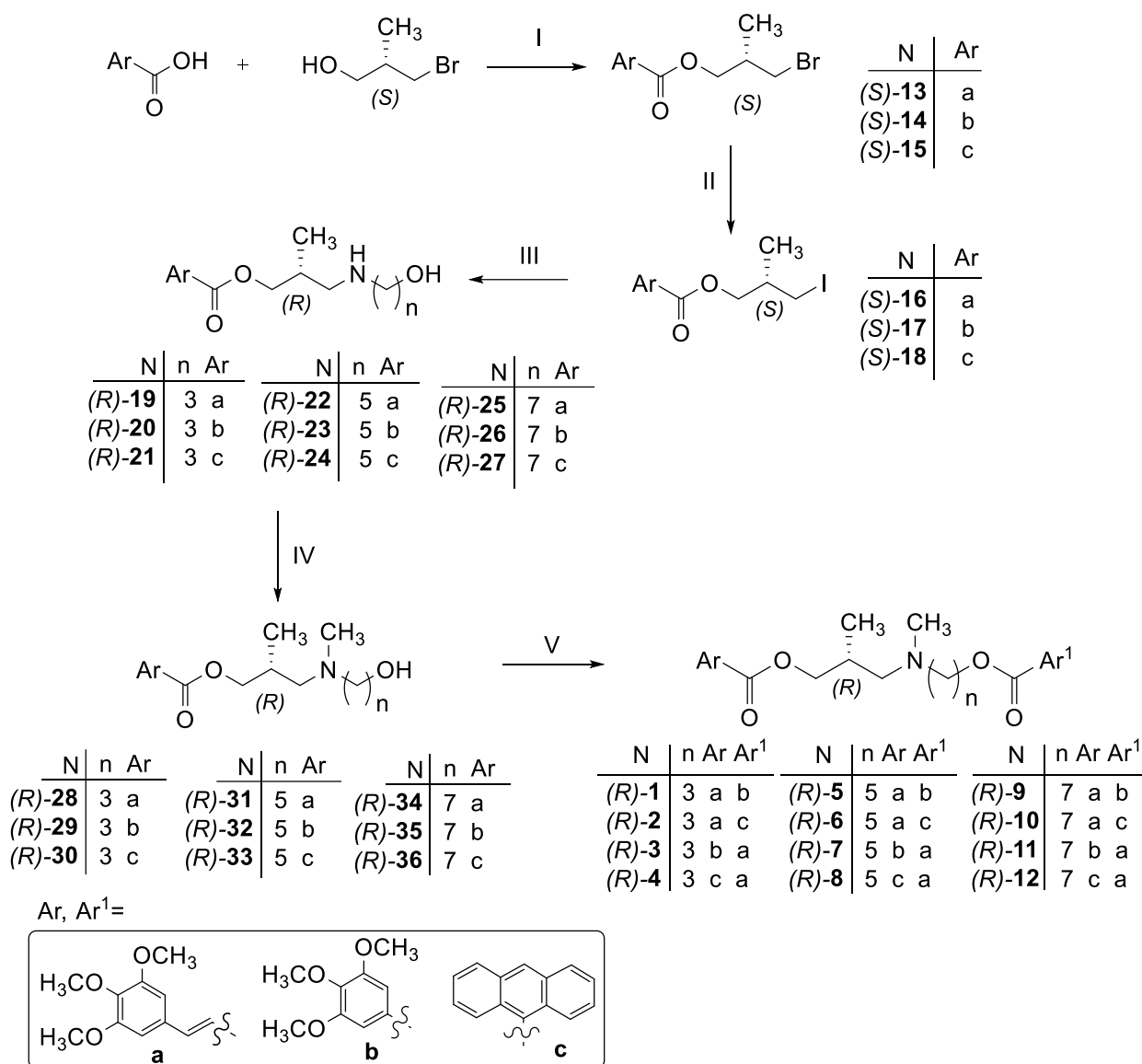
co-administration study in the presence of doxorubicin in MDCK-MDR1 cells. This study has been performed in order to confirm the ability of these compounds to reverse MDR, restoring the access of the chemotherapeutic drug into the cells. Moreover, collateral sensitivity studies were performed for all compounds **1-12** on MDCK-MDR1, MDCK-MRP1 cells and on the parental MDCK cells, in order to evaluate the selective cytotoxicity of the synthesized compounds towards cells overexpressing the two pumps P-gp and MRP1. Two compounds were able to selectively kill MDR cells without cytotoxic effects towards the parental cells. They can be considered as CS-promoting agents and were further studied for their effect on the intracellular ROS level.

Finally, the stability of this series of molecules towards hydrolysis was evaluated. In fact, the presence of the ester groups, in particular, can compromise the stability of these compounds towards plasma enzymes. For this purpose, a series of experiments in phosphate buffer solution (PBS) and human plasma were performed and the degradation profiles were evaluated.

2. Chemistry

The reaction pathways used to synthesize the (*R*) enantiomers of compounds **1-12** are reported in Scheme 1. The (*S*) bromoesters **13-15** were obtained by esterification of the commercially available chiral synthon (*S*)-3-bromo-2-(methyl)propan-1-ol with (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid, 3,4,5-trimethoxybenzoic acid or anthracene-9-carboxylic acid. To obtain the desired ester, the mixture of carboxylic acid and bromoalkyl alcohol was treated with the activating agent EDCI, in the presence of DMAP in anhydrous CH₂Cl₂, or the suitable acid was transformed in the corresponding acyl chloride by reaction with SOCl₂ in ethanol-free CHCl₃ (see Experimental section for details). The (*S*) bromoalkyl esters **13-15** were transformed in the corresponding (*S*) derivatives **16-18** with NaI in acetone, to obtain higher yields in the next reaction. The (*S*) enantiomers **16-18** were then transformed into the (*R*) secondary amines **19-27** by reaction with the commercially available amino alcohols, 3-aminopropan-1-ol and 5-aminopentan-1-ol, or the already reported 7-aminoheptan-1-ol [43], using standard procedures. The absolute configuration assigned to stereocenters changes by passing from iodo to amino derivatives due to the different priority of the groups linked to the stereogenic center. The (*R*) secondary amines were then alkylated by reductive methylation with HCOOH/HCHO to give the corresponding tertiary amines **28-36**. Title compounds (*R*) **1-12** were eventually obtained by reaction of (*R*) **28-36** with the appropriate carboxylic acid in the presence of EDCI and DMAP in anhydrous CH₂Cl₂, or by the corresponding acyl chloride obtained as described before. The proper acids used are the same as described above: (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid, 3,4,5-trimethoxybenzoic acid and anthracene-9-carboxylic acid. The (*S*) enantiomers of compounds **1-12** were obtained starting from (*R*)-3-bromo-2-(methyl)propan-1-ol and following the same procedures described for the (*R*) enantiomers. For biological tests, all compounds were transformed into HCl salts.

The enantiomeric excess (ee) of (*R*) and (*S*) enantiomers of compounds **1-12** was assessed by enantioselective liquid chromatography coupled with diode array detector (LC-DAD) analysis. To perform this analysis, different elution conditions were employed (see Experimental section). All enantiomers showed ee \geq 95%, that is the maximal evaluable value with the used method, except compounds **4**, **7** and **12** 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 (\geq 95%).



Scheme 1. Reagents and conditions: (I) SOCl_2 , ethanol-free CHCl_3 or EDCI, DMAP, an. CH_2Cl_2 ; for details, see the Experimental section; (II) NaI, acetone; (III) $\text{H}_2\text{N}(\text{CH}_2)_n\text{OH}$ ($n = 3, 5, 7$), K_2CO_3 , CH_3CN ; (IV) HCOOH/HCHO ; (V) Ar^1COCl , ethanol-free CHCl_3 or Ar^1COOH , EDCI, DMAP, an. CH_2Cl_2 . The (S) enantiomers of compounds **1-12** were obtained starting from (R)-3-bromo-2-(methyl)propan-1-ol and following the same procedures (see the Experimental section).

3. Results

3.1. Modulation of pirarubicin uptake on K562/DOX cells

Compounds (R) and (S) **1-12** were studied for the P-gp modulating activity by the pirarubicin uptake assay on K562/DOX doxorubicin resistant cells, that overexpress only the membrane glycoprotein P-gp [44-48], following protocols reported in previous papers [49,50].

The results obtained are reported in Table 1 together with those of the corresponding previously synthesized compounds (**I-X**) with an achiral three methylenes chain, and verapamil, used as reference compound. The data reported in Table 1 indicate that all the new compounds inhibited the P-gp activity with high potency since their $[\text{I}]_{0.5}$ values fell in the nanomolar range; in addition, in many cases these compounds completely reversed P-gp-dependent pirarubicin extrusion (α_{max} close to 1). Their potency and affinity values were higher than those of the reference compound verapamil.

A thorough analysis of the results indicated that the activity ($[I]_{0.5}$) is influenced by the length of the linear chain (n value) and only in part by the combination of the aromatic residues. In most cases the two (R) and (S) enantiomers showed different potency values although there is no regular trend in the behavior of the two enantiomers. In fact, the (S) enantiomer was often the most potent, but not in the case of compounds **2** and **3**, that showed an inverted enantioselectivity, and compounds **7**, **9**, **10** and **11** that showed almost equally active (R) and (S) enantiomers. In any case, the influence of the stereogenic center on the activity of these compounds was more or less pronounced depending on the total length of the molecule. In fact, in the set with $n = 3$ (**1-4**), the two enantiomers were in general more potent than the corresponding achiral compound with the same length and residues, particularly in the case of the two enantiomers of **1** (compare **I** $[I]_{0.5} = 0.60 \mu\text{M}$ with (R)-**1** and (S)-**1** $[I]_{0.5} = 0.14$ and $0.09 \mu\text{M}$, respectively). In the set with $n = 5$ (**5-8**), the eutomer (the most active enantiomer) (S)-**5** showed a $[I]_{0.5}$ value lower than that of the corresponding achiral analogue **III** ($[I]_{0.5} = 0.08 \mu\text{M}$ and $0.27 \mu\text{M}$, respectively). In the other cases the activity of the achiral analogues was equally or slightly higher than that of the eutomers (compare **IV** vs (S)-**7**, **V** vs (S)-**6**, **VI** vs (S)-**8**). Regarding the set with $n = 7$ (**9-12**), all the achiral derivatives, **VII-X**, were always more potent than the two enantiomers of the corresponding chiral compounds (**VII** vs (R) and (S) **9**, **VIII** vs (R) and (S) **11**, **IX** vs (R) and (S) **10**, **X** vs (R) and (S) **12**).

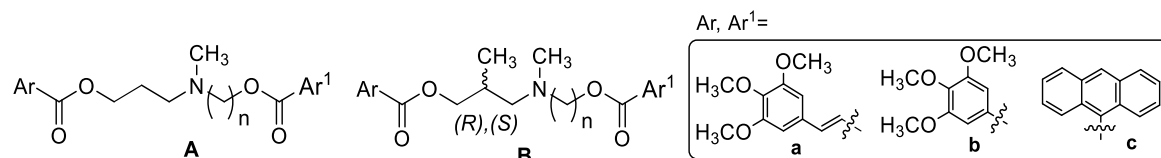
For what concerns the ester groups, the activity is not much affected by the nature of the aromatic groups; in fact, there is no regular trend for the influence of the type and the relative position of the aromatic moieties. Anyway, within each set the combination of the aromatic residues influences the enantioselectivity. In fact, in the set with $n = 3$, the two eutomers of the regioisomers **1** and **3** showed an opposite configuration, (S)-**1** and (R)-**3**, and different enantioselectivity, since the eudismic ratios (ER, here defined for sake of clarity as the ratio between the $[I]_{0.5}$ of the (R) enantiomer and the $[I]_{0.5}$ of the (S) one) are 1.5 and 0.2, respectively. Compounds **2** and **4** showed similar behavior even if, also in this case, the enantioselectivity is inverted and the two eutomers are (R)-**2** and (S)-**4**. In the set with $n = 5$ the eutomers of compounds with the (E)-3-(3,4,5-trimethoxyphenyl)vinyl moiety on the 2-(methyl)propyl chain were more potent than those of the corresponding regioisomers with this group on the 5-methylenes chain (compare (S)-**5** vs (S)-**7** and (S)-**6** vs (S)-**8**). The regioisomers of the set with $n = 7$ showed instead a different behavior since the more potent compounds have the (E)-3-(3,4,5-trimethoxyphenyl)vinyl moiety on the 7-methylenes chain (compare (S)-**9** vs (S)-**11** and (S)-**10** vs (S)-**12**). In particular, the eutomer (S)-**12** was 11 times more potent than (S)-**10** ($[I]_{0.5} = 0.08 \mu\text{M}$ and $0.89 \mu\text{M}$). Moreover, in this set, the two enantiomers were almost equally potent except for compound **12** (ER = 2.5).

As regards the efficacy, these compounds showed α_{max} values between 0.65 and 0.99 indicating that were able to reverse P-gp-dependent pirarubicin extrusion with rather high amount and in some cases the resistance was even completely reversed (α_{max} close to 1).

Altogether, these results suggest that a methyl group at the stereogenic center of the 3-methylenes linker differently influences the activity of these compounds depending on the total length of the molecule. In fact, the presence of this group exerts a favorable effect, in comparison with the corresponding achiral derivatives, in compounds with $n = 3$ (compare **1-4** vs **I** and **II**); on the contrary, this feature appears to have lower influence on the activity for compounds with a 5-methylenes chain (compare **5-8** vs **III-VI**), and is definitely not productive for compounds with a 7-methylenes chain (compare **9-12** vs **VII-X**). On the other hand, it is important to note that, as reported above, the achiral compounds with a total spacer of 10 methylenes (compounds **VII-X**) showed a very good profile both in terms of potency and efficacy with all combinations of aromatic groups; molecular modeling studies indicated that these molecules are able to interact in a productive manner with the P-gp interaction site [42]. In these new molecules the methyl group on the stereogenic center apparently prevents the best interaction of this set of compounds with the protein binding region.

Table 1

MDR-reversing activity of enantiomers of the target compounds **1-12** evaluated on doxorubicin-resistant erythroleukemia K562 cells (K562/DOX) by the pirarubicin uptake assay.



Compd	Struct.	Conf.	n	Ar	Ar ¹	[I] _{0.5} μM ^a	ER ^b	α _{max} ^c	^d ΔG (Kcal/mol)
(R)-1	B	R	3	a	b	0.14±0.03	1.5	0.99±0.01	-15.94
(S)-1	B	S	3	a	b	0.09±0.07		0.75±0.04	-16.66
(R)-3	B	R	3	b	a	0.11±0.02	0.2	0.99±0.01	-16.12
(S)-3	B	S	3	b	a	0.54±0.15		0.89±0.10	-5.90
I ^e	A		3	a	b	0.60±0.15		0.90±0.05	-5.50
(R)-2	B	R	3	a	c	0.08±0.03	0.4	0.99±0.10	-16.80
(S)-2	B	S	3	a	c	0.18±0.02		0.79±0.09	-15.86
(R)-4	B	R	3	c	a	0.17±0.04	4.2	0.80±0.06	-15.90
(S)-4	B	S	3	c	a	0.04±0.01		0.99±0.01	-20.30
II ^e	A		3	a	c	0.18±0.05		0.78±0.03	-15.30
(R)-5	B	R	5	a	b	0.40±0.01	5.0	0.99±0.30	-7.10
(S)-5	B	S	5	a	b	0.08±0.02		0.99±0.01	-16.90
III ^f	A		5	a	b	0.27±0.05		0.97±0.02	-9.10
(R)-7	B	R	5	b	a	0.35±0.10	1.4	0.72±0.07	-9.90
(S)-7	B	S	5	b	a	0.24±0.07		0.75±0.06	-10.20
IV ^f	A		5	b	a	0.12±0.02		0.99±0.01	-16.80
(R)-6	B	R	5	a	c	0.08±0.03	2.6	0.88±0.03	-17.10
(S)-6	B	S	5	a	c	0.03±0.007		0.79±0.01	-22.70
V ^f	A		5	a	c	0.04±0.02		0.94±0.03	-20.00
(R)-8	B	R	5	c	a	0.23±0.08	2.9	0.89±0.06	-9.56
(S)-8	B	S	5	c	a	0.08±0.02		0.75±0.05	-17.50
VI ^f	A		5	c	a	0.04±0.01		0.98±0.02	-19.80
(R)-9	B	R	7	a	b	0.59±0.17	1.5	0.81±0.05	-5.70
(S)-9	B	S	7	a	b	0.38±0.11		0.74±0.05	-8.70
VII ^g	A		7	a	b	0.08±0.03		0.97±0.03	-18.00
(R)-11	B	R	7	b	a	0.12±0.04	1	0.83±0.05	-16.30
(S)-11	B	S	7	b	a	0.13±0.05		0.70±0.04	-16.50
VIII ^g	A		7	b	a	0.06±0.02		0.99±0.01	-19.30
(R)-10	B	R	7	a	c	0.97±0.28	1.1	0.65±0.07	-3.80
(S)-10	B	S	7	a	c	0.89±0.21		0.80±0.05	-4.00
IX ^g	A		7	a	c	0.04±0.01		0.88±0.08	-20.50
(R)-12	B	R	7	c	a	0.20±0.06	2.5	0.93±0.05	-10.10
(S)-12	B	S	7	c	a	0.08±0.02		0.99±0.01	-20.74
X ^g	A		7	c	a	0.01±0.003		0.93±0.04	-25.06
Ver.						1.60±0.30		0.70±0.07	-1.80

^a Potency of the MDR- modulator: concentration of the compound that causes a 50% increase in the nuclear concentration of pirarubicin ($\alpha = 0.5$). ^b ER = eudismic ratio ($[I]_{0.5}$ (R) enantiomer/ $[I]_{0.5}$ (S) enantiomer). ^c Efficacy of the MDR- modulator: maximum increase that can be obtained in the nuclear concentration of pirarubicin in resistant cells; α value varies between 0 (in the absence of the modulator) and 1 (when the amount of pirarubicin in resistant cells is the same as in sensitive cells). Results are expressed as the mean \pm SE of three independent experiments done at least three times. ^d Predicted binding affinity. ^e See ref. 37. ^f See ref. 40. ^g See ref. 42.

3.2. Molecular modeling studies

Docking studies were performed to investigate the interaction mode of the new compounds in the P-gp binding pocket and to elucidate the influence of the stereocenter on their P-gp-inhibitory activity.

For this purpose, MolSoft was used to perform a flexible docking calculation in order to predict the correct binding geometry, the potential binding pocket of the protein, scoring functions and the bond types of the enantiomers with the surrounding amino acid binding pocket [51]. Molecular docking studies were performed into 3D crystal of human P-gp (PDB code 6C0V) [52] and the binding region of our compounds was identified based on 3D crystal structure of *Mus musculus* P-gp in complex with the P-gp inhibitor BDE-100 (PDB code 4XWK) [53]. The binding mode within the P-gp binding region of compounds **1-12** was compared with that of the corresponding achiral analogues **I-X** (Table 1), studied with the same method. The docking energy scores of the tested compounds are listed in Table 1. Figure 1 describes the correlation between the calculated binding free energies (ΔG) and the activities of the compounds on the pirarubicin uptake test, expressed as $p[I]_{0.5}$. The correlation coefficient (R^2) value in Figure 1 suggests that computational analysis results strictly correlate with the biological activities on the pirarubicin uptake test.

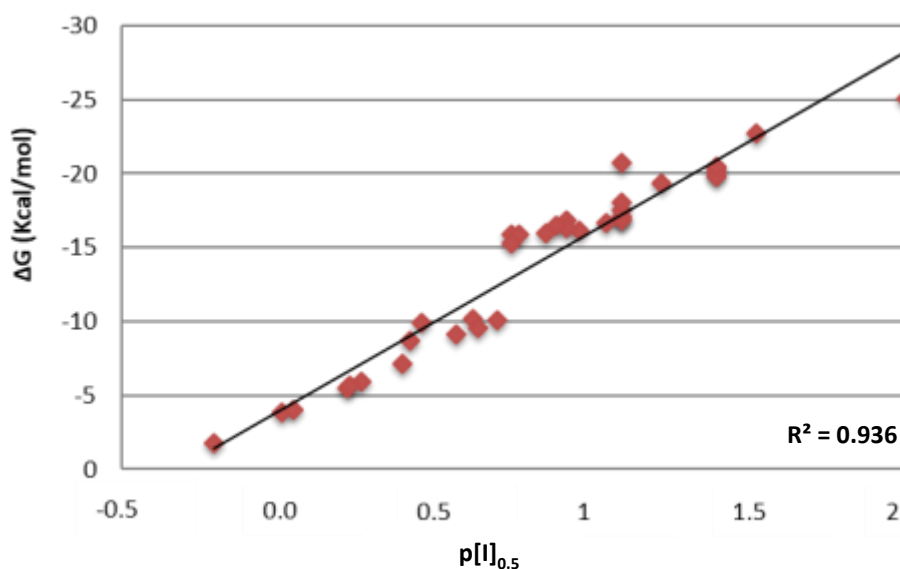


Fig. 1. Correlation between the calculated MolSoft tool binding free energies (ΔG) and the experimental $p[I]_{0.5}$ values, expressed as μM concentration, for P-gp that are reported in Table 1.

MolSoft docking results revealed that all achiral and chiral compounds showed moderate to high binding affinity towards P-gp with binding energy ranging from -3.80 to -25.06 Kcal/mol. They identified a binding region with two pockets: one (site 1) is located in the inner central chamber surrounded by hydrophobic residues, which is the common binding site of ligands in complex crystal structures, while site 2 is a deeper binding site where the residues from transmembrane helices 4, 5, 8 and 9 are able to establish interactions with both hydrophobic and polar residues [54], as showed in Figure 2. The achiral compound **X** showed the lowest binding energy computational value ($\Delta G = -25.06$ Kcal/mol). It interacts at both the P-gp binding sites forming three π -stacking bonds with the three aromatic rings of the anthracene moiety and phenylalanine residues (site 1), and hydrophobic interactions with isoleucine, leucine, phenylalanine and valine residues (sites 1 and 2) (Figure 3 A). On the contrary, molecular docking of chiral compounds showed that they occupy only a part of the binding region establishing interactions with different binding pocket residues than the achiral compounds. In particular, we observed that all chiral compounds do not reach the same binding poses of the best achiral derivative (Figure 3), probably due to the presence of the methyl group on one of the two linkers. Moreover, the presence of the stereocenter influences the pose of the enantiomer couples. As an example, in Figure 3 B we observed how the two enantiomers (*R*)-**6** and (*S*)-**6** achieved similar poses showing comparable binding free energy values (Table 1). Differently, the two enantiomers (*R*)-**5** and (*S*)-**5** showed different poses and binding energies (Figure 3 C and Table 1),

in accordance with the results on the pirarubicin uptake test where the two enantiomers showed an ER = 5.0 (Table 1).

In conclusion, we can therefore state that chiral molecules interact at the P-gp binding pocket in different ways with respect to achiral analogues, in particular, with a 7-methylene chain; the latter derivatives, in fact, can reach easily the end of the pocket forming important interactions with Leu 225, Phe 193, 200 and 204, and Val 338. Anyway, many chiral molecules are able to form strong interactions with binding protein residues that are crucial for the mechanism of action and inhibitory effect proposed by us in this study.

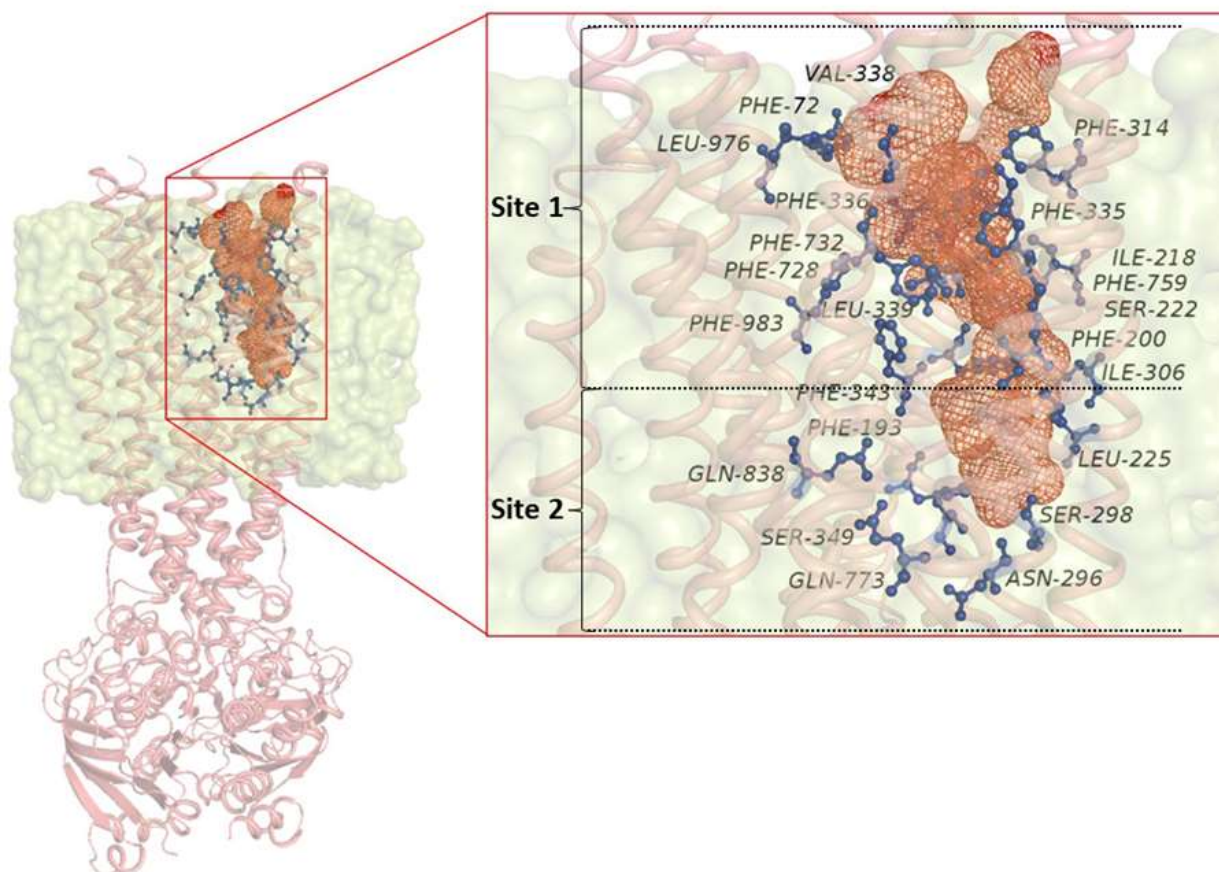


Fig. 2. 3D representation of human P-gp in the lipid bilayer (green). MolSoft program identified two sites inside the protein and predicted binding-pockets are shown in orange surface surrounded by binding region residues represented with blue ball and sticks.

