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New isoform-selective HCN blockers

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(54) Title: ISOFORM-SELECTIVE HCN BLOCKERS

(57) Abstract: The present invention relates to compounds of formula (I) as isoform-selective HCN blockers. In particular said compounds showed diverse capabilities of blocking selectively isoforms HCN1, HCN2 and HCN4 expressed in HEK cells. The invention further relates to the medical use of said compounds or as pharmacological or drug-development tools.

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ISOFORM-SELECTIVE HCN BLOCKERS

FIELD OF INVENTION

The present invention refers to new chemical entities, their synthesis and medical use as pharmacological tools or in the development of new drugs. In particular, the present invention refers to compounds which selectively block HCN (Hyperpolarization-activated Cyclic Nucleotide gated) channel isoforms.

STATE OF THE ART

Cardiovascular disorders represent the first cause of death in western countries. Ischemic cardiomiopathy affects 3-4% of the adult population, and, despite the advances in the medical and surgical fields, it is expected to raise due to aging and to the increasing incidence of hypertension, obesity and diabetes. *Angina pectoris* is typically provoked by an imbalance between myocardial perfusion and oxygen consumption, worsened by increase of heart rate, which mainly determines oxygen demand. It is therefore logical that drugs able to reduce heart rate can potentially relieve the symptoms of the disease.

In addition, twenty years of observations show that the reduction of heart rate is associated to a decreased risk of mortality for cardiovascular problems. Heart rate control is achieved mainly through the administration of beta-blockers and calcium antagonists, but both kinds of drugs cause several side effects, such as depression, hypotension, erectile dysfunction, and respiratory problems, which limit their clinical use. Recently a new class of drugs appeared in therapy, which acts selectively on the ionic mechanism (the funny current) controlling pacemaker function of the sinoatrial node, opening new therapeutic perspectives.

The *funny current* (I_f) plays a primary role in the generation of pacemaker activity and in the regulation of heart rate. I_f is a mixed Na^+/K^+ inward current, activated upon hyperpolarization, directly modulated by cAMP, and regulated by neurotransmitter receptors coupled to cyclic nucleotide second messengers. I_f is mediated by the HCN (Hyperpolarization-activated Cyclic Nucleotide gated) channels: they consist of six transmembrane domains (S1-6), which bear a voltage sensor (S4) and a cyclic nucleotide-binding site. In mammals, the HCN channel family is formed by four members (HCN1-4) which are differently distributed within central and peripheral nervous system and in the

heart. The channels are made up by four subunits; when the isoforms are separately expressed in heterologous cells, they form homomeric channels displaying the main biophysical properties of native I_f but differing from each other mainly with regard to their speed of activation and the extent by which they are modulated by cAMP. The variety of HCN channels in vivo is likely increased since the four subunits can combine into heterotetrameric channels, whose stoichiometry is however not known.

HCN channels have a primary role in the activity of several excitable cells, such as the sinoatrial node, which generate heart rate, neurons in the central and peripheral nervous system, retinal photoreceptors. HCN channels blockers find therapeutic application at the moment only as bradycardic agents in the treatment of stable *angina pectoris*. Nevertheless, several experimental evidences show the involvement of these channels in important pathologies such as epilepsy and neuropathic pain, increasing the interest and the therapeutic potential of modulators of these proteins.

The specificity of action of bradycardic agents is due to the selective blockade of the pacemaker current, i.e. the f-current, by reversibly binding within the ion channel pore. At the state of the art some specific bradycardic agents, able to block If, are known; among them are zatebradine (UL-FS-49), cilobradine (DK-AH-269) and ivabradine ((+)-S 16257). Ivabradine (Procoralan® or Corlentor®, Servier) is the only one available for clinical use.

Other known compounds, structurally related to zatebradine are the following:

- 3-((E)-4-{[2-(3,4-Dimethoxyphenyl)ethyl]methylamino}but-2-enyl)-7,8-dimethoxy-1,3-dihydro-benzo[*d*]azepin-2-one (**EC4**) reported in Romanelli, M. N. et al. Bioorg. Med. Chem. 2005, 581, 97;
- 3-((Z)-4-{[2-(3,4-Dimethoxyphenyl)ethyl]methylamino}but-2-enyl)-7,8-dimethoxy-1,3-dihydro-benzo[*d*]azepin-2-one, (**EC32**) described in E. Cerretini, 1999, graduation thesis in Pharmaceutical Chemistry and Technology (CTF), University of Florence;
- *Cis* 3-(3-{[2-(3,4-Dimethoxyphenyl)ethyl]methylamino}cyclohexyl)-7,8-dimethoxy-1,3,4,5-tetrahydro-benzo[*d*]azepin-2-one, (**EC18**) described in E. Cerretini, 1999 graduation thesis in Pharmaceutical Chemistry and Technology (CTF), University of Florence.

Compounds EC4 and EC32 have shown to block If with potency in the same range of zatebradine; also EC18 showed good negative chronotropic activity on isolated guinea-pig atria.

The chemical structure of the compounds known to the state of the art are reported in figure 1.

Zatebradine, cilobradine and ivabradine have different ability to block cation currents, but they are not able to discriminate among different HCN channel isoforms (Stieber, J. et al. Mol. Pharmacol. 2006, 69, 1328; Lee, Y. T., et al. Eur. J. Pharmacol. 2008, 581, 97).

The lack of selectivity toward channel isoforms, and in particular toward HCN4, the main isoform found in the sinoatrial node may be a possible limitation in the use of these compounds. In fact, the blockade of the neuronal isoform HCN1 may alter vision, causing phenomena called phosphenes, due to the specific function of f-current in retina. It is therefore important that a drug could interact selectively with the isoform which is most abundant or which shows the most relevant function in the target tissue or cell type, with the aim to limit the side effects of the pharmacological treatment. However, no isoform selective substance has been described in the literature: the drugs available for the clinic or under trial block all the isoforms with similar potency

The four HCN channels isoforms are proteins showing a high degree of homology; therefore it is not easy to find substances which selectively interact with one of them.

Aim of the present invention is to provide new molecules able not only to reduce heart rate with a potency at least comparable to that of the reference compounds zatebradine and ivabradine, but showing selectivity toward a specific HCN channel isoform.

DEFINITIONS AND ABBREVIATIONS

Ak = linear or branched alkyl group;

Ak_{0-4}= none or alkyl group containing from 1 to 4 carbon atoms;

Ak_{1-4}= alkyl group containing from 1 to 4 carbon atoms;

Ak_{1-3}= alkyl group containing from 1 to 3 carbon atoms;

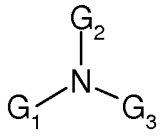
Ak_{1-9}= alkyl group containing from 1 to 9 carbon atoms;

Hal = halogen atom

-* = it represents the point of attachment of the fragment in the chemical structure

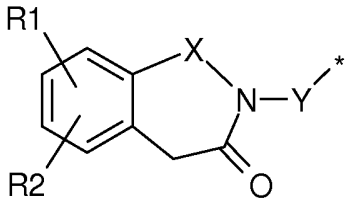
SUMMARY OF THE INVENTION

The present invention solves the above-mentioned problems through new compounds of general formula (I):

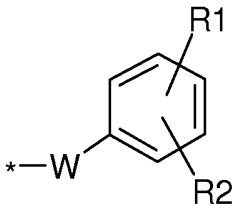
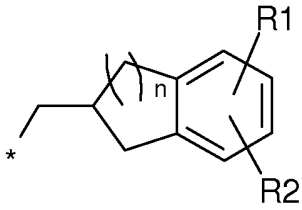


(I)

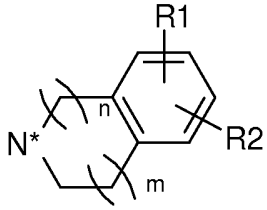
where

G1 = 

G2 = G1 o Ak_{1-4};

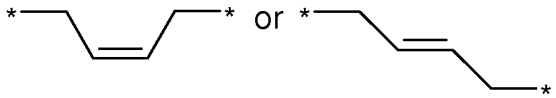
G3 =  or 

or G2 and G3, together with the nitrogen atom to which they are bound, form a cyclic structure such as

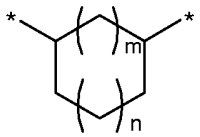


X = CH=CH, CH_2CH_2;