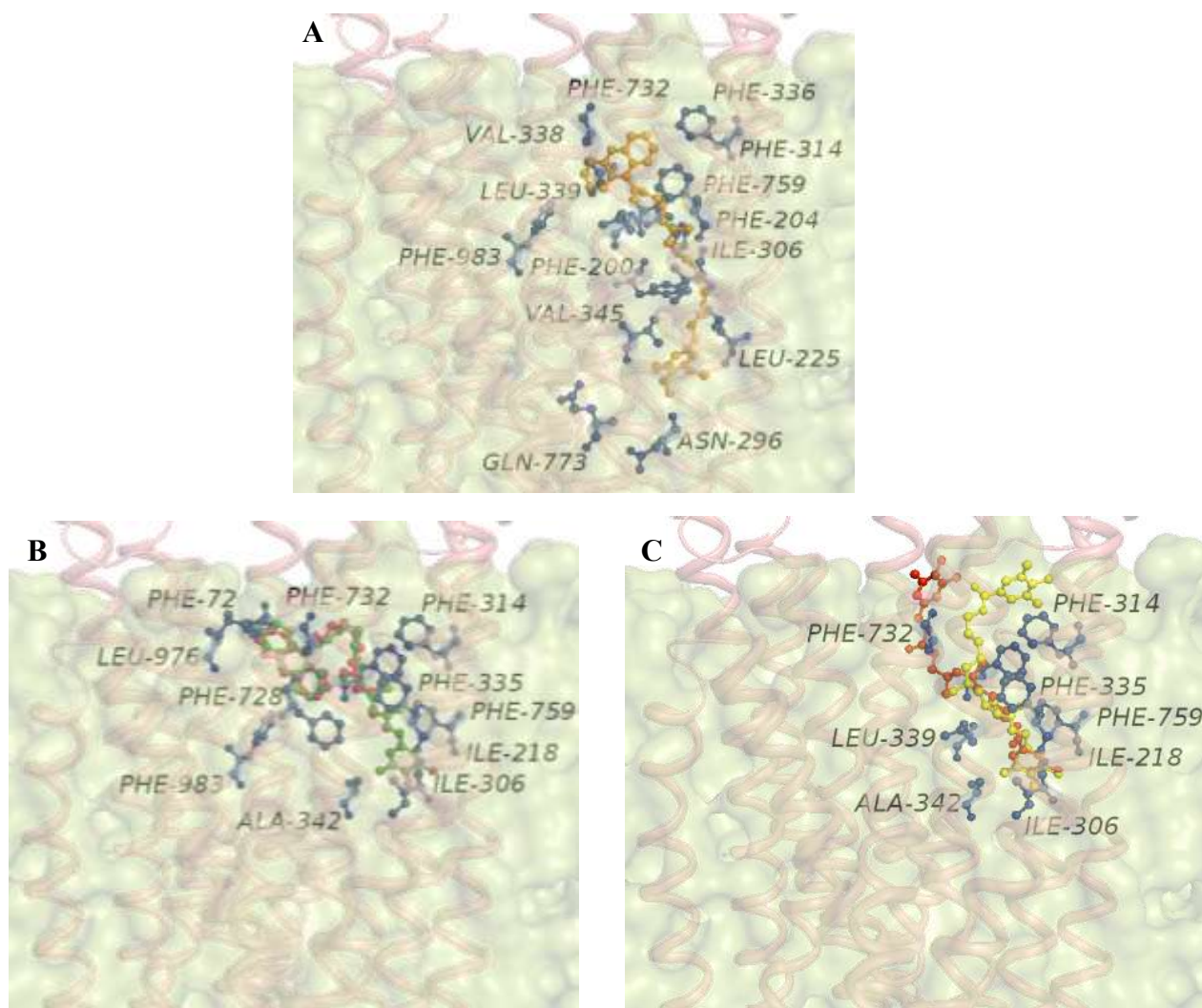


Fig. 3. 3D representation of human P-gp binding region (orange) inside the lipid bilayer (green). (A) Blue balls and sticks represent residues of P-gp binding pocket with the best active achiral compound **X** in orange. (B-C) Blue balls and sticks represent residues of P-gp binding pocket with enantiomers (*R*)-**6** (magenta) and (*S*)-**6** (green), (panel B) and (*R*)-**5** (red) and (*S*)-**5** (yellow), (panel C).

3.3. Characterization of P-gp interacting profile and ABC transporters selectivity on transfected MDCK cells

The new compounds were further studied on MDCK-MDR1 (Madin-Darby Canine Kidney-MDR1) cells overexpressing P-gp, by inhibition of the transport of Calcein-AM, a profluorescent probe that is a P-gp substrate.

The P-gp interaction mechanism of the compounds was also investigated and, besides the P-gp potency assay on MDCK-MDR1 cells, other two combined assays were employed, as previously reported [55]: 1) apparent permeability (P_{app}) determination (BA/AB) in Caco-2 cell monolayer [56] which measures the ratio between two fluxes, BA, from the basolateral to apical compartments, that represents passive diffusion, and AB, from the apical to basolateral compartments, that represents active transport; 2) ATP cell depletion in cells overexpressing the transporter MDCK-MDR1 [57] which measures the ATP consumption due to transport mediated by the pump; generally, a substrate, being transported by the pump, induces ATP cell depletion (unambiguous substrate, category I), while a P-gp inhibitor does not induce ATP consumption. $(BA/AB) < 2$ indicates that the compound can be considered an inhibitor, also taking into account the results of the ATPase assay and Calcein-AM

modulation, $(BA/AB) > 2$ indicates that the compound is classified as a substrate [58,59]. However, another substrate category (known as category IIB3) displaying a P_{app} value > 2 but not inducing an ATP cell depletion is also reported [60].

Finally, the compounds were also studied on cells overexpressing MRP1 (MDCK-MRP1 cells) or BCRP (MDCK-BCRP cells) to evaluate their inhibitory activity toward two other ABC proteins, MRP1 and BCRP, by the inhibition of the transport of Calcein-AM (MRP1 substrate) or the fluorescent probe Hoechst 33342 (BCRP substrate), respectively.

P-gp, MRP1 and BCRP expression levels were periodically analyzed by immunoblotting analysis in MDCK-MDR1, MDCK-MRP1 and MDCK-BCRP cells, respectively, using tubulin as control of equal protein loading. Details of the method and a representative western blot analysis are reported in the Supplementary data.

The results of the assays described above are reported in Table 2 together with those on verapamil, used as reference compound.

As shown in Table 2, all compounds were able to inhibit the P-gp Calcein-AM transport with EC_{50} values ranging from 0.073 to 1.40 μM , indicating, in this assay, high or moderate activities on P-gp. The EC_{50} values are, in some cases ((*R*)-**3**, (*R*)-**7**, (*S*)-**7** and (*S*)-**9**) lower than that of the reference compound verapamil ($EC_{50} = 0.50 \mu\text{M}$).

Stereochemistry does not seem to influence the P-gp inhibition on MDCK-MDR1 cells of these compounds. In fact, in many cases the two enantiomers showed similar EC_{50} values, or with a small difference ranging from 1.5 to 2.8 times, except the two enantiomers (*R*)-**3** and (*S*)-**3** that showed a difference of 7.5 times ($EC_{50} = 0.073 \mu\text{M}$ and $0.55 \mu\text{M}$, respectively). Anyway, also in this test, there is a non-homogeneous *S/R* preference since the eutomers of compounds **1** and **3** showed a *R* absolute configuration while those of compounds **7**, **9** and **10** showed a *S* absolute configuration.

The total length of the molecule does not seem to influence this kind of activity while the nature of the aromatic residues partly affects the activity; in fact, compounds with EC_{50} values lower than, or roughly equal to 0.5 μM ((*R*)-**3**, (*S*)-**3**, (*R*)-**7**, (*S*)-**7**, (*S*)-**9** and (*S*)-**11**) have the combination of the (*E*)-3-(3,4,5-trimethoxyphenyl)vinyl moiety with the 3,4,5-trimethoxyphenyl one, while the presence of the anthracene residue gave higher EC_{50} values ranging from 0.76 to 1.40 μM .

In this test, these compounds showed a lightly different profile than that in the pirarubicin uptake test, but it must be considered that the cell lines used for the two assays are different.

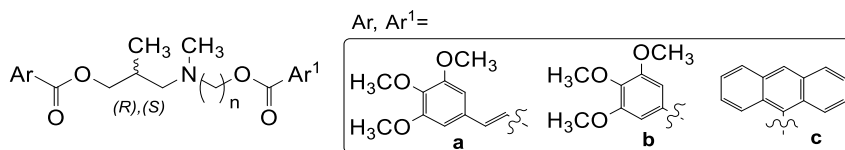
All compounds, except (*R*)-**5**, were able to inhibit BCRP but with activities lower than that of verapamil ($EC_{50} = 0.9 \mu\text{M}$). Also in this case stereochemistry has low influence on the activity with some exception (**1**, **2**, **5**, **6**, **7**). In particular, enantiomer (*S*)-**5** was able to inhibit BCRP ($EC_{50} = 4.2 \mu\text{M}$) while (*R*)-**5** was completely inactive up to a 100 μM .

The inhibitory activity on MRP1 is influenced by the nature of the aromatic residues since all anthracene derivatives were inactive on this transporter; the other compounds were able to inhibit MRP1 Calcein-AM transport with potencies higher than that of verapamil, with the exception of compounds (*S*)-**1**, (*R*)-**3**, (*S*)-**3** and (*S*)-**5**. Also in this case the stereochemistry has little influence on the activity, but not in the case of **1**, **5**, **9** and **3**, where only enantiomer (*R*)-**3** was able to inhibit MRP1 ($EC_{50} = 7.3 \mu\text{M}$) and (*S*)-**3** was completely inactive.

The apparent permeability values (P_{app}) indicated that all compounds had a BA/AB ratio > 2 and were not able to induce ATP consumption, with the exception of compound (*S*)-**1**; therefore, (*S*)-**1** could be considered a P-gp unambiguous substrate (category I) and all the other compounds were not transported substrates (category IIB3) [60].

Table 2

Biological results of enantiomers **1-12**: inhibition activity on MDCK-MDR1, MDCK-MRP1 and MDCK-BCRP cells overexpressing each transporter P-gp, MRP1 and BCRP, ATP cell depletion in MDCK-MDR1 and apparent permeability (P_{app}) determination (BA/AB) in Caco-2 cell monolayer.



Compd	Conf	n	Ar	Ar ¹	EC ₅₀ μM ^a			ATP cell depletion	P _{app} ^b
					P-gp	MRP1	BCRP		
(R)-1	R	3	a	b	0.71±0.10	5.6±1.12	33.5±5.8	no	7.6
(S)-1	S	3	a	b	1.38±0.20	12.7±2.5	3.7±0.70	yes ^c	5
(R)-3	R	3	b	a	0.073±0.01	7.3±1.4	9.5±1.90	no	6.9
(S)-3	S	3	b	a	0.55±0.10	NA	6.4±1.30	no	7.5
(R)-2	R	3	a	c	0.95±0.20	NA	5.9±1.20	no	13
(S)-2	S	3	a	c	0.91±0.20	NA	2.3±0.40	no	30
(R)-4	R	3	c	a	0.96±0.10	NA	6.8±1.36	no	7.5
(S)-4	S	3	c	a	0.91±0.18	NA	11.0±2.0	no	15
(R)-5	R	5	a	b	1.00±0.16	3.9±0.77	NA	no	5
(S)-5	S	5	a	b	1.10±0.20	10.1±2.0	4.2±0.80	no	8
(R)-7	R	5	b	a	0.31±0.05	5.8±1.20	41.0±8.2	no	6.5
(S)-7	S	5	b	a	0.11±0.02	4.4±0.88	8.1±1.62	no	6.6
(R)-6	R	5	a	c	0.72±0.10	NA	2.3±0.46	no	>20
(S)-6	S	5	a	c	0.69±0.14	NA	4.7±0.90	no	>20
(R)-8	R	5	c	a	0.81±0.16	NA	4.6±0.92	no	18
(S)-8	S	5	c	a	0.76±0.10	NA	4.6±0.90	no	15
(R)-9	R	7	a	b	0.82±0.15	3.7±0.72	4.7±0.84	no	16
(S)-9	S	7	a	b	0.32±0.05	1.9±0.32	2.3±0.40	no	17
(R)-11	R	7	b	a	0.65±0.13	3.5±0.70	4.3±0.80	no	10.3
(S)-11	S	7	b	a	0.55±0.10	2.8±0.60	2.5±0.50	no	14.4
(R)-10	R	7	a	c	1.40±0.20	NA	6.4±1.20	no	20
(S)-10	S	7	a	c	0.93±0.18	NA	5.6±1.12	no	23
(R)-12	R	7	c	a	0.99±0.19	NA	6.2±1.24	no	6.1
(S)-12	S	7	c	a	1.04±0.20	NA	7.2±1.40	no	9.2
Ver.					0.50±0.1	6.8±3.0	0.9±0.20	yes ^d	18

^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 Percentage of the effect at a concentration of 10 μM (20%). ^d Percentage of the effect at a concentration of 1 μM (20%). NA = not active.

3.4. Co-administration assay.

Compounds that displayed the best P-gp activity in the test on P-gp transfected cells (MDCK-MDR1) ((R)-3, EC₅₀ = 0.073 μM) or endowed with a good P-gp activity and selectivity profile towards the other two sister proteins ((R)-7, EC₅₀ = 0.31 μM, 5.8 μM, 41 μM vs P-gp, MRP1 and BCRP, respectively), have been tested in co-administration with the antineoplastic drug doxorubicin at 10 μM in MDCK cells overexpressing MDR1. Doxorubicin alone did not exert cytotoxicity effects since it is a P-gp substrate and was unable to overcome MDR1; when doxorubicin was added in the presence of (R)-3 and (R)-7, its activity was restored because of the inhibition of the pump carried out by the two compounds. (R)-3 and (R)-7 were tested at two different doses in a range near their EC₅₀ values (100 nM and 1 μM for (R)-3, and 500 nM and 1 μM for (R)-7) showing a reduction of viability about 30 % at 1 μM and 500 nM, respectively (Figure 4).

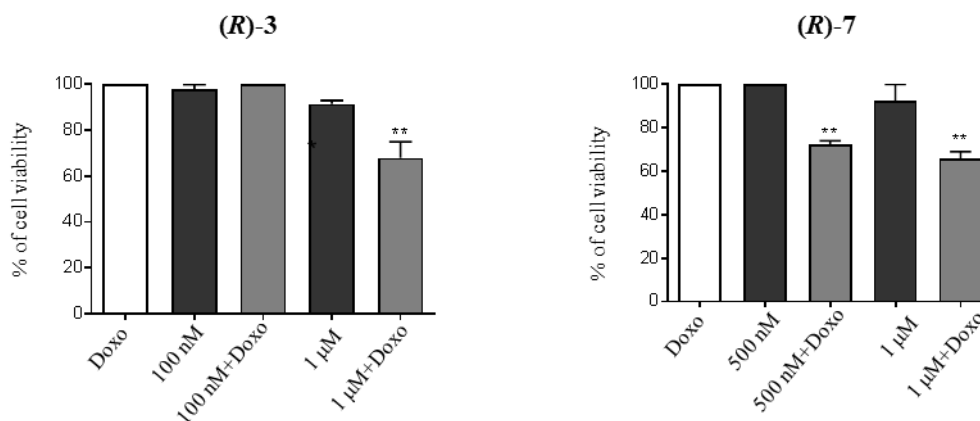


Fig.4. *In vitro* cell growth experiments performed on MDCK-MDR1 cells in the presence of 10 μ M doxorubicin (Doxo) alone (white bar), compounds (R)-3 and (R)-7 at different doses alone and in co-administration with Doxo. Each bar represents the mean \pm SEM of two experiments performed in triplicate. One-way ANOVA analysis: ** $p < 0.005$.

3.5. Collateral sensitivity study and effect on ROS production.

All compounds have been tested for their ability to induce collateral sensitivity (CS) against MDR1 or MRP1. CS is a new strategy to overcome MDR by using compounds able to selectively kill MDR cells without cytotoxic effects towards the parental cells [35,36,61-63]. CS can be quantitatively assessed by measuring the selectivity ratio (SR) between the cytotoxicity of the compound *vs* parental cells and its cytotoxicity *vs* MDR-derived cells (EC_{50} parental/ EC_{50} MDR). $SR < 1$ indicates that MDR cells are resistant to the ligand that is probably transported by the MDR pump; $SR > 1$ indicates that the cytotoxicity of the ligand *vs* MDR cells is higher than that *vs* parental cells. A compound displaying a $SR \geq 2$ is generally defined as CS-promoting agent.

Among the tested series, only compounds (S)-3 and (R)-7 displayed a $SR > 2$ towards MDR pumps. Indeed, (S)-3 has a $SR = 2.5$ *vs* MDCK-MDR1 cells ($EC_{50} = 100$ μ M on MDCK-wt cells and $EC_{50} = 40$ μ M on MDCK-MDR1 cells) and (R)-7 has a $SR = 8.5$ *vs* MDCK-MRP1 cells ($EC_{50} = 100$ μ M on MDCK-wt cells and $EC_{50} = 11.7$ μ M on MDCK-MRP1 cells). Therefore, (S)-3 and (R)-7 can be considered as CS-promoting agents on P-gp and MRP1, respectively.

One of the mechanisms at the basis of CS is the increased production of ROS in cells overexpressing ABC transporters [35]. To investigate whether this mechanism was involved in the sensitizing effects elicited by (S)-3 and (R)-7, the intracellular ROS in MDCK-MDR1, for compound (S)-3, and MDCK-MRP1 cells for compound (R)-7, was measured. (S)-3 and (R)-7 significantly increased intracellular ROS in MDCK-MDR1 (Figure 5 A) and MDCK-MRP1 (Figure 5 C) cells, respectively. To verify whether the increased ROS mediated the reduction in cell viability elicited by the compounds, the cells were co-incubated with (S)-3 or (R)-7 plus the ROS scavenger N-acetyl-cysteine (NAC), at a concentration that abrogated the increase in ROS induced by the compounds (Figures 5 A and C). While (S)-3 or (R)-7 alone significantly decreased cell viability, the co-incubation with NAC partially but significantly rescued cell viability (Figures 5 B and D).

These results suggest that at least part of the mechanism at the basis of the collateral sensitivity of (S)-3 or (R)-7 relies on the increased production of ROS in MDR1- and MRP1-overexpressing cells, respectively.

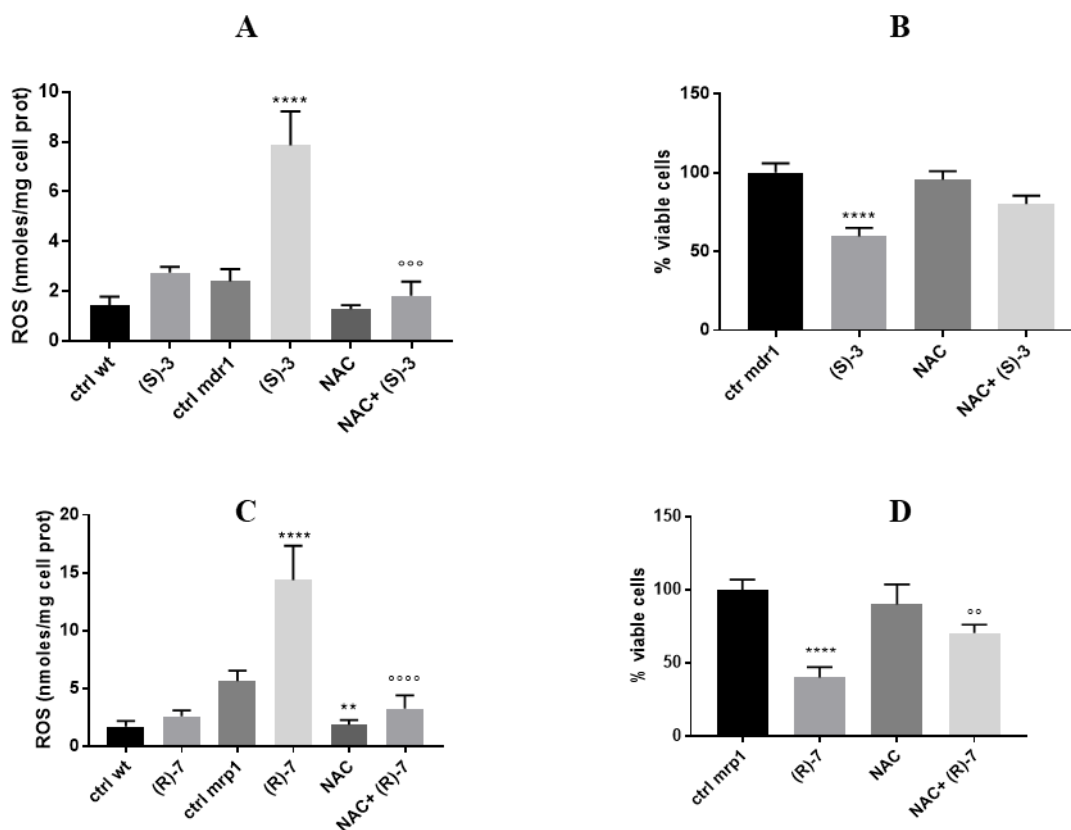


Fig. 5. Effects of (*S*)-**3** and (*R*)-**7** on MDCK-MDR1 and MDCK-MRP1 ROS levels and viability. MDCK-MDR1 cells (panels A and B) were grown for 24 h in fresh medium (ctrl) or with 500 nM (*S*)-**3** alone or in the presence of the ROS scavenger N-acetyl-cysteine (NAC, 10 mM). MDCK-MRP1 cells (panels C and D) were grown for 24 h in fresh medium (ctrl) or with 500 nM (*R*)-**7** alone or in the presence of the ROS scavenger N-acetyl-cysteine (NAC, 10 mM). Panels A and C: ROS were measured fluorimetrically, in duplicates ($n = 3$). Data are presented as means \pm SD. **** $p < 0.0001$ vs ctrl; °°°° $p < 0.0001$: NAC-treated cells vs (*S*)-**3** and (*R*)-**7**, respectively. Panels B and D: cell viability was measured with a chemiluminescence-based assay, in quadruplicates ($n = 3$). Data are presented as means \pm SD. **** $p < 0.0001$: vs ctrl; °° $p < 0.001$: NAC-treated cells vs (*S*)-**3** and (*R*)-**7**, respectively [63].

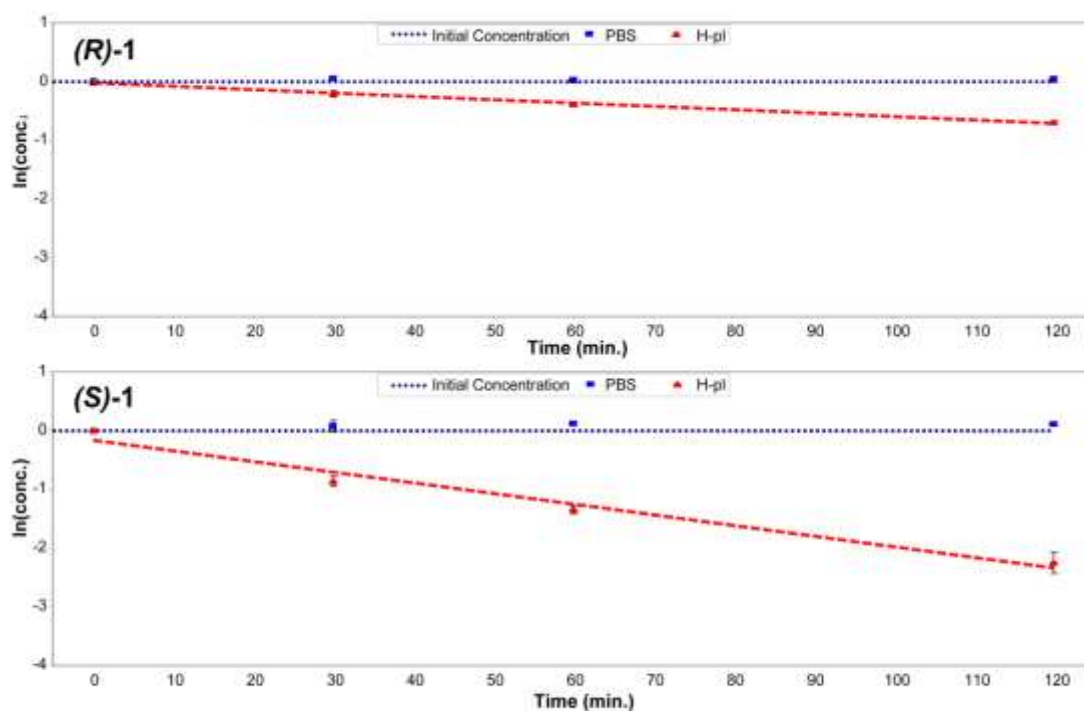
3.6. Chemical stability test

The chemical stability of compounds **1-12** was evaluated in phosphate buffer solution (PBS) and in human plasma samples to verify if spontaneous or enzymatic hydrolysis occurs. The analyses were carried out by LC-MS/MS method operating in Multiple Reaction Monitoring (MRM) mode [64]. The LC-MS/MS system and experimental conditions used are reported in the Supplementary data. The solution stability of each compound was verified by monitoring the variation of analyte concentration at different incubation times in PBS and human plasma samples. The evaluation of the employed LC-MS/MS methods and the processed raw data are reported in the Supplementary data. The obtained results demonstrated that all the compounds were stable in PBS and most of them also in human plasma. In fact, only the degradation plots, in human plasma, of (*R*)-**1**, (*S*)-**1**, (*R*)-**3**, (*R*)-**5**, (*S*)-**5**, (*R*)-**9** and (*S*)-**9** showed a significant decay rate (k value $\leq -2 \cdot 10^{-3} \ln \mu\text{M}/\text{min}$), and their calculated half-life values varied between 18 and 117 min (Table 3). As an example, the human plasma degradation plots of the enantiomer couples (*R*)-**1** and (*S*)-**1** are reported in Figure 6. The plots of all compounds are reported in the Supplementary data (Figures S2-S25). The half-life value of the reference compound ketoprofene ethylester (KEE) indicated that the human batch employed was enzymatically active (half-life < 2 h) [65].