Y = but-2-en-1,4-diyl with structure



or Y is a cycloalkane-diyl such as



W = Ak_{1-9}, $Ak_{1-4}OAk_{0-4}$, $Ak_{1-4}C(=O)Ak_{0-4}$, $Ak_{1-4}SAk_{0-4}$, $Ak_{1-4}N(R1)Ak_{0-4}$, with the condition that, when Y is a but-2-en-1,4-diyl and G2 = Ak_{1-4}, at least one Ak of W is branched and it contains a stereogenic center;

R1 and R2 independently from each other can be H, halogen, -CN, -OH, $-CF_3$, $-Ak_{1-3}$, $-OAk_{1-3}$, $-SAk_{1-3}$, $-Ph-OAk_{1-3}$;

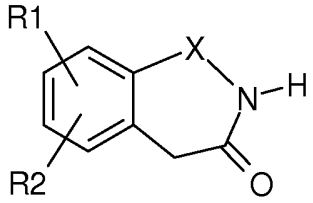
n = 0, 1, 2; m = 0, 1, 2;

with the condition that G2 = Ak_{1-4} when Y is a cycloalkane-diyl as above defined, with the exclusion of compound *cis* 3-(3-{[2-(3,4-dimethoxyphenyl)ethyl]methylamino}cyclohexyl)-7,8-dimethoxy-1,3,4,5-tetrahydrobenzo[*d*]azepin-2-one.

The above substances are useful for medical purpose as active ingredients and therefore can be used for the preparation of pharmaceutical compositions for the treatment of angina, arrhythmia, cardiovascular diseases, neurological disorders such as epilepsy, with inclusion of febrile seizure, neuropathic pain, cognitive dysfunctions.

Surprisingly it has been found that the compounds of formula (I), including *cis* 3-(3-{[2-(3,4-dimethoxyphenyl)ethyl]methylamino}cyclohexyl)-7,8-dimethoxy-1,3,4,5-tetrahydro-benzo[*d*]azepin-2-one, are active at concentration ≤30 μM as isoform-selective HCN channel blockers. In particular, they have different abilities to selectively block HCN1, HCN2 and HCN4 channel isoforms expressed in HEK 293 cells. These substances can therefore be used as pharmacological tools to study the structure and functioning of HCN channels and the physiological and/or pathological processes where they are involved, but they can also be developed as drugs, or they can help the design of drugs showing higher potency and selectivity with respect to the drugs presently known, and with less side effects.

In an aspect this invention concerns processes to prepare the compounds of formula (I) starting from compounds of formula (V)



(V)

where R1, R2 and X are as above defined, the process as described in detail hereinafter.

BRIEF DESCRIPTION OF FIGURES

Figure 1 – Structures of the compounds known to the state of the art.

Figure 2 - Structures of some examples of compounds of the present invention

Figure 3 - Structures of some examples of compounds of the present invention.

Figure 4 - Typical experiment for the study of the compounds of the present invention as If blockers in different channel isoforms. **A**, **B**: Traces of f-current recorded in HEK293 cells expressing HCN1 channel isoform, under control conditions (**A**) or with EC32 10μM (**B**). **C**: Activation curves of I_f recorded in control condition (black) or with EC32 10μM (grey). **D**: Dose-dependence of the block of the f-current evoked by a series of consecutive hyperpolarizing steps at -120 mV in presence of increasing doses (from 1 to 30 μM) of compound EC32

Figure 5: Activation curve of I_f current recorded in HEK293 cells expressing HCN1 (A), HCN2 (B) and HCN4 (C) channel isoform in presence of EC4 10μM (grey) with respect to control (black).

**p< 0.01 vs CTR.

Figure 6: Activation curve of I_f current recorded in HEK293 cells expressing HCN1 (A), HCN2 (B) and HCN4 (C) channel isoform in presence of EC18 10μM (grey) with respect to control (black). *p< 0.05 vs CTR; §p<0.001 vs CTR.

Figure 7: Activation curve of I_f current recorded in HEK293 cells expressing HCN1 (A), HCN2 (B) and HCN4 (C) channel isoform in presence of EC32 10μM (grey) with respect to control (black). *p< 0.05 vs CTR; *p<0.01 vs CTR; §p<0.001 vs CTR.

Figure 8: Activation curve of I_f current recorded in HEK293 cells expressing HCN1 (A), HCN2 (B) and HCN4 (C) channel isoform in presence of MEL57A 10μM (grey) with respect to control (black). § p<0.001 vs CTR.

Figure 9: Ratio between the EC_{50} values on the three channel isoforms of selected compounds representative of the present invention, in comparison with compounds known to the stat of the art..

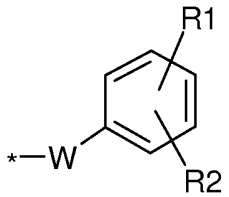
Figure 10: Typical examples of native I_f current traces recorded in SAN single cells of guinea pig in control condition **(A)** and after application of EC32 10 μM **(B)**. **C)** Corresponding I_f activation curves: control condition was reported in black and EC32 10 μM in grey. Plots report I_f current conductance normalized with respect to I_f maximal conductance (G_f/G_{fmax}) versus tested membrane potential (mV) used to evoke current. **D)** Current density calculated at -120 mV after application of EC4, EC18 e EC32 (10 μM). *<0.05 EC4 vs CTR;**<0.01 EC32vs CTR.

Figure 11: Effect of EC4 e EC32 in rabbit sinoatrial node. **A**) Activation curves calculated in control condition (black) and in presence of EC32 10 μM (grey). **B**) I_f current density calculated at -120 mV after application of EC4 and EC32 (10 μM).

DETAILED DESCRIPTION OF THE INVENTION

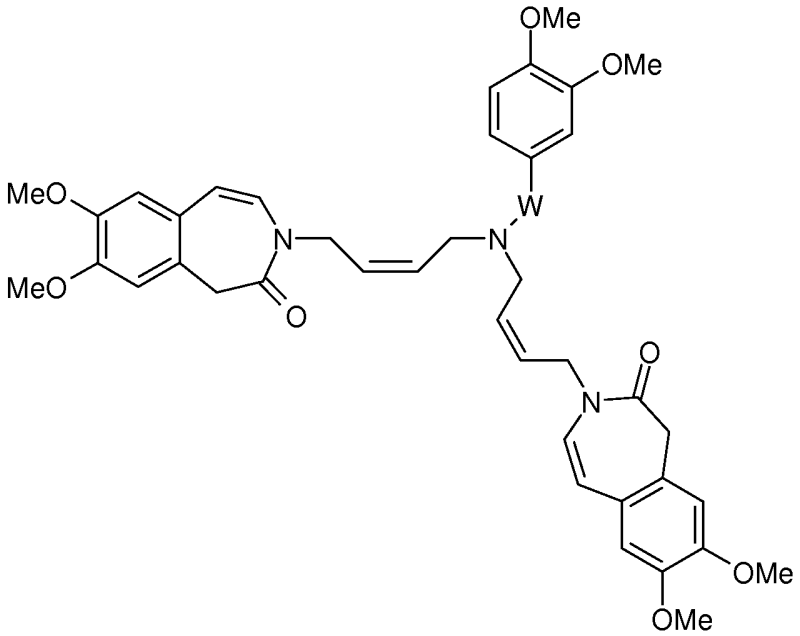
Among the compounds of formula (I) as described above those compounds are preferred which have:

Y = but-2-en-1,4-di-yl;

G3 = 

W = branched Ak_{1-9} which contains at least one stereogenic center.

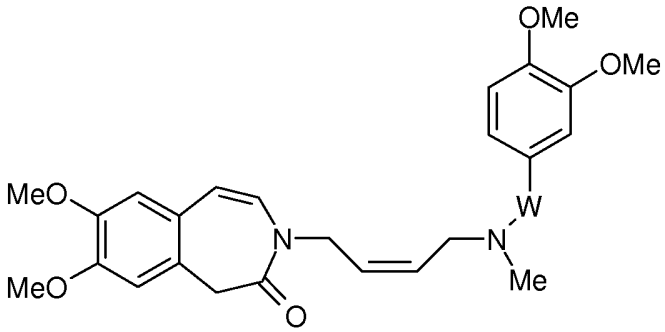
In particular compounds of formula (II) are preferred:



(II)

where W = -CH(CH_3)-CH_2- or -CH_2-CH(CH_3)-.