Table 3Half-life ($t_{1/2}$) values of reference and studied compounds in PBS e human plasma samples.

Compd	PBS $t_{1/2} \pm$ error (min)	Human plasma $t_{1/2} \pm$ error (min)	Compd	PBS $t_{1/2} \pm$ error (min)	Human plasma $t_{1/2} \pm$ error (min)
KEE	n.d.	107 \pm 16			
(R)-1	≥ 240	117 \pm 10	(R)-7	≥ 240	≥ 240
(S)-1	≥ 240	29 \pm 7	(S)-7	≥ 240	≥ 240
(R)-2	≥ 240	≥ 240	(R)-8	≥ 240	≥ 240
(S)-2	≥ 240	≥ 240	(S)-8	≥ 240	≥ 240
(R)-3	≥ 240	54 \pm 12	(R)-9	≥ 240	74 \pm 27
(S)-3	≥ 240	≥ 240	(S)-9	≥ 240	30 \pm 9
(R)-4	≥ 240	≥ 240	(R)-10	≥ 240	≥ 240
(S)-4	≥ 240	≥ 240	(S)-10	≥ 240	≥ 240
(R)-5	≥ 240	29 \pm 15	(R)-11	≥ 240	≥ 240
(S)-5	≥ 240	18 \pm 6	(S)-11	≥ 240	≥ 240
(R)-6	≥ 240	≥ 240	(R)-12	≥ 240	≥ 240
(S)-6	≥ 240	≥ 240	(S)-12	≥ 240	≥ 240

n.d.: not determined

**Fig. 6.** Degradation plots of the enantiomers couples (R)-1 (top), (S)-1 (bottom) in PBS (square blue) and human plasma (red triangle) samples.

The k values of the stable compounds were close to 0; therefore, extremely high half-life values can be calculated for these derivatives. Since 240 min is the maximum time of observation in our experimental protocol, it is possible to assume that their half-life values could be equal or greater than 240 min.

The degradation products of compounds (R)-1, (S)-1, (R)-3, (R)-5, (S)-5, (R)-9 and (S)-9 were also examined to determine the possible mechanism of the enzymatic hydrolysis. The results proved that these compounds were hydrolyzed at the ester group linked to the (E)-3-(3,4,5-

trimethoxyphenyl)vinyl moiety (**a**, see Chart 1), with formation of the corresponding N-alkyl alcohol and of the (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (Supplementary data, Figure S26). Noteworthy is that the hydrolysis takes place only when the (*E*)-3-(3,4,5-trimethoxyphenyl)vinyl ester is linked to the three methylenes 2-(methyl)propyl chain and combined with the 3,4,5-trimethoxybenzoic moiety (**b**, see Chart 1) (e.g. (*R*)-**1** and (*S*)-**1**). As a matter of fact, the enzyme activity is prevented when the (*E*)-3-(3,4,5-trimethoxyphenyl)vinyl moiety is combined with the anthracene residue (**c**, see Chart 1) (i.e. (*R*)-**2** and (*S*)-**2**). This behavior was already highlighted in the previously studied achiral series with a 3-methylenes chain [42] (see the achiral derivative **I**) and it is therefore confirmed also in this chiral series carrying a branched 3-methylenes chain (2-(methyl)propyl chain). However, this feature depends on the configuration of the stereogenic center, in fact, the (*S*) enantiomers always showed a degradation rate higher than the corresponding (*R*) ones (see (*S*)-**1**, (*S*)-**5** and (*S*)-**9**) (Table 3). On the contrary, (*R*)-**3** and (*S*)-**3**, having the (*E*)-3-(3,4,5-trimethoxyphenyl)vinyl moiety linked to the linear three methylenes alkyl chain, showed a different behavior. As a matter of fact, the (*R*) enantiomer suffers a remarkable degradation ($t_{1/2} = 54 \pm 12$ min.) while the (*S*) one did not show a significant enzymatic hydrolysis (Table 3 and Figure 7).

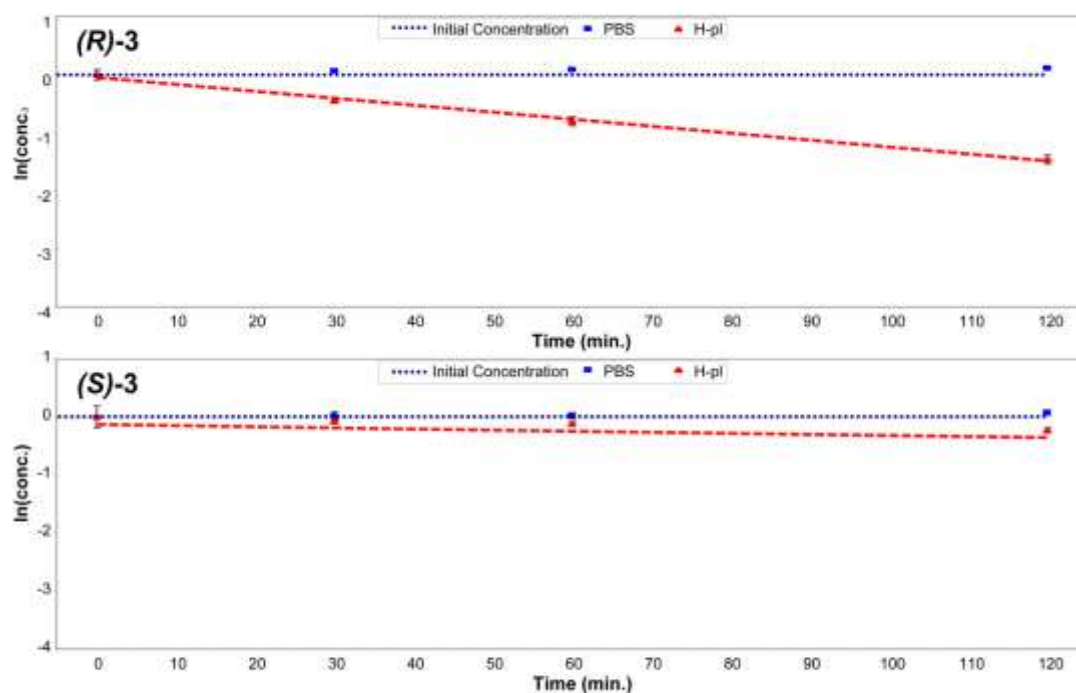


Fig. 7. Degradation plots of the enantiomers couples (*R*)-**3** (top), (*S*)-**3** (bottom) in PBS (square blue) and human plasma (red triangle) samples.

4. Discussion

In this work we reported a new series of *N,N*-bis(alkanol)amine aryl ester derivatives studied as MDR modulators. The new compounds contain a 2-(methyl)propyl chain combined with a 3-, 5- or 7-methylenes long chain and bearing different aromatic ester portions. These compounds represent branched homologues of previously studied derivatives (**I-X**). The introduction of a methyl group in these molecules gives origin to a stereogenic center and consequently to (*R*) and (*S*) enantiomers. The biological behavior of these compounds was studied by various tests that allowed us to highlight different characteristics.

The MDR reversal activity was evaluated by the pirarubicin uptake assay on doxorubicin-resistant erythroleukemia K562 cell line (K562/DOX). In this test these compounds showed good inhibitory activity with potency values ($[I]_{0.5}$) in the nanomolar range and, in general, completely reversed P-gp-dependent pirarubicin extrusion (α_{\max} close to 1). In most cases the (*R*) and (*S*) isomers showed different potency values although there is no regular trend in the behavior of the two enantiomers. In comparison with the corresponding achiral derivatives, the presence of the methyl group on the stereogenic center appears to exert a clear positive effect only in the set with $n = 3$, since these compounds were in general more potent than the corresponding achiral derivatives showing the same length and residues.

Docking simulations were employed to elucidate the influence of the stereocenter on the binding mode of these compounds in the P-gp pocket site. The chiral compounds appeared to have a binding mode partly different from that of achiral ones, forming interactions with different binding pocket residues. In particular, the presence of the methyl group at the stereocenter prevents derivatives with $n = 7$ from reaching the same binding poses of the corresponding most active achiral derivatives; however, the added methyl group is probably able to establish favourable interactions for compounds with $n = 3$.

The P-gp interaction profile and selectivity towards MRP1 and BCRP of the new compounds were investigated on MDCK transfected cells overexpressing the transporters P-gp, MRP1 and BCRP.

In this test, all compounds were able to inhibit P-gp efflux activity and small differences in activity and enantioselectivity were observed throughout the series. These results are lightly different from those found in the pirarubicin uptake test, but it must be kept in mind that the cell lines used in these two tests are different (Madin-Darby Canine Kidney (MDCK) transfected cells and doxorubicin-resistant erythroleukemia K562 cells, respectively).

As for the P-gp-interacting profile, only compound (*S*)-**1** can be considered a P-gp unambiguous substrate (category I) whereas all the other compounds were not transported substrates (category IIB3).

The co-administration assay on the enantiomers (*R*)-**3** and (*R*)-**7** proved that they were able in reversing resistance to doxorubicin since the activity of the anticancer drug in MDCK-MDR1 cells was restored.

All compounds, except (*R*)-**5**, were able to inhibit BCRP, while only molecules bearing the combination of the two aromatic moieties (*E*)-3-(3,4,5-trimethoxyphenyl)vinyl and 3,4,5-trimethoxyphenyl showed MRP1 inhibition activity. These results confirmed the same outcome already found in our previous paper [42], namely that the anthracene residue is not positive for the MRP1 inhibition.

Of great interest was the ability to induce collateral sensitivity showed by (*S*)-**3** and (*R*)-**7**. Both compounds indeed can be considered as CS-promoting agents towards MDR pumps, as they proved to be able to selectively kill MDR cells (MDCK-MDR1 and MDCK-MRP1 cells, respectively) without cytotoxic effects towards the parental cells, displaying a $SR > 2$. The mechanism at the basis of the collateral sensitivity of these two compounds relies at least in part on the increased production of ROS in MDR1- and MRP1-overexpressing cells, respectively.

Finally, most compounds of this new series of MDR inhibitors were stable in PBS and human plasma, despite the presence of the two ester groups. Only (*R*)-**3** and the two enantiomers of compounds **1**, **5** and **9** showed a significant decay rate in the human plasma; the hydrolysis occurs at the ester group linked to the (*E*)-3-(3,4,5-trimethoxyphenyl)vinyl moiety and the degradation rate depends on the configuration of the stereocenter of the molecules.

5. Conclusions

In this study we designed and synthesized a new series of *N,N*-bis(alkanol)amine aryl esters bearing a chiral alkyl chain allowing the identification of interesting MDR reversers. The presence of a methyl group at the stereocenter causes in many cases enantioselectivity, a characteristic that is not common

among MDR transporter protein ligands. Docking studies confirmed the influence of the stereocenter on the interaction of these compounds in the P-gp pocket.

Interestingly, nearly all these compounds were able to inhibit both P-gp and BCRP; since these two transporter proteins were often found co-expressed in several tumors, this behaviour could be an interesting feature of this series of derivatives. The inhibitory activity on MRP1 is influenced by the nature of the aromatic residues since all anthracene derivatives are inactive allowing us to identify the molecular determinants for this activity.

Also noteworthy is the identification of two compounds, (*S*)-**3** and (*R*)-**7**, able to induce collateral sensitivity (CS) against MDR1- and MRP1-overexpressing cells. Therefore, these two compounds can be considered as CS-promoting agents and could represent interesting leads for the development of selective cytotoxic agents for drug-resistant cells. In a biological perspective, these results are remarkable at least for two reasons. First, it was possible to identify two compounds, (*S*)-**3** and (*R*)-**7**, that exhibited anti-cancer activity in the low micromolar/nanomolar range, i.e. a range that are comparable to most common chemotherapeutic drugs. Second, while several sensitizers for MDR1-expressing cells have been designed and tested at preclinical level, the number of sensitizers effective against MRP1-expressing cells is significantly lower. Our study provides a comprehensive view of synthesis, physical-chemical properties and biological efficacy of two new potent and MDR1/MRP1-expressing cells sensitizer compounds.

Taking altogether these results into account, this series of MDR modulators seems very promising and deserves further investigation. Work is underway to expand structure activity relationships and to improve potency and efficacy in this series of molecules.

6. Experimental section

6.1. 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 ^1H NMR, 100 MHz for ^{13}C). ^1H and ^{13}C NMR spectra were measured at room temperature (25°C) in an appropriate solvent. ^1H and ^{13}C chemical shifts are expressed in ppm (δ) referred to TMS. Spectral data are reported using the following abbreviations: s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet, and coupling constants are reported in Hz, followed by integration. Chromatographic separations were performed on a silica gel column by gravity chromatography (Kieselgel 40, 0.063-0.200 mm; Merck) or flash chromatography (Kieselgel 40, 0.040-0.063 mm; Merck). Yields are given after purification, unless otherwise stated.

The enantiomeric excess (ee) and purity of final compounds were determined 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 operating conditions were reported in Supplementary data.

High resolution mass spectrometry (HR-MS) analyses of final compounds were performed with a Thermo Finnigan LTQ Orbitrap mass spectrometer equipped with an electrospray ionization source (ESI). The analyses were carried out introducing, via syringe pump at 10 $\mu\text{L min}^{-1}$, the sample solution (1.0 $\mu\text{g mL}^{-1}$ in mQ water:acetonitrile 50:50), in positive ion mode. These experimental conditions allow the monitoring of protonated molecules of the studied compounds ($[\text{M}+\text{H}]^+$ species), that they were measured with a proper dwell time to achieve 60,000 units of resolution at Full Width at Half Maximum (FWHM). Elemental composition of compounds was calculated on the basis of their measured accurate masses, accepting only results with an attribution error less than 5 ppm and a not integer RDB (double bond/ring equivalents) value, in order to consider only the protonated species [66].

When reactions were performed in anhydrous conditions, the mixtures were maintained under nitrogen. Free bases (*R*) and (*S*) **1-12** were transformed into the hydrochloride by treatment with a

solution of acetyl chloride (1.1 eq) in anhydrous CH₃OH. The salts were crystallized from abs. ethanol/petroleum ether. Compounds were named following IUPAC rules as applied by ChemBioDraw Ultra 14.0 software.

6.1.1. (*S*)-(*E*)-3-Bromo-2-methylpropyl 3-(3,4,5-trimethoxyphenyl)acrylate (*S*)-**13**

To a solution of 467.0 mg (1.96 mmol) of (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid in 6 mL of CHCl₃ (free of ethanol), 0.25 mL (3.9 mmol) of SOCl₂ were added and the solution was stirred for 4 h at 60 °C. The reaction mixture was cooled to rt, and the solvent was removed under reduced pressure; the mixture was then treated twice with cyclohexane and the solvent removed under reduced pressure. The acyl chloride obtained was dissolved in CHCl₃ (free of ethanol), and (*S*)-3-bromo-2-(methyl)propan-1-ol (0.20 mL, 1.96 mmol) was added. The mixture was maintained at room temperature for 18 h and treated with CH₂Cl₂. The resulting organic layer was washed with 10% NaOH solution, dried with Na₂SO₄, and the solvent was removed under reduced pressure. The crude product was then purified by flash chromatography using cyclohexane/ethyl acetate 70:30 as eluent. The desired compound was obtained as an oil. Yield: 98.4%.

¹H NMR (400 MHz, CDCl₃) δ: 7.54 (d, *J*=15.6 Hz, 1H, CH=CH); 6.70 (s, 2H, CH arom.); 6.30 (d, *J*=15.6 Hz, 1H, CH=CH); 4.17-4.12 (m, 1H, OCHH); 4.10-4.05 (m, 1H, OCHH); 3.82 (s, 6H, OCH₃); 3.81 (s, 3H, OCH₃); 3.46-3.38 (m, 2H, CH₂Br); 2.22-2.17 (m, 1H, CH); 1.05 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 166.61 (C=O); 153.40 (C); 145.04 (CH=CH); 140.19 (C); 129.74 (C); 116.89 (CH=CH); 105.28 (CH arom.); 66.99 (CH₂); 60.88 (OCH₃); 56.12 (OCH₃); 36.92 (CH₂); 34.77 (CH); 15.75 (CH₃) ppm.

6.1.2. (*R*)-(*E*)-3-Bromo-2-methylpropyl 3-(3,4,5-trimethoxyphenyl)acrylate (*R*)-**13**

Following the same procedure described for compound (*S*)-**13**, starting from (*R*)-3-bromo-2-(methyl)propan-1-ol, compound (*R*)-**13** was obtained as an oil. Yield: 95.9%.

¹H NMR (400 MHz, CDCl₃) δ: 7.60 (d, *J*=16.0 Hz, 1H, CH=CH); 6.75 (s, 2H, CH arom.); 6.34 (d, *J*=16.0 Hz, 1H, CH=CH); 4.24-4.19 (m, 1H, OCHH); 4.16-4.011 (m, 1H, OCHH); 3.89 (s, 6H, OCH₃); 3.88 (s, 3H, OCH₃); 3.55-3.42 (m, 2H, CH₂Br); 2.35-2.23 (m, 1H, CH); 1.11 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 166.72 (C=O); 153.46 (C); 145.12 (CH=CH); 129.78 (C); 116.94 (CH=CH); 105.31 (CH arom.); 66.60 (CH₂); 60.98 (OCH₃); 56.19 (OCH₃); 36.90 (CH₂); 34.82 (CH); 15.80 (CH₃) ppm.

6.1.3. (*S*)-3-Bromo-2-methylpropyl 3,4,5-trimethoxybenzoate (*S*)-**14**

A solution of 416 mg (1.96 mmol) of 3,4,5-trimethoxybenzoic acid in 15 mL of anhydrous CH₂Cl₂ was cooled at 0 °C and 0.14 mL of (*S*)-3-bromo-2-(methyl)propan-1-ol (1.31 mmol), 128 mg (1.05 mmol) of 4-dimethylaminopyridine (DMAP) and 452 mg (2.36 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) were added in this order. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 18 h and treated with CH₂Cl₂. The organic layer was washed three times with water and twice with a saturated solution of NaHCO₃. After drying with Na₂SO₄, the solvent was removed under reduced pressure. The crude product was then purified by flash chromatography using cyclohexane/ethyl acetate 80:20 as eluent, yielding 426.4 mg (94%) of the desired compound as an oil.

¹H NMR (400 MHz, CDCl₃) δ: 7.28 (s, 2H, CH arom.); 4.33-4.23 (m, 2H, OCH₂); 3.89 (s, 9H, OCH₃); 3.40 (d, *J*=6.4 Hz, 2H, CH₂Br); 2.38-2.32 (m, 1H, CH); 1.03 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.4. (*R*)-3-Bromo-2-methylpropyl 3,4,5-trimethoxybenzoate (*R*)-**14**

Following the same procedure described for compound (*S*)-**14**, starting from (*R*)-3-bromo-2-(methyl)propan-1-ol, compound (*R*)-**14** was obtained as an oil. Yield: 98%.

¹H NMR (400 MHz, CDCl₃) δ: 7.17 (s, 2H, CH arom.); 4.20-4.11 (m, 2H, OCH₂); 3.78 (s, 9H, OCH₃); 3.37 (d, *J*=5.6 Hz, 2H, CH₂Br); 2.26-2.18 (m, 1H, CH); 1.02 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.5. (*S*)-3-Bromo-2-methylpropyl anthracene-9-carboxylate (*S*)-15

Following the same procedure described for compound (*S*)-13, starting from anthracene-9-carboxylic acid and (*S*)-3-bromo-2-(methyl)propan-1-ol, compound (*S*)-15 was obtained as an oil. Yield: 97%.

¹H-NMR (CDCl₃) δ: 8.51 (s, 1H, CH arom.); 8.07-8.01 (m, 4H, CH arom.); 7.58-7.48 (m, 4H, CH arom.); 4.65-4.56 (m, 2H, OCH₂); 3.55-3.46 (m, 2H, CH₂Br); 2.42-2.38 (m, 1H, CH); 1.18 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.6. (*R*)-3-Bromo-2-methylpropyl anthracene-9-carboxylate (*R*)-15

Following the same procedure described for compound (*S*)-13, starting from anthracene-9-carboxylic acid and (*R*)-3-bromo-2-(methyl)propan-1-ol, compound (*R*)-15 was obtained as an oil. Yield: 99%.

¹H NMR (400 MHz, CDCl₃) δ: 8.53 (s, 1H, CH arom.); 8.07-8.02 (m, 4H, CH arom.); 7.58-7.48 (m, 4H, CH arom.); 4.65-4.56 (m, 2H, OCH₂); 3.55-3.46 (m, 2H, CH₂Br); 2.44-2.36 (m, 1H, CH); 1.18 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.7. (*S*) (*E*)-3-Iodo-2-methylpropyl 3-(3,4,5-trimethoxyphenyl)acrylate (*S*)-16

To a solution of 719.4 mg (1.93 mmol) of compound (*S*)-13 in 8 ml of acetone, NaI (1.16 g, 7.70 mmol) was added and the resulting mixture was maintained at reflux in the dark for 18 h. The reaction mixture was cooled to room temperature, and the solvent was removed under reduced pressure; the residue was dissolved in CH₂Cl₂ and washed three times with water. The organic layer was dried with Na₂SO₄, and the solvent was removed under reduced pressure yielding 721.4 mg (89%) of the desired compound (*S*)-16 as a solid which was used as such for the next reaction. Mp: 81-84 °C.

¹H NMR (400 MHz, CDCl₃) δ: 7.59 (d, *J*=16.0 Hz, 1H, CH=CH); 6.75 (s, 2H, CH arom.); 6.34 (d, *J*=16.0 Hz, 1H, CH=CH); 4.19-4.14 (m, 1H, OCHH); 4.10-4.02 (m, 1H, OCHH); 3.88 (s, 6H, OCH₃); 3.87 (s, 3H, OCH₃); 3.33-3.22 (m, 2H, CH₂I); 1.99-1.88 (m, 1H, CH); 1.07 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

6.1.8. (*R*) (*E*)-3-Iodo-2-methylpropyl 3-(3,4,5-trimethoxyphenyl)acrylate (*R*)-16

Following the same procedure described for compound (*S*)-16, starting from 625.6 mg (1.60 mmol) of (*R*)-13, compound (*R*)-16 was obtained as an oil. Yield: 93%.

¹H NMR (400 MHz, CDCl₃) δ: 7.55 (d, *J*=16.0 Hz, 1H, CH=CH); 6.71 (s, 2H, CH arom.); 6.30 (d, *J*=16.0 Hz, 1H, CH=CH); 4.16-4.07 (m, 1H, OCHH); 4.05-3.97 (m, 1H, OCHH); 3.84 (s, 6H, OCH₃); 3.83 (s, 3H, OCH₃); 3.31-3.18 (m, 2H, CH₂I); 1.95-1.85 (m, 1H, CH); 1.03 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.9. (*S*)-3-Iodo-2-methylpropyl 3,4,5-trimethoxybenzoate (*S*)-17

Following the same procedure described for compound (*S*)-16, starting from 214.1 mg (0.62 mmol) of (*S*)-14, compound (*S*)-17 was obtained as an oil. Yield: 99%.

¹H NMR (400 MHz, CDCl₃) δ: 7.27 (s, 2H, CH arom.); 4.25 (dd, *J*=11.2 Hz, *J*=5.6 Hz, 1H, OCHH); 4.16 (dd, *J*=11.2 Hz, *J*=7.2 Hz, 1H, OCHH); 3.88 (s, 9H, OCH₃); 3.31-3.23 (m, 2H, CH₂I); 2.05-1.98 (m, 1H, CH); 1.08 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.10. (*R*)-3-Iodo-2-methylpropyl 3,4,5-trimethoxybenzoate (*R*)-17

Following the same procedure described for compound (*S*)-16, starting from 112.2 mg (0.32 mmol) of (*R*)-14, compound (*R*)-17 was obtained as an oil. Yield: 98%.

¹H NMR (400 MHz, CDCl₃) δ: 7.16 (s, 2H, CH arom.); 4.14 (dd, *J*=11.2 Hz, *J*=5.6 Hz, 1H, OCHH); 4.05 (dd, *J*=11.2 Hz, *J*=5.6 Hz, 1H, OCHH); 3.78 (s, 9H, OCH₃); 3.20-3.12 (m, 2H, CH₂I); 1.96-1.86 (m, 1H, CH); 0.97 (d, *J* = 6.8 Hz, 3H, CH₃) ppm.

6.1.11. (*S*)-3-Iodo-2-methylpropyl anthracene-9-carboxylate (*S*)-18

Following the same procedure described for compound (*S*)-16, starting from 208.6 mg (0.54 mmol) of (*S*)-15, compound (*S*)-18 was obtained as an oil. Yield: 99%.