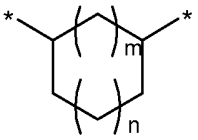
The compounds of formula (II) where W = -CH_2-CH(CH_3)- with the stereogenic centre showing R configuration are particularly potent and selective in vitro in inhibiting dose-dependently f-current carried through HCN1 channels, as described in details afterwards. Other particularly preferred compounds are those of formula (III):



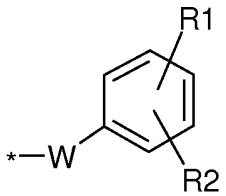
(III)

where W = -CH(CH_3)-CH_2- or -CH_2-CH(CH_3)-. Compounds of formula (III) where W = -CH_2-CH(CH_3)- with the stereogenic center showing R configuration are particularly potent and selective in vitro in inhibiting dose-dependently f-current carried through HCN2 channels, as described in details afterwards.

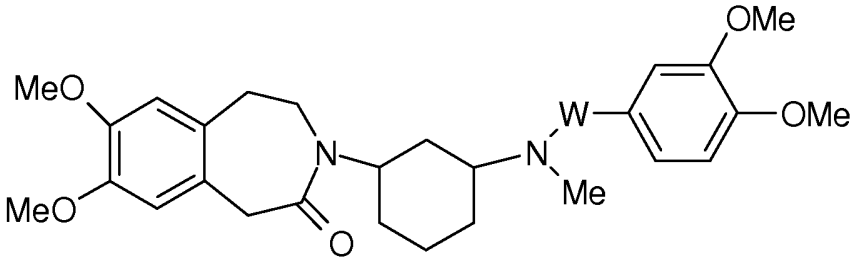
Among the compounds of formula (I) as described above are preferred those compounds having

Y = 

G2 = Ak_{1-4};

G3 = 

In particular compounds of formula (IV) are preferred:



(IV)

wherein W is $-(CH_2)k-$ or $-(CH_2)p-O-$, k=1,2,3, p=1,2. The racemate and the single enantiomers are included. Compounds of formula (IV) in a *cis* configuration and in all the enantiomeric form are useful as selective HCN4 channel isoform inhibitors.

The compounds comprised in the invention may be in the anhydrous or hydrated forms, may be as non-salified molecules or as salts obtained through protonation of the basic moiety with a suitable acid, for instance they can be as chloride, bromide, iodide, sulphate, phosphate or carboxylate salt.

In another aspect, the present invention provides pharmaceutically compositions including a compound of formula (I) as described above, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents, which allow oral or parenteral administration. The additives and/or diluents can be solid, liquid and semisolid

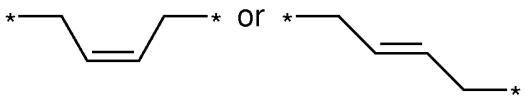
Solid additives can be, with no limitation, starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, silica gel, magnesium stearate, sodium stearate, glycerin monostearate, sodium chloride, powdered milk and similar substances employed in pharmaceutical formulations. Liquid and semisolid additives can be, with no limitation, glycerin, propylene glycol, water, ethanol and animal or vegetal oils. Liquid carriers, particularly suitable for parenteral administration, include water, saline solution, aqueous solution of dextrose and glycols.

A compound with formula (I) can be administered at a dosage level which depends on a variety of factors including the kind and the severity of the pathology, the age and the general health of the patient, the potency of the compound, the administration route and the kind of pharmaceutical formulation. Preferred administration routes are the parenteral administration, for instance the intravenous infusion, and oral administration, for instance capsules, tablets, solutions. Daily effective dosages include dosages from 1 to 20 mg, in one or more administrations.

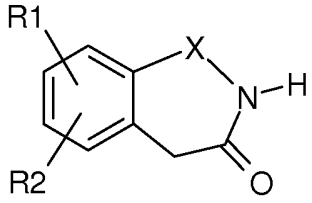
The compounds of the present invention are able to selectively block HCN channel isoforms. These compounds can be used to reduce f-current in tissues showing exaggerated or abnormal rhythmic activity, or automatic non-rhythmic activity, as for instance primary or subsidiary cardiac pacemaker cells, non-pacemaker atrial and ventricular cardiomyocytes with abnormal automaticity, epileptogenic neuronal foci in the central nervous system, and peripheral neurons showing high discharging rate. These mechanisms represent the etiology of worldwide spread pathologies, such as sinus tachycardia, atrial and ventricular arrhythmia, cardiac sudden death, epilepsy and neuropathic pain. Although the role of f-current in these pathologies is not exclusive, nor it is completely clear, the availability of specific isoform-selective blockers can help in the understanding of the cellular basis of normal and abnormal automaticity in excitable cells.

Beside being significant experimental tools, these compounds can be also important at the clinical level, if used alone or in combination with drugs, such as beta-blockers, antiepileptic and antiarrhythmic drugs, presently used to control cardiac arrhythmia, to manage acquired or congenital cardiomyopathy that benefit from therapies aimed to reduce heart rate or to limit the tendency to develop abnormal automaticity in secondary pacemaker centers, and in neurological disorders.

Compounds of formula (I) where

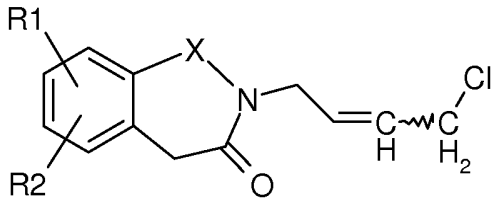
Y= 

can be prepared starting from compounds of formula (V)



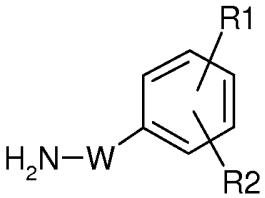
(V)

in a way similar to that previously reported (Bioorg. Med. Chem, 13, 1211-1216, 2005 and E. Cerretini, graduation thesis in Pharmaceutical Chemistry and Technology, 1999) by means of reaction with *cis* or *trans* 1,4-dichloro-but-2-ene obtaining compounds of formula (VI) *cis* or *trans*



(VI)

that can be reacted with suitable amines of formula:



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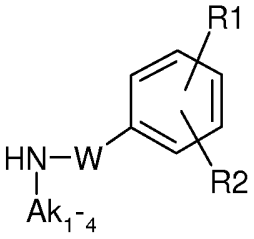

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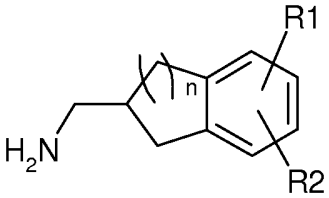
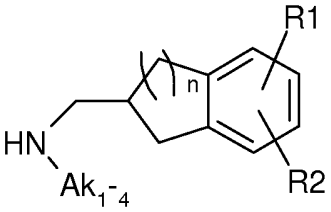
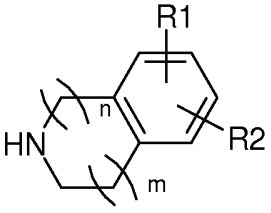




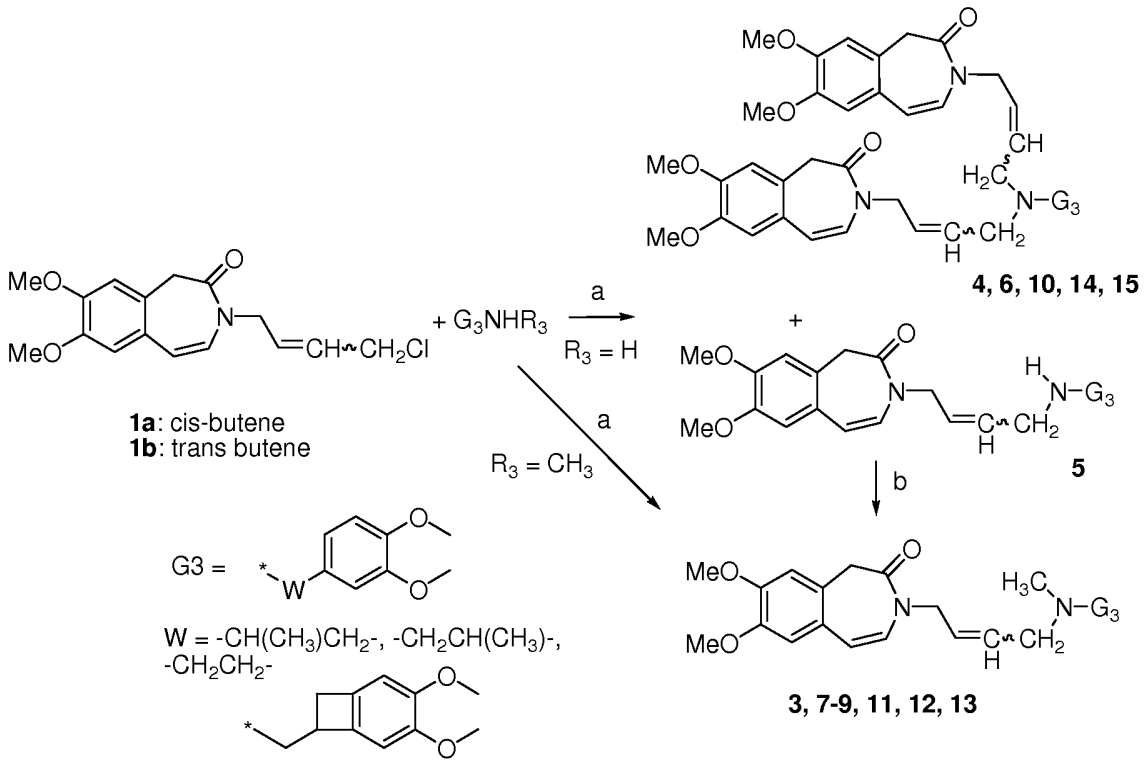
o



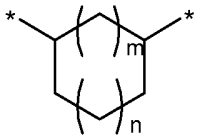
When the reactant is a primary amine, the products of both mono-alkylation and double-alkylation are formed, which can be separated by means of the methods known to the state of the art, for instance by chromatography. The mono-alkylation product can be further alkylated using known methods.

In particular, compounds **4, 6, 14** of formula (II) and the trans analogs **10** and **15**, compounds **3**, **8**, **11**, **13** of formula (III) and the trans analogs **7**, **9**, **12** as described in examples 3-14 can be prepared as described in scheme 1 starting from *cis* or *trans* 3-(4-chloro-but-2-enyl)-7,8-dimethoxy-1,3-dihydro-benzo[*d*]azepin-2-one (respectively **1a** e **1b**) prepared according to the procedure already reported (Bioorg. Med. Chem, 13, 1211-1216, 2005 and E. Cerretini, graduation thesis in Pharmaceutical Chemistry and Technology, 1999). In particular, in step a) of the process the chlorobutenyl derivative is treated with the suitable amine; when the latter is a primary amine the products of both mono and double alkylation are formed, which are separated by chromatography. The secondary amines is then methylated in step b) using formaldehyde and formic acid.

Scheme 1

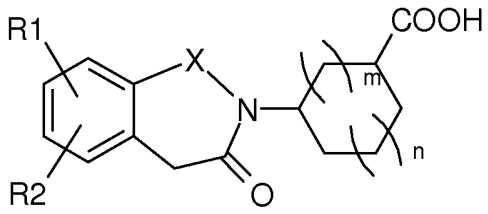


Compounds of formula (I) where

Y = 

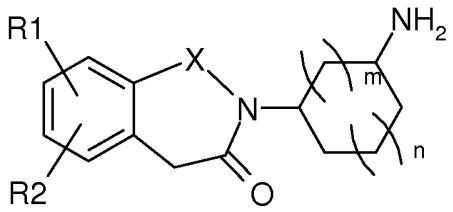
n = 0, 1, 2; m = 0, 1, 2;

can be prepared starting from compounds of formula V in a way similar to what reported in E. Cerretini, graduation thesis in Pharmaceutical Chemistry and Technology, 1999, by means of reaction with Br-cycloalkanecarboxylates to obtain *cis/trans* mixture of compounds of formula (VII) which can be separated with known methods.



(VII)

The carboxylic group of the compounds of formula (VII) can be transformed with known methods in a $-NH_2$ group to obtain compounds of formula (VIII)



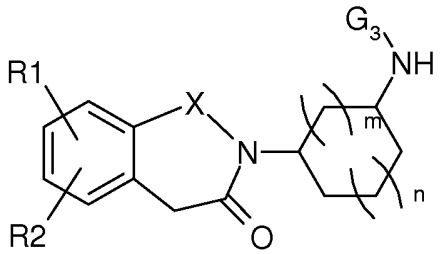
(VIII)

as *cis/trans* mixture which can be separated with known methods.

Cis/trans mixtures can be resolved also at the level of intermediate synthetic derivatives of compounds of formula (VIII) from compounds of formula (VII); as an example, for the preparation of compound *cis* 3-(3-{[2-(3,4-dimethoxyphenyl)ethyl]methylamino}cyclohexyl)-7,8-dimethoxy-1,3,4,5-tetrahydro-

benzo[*d*]azepin-2-one of formula (IV) the separation of the *cis*/*trans* mixture has been done on the corresponding precursors of formula (VII) as carboxylate esters.

The compounds of formula (VIII) are the reacted with a suitable halogen derivative of formula G3-Hal to obtain compounds of formula (IX)

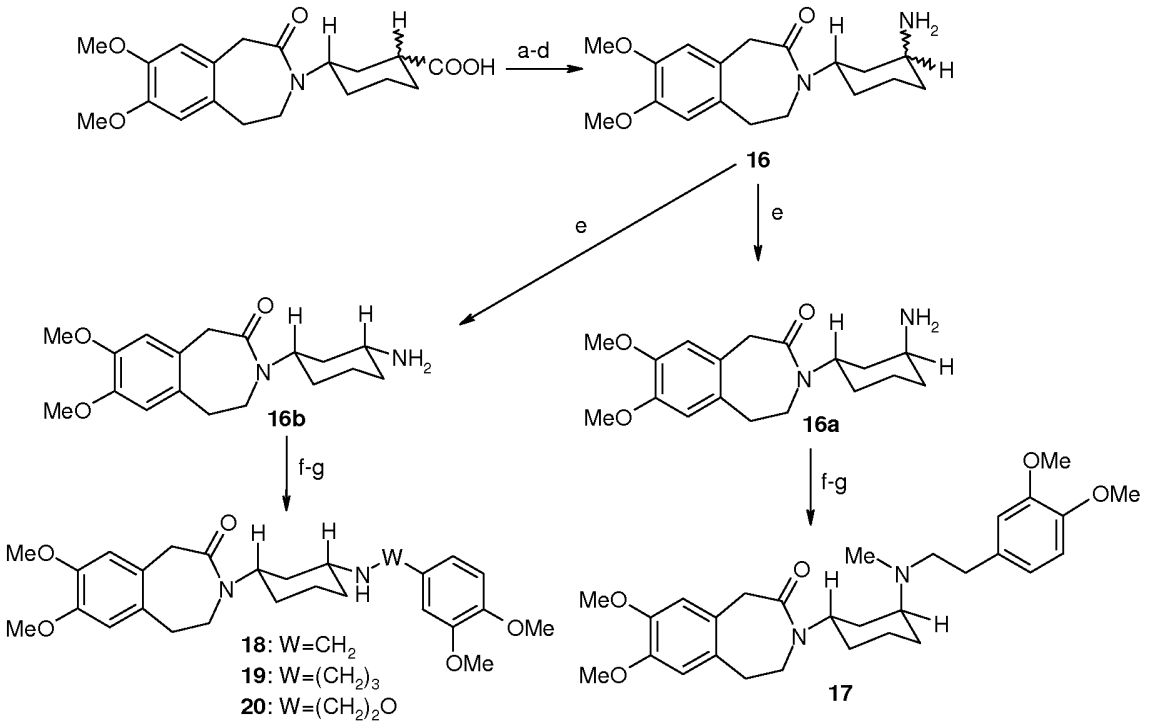


(IX)

that can be reacted with the suitable alkyl halide of formula G2-Hal or with the suitable aldehyde to obtain compounds of formula (I).

To prepare compound **17** of formula (IV) described in example 15 according to the present invention, different synthetic strategies are possible, as illustrated in the following scheme 2.