¹H NMR (400 MHz, CDCl₃) δ: 8.52 (s, 1H, CH arom.); 8.09-8.01 (m, 4H, CH arom.); 7.59-7.48 (m, 4H, CH arom.); 4.61-4.59 (m, 1H, OCHH); 4.47-4.46 (m, 1H, OCHH); 3.32-3.29 (m, 2H, CH₂I); 2.07-1.99 (m, 1H, CH); 1.14 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.12. (*R*)-3-Iodo-2-methylpropyl anthracene-9-carboxylate (*R*)-18

Following the same procedure described for compound (*S*)-16, starting from 112.7 mg (0.32 mmol) of (*R*)-15, compound (*R*)-18 was obtained as an oil. Yield: 96%.

¹H NMR (400 MHz, CDCl₃) δ: 8.54 (s, 1H, CH arom.); 8.07-8.02 (m, 4H, CH arom.); 7.62-7.48 (m, 4H, CH arom.); 4.58 (dd, *J*=11.2 Hz, *J*=5.2 Hz, 1H, OCHH); 4.48 (dd, *J*=11.2 Hz, *J*=7.2 Hz, 1H, OCHH); 3.34-3.23 (m, 2H, CH₂I); 2.08-2.00 (m, 1H, CH); 1.16 (d, *J*=7.2 Hz, 3H, CH₃) ppm.

6.1.13. General procedure for the synthesis of the (*S*) and (*R*) enantiomers of the hydroxyaminoesters 19-27

The appropriate haloester ((*S*) and (*R*)-16, (*S*) and (*R*)-17, (*S*) and (*R*)-18) (1 mmol), K₂CO₃ (1 mmol) and the suitable aminoalkylalcohol (3-aminopropan-1-ol, 5-aminopentan-1-ol or 7-aminoheptan-1-ol [43]) (2 mmol) were dissolved in 1 mL of anhydrous CH₃CN. The mixture was heated at 80 °C for 5-10 h. The reaction mixture was cooled to room temperature, treated with CH₂Cl₂ and the organic layer was washed with 10% NaOH solution. After drying with Na₂SO₄, the solvent was removed under reduced pressure and the residue purified by flash chromatography, yielding a pale-yellow oil.

6.1.13.1. (*R*) (*E*)-3-((3-hydroxypropyl)amino)-2-methylpropyl 3-(3,4,5-trimethoxyphenyl)acrylate (*R*)-19

Starting from (*S*)-16 and 3-aminopropan-1-ol, compound (*R*)-19 was obtained as an oil. Yield: 50%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.1.

¹H NMR (400 MHz, CDCl₃) δ: 7.54 (d, *J*=16.0 Hz, 1H, CH=CH); 6.72 (s, 2H, CH arom.); 6.30 (d, *J*=16.0 Hz, 1H, CH=CH); 4.12-4.03 (m, 2H, OCH₂); 3.84 (s, 6H, OCH₃); 3.82 (s, 3H, OCH₃); 3.75 (t, *J*=5.6 Hz, 2H, OCH₂); 3.30-3.11 (bs, 2H, OH and NH); 2.83 (t, *J*=6.0 Hz, 2H, NCH₂); 2.68-2.59 (m, 1H, NCHH); 2.53-2.45 (m, 1H, NCHH); 2.05-1.97 (m, 1H, CH); 1.71-1.60 (m, 2H, CH₂); 0.97 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.2. (*S*) (*E*)-3-((3-hydroxypropyl)amino)-2-methylpropyl 3-(3,4,5-trimethoxyphenyl)acrylate (*S*)-19

Starting from (*R*)-16 and 3-aminopropan-1-ol, compound (*S*)-19 was obtained as an oil. Yield: 28%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.1.

ESI-MS: 368.3[M+H]⁺.

¹H NMR (400 MHz, CDCl₃) δ: 7.59 (d, 1H, *J*=16.0 Hz, CH=CH); 6.76 (s, 2H, CH arom.); 6.34 (d, *J*=16.0 Hz, 1H, CH=CH); 4.18-4.05 (m, 2H, OCH₂); 3.93-3.85 (m, 9H, OCH₃); 3.81 (t, *J*=5.2 Hz, 2H, OCH₂); 3.08 (bs, 2H, OH and NH); 2.90 (t, *J*=5.6 Hz, 2H, NCH₂); 2.72-2.67 (m, 1H, NCHH); 2.58-2.53 (m, 1H, NCHH); 2.15-2.06 (m, 1H, CH); 1.77-1.68 (m, 2H, CH₂); 1.02 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.3. (*R*)-3-((3-hydroxypropyl)amino)-2-methylpropyl 3,4,5-trimethoxybenzoate (*R*)-20

Starting from (*S*)-17 and 3-aminopropan-1-ol, compound (*R*)-20 was obtained as an oil. Yield: 53%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.1.

¹H NMR (400 MHz, CDCl₃) δ: 7.28 (s, 2H, CH arom.); 4.22 (d, *J*=6.0 Hz, 2H, OCH₂); 3.89 (s, 9H, OCH₃); 3.79 (t, *J*=5.2 Hz, 2H, OCH₂); 2.88-2.85 (m, 2H, NCH₂); 2.68 (dd, *J*=11.8 Hz, *J*=6.4 Hz, 1H, NCHH); 2.56 (dd, *J*=11.8 Hz, *J*=6.8 Hz, 1H, NCHH); 2.15-2.10 (m, 1H, CH); 1.65-1.62 (m, 2H, CH₂); 1.04 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.4. (*S*)-3-((3-hydroxypropyl)amino)-2-methylpropyl 3,4,5-trimethoxybenzoate (*S*)-**20**

Starting from (*R*)-**17** and 3-aminopropan-1-ol, compound (*S*)-**20** was obtained as an oil. Yield: 68%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.1.

¹H NMR (400 MHz, CDCl₃) δ: 7.20 (s, 2H, CH arom.); 4.15 (d, *J*=6.0 Hz, 2H, OCH₂); 3.81 (s, 9H, OCH₃); 3.70 (t, *J*=5.6 Hz, 2H, OCH₂); 3.01 (s, 2H, NH and OH); 2.78 (t, *J*=5.6 Hz, 2H, NCH₂); 2.61 (dd, *J*=12.0 Hz, *J*=6.8 Hz, 1H, NCHH); 2.48 (dd, *J*=12.0 Hz, *J*=6.8 Hz, 1H, NCHH); 2.09-2.01 (m, 1H, CH); 1.64-1.58 (m, 2H, CH₂); 0.96 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.5. (*R*)-3-((3-hydroxypropyl)amino)-2-methylpropyl anthracene-9-carboxylate (*R*)-**21**

Starting from (*S*)-**18** and 3-aminopropan-1-ol, compound (*R*)-**21** was obtained as an oil. Yield: 77%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.1.

¹H NMR (400 MHz, CDCl₃) δ: 8.50 (s, 1H, CH arom.); 8.04-7.98 (m, 4H, CH arom.); 7.56-7.45 (m, 4H, CH arom.); 4.52 (d, *J*=6.0 Hz, 2H, OCH₂); 3.57 (t, *J*=5.2 Hz, 2H, OCH₂); 3.05 (s, 2H, NH and OH); 2.81 (t, *J*=5.6 Hz, 2H, NCH₂); 2.72 (dd, *J*=11.6 Hz, *J*=6.0 Hz, 1H, NCHH); 2.56 (dd, *J*=11.6 Hz, *J*=6.8 Hz, 1H, NCHH); 2.22-2.14 (m, 1H, CH); 1.64-1.60 (m, 2H, CH₂); 1.07 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.6. (*S*)-3-((3-hydroxypropyl)amino)-2-methylpropyl anthracene-9-carboxylate (*S*)-**21**

Starting from (*R*)-**18** and 3-aminopropan-1-ol, compound (*S*)-**21** was obtained as an oil. Yield: 29%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.1.

¹H NMR (400 MHz, CDCl₃) δ: 8.48 (s, 1H, CH arom.); 8.03-7.97 (m, 4H, CH arom.); 7.53-7.44 (m, 4H, CH arom.); 4.50 (d, *J*=6.0 Hz, 2H, OCH₂); 3.75 (t, *J*=5.6 Hz, 2H, OCH₂); 2.92 (s, 2H, NH and OH); 2.80 (t, *J*=5.6 Hz, 2H, NCH₂); 2.71 (dd, *J*=11.6 Hz, *J*=6.4 Hz, 1H, NCHH); 2.56 (dd, *J*=11.6 Hz, *J*=6.8 Hz, 1H, NCHH); 2.22-2.14 (m, 1H, CH); 1.64-1.61 (m, 2H, CH₂); 1.06 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.7. (*R*) (*E*)-3-((5-hydroxypentyl)amino)-2-methylpropyl 3-(3,4,5-trimethoxyphenyl)acrylate (*R*)-**22**

Starting from (*S*)-**16** and 5-aminopentan-1-ol, compound (*R*)-**22** was obtained as an oil. Yield: 47%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:6:0.8.

¹H NMR (400 MHz, CDCl₃) δ: 7.56 (d, *J*=16.0 Hz, 1H, CH=CH); 6.73 (s, 2H, CH arom.); 6.32 (d, *J*=16.0 Hz, 1H, CH=CH); 4.11 (d, *J*=5.6 Hz, 2H, OCH₂); 3.86 (s, 6H, OCH₃); 3.85 (s, 3H, OCH₃); 3.59 (t, *J*=6.8 Hz, 2H, OCH₂); 2.68-2.51 (m, 3H, NCH₂ and NCHH); 2.51-2.42 (m, 1H, NCHH); 2.11-2.01 (m, 1H, CH); 2.00-1.88 (bs, 2H, OH and NH); 1.60-1.45 (m, 4H, CH₂); 1.43-1.32 (m, 2H, CH₂); 0.99 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.8. (*S*) (*E*)-3-((5-hydroxypentyl)amino)-2-methylpropyl 3-(3,4,5-trimethoxyphenyl)acrylate (*S*)-**22**

Starting from (*R*)-**16** and 5-aminopentan-1-ol, compound (*S*)-**22** was obtained as an oil. Yield: 46%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:6:0.8.

¹H NMR (400 MHz, CDCl₃) δ: 7.58 (d, *J*=16.0 Hz, 1H, CH=CH); 6.75 (s, 2H, CH arom.); 6.33 (d, *J*=16.0 Hz, 1H, CH=CH); 4.14 (d, *J*=6.0 Hz, 2H, OCH₂); 3.88 (s, 6H, OCH₃); 3.87 (s, 3H, OCH₃); 3.63 (t, *J*=6.4 Hz, 2H, OCH₂); 2.67-2.53 (m, 3H, NCH₂ and NCHH); 2.52-2.44 (m, 1H, NCHH); 2.41-2.30 (bs, 2H, OH and NH); 2.09-2.00 (m, 1H, CH); 1.59-1.42 (m, 4H, CH₂); 1.41-1.32 (m, 2H, CH₂); 0.98 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

¹³C APT NMR (100 MHz, CDCl₃) δ: 167.08 (C=O); 153.40 (C); 144.87 (CH=CH); 140.13 (C); 129.84 (C); 117.17 (CH=CH); 105.28 (CH arom.); 67.47 (CH₂); 62.28 (CH₂); 60.93 (OCH₃); 56.16 (OCH₃); 52.92 (CH₂); 49.80 (CH₂); 33.10 (CH); 32.35 (CH₂); 29.40 (CH₂); 23.39 (CH₂); 15.50 (CH₃) ppm.

6.1.13.9. (*R*)-3-((5-hydroxypentyl)amino)-2-methylpropyl 3,4,5-trimethoxybenzoate (*R*)-23

Starting from (*S*)-17 and 5-aminopentan-1-ol, compound (*R*)-23 was obtained as an oil. Yield: 63%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.5.

¹H NMR (400 MHz, CDCl₃) δ: 7.28 (s, 2H, CH arom.); 4.29-4.20 (m, 2H, OCH₂); 3.89 (s, 9H, OCH₃); 3.61 (t, *J*=6.4 Hz, 2H, OCH₂); 2.67 (dd, *J*=12.0 Hz, *J*=6.8 Hz, 1H, NCHH); 2.62 (t, *J*=7.2 Hz, 2H, CH₂); 2.55 (dd, *J*=12.0 Hz, *J*=6.8 Hz, 1H, NCHH); 2.17-2.12 (m, 1H, CH); 1.84 (s, 2H, NH and OH); 1.59-1.48 (m, 4H, CH₂); 1.43-1.39 (m, 2H, CH₂); 1.04 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.10. (*S*)-3-((5-hydroxypentyl)amino)-2-methylpropyl 3,4,5-trimethoxybenzoate (*S*)-23

Starting from (*R*)-17 and 5-aminopentan-1-ol, compound (*S*)-23 was obtained as an oil. Yield: 57%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.5.

¹H NMR (400 MHz, CDCl₃) δ: 7.19 (s, 2H, CH arom.); 4.15 (dd, *J*=6.0 Hz, *J*=2.0 Hz, 2H, OCH₂); 3.80 (s, 9H, OCH₃); 3.49 (t, *J*=6.4 Hz, 2H, OCH₂); 2.58 (dd, *J*=12.0 Hz, *J*=6.8 Hz 1H, NCHH); 2.51 (t, *J*=7.2 Hz, 2H, NCH₂); 2.45 (dd, *J*=12.0 Hz, *J*=6.8 Hz, 1H, NCHH); 2.24 (s, 2H, NH and OH); 2.10-2.02 (m, 1H, CH); 1.49-1.38 (m, 4H, CH₂); 1.33-1.27 (m, 2H, CH₂); 0.95 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.11. (*R*)-3-((5-hydroxypentyl)amino)-2-methylpropyl anthracene-9-carboxylate (*R*)-24

Starting from (*S*)-18 and 5-aminopentan-1-ol, compound (*R*)-24 was obtained as an oil. Yield: 50%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:6:0.8.

¹H NMR (400 MHz, CDCl₃) δ: 8.50 (s, 1H, CH arom.); 8.04-7.99 (m, 4H, CH arom.); 7.55-7.45 (m, 4H, CH arom.); 4.55 (d, *J*=6.4 Hz, 2H, OCH₂); 3.54 (t, *J*=6.4 Hz, 2H, OCH₂); 2.73-2.55 (m, 4H, NCH₂); 2.45-2.17 (m, 1H, CH); 1.85 (s, 2H, NH and OH); 1.52-1.39 (m, 4H, CH₂); 1.34-1.27 (m, 2H, CH₂); 1.07 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.12. (*S*)-3-((5-hydroxypentyl)amino)-2-methylpropyl anthracene-9-carboxylate (*S*)-24

Starting from (*R*)-18 and 5-aminopentan-1-ol, compound (*S*)-24 was obtained as an oil. Yield: 44%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:6:0.8.

¹H NMR (400 MHz, CDCl₃) δ: 8.52 (s, 1H, CH arom.); 8.05-8.00 (m, 4H, CH arom.); 7.55-7.46 (m, 4H, CH arom.); 4.56 (d, *J*=6.0 Hz, 2H, OCH₂); 3.57 (t, *J*=6.4 Hz, 2H, OCH₂); 2.75-2.57 (m, 4H, NCH₂); 2.26-2.19 (m, 1H, CH); 1.79 (s, 2H, NH and OH); 1.55-1.42 (m, 4H, CH₂); 1.37-1.30 (m, 2H, CH₂); 1.08 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.13. (*R*) (*E*)-3-((7-hydroxyheptyl)amino)-2-methylpropyl 3-(3,4,5-trimethoxyphenyl)acrylate (*R*)-25

Starting from (*S*)-16 and 7-aminoheptan-1-ol [43], compound (*R*)-25 was obtained as an oil. Yield: 50%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.5.

¹H NMR (400 MHz, CDCl₃) δ: 7.58 (d, *J*=15.6 Hz, 1H, CH=CH); 6.75 (s, 2H, CH arom.); 6.34 (d, *J*=15.6 Hz, 1H, CH=CH); 4.14 (d, *J*=6.0 Hz, 2H, OCH₂); 3.90 (s, 6H, OCH₃); 3.88 (s, 3H, OCH₃); 3.61 (t, *J*=6.6 Hz, 2H, OCH₂); 2.68-2.50 (m, 4H, NCH₂); 2.10-2.06 (m, 1H, CH); 1.76 (s, 2H, NH and OH); 1.54-1.50 (m, 4H, CH₂); 1.45-1.26 (m, 6H, CH₂); 1.01 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.14. (*S*) (*E*)-3-((7-hydroxyheptyl)amino)-2-methylpropyl 3-(3,4,5-trimethoxyphenyl)acrylate (*S*)-25

Starting from (*R*)-16 and 7-aminoheptan-1-ol [43], compound (*S*)-25 was obtained as an oil. Yield: 25%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.5.

¹H NMR (400 MHz, CDCl₃) δ: 7.58 (d, *J*=16.0 Hz, 1H, CH=CH); 6.74 (s, 2H, CH arom.); 6.33 (d, *J*=16.0 Hz, 1H, CH=CH); 4.14 (dd, *J*=6.0 Hz, *J*=2.4 Hz, 2H, OCH₂); 3.86 (s, 6H, OCH₃); 3.85 (s, 3H, OCH₃); 3.60 (t, *J*=6.6 Hz, 2H, OCH₂); 2.65 (dd, *J*=12.0 Hz, *J*=6.8 Hz, 1H, NCHH); 2.58 (t, *J*=7.2 Hz, 2H, NCH₂); 2.51 (dd, *J*=12.0 Hz, *J*=6.8 Hz, 1H, NCHH); 2.11-2.03 (m, 1H, CH); 1.68 (s, 2H, NH and OH); 1.55-1.46 (m, 4H, CH₂); 1.44-1.27 (m, 6H, CH₂); 1.01 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.15. (*R*)-3-((7-hydroxyheptyl)amino)-2-methylpropyl 3,4,5-trimethoxybenzoate (*R*)-**26**

Starting from (*S*)-**17** and 7-aminoheptan-1-ol [43], compound (*R*)-**26** was obtained as an oil. Yield: 41%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.5.

¹H NMR (400 MHz, CDCl₃) δ: 7.21 (s, 2H, CH arom.); 4.19 (d, *J*=6.0 Hz, 2H, OCH₂); 3.82 (s, 9H, OCH₃); 3.50 (t, *J*=6.4 Hz, 2H, OCH₂); 3.44 (s, 2H, NH and OH); 2.70 (dd, *J*=12.4 Hz, *J*=6.8 Hz, 1H, NCHH); 2.64-2.55 (m, 3H, NCH₂ and NCHH); 2.26-2.18 (m, 1H, CH); 1.51-1.42 (m, 4H, CH₂); 1.32-1.18 (m, 6H, CH₂); 1.02 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.16. (*S*)-3-((7-hydroxyheptyl)amino)-2-methylpropyl 3,4,5-trimethoxybenzoate (*S*)-**26**

Starting from (*R*)-**17** and 7-aminoheptan-1-ol [43], compound (*S*)-**26** was obtained as an oil. Yield: 50%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 90:10:1.

¹H NMR (400 MHz, CDCl₃) δ: 7.22 (s, 2H, CH arom.); 4.18 (d, *J*=6.0 Hz, 2H, OCH₂); 3.83 (s, 9H, OCH₃); 3.53 (t, *J*=6.4 Hz, 2H, OCH₂); 2.62 (dd, *J*=12.0 Hz, *J*=6.8 Hz, 1H, NCHH); 2.54-2.46 (m, 3H, NCH₂ and NCHH); 2.12-2.09 (m, 3H, CH, NH and OH); 1.48-1.40 (m, 4H, CH₂); 1.34-1.20 (m, 6H, CH₂); 0.98 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.17. (*R*)-3-((7-hydroxyheptyl)amino)-2-methylpropyl anthracene-9-carboxylate (*R*)-**27**

Starting from (*S*)-**18** and 7-aminoheptan-1-ol [43], compound (*R*)-**27** was obtained as an oil. Yield: 45%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.5.

¹H NMR (400 MHz, CDCl₃) δ: 8.47 (s, 1H, CH arom.); 8.01-7.96 (m, 4H, CH arom.); 7.51-7.42 (m, 4H, CH arom.); 4.52 (d, *J*=5.6 Hz, 2H, OCH₂); 3.52 (t, *J*=6.4 Hz, 2H, OCH₂); 2.68 (dd, *J*=12.0 Hz, 6.8 Hz, 1H, NCHH); 2.57-2.51 (m, 3H, NCH₂ and NCHH); 2.23-2.17 (m, 1H, CH); 1.46-1.38 (m, 4H, CH₂); 1.28-1.15 (m, 6H, CH₂); 1.04 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.13.18. (*S*)-3-((7-hydroxyheptyl)amino)-2-methylpropyl anthracene-9-carboxylate (*S*)-**27**

Starting from (*R*)-**18** and 7-aminoheptan-1-ol [43], compound (*S*)-**27** was obtained as an oil. Yield: 56%. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.5.

¹H NMR (400 MHz, CDCl₃) δ: 8.46 (s, 1H, CH arom.); 8.02-7.95 (m, 4H, CH arom.); 7.51-7.42 (m, 4H, CH arom.); 4.52 (d, *J*=6.0 Hz, 2H, OCH₂); 3.53 (t, *J*=6.4 Hz, 2H, OCH₂); 2.68 (dd, *J*=11.6 Hz, *J*=6.8 Hz, 1H, NCHH); 2.56-2.50 (m, 3H, NCH₂ and NCHH); 2.22-2.15 (m, 1H, CH); 1.97 (s, 2H, NH and OH); 1.48-1.36 (m, 4H, CH₂); 1.27-1.15 (m, 6H, CH₂); 1.04 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.14. General procedure for the synthesis of the (*S*) and (*R*) enantiomers of the (hydroxyalkyl) methylaminoesters **28-36**

A 1 mmol portion of the appropriate hydroxyaminoester ((*R*) and (*S*) **19-27**) was dissolved in 5 mL of anhydrous ethanol and HCOOH (17 mmol) and 37% HCHO solution (5 mmol) were added. The mixture was heated to 80 °C for 4-5 h and concentrated in vacuo. The residue was then dissolved in CH₂Cl₂ and the organic layer was washed with 10% NaOH solution. After drying with Na₂SO₄, the solvent was removed under reduced pressure and the residue purified by flash chromatography, yielding a yellow oil.

6.1.14.1. (*R*)-3-((3-hydroxypropyl)(methyl)amino)-2-methylpropyl (*E*)-3-(3,4,5-trimethoxyphenyl) acrylate (*R*)-**28**

From (*R*)-**19**. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.5. Yield: 64%.

¹H NMR (400 MHz, CDCl₃) δ: 7.57 (d, *J*=15.6 Hz, 1H, CH=CH); 6.73 (s, 2H, CH arom.); 6.33 (d, *J*=15.6 Hz, 1H, CH=CH); 4.20-4.13 (m, 1H, OCHH); 4.08-3.98 (m, 1H, OCHH); 3.86 (s, 6H, OCH₃); 3.85 (s, 3H, OCH₃); 3.77 (t, *J*=5.2 Hz, 2H, OCH₂); 2.61 (t, *J*=5.6 Hz, 2H, NCH₂); 2.44-2.35 (m, 1H, NCHH); 2.34-2.25 (m, 1H, NCHH); 2.29 (s, 3H, NCH₃); 2.18-2.06 (m, 1H, CH); 1.76-1.63 (m, 2H, CH₂); 1.02 (d, *J* = 6.8 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 166.94 (C=O); 153.43 (C); 144.87 (CH=CH); 140.19 (C); 129.87 (C); 117.18 (CH=CH); 105.33 (CH arom.); 67.80 (CH₂); 63.88 (CH₂); 62.05 (CH₂); 60.92 (OCH₃); 58.54 (CH₂); 56.17 (OCH₃); 42.08 (NCH₃); 30.97 (CH); 27.85 (CH₂); 15.65 (CH₃) ppm.

6.1.14.2. *(S)*-3-((3-hydroxypropyl)(methylamino)-2-methylpropyl (*E*)-3-(3,4,5-trimethoxyphenyl) acrylate (*S*)-**28**

From (*S*)-**19**. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.5. Yield: 79%.

¹H NMR (400 MHz, CDCl₃) δ: 7.60 (d, *J*=16.0 Hz, 1H, CH=CH); 6.76 (s, 2H, CH arom.); 6.35 (d, *J*=16.0 Hz, 1H, CH=CH); 4.22-4.16 (m, 1H, OCHH); 4.10-4.02 (m, 1H, OCHH); 3.89 (s, 6H, OCH₃); 3.87 (s, 3H, OCH₃); 3.81 (t, *J*=5.2 Hz, 2H, OCH₂); 2.73-2.65 (m, 2H, NCH₂); 2.51-2.44 (m, 1H, NCHH); 2.43-2.36 (m, 1H, NCHH); 2.35 (s, 3H, NCH₃); 2.26-2.14 (m, 1H, CH); 1.81-1.70 (m, 2H, CH₂); 1.06 (d, *J*=6.4 Hz, 3H, CH₃) ppm

¹³C APT NMR (CDCl₃) δ: 166.95 (C=O); 153.43 (C); 144.87 (CH=CH); 140.16 (C); 129.88 (C); 117.19 (CH=CH); 105.30 (CH arom.); 67.84 (CH₂); 64.03 (CH₂); 62.08 (CH₂); 60.95 (OCH₃); 58.70 (CH₂); 56.17 (OCH₃); 42.12 (NCH₃); 30.99 (CH); 27.84 (CH₂); 15.70 (CH₃) ppm.

6.1.14.3. *(R)*-3-((3-hydroxypropyl)(methylamino)-2-methylpropyl 3,4,5-trimethoxybenzoate (*R*)-**29**

From (*R*)-**20**. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.5. Yield: 82%.