SCHEME 2



A *cis*/*trans* mixture of 3-(7,8-dimethoxy-2-oxo-1,2,4,5-tetrahydro-benzo[*d*]azepin-3-yl)-cyclohexanecarboxylic acid prepared as described in the literature (E. Cerretini, graduation thesis in Pharmaceutical Chemistry and Technology, 1999), is treated with thionyl chloride

and then with sodium azide (steps a and b) to obtain an intermediate acylazide, which undergoes thermal transposition in toluene (step c) and acid hydrolysis (step d) obtaining a *cis/trans* mixture of (3-aminocyclohexyl)-7,8-dimethoxy-1,3,4,5-tetrahydrobenzo[*d*]azepin-2-one which is separated by column chromatography (step e), obtaining separately both the *trans* isomer (**16a**) and the already described *cis* (**16b**) one (S. Betti, graduation thesis in Pharmaceutical Chemistry and Technology, 1998; E. Cerretini, graduation thesis in Pharmaceutical Chemistry and Technology, 1999). Compound **16a** or **16b** is the treated with the suitable alkyl halide (step f) and transformed into the tertiary amine by means of formic acid and formaldehyde (step g).

The present invention will be explained in more details by means of the following examples which are certainly functional to the description while do not limit the range of applications.

EXPERIMENTAL PART

All melting points were taken on a Büchi apparatus. ^{1}H-NMR, ^{13}C-NMR, HSQC and COSY spectra were recordered on a Bruker Avance 400 spectrometer. Infrared spectra were recordered with a Perkin-Elmer 681 spectrofotometer in Nujol mull for solids and neat for liquids. Thin layer chromatography (TLC) were performed on Kieselgel Merck F254 silica gel plates and on F254 neutral alumina plates. Chromatographic separations were performed on a silica gel column by gravity chromatography (Kieselgel 40, 0.063-0. 200 mm; Merck) or flash cromatografy (0.040-0.063 mm; Merck) using the proper eluents. Optical rotation was measured at a concentration of 1g/100mL (c=1) with a Perkin-Elmer polarimeter (accuracy 0.002°). GC-MS analysis were performed on a Perkin-Elmer Turbomass – Autosystem XL. Alternatively, mass spectra were recordered on a Linear Ion Trap (LTQ)-Thermo-Finnigam spectrometer. Compounds were named following IUPAC rules as applied by Beilstein-Institute AutoNom (version 2.1) software for systematic names in organic chemistry.

Example 1:

synthesis of **3-[(Z)-4-(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)but-2-enyl]-7,8-dimethoxy-1,3-dihydro-benzo[d]azepin-2-one** (**2**)

In a two-necked flask under nitrogen, 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (commercially available; 0.65 mmol; 0.15 g) was added to a solution of *cis* 3-(4-chloro-but-2-enyl)-7,8-dimethoxy-1,3-dihydro-benzo[*d*]azepin-2-one **1a** (0.32 mmol; 0.1 g) in dry triethylamine (5 mL). The mixture was stirred for 5 hours at 60 °C. The solvent was removed under vacuum and the residue was taken up with water and extracted with dichloromethane (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by flash chromatography (eluent: dichloromethane-methanol, 9:1) to give **2** as a yellow oil in 53% yields. ^{1}H NMR ($CDCl_3$, δ): 2.74-2.77 (m, 2H); 2.83-2.86 (m, 2H); 3.30 (d, J=6.8 Hz, 2H); 3.45 (s, 2H); 3.60 (s, 2H); 3.83 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.87 (s, 3H, OCH_3); 3.88 (s, 3H, OCH_3); 4.27 (d, J=6.8 Hz, 2H); 5.52-5.58 (m, 1H); 5.76-5.82 (m, 1H); 6.22 (d, J=9.2 Hz, 1H); 6.36 (d, J=9.2 Hz, 1H); 6.50 (s, 1H); 6.59 (s, 1H); 6.73 (s, 1H); 6.78 (s, 1H). ^{13}C NMR ($CDCl_3$, δ, APT): 28.26 (CH_2), 43.26 (CH_2), 44.75 (CH_2), 50.61 (CH_2), 54.45 (CH_2), 55.32 (CH_2), 56.00 (CH_3), 56.09 (CH_3), 109.56 (CH), 109.69 (CH), 111.35 (CH), 111.47 (CH), 117.48 (CH), 124.74 (quat. C), 125.56 (quat. C), 125.73 (quat. C), 126.46 (quat. C), 127.77 (CH), 128.40 (CH), 129.63 (CH), 147.47 (quat. C), 147.85 (quat. C), 148.19 (quat. C), 150.08 (quat. C), 167.62 (CO) ppm.

Example 2:

synthesis of **3-{(Z)-4-[(5,6-dimethoxyindan-2-ylmethyl)methylamino]but-2-enyl}-7,8-dimethoxy-1,3-dihydro-benzo[d]azepin-2-one** (**3**)

In a two-necked flask under nitrogen, a solution of 1-(5,6-dimethoxy-2,3-dihydro-1H-inden-2-yl)-N-methylmethanamine (Eu.Pat. EP 0 534 859 A1) (0.36 mmol; 80 mg) and *cis* 3-(4-chloro-but-2-enyl)-7,8-dimethoxy-1,3-dihydro-benzo[*d*]azepin-2-one **1a** (0.24 mmol; 74 mg) in chloroform (3 mL) and anhydrous triethylamine (3 mL) was stirred for 5 hours at 60 °C. The solvent was removed under vacuum and the residue was dissolved in water and extracted with dichloromethane (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by flash chromatography (eluent: dichloromethane-methanol, 9:1) to give **3** as a clear oil in 15% yields, which was transformed into the oxalate salt: p.f 88-91 °C. **^{1}H NMR** ($CDCl_3$, δ): 2.27 (s, 3H, NCH_3); 2.39 (d, J=7.2 Hz, 2H, CH_2-indane); 2.62 (dd, J=6.4 Hz, J=14.8 Hz, 2H, indane); 2.67-2.73 (m, 1H, indane CH); 3.00 (dd, J=7.2 Hz, J=14.4 Hz, 2H, indane);

3.11 (d, J = 6 Hz, 2H, $NCH_2C=C$); 3.45 (s, 2H, CH_2CO); 3.85 (s, 6H, $2OCH_3$); 3.87 (s, 3H, OCH_3); 3.90 (s, 3H, OCH_3); 4.25 (d, J=6.8 Hz, 2H, CH_2NCO); 5.43-5.49 (m, 1H, CH butene); 5.66-5.72 (m, 1H, CH butene); 6.18 (d, J=9.2 Hz, 1H, CH azepinone); 6.33 (d, J=8.8 Hz, 1H, CH azepinone); 6.72 (s, 1H, aromatics); 6.74 (s, 2H, aromatics); 6.79 (s, 1H, aromatic) ppm. **^{13}C NMR** ($CDCl_3$, δ, APT): 37.57 (CH), 37.65 (CH_2), 42.47 (CH_3), 43.15 (CH), 44.41 (CH), 54.57 (CH), 55.94 (CH_3), 56.00 (CH_3), 62.84 (CH_2), 107.87 (CH), 109.42 (CH), 111.15 (CH), 117.21 (CH), 124.61 (quat. C), 126.32 (quat. C), 127.59 (CH), 134.37 (quat. C), 147.84 (quat. C), 147.98 (quat. C), 149.84 (quat. C), 167.47 (CO) ppm.

Example 3:

synthesis of **(*R*) *N*,*N*-bis-[(Z)-4-(7,8-Dimethoxy-2-oxo-1,3-dihydrobenzo[*d*]azepin-3-yl)but-2-enyl]-2-(3,4-dimethoxyphenyl)propanamine (R-4, MEL57A) e (R) 3-((Z)-4-((2-(3,4-dimethoxyphenyl)propyl)(methyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one (R-5)**

A solution of (*R*)-2-(3,4-dimethoxyphenyl)propan-1-amine [Riggs et al, *J.Med.Chem.*, **1987**, 30, 1914-1918] (0.16 g; 0.01 mol) **1a** (0.18 g; 0.001 mol) in dry CH_3CN (10 mL) and dry triethylamine (0.11 mL; 0.001 mol) was stirred overnight at room temperature under nitrogen atmosphere. Removal of the solvent gave a residue that was dissolved in dichloromethane and washed with 2M NaOH (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum to give a residue that was purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 95:5:0.5). ***(R)*-4** (MEL57A) was eluted first (12%; mp 97-98 °C), the second was ***(R)*-5** (oil, 8% yields).