¹H NMR (400 MHz, CDCl₃) δ: 7.28 (s, 2H, CH arom.); 4.28 (dd, *J*=10.8 Hz, *J*=5.2 Hz, 1H, OCHH); 4.13 (dd, *J*=10.8 Hz, *J*=6.0 Hz, 1H, OCHH); 3.88 (s, 9H, OCH₃); 3.77 (t, *J*=5.2 Hz, 2H, OCH₂); 2.57 (t, *J*=5.2 Hz, 2H, NCH₂); 2.39-2.35 (m, 1H, NCHH); 2.28-2.16 (m, 2H, NCHH and CH); 2.24 (s, 3H, NCH₃); 1.71-1.67 (m, 2H, CH₂); 1.02 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

6.1.14.4. *(S)*-3-((3-hydroxypropyl)(methylamino)-2-methylpropyl 3,4,5-trimethoxybenzoate (*S*)-**29**

From (*S*)-**20**. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 95:5:0.5. Yield: 84%.

¹H NMR (400 MHz, CDCl₃) δ: 7.23 (s, 2H, CH arom.); 4.23 (dd, *J*=10.8 Hz, *J*=5.2 Hz, 1H, OCHH); 4.07 (dd, *J*=10.8 Hz, *J*=6.4 Hz, 1H, OCHH); 3.83 (s, 9H, OCH₃); 3.71 (t, *J*=5.2 Hz, 2H, CH₂); 2.52 (t, *J*=5.6 Hz, 2H, CH₂); 2.32 (dd, *J*=12.0 Hz, *J*=6.4 Hz, 1H, NCHH); 2.23-2.11 (m, 2H, NCHH and CH); 2.19 (s, 3H, NCH₃); 1.65-1.62 (m, 2H, CH₂); 0.97 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

6.1.14.5. *(R)*-3-((3-hydroxypropyl)(methylamino)-2-methylpropyl anthracene-9-carboxylate (*R*)-**30**

From (*R*)-**21**. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 90:10:1. Yield: 98%.

¹H NMR (400 MHz, CDCl₃) δ: 8.52 (s, 1H, CH arom.); 8.06-8.01 (m, 4H, CH arom.); 7.56-7.47 (m, 4H, CH arom.); 4.61 (dd, *J*=10.8 Hz, *J*=6.8 Hz, 1H, OCHH); 4.48 (dd, *J*=10.8 Hz, *J*=5.2 Hz, 1H, OCHH); 3.80 (t, *J*=5.2 Hz, 2H, OCH₂); 2.58 (t, *J*=5.2 Hz, 2H, NCH₂); 2.46-2.42 (m, 1H, CH); 2.35-2.26 (m, 2H, NCH₂); 2.27 (s, 3H, NCH₃); 1.72-1.68 (m, 2H, CH₂); 1.10 (d, *J*=5.6 Hz, 3H, CH₃) ppm.

6.1.14.6. *(S)*-3-((3-hydroxypropyl)(methylamino)-2-methylpropyl anthracene-9-carboxylate (*S*)-**30**

From (*S*)-**21**. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 90:10:1. Yield: 92%.

¹H NMR (400 MHz, CDCl₃) δ: 8.48 (s, 1H, CH arom.); 8.00 (m, 4H, CH arom.); 7.52-7.43 (m, 4H, CH arom.); 4.58 (dd, *J*=10.8 Hz, *J*=4.8 Hz, 1H, OCHH); 4.45 (dd, *J*=10.8 Hz, *J*=5.6 Hz, 1H, OCHH); 3.77 (t, *J*=5.2 Hz, 2H, OCH₂); 2.53 (t, *J*=6.0 Hz, 2H, NCH₂); 2.32 (dd, *J*=15.2 Hz, *J*=10.0 Hz, 1H, NCHH); 2.26-2.23 (m, 2H, NCHH and CH); 2.23 (s, 3H, NCH₃); 1.69-1.63 (m, 2H, CH₂); 1.07 (d, *J*=6.0 Hz, 3H, CH₃) ppm.

6.1.14.7. *(R)*-3-((5-hydroxypentyl)(methylamino)-2-methylpropyl (*E*)-3-(3,4,5-trimethoxyphenyl) acrylate (*R*)-**31**

From (*R*)-**22**. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 90:10:1. Yield: 93%.

¹H NMR (400 MHz, CDCl₃) δ: 7.57 (d, *J*=15.6 Hz, 1H, CH=CH); 6.74 (s, 2H, CH arom.); 6.33 (d, *J*=16.0 Hz, 1H, CH=CH); 4.28-4.21 (m, 1H, OCHH); 4.06-3.98 (m, 1H, OCHH); 3.87 (s, 6H, OCH₃); 3.86 (s, 3H, OCH₃); 3.61 (t, *J*=6.4 Hz, 2H, OCH₂); 2.36-2.26 (m, 3H, NCH₂ and NCHH); 2.18 (s, 3H, NCH₃) ppm.

3H, NCH₃); 2.17-2.10 (m, 1H, NCHH); 2.09-2.01 (m, 1H, CH); 1.98 (bs, 1H, OH); 1.60-1.52 (m, 2H, CH₂); 1.51-1.41 (m, 2H, CH₂); 1.41-1.32 (m, 2H, CH₂); 0.98 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 167.16 (C=O); 153.43 (C); 144.62 (CH=CH); 139.81 (C); 129.94 (C); 117.44 (CH=CH); 105.27 (CH arom.); 68.18 (CH₂); 62.83 (CH₂); 61.32 (CH₂); 60.95 (OCH₃); 58.10 (CH₂); 56.18 (OCH₃); 42.74 (NCH₃); 32.55 (CH₂); 31.27 (CH); 26.99 (CH₂); 23.47 (CH₂); 15.91 (CH₃) ppm.

6.1.14.8. (S)-3-((5-hydroxypentyl)(methylamino)-2-methylpropyl (E)-3-(3,4,5-trimethoxyphenyl) acrylate (S)-31

From (S)-22. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 90:10:1. Yield: 94%.

¹H NMR (400 MHz, CDCl₃) δ: 7.58 (d, *J*=15.6 Hz, 1H, CH=CH); 6.74 (s, 2H, CH arom.); 6.34 (d, *J*=16.0 Hz, 1H, CH=CH); 4.28-4.19 (m, 1H, OCHH); 4.07-3.98 (m, 1H, OCHH); 3.87 (s, 6H, OCH₃); 3.86 (s, 3H, OCH₃); 3.61 (t, *J*=6.4 Hz, 2H, OCH₂); 2.37-2.25 (m, 3H, NCH₂ and NCHH); 2.19 (s, 3H, NCH₃); 2.18-2.11 (m, 1H, NCHH); 2.10-2.01 (m, 1H, CH); 1.60-1.50 (m, 2H, CH₂); 1.50-1.42 (m, 2H, CH₂); 1.41-1.32 (m, 2H, CH₂); 0.99 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

6.1.14.9. (R)-3-((5-hydroxypentyl)(methylamino)-2-methylpropyl 3,4,5-trimethoxybenzoate (R)-32

From (R)-23. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 90:10:1. Yield: 84%.

¹H NMR (400 MHz, CDCl₃) δ: 7.28 (s, 2H, CH arom.); 4.33 (dd, *J*=10.8 Hz, *J*=4.4 Hz, 1H, OCHH); 4.11 (dd, *J*=10.8 Hz, *J*=6.4 Hz, 1H, OCHH); 3.88 (s, 9H, OCH₃); 3.59 (t, *J*=6.4 Hz, 2H, OCH₂); 2.32-2.22 (m, 3H, NCH₂ and NCHH); 2.18 (s, 3H, NCH₃); 2.16-2.11 (m, 2H, NCHH and CH); 1.54-1.41 (m, 4H, CH₂); 1.38-1.34 (m, 2H, CH₂); 1.00 (d, *J*=6.0 Hz, 3H, CH₃) ppm.

6.1.14.10. (S)-3-((5-hydroxypentyl)(methylamino)-2-methylpropyl 3,4,5-trimethoxybenzoate (S)-32

From (S)-23. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 90:10:1. Yield: 88%.

¹H NMR (400 MHz, CDCl₃) δ: 7.23 (s, 2H, CH arom.); 4.28 (dd, *J*=10.8 Hz, *J*=4.8 Hz, 1H, OCHH); 4.06 (dd, *J*=10.8 Hz, *J*=6.4 Hz, 1H, OCHH); 3.83 (s, 9H, OCH₃); 3.53 (t, *J*=6.4 Hz, 2H, OCH₂); 2.46 (s, 1H, OH); 2.31-2.25 (m, 3H, NCH₂ and NCHH); 2.14 (s, 3H, NCH₃); 2.13-2.05 (m, 2H, NCHH and CH); 1.51-1.37 (m, 4H, CH₂); 1.33-1.27 (m, 2H, CH₂); 0.96 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

6.1.14.11. (R)-3-((5-hydroxypentyl)(methylamino)-2-methylpropyl anthracene-9-carboxylate (R)-33

From (R)-24. Chromatographic eluent: CH₂Cl₂/CH₃OH 90:10. Yield: 99%.

¹H NMR (400 MHz, CDCl₃) δ: 8.51 (s, 1H, CH arom.); 8.07-8.00 (m, 4H, CH arom.); 7.55-7.46 (m, 4H, CH arom.); 4.66 (dd, *J*=10.8 Hz, *J*=4.4 Hz, 1H, OCHH), 4.48 (dd, *J*=10.8 Hz, *J*=6.4 Hz, 1H, OCHH); 3.59 (t, *J*=6.4 Hz, 2H, OCH₂); 2.41-2.30 (m, 3H, NCH₂ and NCHH); 2.23 (s, 3H, NCH₃); 2.20-2.16 (m, 2H, NCHH and CH); 1.56-1.43 (m, 4H, CH₂); 1.39-1.33 (m, 2H, CH₂); 1.06 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

6.1.14.12. (S)-3-((5-hydroxypentyl)(methylamino)-2-methylpropyl anthracene-9-carboxylate (S)-33

From (S)-24. Chromatographic eluent: CH₂Cl₂/CH₃OH 90:10. Yield: 95%.

¹H NMR (400 MHz, CDCl₃) δ: 8.51 (s, 1H, CH arom.); 8.07-8.00 (m, 4H, CH arom.); 7.55-7.46 (m, 4H, CH arom.); 4.66 (dd, *J*=10.8 Hz, *J*=4.4 Hz, 1H, OCHH), 4.48 (dd, *J*=10.8 Hz, *J*=6.0 Hz, 1H, OCHH); 3.59 (t, *J*=6.4 Hz, 2H, OCH₂); 2.42-2.25 (m, 3H, NCH₂ and NCHH); 2.22 (s, 3H, NCH₃); 2.20-2.16 (m, 2H, NCHH and CH); 1.57-1.44 (m, 4H, CH₂); 1.39-1.33 (m, 2H, CH₂); 1.06 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

6.1.14.133. (R)-3-((7-hydroxyheptyl)(methylamino)-2-methylpropyl (E)-3-(3,4,5-trimethoxyphenyl) acrylate (R)-34

From (R)-25. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 90:10:1. Yield: 89%.

¹H NMR (400 MHz, CDCl₃) δ: 7.58 (d, *J*=16.0 Hz, 1H, CH=CH); 6.73 (s, 2H, CH arom.); 6.34 (d, *J*=16.0 Hz, 1H, CH=CH); 4.12 (m, 2H, OCH₂); 3.88 (s, 6H, OCH₃); 3.86 (s, 3H, OCH₃); 3.61 (t,

$J=6.6$ Hz, 2H, OCH₂); 2.32-2.27 (m, 3H, NCH₂ and NCHH); 2.18 (s, 3H, NCH₃); 2.19-2.00 (m, 2H, NCHH and CH); 1.53 (s, 2H, NH and OH); 1.45-1.36 (m, 4H, CH₂); 1.34-1.18 (m, 6H, CH₂); 0.98 (d, $J=6.4$ Hz, 3H, CH₃) ppm.

6.1.14.14. (*S*)-3-((7-hydroxyheptyl)(methyl)amino)-2-methylpropyl (*E*)-3-(3,4,5-trimethoxyphenyl) acrylate (*S*)-**34**

From (*S*)-**25**. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 90:10:1. Yield: 97%.

¹H NMR (400 MHz, CDCl₃) δ: 7.58 (d, $J=16.0$ Hz, 1H, CH=CH); 6.73 (s, 2H, CH arom.); 6.34 (d, $J=16.0$ Hz, 1H, CH=CH); 4.12 (m, 2H, OCH₂), 3.87 (s, 6H, OCH₃); 3.85 (s, 3H, OCH₃); 3.61 (t, $J=6.6$ Hz, 2H, OCH₂); 2.32-2.27 (m, 3H, NCH₂ and NCHH); 2.18 (s, 3H, NCH₃); 2.19-2.00 (m, 2H, NCHH and CH); 1.55-1.51 (m, 2H, CH₂); 1.50-1.40 (m, 2H, CH₂); 1.28-1.15 (m, 6H, CH₂); 0.98 (d, $J=6.4$ Hz, 3H, CH₃) ppm.

6.1.14.15. (*R*)-3-((7-hydroxyheptyl)(methyl)amino)-2-methylpropyl 3,4,5-trimethoxybenzoate (*R*)-**35**

From (*R*)-**26**. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 90:10:1. Yield: 91%.

¹H NMR (400 MHz, CDCl₃) δ: 7.21 (s, 2H, CH arom.); 4.25 (dd, $J=10.4$ Hz, $J=4.4$ Hz, 1H, OCHH); 4.04 (dd, $J=10.8$ Hz, $J=6.4$ Hz, 1H, OCHH); 3.81 (s, 9H, OCH₃); 3.50 (t, $J=6.8$ Hz, 2H, OCH₂); 2.29-2.19 (m, 4H, NCHH, CH₂ and OH); 2.14-2.03 (m, 2H, NCHH and CH); 2.11 (s, 3H, NCH₃); 1.45-1.34 (m, 4H, CH₂); 1.26-1.12 (m, 6H, CH₂); 0.93 (d, $J=6.4$ Hz, 3H, CH₃) ppm.

6.1.14.16. (*S*)-3-((7-hydroxyheptyl)(methyl)amino)-2-methylpropyl 3,4,5-trimethoxybenzoate (*S*)-**35**

From (*S*)-**26**. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 90:10:1. Yield: 97%.

¹H NMR (400 MHz, CDCl₃) δ: 7.24 (s, 2H, CH arom.); 4.29 (dd, $J=10.8$ Hz, 4.4 Hz, 1H, OCHH); 4.07 (dd, $J=10.8$ Hz, $J=6.4$ Hz, 1H, OCHH); 3.84 (s, 9H, OCH₃); 3.55 (t, $J=6.8$ Hz, 2H, OCH₂); 2.32-2.23 (m, 3H, NCHH and CH₂); 2.17-2.06 (m, 2H, NCHH and CH); 2.15 (s, 3H, NCH₃); 1.50-1.38 (m, 4H, CH₂); 1.28-1.15 (m, 6H, CH₂); 0.97 (d, $J=6.4$ Hz, 3H, CH₃) ppm.

6.1.14.17. (*R*)-3-((7-hydroxyheptyl)(methyl)amino)-2-methylpropyl anthracene-9-carboxylate (*R*)-**36**

From (*R*)-**27**. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 93:7:0.3. Yield: 90%.

¹H NMR (400 MHz, CDCl₃) δ: 8.48 (s, 1H, CH arom.); 8.04-7.97 (m, 4H, CH arom.); 7.52-7.43 (m, 4H, CH arom.); 4.61 (dd, $J=10.4$ Hz, $J=4.0$ Hz, 1H, OCHH); 4.46 (dd, $J=10.8$ Hz, $J=6.4$ Hz, 1H, OCHH); 3.57 (t, $J=6.8$ Hz, 2H, OCH₂); 2.38-2.27 (m, 3H, NCHH and CH₂); 2.19 (s, 3H, NCH₃); 2.19-2.13 (m, 2H, NCHH and CH); 1.50-1.42 (m, 4H, CH₂); 1.34-1.20 (m, 6H, CH₂); 1.04 (d, $J=6.4$ Hz, 3H, CH₃) ppm.

6.1.14.18. (*S*)-3-((7-hydroxyheptyl)(methyl)amino)-2-methylpropyl anthracene-9-carboxylate (*S*)-**36**

From (*S*)-**27**. Chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 90:10:1. Yield: 92%.

¹H NMR (400 MHz, CDCl₃) δ: 8.47 (s, 1H, CH arom.); 8.05-7.96 (m, 4H, CH arom.); 7.52-7.43 (m, 4H, CH arom.); 4.63 (dd, $J=10.8$ Hz, $J=4.4$ Hz, 1H, OCHH); 4.46 (dd, $J=10.8$ Hz, $J=6.4$ Hz, 1H, OCHH); 3.56 (t, $J=6.8$ Hz, 2H, OCH₂); 2.37-2.27 (m, 3H, NCHH and CH₂); 2.19 (s, 3H, NCH₃); 2.19-2.14 (m, 2H, NCHH and CH); 1.95 (s, 1H, OH); 1.50-1.41 (m, 4H, CH₂); 1.32-1.18 (m, 6H, CH₂); 1.04 (d, $J=6.0$ Hz, 3H, CH₃) ppm.

6.1.15. General procedures for the synthesis of the (*S*) and (*R*) enantiomers of the diesters **1-12**

Diester compounds were synthesized using two different general procedures:

General procedure A.

A solution of the suitable (hydroxyalkyl)methylaminoester (0.250 mmol) in 5 mL of an. CH₂Cl₂ was cooled at 0°C and 0.360 mmol of the appropriate carboxylic acid ((*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid or 3,4,5-trimethoxybenzoic acid), 0.083 mmol of 4-dimethylaminopyridine (DMAP) and 0.500 mmol of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) were added. The reaction mixture was stirred for 1 h at 0 °C and 48 h at room

temperature. Then CH₂Cl₂ was added and the organic layer was washed twice with a saturated solution of NaHCO₃. After drying with Na₂SO₄, the solution was concentrated to dryness and the crude product was purified by flash chromatography with the eluting systems described below. The pure compounds were transformed into the corresponding white solid hydrochloride salts that were crystallized from abs. ethanol/petroleum ether.

General procedure B.

A 0.250 mmol portion of the appropriate carboxylic acid ((*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid or anthracene-9-carboxylic acid) was transformed into the acyl chloride by reaction with SOCl₂ (2 eq) in 3 mL of CHCl₃ (free of ethanol) at 60 °C for 4-5 h.

After cooling to room temperature, the solution was concentrated to dryness; the mixture was washed twice with cyclohexane and then organic layer was removed by a rotary evaporation. The acyl chloride obtained was dissolved in CHCl₃ (free of ethanol), and the appropriate (hydroxyalkyl)methylaminoester (1 eq) was added. The mixture was heated to 60 °C for 4 h, cooled to room temperature and then CH₂Cl₂ was added. The organic phase was washed with 10% NaOH solution, dried with Na₂SO₄ and then concentrated to dryness. The crude products obtained were purified by flash chromatography with the eluting systems described below, and the desired compounds were obtained as oil.

All the compounds were transformed into the corresponding hydrochloride as white solid. The salts were crystallized from abs. ethanol/petroleum ether.

6.1.15.1. (*R*) (*E*)-3-(methyl(2-methyl-3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)propyl 3,4,5-trimethoxybenzoate (*R*)-**1**

Procedure A, starting from (*R*)-**28** and 3,4,5-trimethoxybenzoic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 98:2:0.2. Yield: 74%.

¹H NMR (400 MHz, CDCl₃) δ: 7.56 (d, *J*=16.0 Hz, 1H, CH=CH); 7.26 (s, 2H, CH arom.); 6.72 (s, 2H, CH arom.); 6.31 (d, *J*=16.0 Hz, 1H, CH=CH); 4.36 (t, *J*=6.4 Hz, 2H, OCH₂); 4.22 (dd, *J*=10.8 Hz, *J*=5.2 Hz, 1H, OCHH); 4.03 (dd, *J*=10.8 Hz, *J*=6.4 Hz, 1H, OCHH); 3.86 (s, 9H, OCH₃); 3.85 (s, 6H, OCH₃); 3.84 (s, 3H, OCH₃); 2.63-2.50 (m, 2H, NCH₂); 2.49-2.38 (m, 1H, NCHH); 2.30 (s, 3H, NCH₃); 2.29-2.20 (m, 1H, NCHH); 2.18-2.03 (m, 1H, CH); 2.02-1.90 (m, 2H, CH₂); 1.01 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 167.00 (C=O); 166.16 (C=O); 153.41 (C); 152.89 (C); 144.61 (CH=CH); 142.15 (C); 140.09 (C); 129.90 (C); 125.42 (C); 117.34 (CH=CH); 106.78 (CH arom.); 105.24 (CH arom.); 67.96 (CH₂); 63.47 (CH₂); 61.34 (CH₂); 60.92 (OCH₃); 60.85 (OCH₃); 56.19 (OCH₃); 56.14 (OCH₃); 54.59 (CH₂); 42.64 (NCH₃); 31.34 (CH); 26.76 (CH₂); 15.79 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₀H₄₂NO₁₀= 576.2803, found 576.2797.

Hydrochloride: mp 50-55 °C.

6.1.15.2. (*S*) (*E*)-3-(methyl(2-methyl-3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)propyl 3,4,5-trimethoxybenzoate (*S*)-**1**

Procedure A, starting from (*S*)-**28** and 3,4,5-trimethoxybenzoic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 98:2:0.2. Yield: 61%.

¹H NMR (400 MHz, CDCl₃) δ: 7.55 (d, *J*=16.0 Hz, 1H, CH=CH); 7.25 (s, 2H, CH arom.); 6.72 (s, 2H, CH arom.); 6.31 (d, *J*=16.0 Hz, 1H, CH=CH); 4.34 (t, *J*=6.8 Hz, 2H, OCH₂); 4.22 (dd, *J*=10.8 Hz, *J*=4.8 Hz, 1H, OCHH); 4.00 (dd, *J*=10.8 Hz, *J*=6.8 Hz, 1H, OCHH); 3.86 (s, 9H, OCH₃); 3.85 (s, 6H, OCH₃); 3.84 (s, 3H, OCH₃); 2.50-2.44 (m, 2H, NCH₂); 2.33 (dd, *J*=12.0 Hz, *J*=7.6 Hz, 1H, NCHH); 2.22 (s, 3H, NCH₃); 2.19-2.14 (m, 1H, NCHH); 2.10-2.01 (m, 1H, CH); 1.93-1.86 (m, 2H, CH₂); 0.97 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 166.96 (C=O); 166.17 (C=O); 153.42 (C); 152.91 (C); 144.79 (CH=CH); 142.22 (C); 140.14 (C); 129.85 (C); 125.28 (C); 117.18 (CH=CH); 106.81 (CH arom.); 105.26 (CH

arom.); 67.79 (CH₂); 63.25 (CH₂); 61.10 (CH₂); 60.93 (OCH₃); 60.87 (OCH₃); 56.22 (OCH₃); 56.15 (OCH₃); 54.55 (CH₂); 42.44 (NCH₃); 31.12 (CH); 26.39 (CH₂); 15.89 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₀H₄₂NO₁₀= 576.2803, found 576.2804.

Hydrochloride: mp 46-50 °C.

6.1.15.3. (R) (E)-3-(methyl(2-methyl-3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)propyl anthracene-9-carboxylate (R)-2

Procedure B, starting from (R)-**28** and anthracene-9-carboxylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 98:2:0.2. Yield: 37%.

¹H NMR (400 MHz, CDCl₃) δ: 8.50 (s, 1H, CH arom.); 8.04-7.99 (m, 4H, CH arom.); 7.56 (d, *J*=16.0 Hz, 1H, CH=CH); 7.53-7.45 (m, 4H, CH arom.); 6.69 (s, 2H, CH arom.); 6.32 (d, *J*=16.0 Hz, 1H, CH=CH); 4.69 (t, *J*=6.4 Hz, 2H, OCH₂); 4.25 (dd, *J*=10.8 Hz, *J*=4.8 Hz, 1H, OCHH); 4.08 (dd, *J*=10.8 Hz, *J*=6.0 Hz, 1H, OCHH); 3.86 (s, 3H, OCH₃); 3.82 (s, 6H, OCH₃); 2.72-2.58 (m, 2H, NCH₂); 2.50-2.40 (m, 1H, NCHH); 2.31 (s, 3H, NCH₃); 2.30-2.22 (m, 1H, NCHH); 2.20-2.03 (m, 3H, CH₂ and CH); 1.04 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 169.69 (C=O); 167.10 (C=O); 153.37 (C); 144.65 (CH=CH); 142.65 (C); 130.98 (C); 129.92 (C); 129.23 (CH arom.); 128.61 (CH arom.); 128.38 (C); 128.08 (C); 126.93 (CH arom.); 125.46 (CH arom.); 125.00 (CH arom.); 117.38 (CH=CH); 105.21 (CH arom.); 67.86 (CH₂); 64.16 (CH₂); 61.25 (CH₂); 60.95 (OCH₃); 56.11 (OCH₃); 54.78 (CH₂); 42.57 (NCH₃); 31.30 (CH); 26.75 (CH₂); 15.83 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₅H₄₀NO₇= 586.2799, found 586.2791.

Hydrochloride: mp 87-92 °C.

6.1.15.4. (S) (E)-3-(methyl(2-methyl-3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)propyl anthracene-9-carboxylate (S)-2

Procedure B, starting from (S)-**28** and anthracene-9-carboxylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 98:2:0.2. Yield: 32%.