***(R)*-4** **^{1}H NMR** ($CDCl_3$, δ): 1.21 (d, J=6.8 Hz, 3H, CCH_3); 2.45 (dd, J = 7.4, 12.6 Hz, 1H) and 2.56 (dd, J = 7.4, 12.6 Hz, 1H)(NCH_2CAr); 2.83.2.89 (m, 1H, CHAr); 3.11-3.15 (m, 4H, $NCH_2C=$); 3.44 (s, 4H, CH_2CO); 3.83 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.86 (s, 6H, 2 OCH_3); 3.88 (s, 6H, 2 OCH_3); 4.12-4.24 (m, 4H, CH_2NCO); 5.39-5.45 (m, 2H, CH butene); 5.56-5.62 (m, 2H, CH butene); 6.13 (d, J=9.2 Hz, 2H, CH azepinone); 6.31 (d, J=9.2 Hz, 2H, CH azepinone); 6.70-6.72 (m, 4H) and 6.77-6.79 (m, 3H) (aromatics) ppm. **^{13}C NMR** ($CDCl_3$, δ, APT): 20.38 (CH_3), 37.87 (CH), 43.14 (CH_2), 44.53 (CH_2), 51.00 (CH_2), 55.80 (OCH_3), 55.82 (OCH_3), 55.92 (OCH_3), 61.70 (CH_2), 109.50 (CH), 110.58 (CH), 111.09 (CH), 111.17 (CH), 117.10 (CH), 119.00 (CH), 124.61 (C quat.), 126.34 (C quat.), 127.27 (CH), 127.63 (CH), 131.01 (CH), 138.71 (quat. C), 147.26 (quat. C), 147.99

(quat. C), 148. (quat. C), 149.84 (quat. C), 167.41 (CO) ppm. **(R)-4** (hydrochloride): $[\alpha]^{25}_{Na}$ -22.25° (c=0.5; CH_3OH).

***(R)*-5 ^{1}H NMR** ($CDCl_3$, δ): 1.24 (d, J=6.8 Hz, 3H, CH_3); 2.73-2.75 (m, 2H, $ArCCH_2$); 2.86-2.89 (m, 1H, CHAr); 3.29 (d, J=6.8 Hz, 2H, $NHCH_2C$=); 3.42 (s, 2H, CH_2CO); 3.86 (s, 3H, OCH_3); 3.87 (s, 3H, OCH_3); 3.88 (s, 3H, OCH_3); 3.89 (s, 3H, OCH_3); 4.18 (d. J=6.8 Hz, 2H, NCH_2C=); 5.36-5.39 (m, 1H, CH butene); 5.58-5.61 (m, 1H, CH butene); 6.13 (d, J=9.2 Hz, 1H, CH azepinone); 6.31 (d, J=9.2 Hz, 1H, CH azepinone); 6.72-6.81 (m, 5H, aromatics) ppm. **^{13}C NMR** ($CDCl_3$, δ, APT): 20.29 (CH_3), 39.64 (CH), 43.15 (CH_2), 44.39 (CH_2), 46.11 (CH_2), 55.84 (OCH_3), 55.89 (OCH_3), 55.96 (OCH_3), 56.73 (CH_2), 109.48 (CH), 110.44 (CH), 111.20 (CH), 111.36 (CH), 117.22 (CH), 118.89 (CH), 124.63 (quat. C), 126.26 (CH), 126.32 (quat. C), 127.54 (CH), 131.92 (CH), 137.72 (quat. C), 147.52 (quat. C), 148.01 (quat. C), 148. (quat. C), 149.89 (quat. C), 167.46 (CO) ppm. $[\alpha]^{25}_{Na}$ -1.70° (c=1; $CHCl_3$) (free amine).

Example 4:

synthesis of **(*S*) *N,N*-bis-[(Z)-4-(7,8-Dimethoxy-2-oxo-1,3-dihydrobenzo[*d*]azepin-3-yl)but-2-enyl]-2-(3,4-dimethoxyphenyl)propanamine, (S-4) and (S) 3-{(Z)-4-[2-(3,4-dimethoxyphenyl) propylamino]buten-2-yl}-7,8-dimethoxy-2-oxo-1,3-dihydro-benzo[d]azepin-2-one, (S-5).**

They were synthesized following the procedure described for ***(R)*-4** and ***(R)*-5**, starting from (S)-2-(3,4-dimethoxyphenyl)propan-1-amine [(Riggs et al, J.Med.Chem., 1987, 30, 1914-1918] **(S)-4** (yield: 20%; m.p. 98-102 °C) and **(S)-5** (oil, yield: 21%) were obtained.

***(S)*-4:** 0.13 mmol; 60 mg; yellow oil; 21%); $[\alpha]^{20}_{D}$ = +1.58° (c=1; $CHCl_3$.

***(S)*-5:** 0.122 mmol; 90 mg; 20%; yellow solid; mp 98-102 °C; ***(S)*-5** (hydrochloride): $[\alpha]^{20}_{D}$ = +25.13° (c=0.5; CH_3OH).

Their NMR spectra were identical to those of their enantiomers.

Example 5:

synthesis of **3-((Z)-4-{[(Z)-4-(7,8-Dimethoxy-2-oxo-1,3-dihydro-benzo[d]azepin-3-yl)buten-2-yl]-[2-(3,4-dimethoxyphenyl)ethyl]amino}buten-2-yl)-7,8-dimethoxy-2-oxo-1,3-dihydro-benzo[d]azepin-2-one, 6**

Under nitrogen atmosphere, a solution of **1a** (0.17 g; 0.55 mmol), dry triethylamine (1 eq) and homoveratrylamine (0.05 g; 0.28 mmol) in dry CH_3CN (10 mL) was stirred under reflux for 16 hours. The solvent was removed under vacuum and the residue was taken up with dichloromethane and washed with 2M NaOH (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 97:3:0.3). Compound **6** was obtained as a pale yellow solid: mp 60-62 °C

^{1}H NMR ($CDCl_3$, δ): 2.69 (bs, 4H, CH_2CH_2); 3.22 (d, J = 6.4 Hz, 4H, =CCH_2N); 3.42 (s, 4H, CH_2CO); 3.81 (s, 3H, OCH_3); 3.83 (s, 6H, 2 OCH_3); 3.85 (s, 6H, 2 OCH_3); 3.87 (s, 3H, OCH_3); 4.22 (d, J = 6.8 Hz, 4H, CH_2NCO); 5.42-5.48 (m, 2H, CH butene); 5.62-5.68 (m, 2H, CH butene); 6.16 (d, J=9.2 Hz, 2H, CH azepinone); 6.31 (d, J=9.2 Hz, 2H, CH azepinone); 6.68-6.78 (m, 7H, aromatics) ppm. **^{13}C NMR** ($CDCl_3$, δ, APT): 33.05 (CH_2), 43.08 (CH_2), 44.52 (CH_2), 50.55 (CH_2), 55.63 (CH_2), 55.79 (OCH_3), 55.83 (OCH_3), 55.88 (OCH_3), 109.50 (CH), 111.15 (CH), 111.19 (CH), 112.04 (CH), 117.18 (CH), 120.46 (CH), 124.56 (quat. C), 126. (quat. C), 127.45 (CH), 127.55 (CH), 130.55 (CH), 132.79 (quat. C), 147.26 (quat. C), 147.97 (quat. C), 148.74 (quat. C), 149.84 (quat. C), 167.39 (CO) ppm.

Example 6:

synthesis of **(S) 3-{(E)-4-[(3,4-Dimethoxy-bicyclo[4.2.0]octa-1(6),2,4-trien-7-ylmethyl) methylamino]buten-2-yl}-7,8-dimethoxy-2-oxo-1,3-dihydro-benzo[d]azepin-2-one (S)-7**

Under nitrogen atmosphere, a solution of 3-((E)-4-chloro-buten-2-yl)-7,8-dimethoxy-1,3-dihydro-benzo[*d*]azepin-2-one **1b** (Romanelli M. N. et al BMC 2005) (0.49 mmol, 151 mg), dry triethylamine (1 eq) and (S) (3,4-dimethoxybicyclo[4.2.0]octa-1(6),2,4-trien-7-ylmethyl)methylamine (Lerestif, J.-M., EP 1598333, 2005) (0.49 mmol; 102 mg) in dry CH_3CN (4 mL) was stirred for 48 hours at room temperature. The solvent was removed under vacuum and the residue was dissolved in ethyl acetate and washed with aqueous $NaHCO_3$ (saturated solution; 3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 95:5:0.5). ***(S)*-7** was obtained as a yellow solid in 40% yields (mp 60-62 °C; $[\alpha]^{20}_D$ = +1.96° (c=1; $CHCl_3$), and

it was transformed into the hydrochloride salt (m.p 86-89 °C). **^{1}H NMR** ($CDCl_3$, δ): 2.27 (s, 3H, NCH_3); 2.53 (dd, J=12.8 Hz, 8.8 Hz, 1H); 2.68-2.75 (m, 2H, cyclobutane-NC*HH*+ C*H*H cyclobutane); 3.00-3.09 (m, 2H, =CCH_2N); 3.25 (dd, J=12.8 Hz, J=5.2 Hz, 1H); 3.43 (s, 2H, CH_2CO); 3.51-3.58 (m, 1H, CH cyclobutane); 3.82 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.87 (s, 3H, OCH_3); 4.14 (d, J=5.2 Hz, 2H, CH_2NCO); 5.52-5.65 (m, 2H, =CH butene); 6.14 (d, J=9.2 Hz, 1H, CH azepinone); 6.29 (d, J=9.2 Hz, 1H, CH azepinone); 6.68 (s, 2H), 6.70 (s, 1H) and 6.77 (s, 1H) (aromatics) ppm. **^{13}C NMR** ($CDCl_3$, δ, APT): 35.39 (CH_2), 40.77 (ArCH), 42.48 (NCH_3), 43.25 ($COCH_2$), 49.97 ($CONCH_2$), 56.07 (OCH_3), 56.34 (OCH_3), 56.42 (OCH_3), 59.67 (butene-CH_2N), 61.35 (CH_2), 106.85 (aromatic CH), 107.53 (aromatic CH), 109.55 (aromatic CH), 111.30 (aromatic CH), 117.20 (azepinone CH), 124.75 (quat. C), 126.49 (quat. C), 127.98 (azepinone CH), 128.46 (butene CH), 130.28 (butene CH), 135.06 (quat. C), 138.76 ((quat. C), 148.11 (quat. C), 149.43 (quat. C), 149.96 (quat. C), 167.60 (CO) ppm.

Example 7:

synthesis of **(S) 3-{(Z)-4-[(3,4-Dimethoxy-bicyclo[4.2.0]octa-1(6),2,4-trien-7-ylmethyl)methylamino]-buten-2-yl}-7,8-dimethoxy-1,3-dihydro-benzo[d]azepin-2-one (S)-8**

It was synthesized following the procedure described for ***(S)*-7**, starting from **1a** (0.49 mmol, 101 mg), and (S) (3,4-dimethoxybicyclo[4.2.0]octa-1(6),2,4-trien-7-ylmethyl)methylamine (Lerestif, J.-M., EP 1598333, 2005) (0.15 g; 0.49 mmol). ***(S)*-8** was obtained as a pale yellow solid (m.p. 61-63 ^{0}C) in 26% yields and transformed into the hydrochloride salt (m.p. 91-94 °C). **^{1}H NMR** ($CDCl_3$, δ): 2.31 (s, 3H, NCH_3); 2.58 (dd, J=12.8 Hz, 8.8 Hz, 1H); 2.71-2.79 (m, 2H, NC*HH*- cyclobutane + cyclobutane C*H*H); 3.17 (d, J = 6.4 Hz, 2H, =CCH_2N); 3.27 (dd, J=12.8 Hz, J=5.2 Hz, 1H); 3.43 (s, 2H, CH_2CO); 3.55-3.62 (m, 1H, CH cyclobutane); 3.83 (s, 6H, 2 OCH_3); 3.86 (s, 3H, OCH_3); 3.87 (s, 3H, OCH_3); 4.18-4.29 (m, 2H, CH_2NCO); 5.42-5.52 (m, 1H, =CH butene); 5.65-5.75 (m, 1H, =CH butene); 6.17 (d, J=9.2 Hz, 1H, CH azepinone); 6.32 (d, J=9.2 Hz, 1H, CH azepinone); 6.67 (s, 1H), 6.70 (s, 1H), 6.71 (s, 1H), 6.76 (s, 1H) (aromatics) ppm. **^{13}C NMR** ($CDCl_3$, δ, APT): 35.25 (CH_2), 40.62 (ArCH), 42.49 (NCH_3), 43.17 ($COCH_2$), 44.53 ($CONCH_2$), 54.63 (butene-CH_2N), 55.98 (OCH_3), 56.25 (OCH_3), 56.33 (OCH_3), 61.55 (CH_2), 106.78 (aromatic CH), 107.46 (aromatic CH), 109.52 (aromatic CH), 111.21

(aromatic CH), 117.32 (azepinone CH), 124.63 (quat. C), 126.35 (quat. C), 127.63 (azepinone CH), 129.21 (butene CH), 130.44 (butene CH), 134.96 (quat. C), 138.63 (quat. C), 148.05 (quat. C), 149.36 (quat. C), 149.92 (quat. C), 167.51 (CO) ppm. $[\alpha]^{25}_{Na}$ +13.8° (c=1; $CHCl_3$)

Example 8:

synthesis of **(R) 3-((E)-4-{[2-(3,4-Dimethoxyphenyl)propyl]methylamino}buten-2-yl)-7,8-dimethoxy-1,3-dihydro-benzo[d]azepin-2-one (R)-9**

It was synthesized following the procedure described for ***(S)*-7**, starting from 3-((E)-4-chloro-buten-2-yl)-7,8-dimethoxy-1,3-dihydro-benzo[*d*]azepin-2-one **1b** (Romanelli M. N. et al BMC 2005) (1.04 mmol; 320 mg) and (R)-2-(3,4-dimethoxyphenyl)-N-methylpropan-1-amine (Jullian et al *Eur. J. Org. Chem* 1319, 2000) (1.04 mmol; 203 mg). ***(R)*-9** was obtained as a pale yellow solid in 54% yields (mp 59-61 ^{0}C; $[\alpha]^{20}_D$ = -11.4° (c=1; $CHCl_3$) and transformed into the hydrochloride salt (m.p. 71-73 °C). **^{1}H NMR** ($CDCl_3$, δ): 1.14 (d, J=6.8 Hz, 3H, CCH_3); 2.11 (s, 3H, NCH_3); 2.28-2.40 (m, 2H, $ArCCH_2$); 2.74-2.96 (m, 3H, CHAr and $C{=}CCH_2N$); 3.39 (s, 2H, CH_2CO); 3.77 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 3.81 (s, 6H, 2 OCH_3); 4.02-4.15 (m, 2H, CH_2NCO); 5.39-5.53 (m, 2H, butene CH); 6.12 (d, J=9.2 Hz, 1H, azepinone CH); 6.30 (d, J=9.2 Hz, 1H, azepinone CH); 6.65-6.75 (m, 5H, aromatics) ppm.

^{13}C NMR ($CDCl_3$, δ, APT): 20.28 (CH_3), 37.43 (CH), 42.43 (NCH_3), 43.02 ($COCH_2$), 48.76 ($CONCH_2$), 55.76 (OCH_3), 55.81 (OCH_3), 56.00 (OCH_3), 59.39 (butene-CH_2N), 64.43 (NCH_2), 109.37 (aromatic CH), 110.50 (aromatic CH), 111.08 (aromatic CH), 111.12 (aromatic CH), 116.89 (azepinone CH), 118.70 (aromatic CH), 124.51 (quat. C), 126.29 (quat. C), 127.61 (azepinone CH), 127.68 (butene CH), 130.42 (butene CH), 138.59 (quat. C), 147.18 (quat. C), 147.87 (quat. C), 148.65 (quat. C), 149.71 (quat. C), 167.28 (CO) ppm.