¹H NMR (400 MHz, CDCl₃) δ: 8.50 (s, 1H, CH arom.); 8.03 (d, *J*=8.8 Hz, 2H, CH arom.); 8.00 (d, *J*=8.8 Hz, 2H, CH arom.); 7.56 (d, *J*=16.0 Hz, 1H, CH=CH); 7.53-7.45 (m, 4H, CH arom.); 6.69 (s, 2H, CH arom.); 6.33 (d, *J*=16.0 Hz, 1H, CH=CH); 4.69 (t, *J*=6.4 Hz, 2H, OCH₂); 4.26 (dd, *J*=10.8 Hz, *J*=4.8 Hz, 1H, OCHH); 4.08 (dd, *J*=10.8 Hz, *J*=6.0 Hz, 1H, OCHH); 3.86 (s, 3H, OCH₃); 3.82 (s, 6H, OCH₃); 2.56 (t, *J*=6.8 Hz, 2H, NCH₂); 2.41 (dd, *J*=12.4 Hz, *J*=7.6 Hz, 1H, NCHH); 2.27 (s, 3H, NCH₃); 2.22 (dd, *J*=12.4 Hz, *J*=7.2 Hz, 1H, NCHH); 2.12-2.02 (m, 3H, NCH₂ and CH); 1.02 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 169.59 (C=O); 167.01 (C=O); 153.38 (C); 144.88 (CH=CH); 142.55 (C); 130.96 (C); 129.84 (C); 129.33 (CH arom.); 128.64 (CH arom.); 128.38 (C); 128.25 (C); 127.00 (CH arom.); 125.49 (CH arom.); 124.93 (CH arom.); 117.15 (CH=CH); 105.24 (CH arom.); 67.61 (CH₂); 63.86 (CH₂); 60.96 (OCH₃); 60.92 (CH₂); 56.12 (OCH₃); 54.72 (CH₂); 42.30 (NCH₃); 30.99 (CH); 26.22 (CH₂); 15.94 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₅H₄₀NO₇= 586.2799, found 586.2792.

Hydrochloride: mp 80-82 °C.

6.1.15.5. (R) (E)-2-methyl-3-(methyl(3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)propyl 3,4,5-trimethoxybenzoate (R)-3

Procedure A, starting from (R)-**29** and ((E)-3-(3,4,5-trimethoxyphenyl)acrylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH 96:4. Yield: 86%.

¹H NMR (400 MHz, CDCl₃) δ: 7.57 (d, *J*=16.0 Hz, 1H, CH=CH); 7.28 (s, 2H, CH arom.); 6.74 (s, 2H, CH arom.); 6.32 (d, *J*=16.0 Hz, 1H, CH=CH); 4.36 (dd, *J*=10.4 Hz, *J*=4.8 Hz, 1H, OCHH); 4.25 (t, *J*=6.4 Hz, 2H, OCH₂); 4.14 (dd, *J*=10.4 Hz, *J*=6.4 Hz, 1H, OCHH); 3.88 (s, 9H, OCH₃); 3.87 (s, 9H, OCH₃); 2.58-2.35 (m, 3H, NCHH and NCH₂); 2.25 (s, 3H, NCH₃); 2.24-2.10 (m, 2H, NCHH and CH); 1.90-1.80 (m, 2H, CH₂); 1.03 (d, *J*=6.0 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 166.96 (C=O); 166.22 (C=O); 153.43 (C); 152.93 (C); 144.70 (CH=CH); 129.90 (C); 125.47 (C); 117.32 (CH=CH); 106.78 (CH arom.); 105.23 (CH arom.); 68.44 (CH₂); 62.87 (CH₂); 61.32 (CH₂); 60.97 (OCH₃); 60.91 (OCH₃); 56.21 (OCH₃); 56.16 (OCH₃); 54.74 (CH₂); 42.66 (NCH₃); 31.41 (CH); 26.73 (CH₂); 15.90 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₀H₄₂NO₁₀= 576.2803, found 576.2802.

Hydrochloride: mp 49-53 °C.

6.1.15.6. (S) (E)-2-methyl-3-(methyl(3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)propyl 3,4,5-trimethoxybenzoate (S)-3

Procedure A, starting from (S)-29 and ((E)-3-(3,4,5-trimethoxyphenyl)acrylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH 96:4. Yield: 81%.

¹H NMR (400 MHz, CDCl₃) δ: 7.54 (d, *J*=16.0 Hz, 1H, CH=CH); 7.25 (s, 2H, CH arom.); 6.71 (s, 2H, CH arom.); 6.28 (d, *J*=16.0 Hz, 1H, CH=CH); 4.32 (dd, *J*=10.8 Hz, *J*=4.8 Hz, 1H, OCHH); 4.22 (t, *J*=6.8 Hz, 2H, OCH₂); 4.11 (dd, *J*=10.8 Hz, *J*=6.4 Hz, 1H, OCHH); 3.85 (s, 9H, OCH₃); 3.84 (s, 6H, OCH₃); 3.83 (s, 3H, OCH₃); 2.44 (t, *J*=6.8 Hz, 2H, NCH₂); 2.35 (dd, *J*=11.6 Hz, *J*=7.2 Hz, 1H, NCHH); 2.21 (s, 3H, NCH₃); 2.19-2.10 (m, 2H, NCHH and CH); 1.86-1.79 (m, 2H, CH₂); 0.99 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 166.91 (C=O); 166.19 (C=O); 153.45 (C); 152.94 (C); 144.65 (CH=CH); 140.22 (C); 129.91 (C); 125.49 (C); 117.35 (CH=CH); 106.88 (CH arom.); 105.34 (CH arom.); 68.43 (CH₂); 62.87 (CH₂); 61.37 (CH₂); 60.92 (OCH₃); 60.86 (OCH₃); 56.21 (OCH₃); 56.17 (OCH₃); 54.76 (CH₂); 42.66 (NCH₃); 31.47 (CH); 26.78 (CH₂); 15.86 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₀H₄₂NO₁₀= 576.2803, found 576.2805.

Hydrochloride: mp 50-55 °C.

6.1.15.7. (R) (E)-2-methyl-3-(methyl(3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)propyl anthracene-9-carboxylate (R)-4

Procedure A, starting from (R)-30 and ((E)-3-(3,4,5-trimethoxyphenyl)acrylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH 98:2. Yield: 67%.

¹H NMR (400 MHz, CDCl₃) δ: 8.49 (s, 1H, CH arom.); 8.04 (d, *J*=8.8 Hz, 2H, CH arom.); 8.00 (d, *J*=8.8 Hz, 2H, CH arom.); 7.56 (d, *J*=16.0 Hz, 1H, CH=CH); 7.52-7.44 (m, 4H, CH arom.); 6.68 (s, 2H, CH arom.); 6.31 (d, *J*=16.0 Hz, 1H, CH=CH); 4.66 (dd, *J*=10.8 Hz, *J*=4.0 Hz, 1H, OCHH); 4.50 (dd, *J*=10.8 Hz, *J*=5.6 Hz, 1H, OCHH); 4.27 (t, *J*=6.0 Hz, 2H, OCH₂); 3.86 (s, 3H, OCH₃); 3.81 (s, 6H, OCH₃); 2.60-2.39 (m, 3H, NCHH and NCH₂); 2.28 (s, 3H, NCH₃); 2.27-2.17 (m, 2H, NCHH and CH); 1.98-1.82 (m, 2H, CH₂); 1.09 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 169.75 (C=O); 167.01 (C=O); 153.37 (C); 144.74 (CH=CH); 140.32 (C); 130.99 (C); 129.81 (C); 129.20 (CH=CH); 128.60 (CH arom); 128.41 (C); 126.90 (CH arom); 125.46 (CH arom); 125.06 (CH arom); 117.29 (CH=CH); 105.26 (CH arom.); 69.12 (CH₂); 62.64 (CH₂); 61.18 (CH₂); 60.93 (OCH₃); 56.10 (OCH₃); 54.49 (CH₂); 42.60 (NCH₃); 31.21 (CH); 26.65 (CH₂); 16.04 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₅H₄₀NO₇= 586.2799, found 586.2793.

Hydrochloride: mp 96-99 °C.

6.1.15.8. (S) (E)-2-methyl-3-(methyl(3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)propyl anthracene-9-carboxylate (S)-4

Procedure A, starting from (R)-30 and ((E)-3-(3,4,5-trimethoxyphenyl)acrylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH 98:2. Yield: 70%.

¹H NMR (400 MHz, CDCl₃) δ: 8.46 (s, 1H, CH arom.); 8.02 (d, *J*=8.8 Hz, 2H, CH arom.); 7.97 (d, *J*=8.8 Hz, 2H, CH arom.); 7.53 (d, *J*=16.0 Hz, 1H, CH=CH); 7.49-7.42 (m, 4H, CH arom.); 6.65 (s, 2H, CH arom.); 6.29 (d, *J*=16.0 Hz, 1H, CH=CH); 4.63 (dd, *J*=10.8 Hz, *J*=4.0 Hz, 1H, OCHH); 4.46 (dd, *J*=10.8 Hz, *J*=6.0 Hz, 1H, OCHH); 4.25 (t, *J*=6.4 Hz, 2H, OCH₂); 3.83 (s, 3H, OCH₃); 3.78 (s,

6H, OCH₃); 2.51-2.40 (m, 3H, NCHH and NCH₂); 2.23 (s, 3H, NCH₃); 2.23-2.18 (m, 2H, NCHH and CH); 1.88-1.81 (m, 2H, CH₂); 1.04 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 169.87 (C=O); 167.01 (C=O); 153.34 (C); 144.69 (CH=CH); 141.85 (C); 130.97 (C); 129.89 (C); 129.18 (CH arom.); 128.60 (CH arom.); 128.38 (C); 126.89 (CH arom.); 125.46 (CH arom.); 125.08 (CH arom.); 117.36 (CH=CH); 105.14 (CH arom.); 69.27 (CH₂); 62.78 (CH₂); 61.31 (CH₂); 60.96 (OCH₃); 56.16 (OCH₃); 56.07 (OCH₃); 54.59 (CH₂); 42.62 (NCH₃); 31.28 (CH); 26.68 (CH₂); 16.00 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₅H₄₀NO₇= 586.2799, found 586.2792.

Hydrochloride: mp 88-92 °C.

6.1.15.9. (R) (E)-5-(methyl(2-methyl-3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)pentyl 3,4,5-trimethoxybenzoate (R)-5

Procedure A, starting from (R)-**31** and 3,4,5-trimethoxybenzoic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 98:2:0.2. Yield: 70.5%.

¹H NMR (400 MHz, CDCl₃) δ: 7.58 (d, *J*=16.0 Hz, 1H, CH=CH); 7.28 (s, 2H, CH arom.); 6.74 (s, 2H, CH arom.); 6.34 (d, *J*=16.0 Hz, 1H, CH=CH); 4.30 (t, *J*=6.8 Hz, 2H, OCH₂); 4.24 (dd, *J*=10.8 Hz, *J*=4.8 Hz, 1H, OCHH); 4.02 (dd, *J*=10.8 Hz, *J*=6.8 Hz, 1H, OCHH); 3.89 (s, 9H, OCH₃); 3.88 (s, 6H, OCH₃); 3.87 (s, 3H, OCH₃); 2.37-2.30 (m, 3H, NCHH and NCH₂); 2.21 (s, 3H, NCH₃); 2.20-2.12 (m, 1H, NCHH); 2.11-2.04 (m, 1H, CH); 1.82-1.72 (m, 2H, CH₂); 1.59-1.40 (m, 4H, CH₂); 0.99 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 167.04 (C=O); 166.25 (C=O); 153.45 (C); 152.93 (C); 144.62 (CH=CH); 142.23 (C); 140.17 (C); 129.93 (C); 125.49 (C); 117.40 (CH=CH); 106.86 (CH arom.); 105.30 (CH arom.); 68.09 (CH₂); 65.16 (CH₂); 61.30 (CH₂); 60.95 (OCH₃); 60.89 (OCH₃); 58.12 (CH₂); 56.25 (OCH₃); 56.18 (OCH₃); 42.69 (NCH₃); 31.28 (CH); 28.72 (CH₂); 26.96 (CH₂); 23.84 (CH₂); 15.88 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₂H₄₆NO₁₀= 604.3116, found 604.3115.

Hydrochloride: mp 95-98 °C.

6.1.15.10. (S) (E)-5-(methyl(2-methyl-3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)pentyl 3,4,5-trimethoxybenzoate (S)-5

Procedure A, starting from (S)-**31** and 3,4,5-trimethoxybenzoic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 98:2:0.2. Yield: 28.5%.

¹H NMR (400 MHz, CDCl₃) δ: 7.57 (d, *J*=16.0 Hz, 1H, CH=CH); 7.27 (s, 2H, CH arom.); 6.74 (s, 2H, CH arom.); 6.33 (d, *J*=16.0 Hz, 1H, CH=CH); 4.29 (t, *J*=6.4 Hz, 2H, OCH₂); 4.23 (dd, *J*=10.8 Hz, *J*=4.8 Hz, 1H, OCHH); 4.00 (dd, *J*=10.8 Hz, *J*=6.8 Hz, 1H, OCHH); 3.88 (s, 9H, OCH₃); 3.87 (s, 6H, OCH₃); 3.86 (s, 3H, OCH₃); 2.38-2.27 (m, 3H, NCHH and NCH₂); 2.19 (s, 3H, NCH₃); 2.18-2.14 (m, 1H, NCHH); 2.10-2.00 (m, 1H, CH); 1.80-1.72 (m, 2H, CH₂); 1.54-1.40 (m, 4H, CH₂); 0.98 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 167.05 (C=O); 166.24 (C=O); 153.43 (C); 152.91 (C); 144.58 (CH=CH); 142.17 (C); 140.12 (C); 129.93 (C); 125.50 (C); 117.43 (CH=CH); 106.82 (CH arom.); 105.25 (CH arom.); 68.12 (CH₂); 65.18 (CH₂); 61.34 (CH₂); 60.94 (OCH₃); 60.89 (OCH₃); 58.14 (CH₂); 56.23 (OCH₃); 56.16 (OCH₃); 42.73 (NCH₃); 31.30 (CH); 28.72 (CH₂); 27.05 (CH₂); 23.84 (CH₂); 15.86 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₂H₄₆NO₁₀= 604.3116, found 604.3125.

Hydrochloride: mp 90-95 °C.

6.1.15.11. (R) (E)-5-(methyl(2-methyl-3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)pentyl anthracene-9-carboxylate (R)-6

Procedure B, starting from (R)-**31** and anthracene-9-carboxylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 98:2:0.2. Yield: 54%.

¹H NMR (400 MHz, CDCl₃) δ: 8.51 (s, 1H, CH arom.); 8.04-8.00 (m, 4H, CH arom.); 7.58 (d, *J*=16.0 Hz, 1H, CH=CH); 7.55-7.45 (m, 4H, CH arom.); 6.73 (s, 2H, CH arom.); 6.34 (d, *J*=16.0 Hz, 1H, CH=CH); 4.61 (t, *J*=6.4 Hz, 2H, OCH₂); 4.23 (dd, *J*=10.8 Hz, *J*=4.8 Hz, 1H, OCHH); 4.03 (dd, *J*=10.8 Hz, *J*=6.4 Hz, 1H, OCHH); 3.87 (s, 3H, OCH₃); 3.85 (s, 6H, OCH₃); 2.41-2.29 (m, 3H, NCHH and NCH₂); 2.21 (s, 3H, NCH₃); 2.20-2.11 (m, 1H, NCHH); 2.10-2.04 (m, 1H, CH); 1.94-1.87 (m, 2H, CH₂); 1.59-1.46 (m, 4H, CH₂); 0.99 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 169.73 (C=O); 167.06 (C=O); 153.42 (C); 144.64 (CH=CH); 131.00 (C); 129.93 (C); 129.22 (CH arom.); 128.61 (CH arom.); 128.38 (C); 126.92 (CH arom.); 125.46 (CH arom.); 125.00 (CH arom.); 117.41 (CH=CH); 105.26 (CH arom.); 68.05 (CH₂); 65.84 (CH₂); 61.22 (CH₂); 60.96 (OCH₃); 58.03 (CH₂); 56.16 (OCH₃); 42.65 (NCH₃); 31.25 (CH); 28.70 (CH₂); 26.79 (CH₂); 23.90 (CH₂); 15.89 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₇H₄₄NO₇= 614.3112, found 614.3102.

Hydrochloride: mp 79-83 °C.

6.1.15.12. (*S*) (*E*)-5-(methyl(2-methyl-3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)pentyl anthracene-9-carboxylate (*S*)-6

Procedure B, starting from (*S*)-**31** and anthracene-9-carboxylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH/NH₄OH 98:2:0.2. Yield: 71%.

ESI-MS: 614.2 [M+H]⁺.

¹H NMR (400 MHz, CDCl₃) δ: 8.50 (s, 1H, CH arom.); 8.04-7.99 (m, 4H, CH arom.); 7.58 (d, *J*=16.0 Hz, 1H, CH=CH); 7.55-7.45 (m, 4H, CH arom.); 6.73 (s, 2H, CH arom.); 6.34 (d, *J*=16.0 Hz, 1H, CH=CH); 4.62 (t, *J*=6.8 Hz, 2H, OCH₂); 4.24 (dd, *J*=10.8 Hz, *J*=4.8 Hz, 1H, OCHH); 4.03 (dd, *J*=10.8 Hz, *J*=6.4 Hz, 1H, OCHH); 3.87 (s, 3H, OCH₃); 3.85 (s, 6H, OCH₃); 2.37-2.29 (m, 3H, NCHH and NCH₂); 2.20 (s, 3H, NCH₃); 2.18-2.13 (m, 1H, NCHH); 2.11-2.04 (m, 1H, CH); 1.94-1.86 (m, 2H, CH₂); 1.59-1.46 (m, 4H, CH₂); 0.98 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 169.73 (C=O); 167.07 (C=O); 153.42 (C); 144.59 (CH=CH); 131.00 (C); 129.95 (C); 129.21 (CH arom.); 128.61 (CH arom.); 128.38 (C); 128.16 (C); 126.91 (CH arom.); 125.45 (CH arom.); 125.01 (CH arom.); 117.46 (CH=CH); 105.24 (CH arom.); 68.11 (CH₂); 65.86 (CH₂); 61.31 (CH₂); 60.96 (OCH₃); 58.09 (CH₂); 56.15 (OCH₃); 42.73 (NCH₃); 31.32 (CH); 28.72 (CH₂); 26.95 (CH₂); 23.91 (CH₂); 15.87 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₇H₄₄NO₇= 614.3112, found 614.3104.

Hydrochloride: mp 76-80 °C.

6.1.15.13. (*R*) (*E*)-2-methyl-3-(methyl(5-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)pentyl)amino)propyl 3,4,5-trimethoxybenzoate (*R*)-7

Procedure B, starting from (*R*)-**32** and ((*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH 96:4. Yield: 78%.

¹H NMR (400 MHz, CDCl₃) δ: 7.57 (d, *J*=16.0 Hz, 1H, CH=CH); 7.28 (s, 2H, CH arom.); 6.74 (s, 2H, CH arom.); 6.33 (d, *J*=16.0 Hz, 1H, CH=CH); 4.34 (dd, *J*=10.8 Hz, *J*=4.4 Hz, 1H, OCHH); 4.17 (t, *J*=6.4 Hz, 2H, OCH₂); 4.13 (dd, *J*=10.8 Hz, *J*=6.4 Hz, 1H, OCHH); 3.88 (s, 9H, OCH₃); 3.87 (s, 9H, OCH₃); 2.36-2.31 (m, 3H, NCHH and NCH₂); 2.22 (s, 3H, NCH₃); 2.21-2.10 (m, 2H, NCHH and CH); 1.71-1.65 (m, 2H, CH₂); 1.56-1.35 (m, 4H, CH₂); 1.02 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 167.04 (C=O); 166.27 (C=O); 153.89 (C); 152.94 (C); 144.61 (CH=CH); 142.19 (C); 129.93 (C); 125.48 (C); 117.42 (CH=CH); 106.81 (CH arom.); 105.24 (CH arom.); 68.49 (CH₂); 64.55 (CH₂); 60.96 (CH₂); 60.90 (OCH₃); 58.14 (CH₂); 56.25 (OCH₃); 56.23 (OCH₃); 42.77 (NCH₃); 31.31 (CH); 28.68 (CH₂); 27.00 (CH₂); 23.80 (CH₂); 15.99 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₂H₄₆NO₁₀= 604.3116, found 604.3110.

Hydrochloride: mp 53-57 °C.

6.1.15.14. (*S*) (*E*)-2-methyl-3-(methyl(5-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)pentyl)amino)propyl 3,4,5-trimethoxybenzoate (*S*)-7

Procedure B, starting from (*S*)-**32** and ((*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH 96:4. Yield: 67%.

¹H NMR (400 MHz, CDCl₃) δ: 7.51 (d, *J*=16.0 Hz, 1H, CH=CH); 7.22 (s, 2H, CH arom.); 6.68 (s, 2H, CH arom.); 6.27 (d, *J*=16.0 Hz, 1H, CH=CH); 4.28 (dd, *J*=10.8 Hz, *J*=4.4 Hz, 1H, OCHH); 4.12-4.05 (m, 3H, OCHH and OCH₂); 3.82 (s, 9H, OCH₃); 3.80 (s, 6H, OCH₃); 3.79 (s, 3H, OCH₃); 2.29-2.20 (m, 3H, NCHH and NCH₂); 2.14 (s, 3H, NCH₃); 2.13-2.06 (m, 2H, NCHH and CH); 1.66-1.55 (m, 2H, CH₂); 1.46-1.30 (m, 4H, CH₂); 0.96 (d, *J*=6.0 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 166.98 (C=O); 166.18 (C=O); 153.39 (C); 152.89 (C); 144.56 (CH=CH); 142.13 (C); 140.03 (C); 129.93 (C); 125.50 (C); 117.43 (CH=CH); 106.74 (CH arom.); 105.19 (CH arom.); 68.50 (CH₂); 64.55 (CH₂); 61.32 (CH₂); 60.91 (OCH₃); 60.86 (OCH₃); 58.13 (CH₂); 56.17 (OCH₃); 56.11 (OCH₃); 42.78 (NCH₃); 31.33 (CH); 28.66 (CH₂); 27.01 (CH₂); 23.78 (CH₂); 15.93 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₂H₄₆NO₁₀= 604.3116, found 604.3106.

Hydrochloride: mp 59-62 °C.

6.1.15.15. (*R*) (*E*)-2-methyl-3-(methyl(5-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)pentyl)amino)propyl anthracene-9-carboxylate (*R*)-**8**

Procedure A, starting from (*R*)-**33** and ((*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH 97:3. Yield: 78%.

¹H NMR (400 MHz, CDCl₃) δ: 8.51 (s, 1H, CH arom.); 8.05 (d, *J*=8.8 Hz, 2H, CH arom.); 8.01 (d, *J*=8.8 Hz, 2H, CH arom.); 7.58 (d, *J*=16.0 Hz, 1H, CH=CH); 7.54-7.45 (m, 4H, CH arom.); 6.72 (s, 2H, CH arom.); 6.35 (d, *J*=16.0 Hz, 1H, CH=CH); 4.65 (dd, *J*=10.4 Hz, *J*=4.0 Hz, 1H, OCHH); 4.49 (dd, *J*=10.4 Hz, *J*=6.0 Hz, 1H, OCHH); 4.19 (t, *J*=6.4 Hz, 2H, OCH₂); 3.86 (s, 3H, OCH₃); 3.84 (s, 6H, OCH₃); 2.43-2.30 (m, 3H, NCHH and NCH₂); 2.23 (s, 3H, NCH₃); 2.22-2.17 (m, 2H, NCHH and CH); 1.72-1.65 (m, 2H, CH₂); 1.53-1.42 (m, 4H, CH₂); 1.06 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 169.83 (C=O); 167.10 (C=O); 153.37 (C); 144.63 (CH=CH); 139.95 (C); 131.00 (C); 129.92 (C); 129.20 (CH arom.); 128.63 (CH arom.); 128.38 (C); 126.86 (CH arom.); 125.42 (CH arom.); 125.15 (CH arom.); 117.47 (CH=CH); 105.13 (CH arom.); 69.33 (CH₂); 64.61 (CH₂); 61.28 (CH₂); 60.98 (OCH₃); 58.13 (CH₂); 56.13 (OCH₃); 42.72 (NCH₃); 31.22 (CH); 28.73 (CH₂); 27.00 (CH₂); 23.83 (CH₂); 16.10 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₇H₄₄NO₇= 614.3112, found 614.3115.

Hydrochloride: mp 129-132 °C.

6.1.15.16. (*S*) (*E*)-2-methyl-3-(methyl(5-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)pentyl)amino)propyl anthracene-9-carboxylate (*S*)-**8**

Procedure A, starting from (*S*)-**33** and ((*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH 97:3. Yield: 72%.

¹H NMR (400 MHz, CDCl₃) δ: 8.50 (s, 1H, CH arom.); 8.05 (d, *J*=8.8 Hz, 2H, CH arom.); 8.00 (d, *J*=8.8 Hz, 2H, CH arom.); 7.58 (d, *J*=16.0 Hz, 1H, CH=CH); 7.54-7.45 (m, 4H, CH arom.); 6.72 (s, 2H, CH arom.); 6.35 (d, 1H, *J*=16.0 Hz, CH=CH); 4.65 (dd, *J*=10.8 Hz, *J*=4.4 Hz, 1H, OCHH); 4.49 (dd, *J*=10.8 Hz, *J*=6.0 Hz, 1H, OCHH); 4.19 (t, *J*=6.4 Hz, 2H, OCH₂); 3.86 (s, 3H, OCH₃); 3.83 (s, 6H, OCH₃); 2.42-2.30 (m, 3H, NCHH and NCH₂); 2.22 (s, 3H, NCH₃); 2.25-2.15 (m, 2H, NCHH and CH); 1.72-1.65 (m, 2H, CH₂); 1.53-1.42 (m, 4H, CH₂); 1.06 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 169.86 (C=O); 167.08 (C=O); 153.38 (C); 144.62 (CH=CH); 139.97 (C); 130.98 (C); 129.95 (C); 129.17 (CH arom.); 128.61 (CH arom.); 128.39 (C); 126.88 (CH arom.); 125.47 (CH arom.); 125.11 (CH arom.); 117.47 (CH=CH); 105.15 (CH arom.); 69.32 (CH₂); 64.60 (CH₂); 61.25 (CH₂); 60.97 (OCH₃); 58.13 (CH₂); 56.10 (OCH₃); 42.76 (NCH₃); 31.23 (CH); 28.70 (CH₂); 26.99 (CH₂); 23.85 (CH₂); 16.08 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₇H₄₄NO₇= 614.3112, found 614.3117.

Hydrochloride: mp 88-99 °C.

6.1.15.17. (R) (E)-7-(methyl(2-methyl-3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)heptyl 3,4,5-trimethoxybenzoate (R)-9

Procedure B, starting from (R)-34 and 3,4,5-trimethoxybenzoic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH 97:3. Yield: 21%.

¹H NMR (400 MHz, CDCl₃) δ: 7.58 (d, *J*=15.6 Hz, 1H, CH=CH); 7.28 (s, 2H, CH arom.); 6.74 (s, 2H, CH arom.); 6.33 (d, *J*=15.6 Hz, 1H, CH=CH); 4.28 (t, *J*=6.8 Hz, 2H, OCH₂); 4.23 (dd, *J*=10.8 Hz, *J*=5.2 Hz, 1H, OCHH); 4.05 (dd, *J*=10.8 Hz, *J*=6.4 Hz, 1H, OCHH); 3.89 (s, 9H, OCH₃); 3.88 (s, 6H, OCH₃); 3.87 (s, 3H, OCH₃); 2.47-2.43 (m, 3H, NCHH and NCH₂); 2.31 (s, 3H, NCH₃); 2.30-2.26 (m, 1H, NCHH); 2.19-2.09 (m, 1H, CH); 1.70-1.59 (m, 2H, CH₂); 1.48-1.29 (m, 8H, CH₂); 1.03 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 167.00 (C=O); 166.27 (C=O); 153.45 (C); 152.92 (C); 144.77 (CH=CH); 142.78 (C); 129.88 (C); 125.51 (C); 117.26 (CH=CH); 106.83 (CH arom.); 105.29 (CH arom.); 67.92 (CH₂); 65.18 (CH₂); 60.96 (CH₂); 60.67 (OCH₃); 57.76 (CH₂); 56.10 (OCH₃); 42.19 (NCH₃); 30.87 (CH); 29.16 (CH₂) 28.69 (CH₂) 27.26 (CH₂) 25.97 (CH₂) 16.03 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₄H₅₀NO₁₀= 632.3429, found 632.3434.

Hydrochloride: mp 67-70 °C.

6.1.15.18. (S) (E)-7-(methyl(2-methyl-3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)heptyl 3,4,5-trimethoxybenzoate (S)-9

Procedure A, starting from (S)-34 and 3,4,5-trimethoxybenzoic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH 95:5. Yield: 77%.

¹H NMR (400 MHz, CDCl₃) δ: 7.58 (d, *J*=16.0 Hz, 1H, CH=CH); 7.28 (s, 2H, CH arom.); 6.75 (s, 2H, CH arom.); 6.34 (d, *J*=16.0 Hz, 1H, CH=CH); 4.29 (t, *J*=6.8 Hz, 2H, OCH₂); 4.24 (dd, *J*=10.8 Hz, *J*=4.4 Hz, 1H, OCHH); 4.02 (dd, *J*=10.8 Hz, *J*=6.8 Hz, 1H, OCHH); 3.90 (s, 9H, OCH₃); 3.88 (s, 6H, OCH₃); 3.87 (s, 3H, OCH₃); 2.40-2.25 (m, 3H, NCHH and NCH₂); 2.20 (s, 3H, NCH₃); 2.19-2.03 (m, 2H, NCHH and CH); 1.77-1.71 (m, 2H, CH₂); 1.50-1.24 (m, 8H, CH₂); 1.00 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 167.04 (C=O); 166.27 (C=O); 153.45 (C); 152.92 (C); 144.61 (CH=CH); 142.80 (C); 140.30 (C); 129.93 (C); 125.54 (C); 117.42 (CH=CH); 106.83 (CH arom.); 105.27 (CH arom.); 68.11 (CH₂); 65.23 (CH₂); 61.23 (CH₂); 60.96 (OCH₃); 60.90 (OCH₃); 58.25 (CH₂); 56.25 (OCH₃); 56.18 (OCH₃); 42.71 (NCH₃); 31.22 (CH); 29.23 (CH₂); 28.72 (CH₂); 27.30 (CH₂); 26.00 (CH₂); 15.93 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₄H₅₀NO₁₀= 632.3429, found 632.3430.

Hydrochloride: mp 71-75 °C.

6.1.15.19. (R) (E)-7-(methyl(2-methyl-3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)heptyl anthracene-9-carboxylate (R)-10

Procedure B, starting from (R)-34 and anthracene-9-carboxylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH 97:3. Yield: 58%.

¹H NMR (400 MHz, CDCl₃) δ: 8.51 (s, 1H, CH arom.); 8.05-8.00 (m, 4H, CH arom.); 7.59 (d, *J*=16.0 Hz, 1H, CH=CH); 7.55-7.46 (m, 4H, CH arom.); 6.74 (s, 2H, CH arom.); 6.35 (d, *J*=16.0 Hz, 1H, CH=CH); 4.60 (t, *J*=6.4 Hz, 2H, OCH₂); 4.24 (dd, *J*=10.8 Hz, *J*=4.8 Hz, 1H, OCHH); 4.03 (dd, *J*=10.8 Hz, *J*=6.4 Hz, 1H, OCHH); 3.87 (s, 3H, OCH₃); 3.86 (s, 6H, OCH₃); 2.33-2.28 (m, 3H, NCH₂ and NCHH); 2.18 (s, 3H, NCH₃); 2.17-2.06 (m, 2H, NCHH and CH); 1.91-1.83 (m, 2H, CH₂); 1.53-1.30 (m, 8H, CH₂); 1.00 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 167.08 (C=O); 153.41 (C); 144.52 (CH=CH); 131.00 (C); 129.95 (C); 129.17 (CH arom.); 128.63 (CH arom.); 128.42 (C); 126.88 (CH arom.); 125.43 (CH arom.); 125.04 (CH arom.); 117.51 (CH=CH); 105.22 (CH arom.); 68.25 (CH₂); 65.90 (CH₂); 61.32 (CH₂); 60.99 (OCH₃); 58.25 (CH₂); 56.21 (OCH₃); 42.72 (NCH₃); 31.28 (CH); 29.25 (CH₂); 28.75 (CH₂); 27.35 (CH₂); 26.12 (CH₂); 15.89 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₉H₄₈NO₇= 642.3425, found 642.3425.

Hydrochloride: mp 100-103 °C.

6.1.15.20. (*S*) (*E*)-7-(methyl(2-methyl-3-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)propyl)amino)heptyl anthracene-9-carboxylate (*S*)-**10**

Procedure B, starting from (*S*)-**34** and anthracene-9-carboxylic acid.

Free base: chromatographic eluent: CH₂Cl₂/MeOH/NH₄OH 93:7:0.3. Yield: 46%.

¹H NMR (400 MHz, CDCl₃) δ: 8.51 (s, 1H, CH arom.); 8.05-8.00 (m, 4H, CH arom.); 7.59 (d, *J*=16.0 Hz, 1H, CH=CH); 7.55-7.46 (m, 4H, CH arom.); 6.74 (s, 2H, CH arom.); 6.35 (d, *J*=16.0 Hz, 1H, CH=CH); 4.61 (t, *J*=6.8 Hz, 2H, OCH₂); 4.25 (dd, *J*=10.8 Hz, *J*=4.8 Hz, 1H, OCHH); 4.03 (dd, *J*=10.8 Hz, *J*=6.8 Hz, 1H, OCHH); 3.87 (s, 3H, OCH₃); 3.86 (s, 6H, OCH₃); 2.35-2.28 (m, 3H, NCHH and NCH₂); 2.19 (s, 3H, NCH₃); 2.17-2.02 (m, 2H, NCHH and CH); 1.91-1.84 (m, 2H, CH₂); 1.53-1.30 (m, 8H, CH₂); 0.99 (d, *J*=6.8 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 167.07 (C=O); 153.44 (C); 144.55 (CH=CH); 131.01 (C); 129.96 (C); 129.19 (CH arom.); 128.61 (CH arom.); 128.39 (C); 126.90 (CH arom.); 125.45 (CH arom.); 125.03 (CH arom.); 117.49 (CH=CH); 105.25 (CH arom.); 68.20 (CH₂); 65.92 (CH₂); 61.30 (CH₂); 60.97 (OCH₃); 58.28 (CH₂); 56.17 (OCH₃); 42.78 (NCH₃); 31.30 (CH); 29.21 (CH₂); 28.75 (CH₂); 27.32 (CH₂); 26.10 (CH₂); 15.91 (CH₃) ppm.

ESI-HRMS (*m/z*) calculated for [M+H]⁺ ion species C₃₉H₄₈NO₇= 642.3425, found 642.3437.

Hydrochloride: mp 125-127°C.

6.1.15.21. (*R*) (*E*)-2-methyl-3-(methyl(7-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)heptyl)amino)propyl 3,4,5-trimethoxybenzoate (*R*)-**11**

Procedure B, starting from (*R*)-**35** and ((*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH 98:2. Yield: 41%.

¹H NMR (400 MHz, CDCl₃) δ: 7.54 (d, *J*=15.6 Hz, 1H, CH=CH); 7.25 (s, 2H, CH arom.); 6.71 (s, 2H, CH arom.); 6.30 (d, *J*=15.6 Hz, 1H, CH=CH); 4.30 (dd, *J*=10.8 Hz, *J*=4.8 Hz, 1H, OCHH); 4.15-4.07 (m, 3H, OCH₂ and OCHH); 3.85 (s, 9H, OCH₃); 3.83 (s, 6H, OCH₃); 3.82 (s, 3H, OCH₃); 2.33-2.27 (m, 3H, NCH₂ and NCHH); 2.18 (s, 3H, NCH₃); 2.17-2.11 (m, 2H, NCHH and CH); 1.65-1.59 (m, 2H, CH₂); 1.42-1.20 (m, 8H, CH₂); 0.98 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 167.02 (C=O); 166.21 (C=O); 153.42 (C); 152.92 (C); 144.55 (CH=CH); 129.95 (C); 125.51 (C); 117.49 (CH=CH); 106.79 (CH arom.); 105.22 (CH arom.); 68.53 (CH₂); 64.63 (CH₂); 61.28 (CH₂); 60.94 (OCH₃); 60.89 (OCH₃); 58.30 (CH₂); 56.21 (OCH₃); 56.15 (OCH₃); 42.78 (NCH₃); 31.30 (CH); 29.21 (CH₂); 28.70 (CH₂); 27.29 (CH₂); 27.18 (CH₂); 25.95 (CH₂); 15.98 (CH₃) ppm.

ESI-HRMS (*m/z*) calculated for [M+H]⁺ ion species C₃₄H₅₀NO₁₀= 632.3429, found 632.3420.

Hydrochloride: mp 81-85 °C.

6.1.15.22. (*S*) (*E*)-2-methyl-3-(methyl(7-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)heptyl)amino)propyl 3,4,5-trimethoxybenzoate (*S*)-**11**

Procedure B, starting from (*S*)-**35** and ((*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH 98:2. Yield: 27%.

¹H NMR (400 MHz, CDCl₃) δ: 7.55 (d, *J*=16.0 Hz, 1H, CH=CH); 7.26 (s, 2H, CH arom.); 6.72 (s, 2H, CH arom.); 6.31 (d, *J*=16.0 Hz, 1H, CH=CH); 4.31 (dd, *J*=10.8 Hz, *J*=4.8 Hz, 1H, OCHH); 4.16-4.09 (m, 3H, OCH₂ and OCHH); 3.86 (s, 9H, OCH₃); 3.85 (s, 6H, OCH₃); 3.84 (s, 3H, OCH₃); 2.40-2.30 (m, 3H, NCH₂ and NCHH); 2.22 (s, 3H, NCH₃); 2.21-2.10 (m, 2H, NCHH and CH); 1.66-1.61 (m, 2H, CH₂); 1.50-1.40 (m, 2H, CH₂); 1.39-1.20 (m, 6H, CH₂); 1.01 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 167.05 (C=O); 166.22 (C=O); 153.43 (C); 152.94 (C); 144.57 (CH=CH); 129.96 (C); 125.48 (C); 117.49 (CH=CH); 106.82 (CH arom.); 105.23 (CH arom.); 68.50 (CH₂); 64.64 (CH₂); 61.18 (CH₂); 60.96 (OCH₃); 60.91 (OCH₃); 58.22 (CH₂); 56.23 (OCH₃); 56.16 (OCH₃); 42.69 (NCH₃); 31.24 (CH); 29.21 (CH₂); 28.71 (CH₂); 27.29 (CH₂); 25.95 (CH₂); 16.03 (CH₃) ppm.

ESI-HRMS (*m/z*) calculated for [M+H]⁺ ion species C₃₄H₅₀NO₁₀= 632.3429, found 632.3429.

Hydrochloride: mp 86-93 °C.

6.1.15.23. (*R*) (*E*)-2-methyl-3-(methyl(7-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)heptyl)amino)propyl anthracene-9-carboxylate (*R*)-**12**

Procedure B, starting from (*R*)-**36** and ((*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid.

Free base: chromatographic eluent: CH₂Cl₂/MeOH/NH₄OH 93:7:0.3. Yield: 77%.

¹H NMR (400 MHz, CDCl₃) δ: 8.48 (s, 1H, CH arom.); 8.02 (d, *J*=8.4 Hz, 2H, CH arom.); 7.98 (d, *J*=8.4 Hz, 2H, CH arom.); 7.56 (d, *J*=16.0 Hz, 1H, CH=CH); 7.51-7.43 (m, 4H, CH arom.); 6.71 (s, 2H, CH arom.); 6.31 (d, *J*=16.0 Hz, 1H, CH=CH); 4.60 (dd, *J*=10.8 Hz, *J*=4.4 Hz, 1H, OCHH); 4.47 (dd, *J*=10.8 Hz, *J*=5.6 Hz, 1H, OCHH); 4.15 (t, *J*=6.8 Hz, 2H, OCH₂); 3.84 (s, 3H, OCH₃); 3.83 (s, 6H, OCH₃); 2.45-2.35 (m, 3H, NCH₂ and NCHH); 2.24 (s, 3H, NCH₃); 2.24-2.22 (m, 2H, NCHH and CH); 1.66-1.61 (m, 2H, CH₂); 1.48-1.37 (m, 2H, CH₂); 1.36-1.20 (m, 6H, CH₂); 1.06 (d, *J*=6.4 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 169.80 (C=O); 167.06 (C=O); 153.42 (C); 144.57 (CH=CH); 131.01 (C); 129.96 (C); 129.20 (CH arom.); 128.61 (CH arom.); 128.42 (C); 126.89 (CH arom.); 125.47 (CH arom.); 125.11 (CH arom.); 117.51 (CH=CH); 105.23 (CH arom.); 69.26 (CH₂); 64.66 (CH₂); 60.95 (CH₂); 58.12 (CH₂); 56.15 (OCH₃); 42.56 (NCH₃); 31.09 (CH); 29.18 (CH₂); 28.72 (CH₂); 27.29 (CH₂); 26.80 (CH₂); 25.95 (CH₂); 16.15 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₉H₄₈NO₇= 642.3425, found 642.3425.

Hydrochloride: mp 67-73 °C.

6.1.15.24. (*S*) (*E*)-2-methyl-3-(methyl(7-((3-(3,4,5-trimethoxyphenyl)acryloyl)oxy)heptyl)amino)propyl anthracene-9-carboxylate (*S*)-**12**

Procedure B, starting from (*R*)-**36** and ((*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid.

Free base: chromatographic eluent: CH₂Cl₂/CH₃OH 98:2. Yield: 29%.

¹H NMR (400 MHz, CDCl₃) δ: 8.47 (s, 1H, CH arom.); 8.01 (d, *J*=8.4 Hz, 2H, CH arom.); 7.97 (d, *J*=8.4 Hz, 2H, CH arom.); 7.56 (d, *J*=16.0 Hz, 1H, CH=CH); 7.51-7.42 (m, 4H, CH arom.); 6.70 (s, 2H, CH arom.); 6.31 (d, *J*=16.0 Hz, 1H, CH=CH); 4.60 (dd, *J*=10.8 Hz, *J*=4.4 Hz, 1H, OCHH); 4.46 (dd, *J*=10.8 Hz, *J*=6.0 Hz, 1H, OCHH); 4.15 (t, *J*=6.8 Hz, 2H, OCH₂); 3.84 (s, 3H, OCH₃); 3.82 (s, 6H, OCH₃); 2.45-2.30 (m, 3H, NCH₂ and NCHH); 2.22 (s, 3H, NCH₃); 2.22-2.20 (m, 2H, NCHH and CH); 1.67-1.61 (m, 2H, CH₂); 1.48-1.20 (m, 8H, CH₂); 1.05 (d, *J*=6.0 Hz, 3H, CH₃) ppm.

¹³C APT NMR (CDCl₃) δ: 169.79 (C=O); 167.04 (C=O); 153.42 (C); 144.55 (CH=CH); 131.00 (C); 129.97 (C); 129.18 (CH arom.); 128.60 (CH arom.); 128.41 (C); 128.28 (C); 126.87 (CH arom.); 125.46 (CH arom.); 125.11 (CH arom.); 117.52 (CH=CH); 105.24 (CH arom.); 69.27 (CH₂); 64.65 (CH₂); 61.13 (CH₂); 60.95 (OCH₃); 58.27 (CH₂); 56.14 (OCH₃); 42.69 (NCH₃); 31.17 (CH); 29.19 (CH₂); 28.73 (CH₂); 27.28 (CH₂); 25.96 (CH₂); 16.12 (CH₃) ppm.

ESI-HRMS (m/z) calculated for [M+H]⁺ ion species C₃₉H₄₈NO₇= 642.3425, found 642.3415.

Hydrochloride: mp 84-89 °C.

6.1.16. 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 **1**, **2**, **3**, **5**, **6**, **8**, **9**, **10** and **11** are reported in Table 4 and the Retention Time (TR), *ee* values and resolution (*R*) between the enantiomers of the same compounds are reported in Table 5.

Table 4

Elution conditions employed to carry out the resolution of racemic mixtures of compounds **1**, **2**, **3**, **5**, **6**, **8**, **9**, **10** and **11**.

Racemic mixture	Mobile phase ^a	Flow (mL min ⁻¹)	T (°C)	UV (nm)
(<i>R</i>)- 1 / <i>(S)</i> - 1 , (<i>R</i>)- 3 / <i>(S)</i> - 3 , (<i>R</i>)- 5 / <i>(S)</i> - 5 , (<i>R</i>)- 9 / <i>(S)</i> - 9 , (<i>R</i>)- 11 / <i>(S)</i> - 11	A	0.5	20	300
(<i>R</i>)- 2 / <i>(S)</i> - 2	B	0.8	10	250
(<i>R</i>)- 6 / <i>(S)</i> - 6 , (<i>R</i>)- 10 / <i>(S)</i> - 10	C	0.8	20	250
(<i>R</i>)- 8 / <i>(S)</i> - 8	D	0.5	20	250

^a A: methanol:isopropanol 60:40 (v/v), with 0.1% of DEA; B: methanol:acetonitrile 95:5 (v/v), with 0.1% of DEA; C: methanol:acetonitrile 90:10 (v/v), with 0.1% of DEA; D: methanol:acetonitrile 98:2 (v/v), with 0.1% of DEA.

Table 5

Retention time (TR), ee values and resolution (R) between the enantiomers of compounds **1**, **2**, **3**, **5**, **6**, **8**, **9**, **10** and **11**.

Compd	TR (min)	ee %	R value	Compd	TR (min)	ee %	R value
(<i>R</i>)- 1	22.28 ± 0.02	≥ 95	2.18 ± 0.04	(<i>R</i>)- 6	12.02 ± 0.01	≥ 95	3.88 ± 0.01
(<i>S</i>)- 1	19.36 ± 0.03	≥ 95		(<i>S</i>)- 6	9.27 ± 0.01	≥ 95	
(<i>R</i>)- 2	16.47 ± 0.05	≥ 95	1.22 ± 0.04	(<i>R</i>)- 8	23.22 ± 0.06	≥ 95	1.18 ± 0.01
(<i>S</i>)- 2	14.73 ± 0.04	≥ 95		(<i>S</i>)- 8	24.80 ± 0.07	≥ 95	
(<i>R</i>)- 3	19.70 ± 0.20	≥ 95	1.43 ± 0.03	(<i>R</i>)- 9	22.72 ± 0.02	≥ 95	1.89 ± 0.05
(<i>S</i>)- 3	21.70 ± 0.20	≥ 95		(<i>S</i>)- 9	20.13 ± 0.02	≥ 95	
(<i>R</i>)- 5	22.70 ± 0.60	≥ 95	2.78 ± 0.05	(<i>R</i>)- 10	11.01 ± 0.01	≥ 95	3.12 ± 0.01
(<i>S</i>)- 5	19.10 ± 0.50	≥ 95		(<i>S</i>)- 10	9.11 ± 0.01	≥ 95	

6.2. Biological assay

6.2.1. Cell lines and cultures

The K562 is an undifferentiated erythroleukemia cell line originally derived from a patient with chronic myelogenous leukemia [48]. The K562 leukemia cells and the P-gp over-expressing K562/DOX cells were obtained from Prof. J.P. Marie (Hopital Hotel-Dieu, Paris, France). The cells were cultured following a previously reported protocol [67].

MDCK-MDR1, MDCK-MRP1 and MDCK-BCRP cells are a gift of Prof. P. Borst, NKI-AVL Institute, Amsterdam, Nederland. MDCK cells were grown in DMEM high glucose 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 % CO₂ atmosphere. Caco-2 cells were a gift of Dr. Aldo Cavallini and Dr. Caterina Messa from the Laboratory of Biochemistry, National Institute for Digestive Diseases, “S. de Bellis”, Bari (Italy).

6.2.2. Drugs and materials

Purified verapamil and pirarubicin were purchased by Sigma-Aldrich (Milan - Italy). Concentrations were determined by diluting stock solutions to approximately 10⁻⁵ M and using ε₄₈₀ = 11500 M⁻¹ cm⁻¹. Stock solutions were prepared just before use. Buffer solutions were HEPES buffer containing 5 mM HEPES, 132 mM NaCl, 3.5 mM CaCl₂, 5 mM glucose, at pH 7.3.

Cell culture reagents were purchased from Celbio s.r.l. (Milano, Italy). CulturePlate 96/wells plates were purchased from PerkinElmer Life Science; Calcein-AM, bisBenzimide H 33342 trihydrochloride were obtained from Sigma-Aldrich (Milan, Italy).

6.2.3. Modulation of pirarubicin uptake on K562/DOX cells

The uptake of pirarubicin in cells was followed by monitoring the decrease in the fluorescence signal at 590 nm ($\lambda_{\text{ex}} = 480$ nm) according to the previously described method [68,69].

6.2.4. Characterization of P-gp interacting profile and ABC transporters selectivity on transfected MDCK cells

Calcein-AM and Hoechst 33342 experiments, ATPlite assay and Permeability experiments were carried out as previously described [42]. Details are reported in the Supplementary data.

6.2.5. Antiproliferative assay

Determination of cell growth was performed using the MTT assay at 48 h and 72 h [70]. Data were analyzed by one-way ANOVA for repeated measures. Results are expressed as mean \pm SD of at 2-3 independent experiments in triplicates. Statistical significance was accepted at a level of $P < 0.05$. Details are reported in the Supplementary data.

6.2.6. Co-administration assay

The co-administration assay with doxorubicin was performed in MDCK-MDR1 cells at 48 h as reported with minor modification [71]. Details are reported in the Supplementary data.

6.2.7. Collateral Sensitivity (CS) Study

All the compounds have been tested for their cytotoxic activity at 24 h on MDCK-MDR1 and MDCK-MRP1 cells and on the parental MDCK cells by MTT assay.

To evaluate the mechanisms involved in CS, reactive oxygen species (ROS) were measured using the fluorescent probe 5-(and-6)-chloromethyl-2',7'-dichlorodihydro-fluorescein diacetate-acetoxymethyl ester (DCFDA-AM), as previously reported [72]. The results were expressed as nmoles/mg cellular proteins. Cell viability with the compounds and ROS scavenger was measured by the ATPlite Luminescence Assay System (PerkinElmer, Waltham, MA), as per manufacturer's instructions, using a Synergy HT Multi-Detection Microplate Reader (BioTek Instruments, Winooski, VT). The relative luminescence units (RLU) of untreated cells were considered as 100% viability; results were expressed as a percentage of viable cells versus untreated cells.

6.3. Molecular docking studies

The crystal structure of human P-gp with in the ATP-bound, outward-facing conformation (PDB ID: 6C0V) [52], was downloaded from PDB database.

The molecular structures of synthesized compounds were designed with Chemdraw program, and then optimized prior to docking procedure, by using the Dock Prep application.

In order to prepare the ligands and receptor for docking calculation, MolSoft was used [51], then for each compound, the molecular docking study was carried out by the software. The grid box was set to 20 Å \times 20 Å \times 28 Å with a grid space value of 1 Å. The binding box was centred at the previously reported helix TM4 (W232), TM5 (R296, I299, I306), TM6 (Y310, F336), TM7 (F928), TM8 (F303, Y307, F770) and TM12 (M986, Q990, F994), involved in the substrate-binding region [73-76]. MolSoft program was used setting default parameters and a maximum of 50 poses per ligand were collected. The results were analysed using PyMOL v2.0 [77]. To find network of interaction we used Ligplot+ v.1.4.5 [78] and Protein-Ligand Interaction Profiler [79] all the sets were by default.

6.4. Stability test

6.4. 1. Chemicals

Acetonitrile (Chromasolv), formic acid and ammonium formate (MS grade), NaCl, KCl, Na₂HPO₄ 2H₂O, KH₂PO₄ (Reagent grade) and verapamil hydrochloride (analytical standard, used as internal

standard), ketoprofen and enalapril (analytical standard) were purchased by Sigma-Aldrich (Milan, Italy). Ketoprofen Ethyl Ester (KEE) were obtained by Fisher's reaction from ketoprofen and ethanol. MilliQ water $18 \text{ M}\Omega \text{ cm}^{-1}$ was obtained from Millipore's Simplicity system (Milan - Italy). 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 $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ and 0.27 g L^{-1} of KH_2PO_4 . Human plasma was collected from healthy male volunteer and kept at $-80 \text{ }^\circ\text{C}$ until use.

6.4. 2. Preparation of samples

Each sample was prepared adding $10 \mu\text{L}$ of working solution 1 to $100 \mu\text{L}$ of tested matrix (PBS or rat plasma or human plasma) in microcentrifuge tubes. The obtained solutions correspond to $1 \mu\text{M}$ of analyte.

Each set of samples was incubated in triplicate at four different times, 0, 30, 60 and 120 min at $37 \text{ }^\circ\text{C}$. Therefore, the degradation profile of each analyte was represented by a batch of 12 samples (4 incubation times \times 3 replicates). After the incubation, the samples were added with $300 \mu\text{L}$ of ISTD solution and centrifuged (room temperature for 5 min at 10000 rpm). The supernatants were transferred in autosampler 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 method described in the Supplementary data.

Acknowledgements

This work was supported by grants from the University of Florence (Fondo Ricerca Ateneo RICATEN16 and RICATEN17) and by Italian Association of Cancer Research (AIRC; IG21408 to CR).

Supplementary data

Supplementary data to this article can be found online.

$^1\text{H-NMR}$ (400 MHz), $^{13}\text{C-APT-NMR}$ (100 MHz) spectra of (*S*) enantiomers of compounds **1-12**. Immunoblotting analysis of P-gp, MRP1 and BCRP expression in MDCK, MDCK-MDR1, MDCK-MRP1 and MDCK-BCRP cells. Characterization of P-gp interacting profile and ABC transporters selectivity on transfected MDCK cells. Antiproliferative assay. Co-administration assay. Chemical stability data of compounds **1-12**. Chromatographic profiles of LC-DAD analysis and corresponding UV spectra of compounds **1-12**.

References

- [1] L.A. Mitcher, S.P. Pillai, E.J. Gentry, D.M. Shankel, Multiple Drug Resistance, *Med. Res. Rev.* 19 (1999) 477-496.
- [2] C.F. Higgins, ABC transporters: from microorganisms to man, *Annu. Rev. Cell Biol.* 8 (1992) 67-113.
- [3] I.B. Holland, M.A. Blight, ABC-ATPases, adaptable energy generators fuelling transmembrane movement of a variety of molecules in organisms from bacteria to humans, *J. Mol. Biol.* 293 (1999) 381-397.
- [4] G.A. Altenberg, Structure of multidrug-resistance proteins of the ATP-binding cassette (ABC) superfamily, *Curr. Med. Chem. Anticancer Agents* 4 (2004) 53-62.
- [5] W. Li, H. Zhang, Y.G. Assaraf, K. Zhao, X. Xu, J. Xie, D.-H. Yang, Z.-S. Chen, Overcoming ABC transporter-mediated multidrug resistance: molecular mechanisms and novel therapeutic drug strategies, *Drug Resist. Updates* 27 (2016) 14-29.
- [6] M. Videira, R.L. Reis, M.A Brito, Deconstructing breast cancer cell biology and the mechanisms of multidrug resistance, *BBA Rev. Can.* 1846 (2014) 312-325.
- [7] E.M. Leslie, R.G. Deeley, S.P. Cole, Multidrug resistance proteins: role of Pglycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense, *Toxicol. Appl. Pharmacol.* 204 (2005) 216-237.

- [8] R.L. Juliano, V. Ling, A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants, *Biochim. Biophys. Acta.* 455 (1976) 152-162.
- [9] A.A. Ruefli, M.J. Smyth, R.W. Johnstone, HMBA induces activation of a caspase-independent cell death pathway to overcome P-glycoprotein-mediated multidrug resistance, *Blood* 95 (2000) 2378–2385.
- [10] J.M. Croop, B.C. Guild, P. Gros, D.E. Housman, Genetics of multidrug resistance: relationship of a cloned gene to the complete multidrug resistant phenotype, *Cancer Res.* 47 (1987) 5982-5988.
- [11] S. Li, W. Zhang, X. Yin, S. Xing, H.Q. Xie, Z. Cao, B. Zhao, Mouse ATP-Binding cassette (ABC) transporters conferring multi-drug resistance, *Anticancer Agents Med. Chem.* 15 (2015) 423-432.
- [12] E. Baiceanu, K.A. Nguyen, L. Gonzalez-Lobato, R. Nasr, H. Baubichon-Cortay, F. Loghin, M. Le Borgne, L. Chow, A. Boumendjel, M. Peuchmaur, P. Falson, 2-Indolylmethylenbenzofuranones as first effective inhibitors of ABCC2, *Eur. J. Med. Chem.* 122 (2016) 408–418.
- [13] A.J. Horsey, M.H. Cox, S. Sarwat, I.D. Kerr, The multidrug transporter ABCG2: still more questions than answers, *Biochem. Soc. Trans.* 44 (2016) 824-830.
- [14] K.D. Bunting, ABC transporters as phenotypic markers and functional regulators of stem cells, *Stem Cells* 20 (2002) 11–20. Erratum in: *Stem Cells* 20 (2002) 274.
- [15] A.E. Stacy, P.J. Jansson, D.R. Richardson, Molecular pharmacology of ABCG2 and its role in chemoresistance, *Mol. Pharmacol.* 84 (2013) 655–669.
- [16] M. Kühnle, M. Egger, C. Müller, A. Mahringer, G. Bernhardt, G. Fricker, B. König, A. Buschauer, Potent and selective inhibitors of breast cancer resistance protein (ABCG2) derived from the p-glycoprotein (ABCB1) modulator tariquidar, *J. Med. Chem.* 52 (2009) 1190-1197.
- [17] A.H. Schinkel, J.J. Smit, O. van Tellingen, J.H. Beijnen, E. Wagenaar, L. van Deemeter, C.A. Mol, M.A. van der Valk, E.C. Robanus-Maandag, H.P. te Riele, Disruption of the mouse *mdr1a* P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs, *Cell.* 77 (1994) 491–502.
- [18] N.A. Colabufo, F. Berardi, M. Cantore, M. Contino, C. Inglese, M. Niso, R. Perrone, Perspectives of P-glycoprotein modulating agents in oncology and neurodegenerative diseases: pharmaceutical, biological, and diagnostic potentials, *J. Med. Chem.* 53 (2010) 1883-1897.
- [19] R.J. Kathawala, P. Gupta, Jr C.R. Ashby, Z.S. Chen, The modulation of ABC transporter-mediated multidrug resistance in cancer: A review of the past decade, *Drug Resist. Updat.* 18 (2015) 1-17.
- [20] A. Palmeira, E. Sousa, M.H. Vasconcelos, M.M. Pinto, Three decades of P-gp inhibitors: skimming through several generations and scaffolds, *Curr. Med. Chem.* 19 (2012) 1946-2025.
- [21] D. Waghray, Q. Zhang, Inhibit or Evade Multidrug Resistance P-Glycoprotein in Cancer Treatment, *J. Med. Chem.* 61 (2018) 5108-5121.
- [22] B. Wang, B. Zhao, Z.S. Chen, L.P. Pang, Y.D. Zhao, Q. Guo, X.H. Zhang, Y. Liu, G.Y. Liu, H. Zhang, X.Y. Zhang, L.Y. Ma, H.M. Liu, Exploration of 1,2,3-triazole-pyrimidine hybrids as potent reversal agents against ABCB1-mediated multidrug resistance, *Eur. J. Med. Chem.* 143 (2018) 1535-1542.
- [23] Li, X. Q.; Wang, L.; Lei, Y.; Hu, T.; Zhang, F. L.; Cho, C. H.; To, K. K. Reversal of P-gp and BCRP-mediated MDR by tariquidar derivatives. *Eur. J. Med. Chem.* **2015**, *101*, 560-572.
- [24] M. Baekelandt, G. Lehne, C.G. Trope, I. Szanto, P.; Pfeiffer, B. Gustavsson, G.B. Kristensen, Phase I/II trial of the multidrug-resistance modulator valspodar combined with cisplatin and doxorubicin in refractory ovarian cancer, *J. Clin. Oncol.* 19 (2001) 2983–2993.
- [25] P.M. Fracasso, M.F. Brady, D.H. Moore, J.L. Walker, P.G. Rose, L. Letvak, T.M. Grogan, W.P. McGuire, Phase II study of paclitaxel and valspodar (PSC 833) in refractory ovarian carcinoma: a gynecologic oncology group study, *J. Clin. Oncol.* 19 (2001) 2975–2982.

- [26] M.V. Seiden, K.D. Swenerton, U. Matulonis, S. Campos, P. Rose, G. Batist, E. Ette, V. Garg, A. Fuller, M.W. Harding, D. Charpentier, A phase II study of the MDR inhibitor biricodar (INCEL, VX-710) and paclitaxel in women with advanced ovarian cancer refractory to paclitaxel therapy, *Gynecol. Oncol.* 86 (2002) 302–310.
- [27] C. Lhomme, F. Joly, J.L. Walker, A.A. Lissoni, M.O. Nicoletto, G.M. Manikhas, M.M. Baekelandt, A.N. Gordon, P.M. Fracasso, W.L. Mietlowski, G.J. Jones, M.H. Dugan, Phase III study of valspodar (PSC 833) combined with paclitaxel and carboplatin compared with paclitaxel and carboplatin alone in patients with stage IV or suboptimally debulked stage III epithelial ovarian cancer or primary peritoneal cancer, *J. Clin. Oncol.* 26 (2008) 2674–2682.
- [28] S. Modok, H.R. Mellor, R. Callaghan, Modulation of multidrug resistance efflux pump activity to overcome chemoresistance in cancer, *Curr. Opin. Pharmacol.* 6 (2006) 350-354.
- [29] A. Seelig, E. Landwojtowicz, Structure-activity relationship of P-glycoprotein substrates and modifiers, *Eur. J. Pharm. Sci.* 12 (2000) 31-40.
- [30] M.M. Gottesman, T. Fojo, S.E. Bates, Multidrug resistance in cancer: role of ATP-dependent transporters, *Nature Rev. Cancer.* 2 (2002) 48-58.
- [31] M.A. Schumacher, M.C. Miller, R.G. Brennan, Structural mechanism of the simultaneous binding of two drugs to a multidrug binding protein, *EMBO J.* 23 (2004) 2923-2930.
- [32] G. Szakács, J.P. Annereau, S. Lababidi, U. Shankavaram, A. Arciello, K.J. Bussey, W. Reinhold, Y. Guo, G.D. Kruh, M. Reimers, J.N. Weinstein, M.M. Gottesman, Predicting drug sensitivity and resistance: profiling ABC transporter genes in cancer cells, *Cancer Cell.* 6 (2004) 129-137.
- [33] D. Türk, M.D. Hall, B.F. Chu, J.A. Ludwig, H.M. Fales, M.M. Gottesman, G. Szakács, Identification of compounds selectively killing multidrug-resistant cancer cells, *Cancer Res.* 69 (2009) 8293-82301.
- [34] M.D. Hall, N.K. Salam, J.L. Hellawell, H.M. Fales, C.B. Kensler, J.A. Ludwig, G. Szakács, D.E. Hibbs, M.M. Gottesman, Synthesis, activity, and pharmacophore development for isatin-beta-thiosemicarbazones with selective activity toward multidrug-resistant cells, *J. Med. Chem.* 52 (2009) 3191-3204.
- [35] K.M. Pluchino, M.D. Hall, A.S. Goldsborough, R. Callaghan, M.M. Gottesman, Collateral sensitivity as a strategy against cancer multidrug resistance, *Drug Resist. Updat.* 15 (2012) 98-105.
- [36] G. Szakács, M.D. Hall, M.M. Gottesman, A. Boumendjel, R. Kachadourian, B.J. Day, H. Baubichon-Cortay, A. Di Pietro, Targeting the Achilles heel of multidrug-resistant cancer by exploiting the fitness cost of resistance, *Chem. Rev.* 114 (2014) 5753-5774.
- [37] E. Teodori, S. Dei, A. Garnier-Suillerot, F. Gualtieri, D. Manetti, C. Martelli, M.N. Romanelli, S. Scapecchi, P. Sudwan, M. Salerno, Exploratory chemistry toward the identification of a new class of multidrug resistance reverters inspired by pervilleine and verapamil models, *J. Med. Chem.* 48 (2005) 7426-7436.
- [38] E. Teodori, C. Martelli, M. Salerno, N. Darghal, S. Dei, A. Garnier-Suillerot, F. Gualtieri, D. Manetti, S. Scapecchi, M. N. Romanelli, Isomeric *N,N*-bis(cyclohexanol)amine aryl esters: the discovery of a new class of highly potent Pgp-dependent MDR inhibitors, *J. Med. Chem.* 50 (2007) 599-602.
- [39] C. Martelli, M. Coronello, S. Dei, D. Manetti, F. Orlandi, S. Scapecchi, M.N. Romanelli M. Salerno, E. Mini, E. Teodori, Structure-activity relationships studies in a series of *N,N*-bis(alkanol)amine aryl esters as P-glycoprotein (Pgp) dependent multidrug resistance (MDR) inhibitors, *J. Med. Chem.* 53 (2010) 1755-1762.
- [40] S. Dei, M. Coronello, E. Floriddia, G. Bartolucci, C. Bellucci, L. Guandalini, D. Manetti, M.N. Romanelli, M. Salerno, I. Bello, E. Mini, E. Teodori Multidrug Resistance (MDR) reversers: high activity and efficacy in a series of asymmetrical *N,N*-bis(arylalkanol)amine aryl esters, *Eur. J. Med. Chem.* 87 (2014) 398-412.

- [41] S. Dei, D. Manetti, M.N. Romanelli, N. Chiamonte, M. Salerno, E. Teodori, Design and synthesis of aminoester heterodimers containing flavone or chromone moieties as modulators of P-glycoprotein-based multidrug resistance (MDR), *Bioorg. Med. Chem.* 26 (2018) 50-64.
- [42] S. Dei, L. Braconi, A. Trezza, M. Menicatti, N. Chiamonte, D. Manetti, M.N. Romanelli, C. Udomtanakunchai, G. Bartolucci, O. Spiga, M. Salerno, E. Teodori, Modulation of the spacer in N,N-bis(alkanol)amine aryl ester heterodimers led to the discovery of a series of highly potent P-glycoprotein-based multidrug resistance (MDR) modulators, *Eur. J. Med. Chem.* 172 (2019) 71-94.
- [43] H. Kromann, S. Krikstolaityte, A.J. Andersen, K. Andersen, P. Krogsgaard-Larsen, J.W. Jaroszewski, J. Egebjerg, K. Strømgaard, Solid-phase synthesis of polyamine toxin analogues: potent and selective antagonists of Ca²⁺-permeable AMPA receptors, *J. Med. Chem.* 45 (2002) 5743-5754.
- [44] J. Vergote, J.L. Moretti, E.G.E. De Vries, A. Garnier-Suillerot, Comparison of the kinetics of active efflux of ^{99m}Tc-MIBI in cells with P-glycoprotein-mediated and multidrug-resistance protein-associated multidrug-resistance phenotype, *Eur. J. Biochem.* 252 (1998) 140-146.
- [45] P. Reungpatthanaphong, C. Marbeuf-Gueye, L. Le Moyec, M. Salerno, A. Garnier-Suillerot, Decrease of P-glycoprotein activity in K562/ADR cells by M β CD and filipin and lack of effect induced by cholesterol oxidase indicate that this transporter is not located in rafts, *J. Bioenerg. Biomembr.* 36 (2004) 533-543.
- [46] L. Yalçintepe, E. Halis, S. Ulku, Effect of CD38 on the multidrug resistance of human chronic myelogenous leukemia K562 cells to doxorubicin, *Oncol. Lett.* 11 (2016) 2290-2296.
- [47] T. Saeki, T. Tsuruo, W. Sato, K. Nishikawa Drug resistance in chemotherapy for breast cancer, *Cancer Chemother. Pharmacol.* 56 (2005) Suppl 1, 84-89.
- [48] C.B. Lozzio, B.B. Lozzio, Human chronic myelogenous leukemia cell line positive philadelphia chromosome, *Blood* 45 (1975) 321-334.
- [49] E. Teodori, S. Dei, P. Quidu, R. Budriesi, A. Chiarini, A. Garnier-Suillerot, F. Gualtieri, D. Manetti, M.N. Romanelli, S. Scapecchi, Design, synthesis, and in vitro activity of catamphiphilic reverters of multidrug resistance: discovery of a selective, highly efficacious chemosensitizer with potency in the nanomolar range, *J. Med. Chem.* 42 (1999) 1687-1697.
- [50] E. Pereira, A. Garnier-Suillerot, Correlation between the short-term measurements of drug accumulation in living cells and the long-term growth inhibition, *Biochem. Pharmacol.* 47 (1994) 1851-1857.
- [51] J. Fernandez-Recio, M. Totrov, R. Abagyan, Soft Protein-Protein Docking in Internal Coordinates, *Protein Science* 11 (2002) 280-291.
- [52] Y. Kim, J. Chen Molecular structure of human P-glycoprotein in the ATP-bound, outward-facing conformation, *Science* 359 (2018) 915-919.
- [53] S.C. Nicklisch, S.D. Rees, A.P. McGrath, T. Gökirmak, L.T. Bonito, L.M. Vermeer, C. Cregger, G. Loewen, S. Sandin, G. Chang, A. Hamdoun, Global marine pollutants inhibit P-glycoprotein: Environmental levels, inhibitory effects, and cocrystal structure, *Sci. Adv.* 2 (2016) 2(4): e1600001.
- [54] L. Chang, M. Xiao, L. Yang, S. Wang, S.Q. Wang, A. Bender, A. Hu, Z.S. Chen, B. Yu, H.M. Liu, Discovery of a non-toxic [1,2,4]triazolo[1,5-a]pyrimidin-7-one (WS-10) that modulates ABCB1-mediated multidrug resistance (MDR), *Bioorg. Med. Chem.* 26 (2018) 5974-5985.
- [55] J.W. Polli, S.A. Wring, J.E. Humphreys, L. Huang, J.B. Morgan, L.O. Webster, C.S. Serabjit-Singh, Rational use of in vitro P-glycoprotein assays in drug discovery. *J. Pharmacol. Exp. Ther.* 299 (2001) 620-628.
- [56] C. Inglese, M.G. Perrone, F. Berardi, R. Perrone, N.A. Colabufo, Modulation and absorption of xenobiotics: the synergistic role of CYP450 and P-gp activities in cancer and neurodegenerative disorders, *Curr. Drug Metab.* 12 (2011) 702-712.
- [57] L. Kangas, M. Grönroos, A. L. Nieminem, Bioluminescence of cellular ATP: a new method for evaluating cytotoxic agents in vitro, *Med. Biol.* 62 (1984) 338-343.

- [58] N.A. Colabufo, F. Berardi, M. Cantore, M.G. Perrone, M. Contino, C. Inglese, M. Niso, R. Perrone, A. Azzariti, G.M. Simone, L. Porcelli, A. Paradiso, Small P-gp modulating molecules: SAR studies on tetrahydroisoquinoline derivatives, *Bioorg. Med. Chem.* 16 (2008) 362–373.
- [59] B. Feng, J.B. Mills, R.E. Davidson, R.J. Mireles, J.S. Janiszewski, M.D. Troutman, S.M. de Morais, In vitro P-glycoprotein assays to predict the in vivo interactions of P-glycoprotein with drugs in the central nervous system, *Drug Metab. Dispos.* 36 (2008) 268–275.
- [60] N.A. Colabufo, F. Berardi, M. Cantore, M.G. Perrone, M. Contino, C. Inglese, M. Niso, R. Perrone, A. Azzariti, G.M. Simone, A. Paradiso, 4-Biphenyl and 2-naphthyl substituted 6,7-dimethoxytetrahydroisoquinoline derivatives as potent P-gp modulators, *Bioorg. Med. Chem.* 16 (2008) 3732-3743.
- [61] M.D. Hall, M.D. Handley, M.M. Gottesman, Is resistance useless? Multidrug resistance and collateral sensitivity, *Trends Pharmacol. Sci.* 30 (2009) 546-556.
- [62] M. Niso, C. Abate, M. Contino, S. Ferorelli, A. Azzariti, R. Perrone, N.A. Colabufo, F. Berardi, Sigma-2 receptor agonists as possible antitumor agents in resistant tumors: hints for collateral sensitivity, *ChemMedChem.* 8 (2013) 2026-2035.
- [63] D. Lorendeau, L. Dury, R.I. Nasr, A. Boumendjel, E. Teodori, M. Gutschow, P. Falson, A. Di Pietro, H. Baubichon-Cortay, MRP1-dependent Collateral Sensitivity of Multidrug-resistant Cancer Cells: Identifying Selective Modulators Inducing Cellular Glutathione Depletion, *Curr. Med. Chem.* 24 (2017) 1186-1213.
- [64] M. Menicatti, L. Guandalini, S. Dei, E. Floriddia, E. Teodori, P. Traldi, G. Bartolucci, The power of energy-resolved tandem mass spectrometry experiments for resolution of isomers: the case of drug plasma stability investigation of multidrug resistance inhibitors, *Rapid Commun. Mass Spectrom.* 30 (2016) 423-432.
- [65] E. Teodori, S. Dei, M. Coronello, E. Floriddia, G. Bartolucci, D. Manetti, D. Santo Domingo Porqueras, M. Salerno, *N*-alkanol-*N*-cyclohexanol amine aryl esters: Multidrug resistance (MDR) reversing agents with high potency and efficacy, *Eur. J. Med. Chem.* 127 (2017) 586-598.
- [66] A.G. Marshall, C.L. Hendrickson, High-resolution mass spectrometers, *Annu. Rev. Anal. Chem.* 1 (2008) 579-599.
- [67] C. Martelli, D. Alderighi, M. Coronello, S. Dei, M. Frosini, B. Le Bozec, D. Manetti, A. Neri, M.N. Romanelli, M. Salerno, S. Scapecchi, E. Mini, G. Sgaragli, E. Teodori, *N,N*-bis(cyclohexanol)amine aryl esters: a new class of highly potent transporter-dependent multidrug resistance inhibitors, *J. Med. Chem.* 52 (2009) 807-817.
- [68] S. Mankhetkorn, A. Garnier-Suillerot, The ability of verapamil to restore intracellular accumulation of anthracyclines in multidrug resistant cells depends on the kinetics of their uptake, *Eur J Pharmacol.* 343 (1998) 313-321.
- [69] C. Marbeuf-Gueye, M. Salerno, P. Quidu, A. Garnier-Suillerot, Inhibition of the P-glycoprotein- and multidrug resistance protein-mediated efflux of anthracyclines and calceinacetoxymethyl ester by PAK-104P. *Eur J Pharmacol.* 391 (2000) 207-216.
- [71] N.A. Colabufo, M. Contino, M. Cantore, E. Capparelli, M.G. Perrone, G. Cassano, G. Gasparre, M. Leopoldo, F. Berardi, R. Perrone, Naphthalenyl derivatives for hitting P-gp/MRP1/BCRP transporters, *Bioorg. Med. Chem.* 21 (2013) 1322-1330.
- [71] M. Contino, S. Guglielmo, M.G. Perrone, R. Giampietro, B. Rolando, A. Carrieri, D. Zaccaria, K. Chegaev, V. Borio, C. Riganti, K. Zabielska-Koczywaś, N.A. Colabufo, R. Fruttero, New tetrahydroisoquinoline based P-glycoprotein modulators: decoration of the biphenyl core gives selective ligands, *MedChemComm.* 9 (2018) 862- 869.
- [72] C. Riganti, E. Gazzano, G.R. Gulino, M. Volante, D. Ghigo, J. Kopecka, Two repeated low doses of doxorubicin are more effective than a single high dose against tumors overexpressing P-glycoprotein, *Cancer Lett.* 360 (2015) 219-226.
- [73] J.W. McCormick, P.D. Vogel, J.G. Wise, Multiple Drug Transport Pathways through Human P-Glycoprotein, *Biochemistry* 54 (2015) 4374-4390.

- [74] A. Bernini, O.M. Spiga, A. Ciutti, S. Chiellini, L. Bracci, X. Yan, B. Zheng, J. Huang, M.L. He, H.D. Song, P. Hao, G. Zhao, N. Niccolai, Prediction of quaternary assembly of SARS coronavirus peplomer, *Biochem Biophys Res Commun.* 325 (2004) 1210-1214.
- [75] O. Spiga, A. Bernini, M. Scarselli, A. Ciutti, L. Bracci, L. Lozzi, B. Lelli, D. Di Maro, D. Calamandrei, N. Niccolai, Peptide-protein interactions studied by surface plasmon and nuclear magnetic resonances, *FEBS Lett.* 511 (2002) 33-35.
- [76] F. Fusi, O. Spiga, A. Trezza, G. Sgaragli, S. Saponara, The surge of flavonoids as novel, fine regulators of cardiovascular Ca(v) channels, *Eur J Pharmacol.* 796 (2017) 158-174.
- [77] The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.
- [78] A.C. Wallace, R.A. Laskowski, J.M. Thornton. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions, *Protein Eng.* 8 (1995) 127–134.
- [79] S. Salentin, S. Schreiber, V.J. Haupt, M.F. Adasme, M. Schroeder, PLIP: fully automated protein–ligand interaction profiler. *Nucleic Acids Res.* (2015) W443–W447.