Example 9:

synthesis of **(S) 3-((E)-4-{[2-(3,4-dimethoxyphenyl)propyl]methylamino}-buten-2-yl)-7,8-dimethoxy-1,3-dihydro-benzo[d]azepin-2-one (S)-9**

It was synthesized following the procedure described for ***(S)*-7**, starting from 3-((E)-4-chloro-buten-2-yl)-7,8-dimethoxy-1,3-dihydro-benzo[*d*]azepin-2-one **1b** (1.04 mmol, 320

mg) and (S)-2-(3,4-dimethoxyphenyl)-N-methylpropan-1-amine (Vicario et al *Tet. Asimmetry* 11, 3779, 2000) (1.02 mmol; 198 mg). ***(S)*-9** was obtained in 9% yields ($[\alpha]^{20}_{D}$ = +6.4°, c=1; $CHCl_3$) and transformed into the hydrochloride salt (m.p. 72-75 °C). The NMR spectrum is identical to that of its enantiomer.

Example 10:

synthesis of **(R)** ***N,N-bis*-[(E)-4-(7,8-dimethoxy-2-oxo-1,3-dihydrobenzo[*d*]azepin-3-yl)but-2-enyl]-2-(3,4-dimethoxyphenyl)propanamine (R)-10**

It was synthesized following the procedure described for ***(S)*-7**, starting from **1b** (1.45 mmol; 445 mg) and (R)-2-(3,4-dimethoxyphenyl)propan-1-amine [(Riggs et al, J.Med.Chem., 1987, 30, 1914-1918] (0.141 g; 0.72 mmol). ***(R)*-10** was obtained in7% yields, and transformed into the hydrochloride salt, mp 85-87 ^{0}C.

^{1}H NMR (as hydrochloride) (CD_3OD, δ): 1.13 (d, J=6.0 Hz, 3H, CCH_3); 2.87-3.10 (m, 3H, NCH_2CHAr); 3.19-3.49 (m, 4H, NCH_2C=); 3.45 (s, 4H, CH_2CO); 3.79 (s), 3.81 (s), 3.82 (s), 3.83 (s) and 3.85 (s) (18H, 6 OCH_3); 4.01-4.23 (m, 4H, CH_2NCO); 5.08-5.26 (m, 2H); 5.51-5.61 (m, 1H) and 5.66-5.74 (m, 1H) (CH butene); 6.24-6.29 (m, 2H), 6.49-6.52 (m, 1H) and 6.65 (d, 1H, J = 8 Hz) (azepinone CH); 6.82-6.91 (m, 7H, aromatici) ppm.

^{13}C NMR (CD_3OD, δ, APT): 21.38 (CH_3), 36.65 (CH), 43.34 ($CO\mathit{C}H_2$), 49.96 ($CON\mathit{C}H_2$), 54.85 (butene-$\mathit{C}H_2N$), 56.57 (OCH_3), 56.64 (OCH_3), 56.78 (OCH_3), 59.01 ($N\mathit{C}H_2CAr$), 111.51 (aromatic CH), 112.29 (aromatic CH), 112.64 (aromatic CH), 113.70 (aromatic CH), 119.51 (azepinone CH), 119.59 (azepinone CH), 119.83 (azepinone CH), 120.49 (butene CH), 126.19 (quat. C), 127.86 (quat. C), 129.20 (azepinone CH), 135.45 (quat. C), 138.79 (butene CH), 138.84 (butene CH), 149.81 (quat. C), 150.13 (quat. C), 151.15 (quat. C), 161.70 (quat. C), 169.48 (CO) ppm.

$[\alpha]^{25}_{Na}$ -14.7° (c=1; $CHCl_3$)

Example 11:

synthesis of **(S)** ***N,N-bis*-[(Z)-4-(7,8-dimethoxy-2-oxo-1,3-dihydrobenzo[*d*]azepin-3-yl)but-2-enyl]-2-(3,4-dimethoxyphenyl)propanamine (S)-10**

It was synthesized following the procedure described for ***(S)*-7**, starting from **1b** (0.445 g; 01.45 mmol) and (S)-2-(3,4-dimethoxyphenyl)propan-1-amine [(Riggs et al, J.Med.Chem., 1987, 30, 1914-1918] (0.141 g; 0.72 mmol). ***(S)*-10** was obtained in 6% yields and

transformed into the hydrochloride salt (m.p. 89-93 ^{0}C). NMR spectra were identical to those of ***(R)*-10**. $[\alpha]^{25}_{Na}$ +11.5° (c=1; $CHCl_3$)

Example 12:

synthesis of **(S)** and **(R)** **3-((Z)-4-((1-(3,4-dimethoxyphenyl)propan-2-y)(methyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one** [**(S)-11** and (R)-**11**]

Following the procedure described for ***(S)*-7**, starting from (S)-[3-(3,4-dimethoxyphenyl)-2-propyl]methylamine (0.10 g, 0.49 mmol) and **1a** (0.15 g; 0.49 mmol), ***(S)*-11** was obtained in 36% yields and transformed into the hydrochloride salt.

^{1}H NMR ($CDCl_3$, δ): 0.87 (d, J=6.4 Hz, 3H, CCH_3); 2.20 (s, 3H, NCH_3); 2.32 (dd, J = 13.8 Hz, 10.6 Hz, 1H, CH*H*Ar); 2.82-2.89 (m, 2H, C*H*HAr + CHMe); 3.00-3.10 (m, 2H, C=CCH_2N); 3.44 (s, 2H, CH_2CO); 3.84 (s) and 3.87 (s) (12H, 4OCH_3); 4.09-4.19 (m, 2H, CH_2NCO); 5.50-5.62 (m, 2H, butene CH); 6.17 (d, J=9.2 Hz, 1H, azepinone CH); 6.31 (d, J=9.2 Hz, 1H, azepinone CH); 6.66-6.69 (m, 3H) and 6.76-6.78 (m, 2H) (aromatici) ppm.

^{13}C NMR ($CDCl_3$, δ, APT): 13.84 (CH_3), 36.73 (CH_3), 39.09 (CH), 43.27 (CH_2), 44.54 (CH_2), 54.03 (CH_2), 55.42 (OCH_3), 55.97 (OCH_3), 56.06 (OCH_3), 56.22 (OCH_3), 59.87 (CH), 109.62 (CH), 111.27 (CH), 111.35 (CH), 112.63 (CH), 117.11 (CH), 121.18 (CH), 124.78 (quat. C), 126.02 (quat. C), 127.27 (CH), 127.87 (CH), 131.94 (CH), 133.22 (quat. C), 147.35 (quat. C), 148.13 (quat. C), 148.83 (quat. C), 149.98 (quat. C), 167.56 (CO) ppm. $[\alpha]^{25}_{Na}$ +2.2°, (c=0.5; $CHCl_3$)

With the same procedure, starting from **1a** and (R)-[3-(3,4-dimethoxyphenyl)-2-propyl]methylamine, ***(R)*-11** was obtained in 41% yields and transformed into the hydrochloride salt. NMR spectra were identical to those of its enantiomer

Example 13:

synthesis of **(S)** **e** **(R)** **3-((E)-4-((1-(3,4-dimethoxyphenyl)propan-2-yl)(methyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one** [**(S)-12** e (**R**)-**12**]

Following the procedure described for ***(S)*-7**, starting from (S)-[3-(3,4-dimethoxyphenyl)-2-propyl]methylamine (0.10 g, 0.49 mmol) and **1b** (0.15 g; 0.49 mmol), ***(S)*-11** was obtained in 56% yields and transformed into the hydrochloride salt.

^{1}H NMR ($CDCl_3$, δ): 0.93 (d, J=6.8 Hz, 3H, CCH_3); 2.25 (s, 3H, NCH_3); 2.37 (dd, J = 12.8 Hz, 9.2 Hz, 1H, CH*H*Ar); 2.84-2.95 (m, 2H, C*H*HAr + CHMe); 3.17 (d, J = 6.4 Hz, 2H, C=CCH_2N); 3.45 (s, 2H, CH_2CO); 3.85 (s, 3H, OCH_3); 3.86 (s, 3H, OCH_3); 3.86 (s, 3H, OCH_3); 3.89 (s, 3H, OCH_3) ; 4.24 (d, J = 14.0 Hz, 2H, CH_2NCO); 5.41-5.47 (m, 1H, butene CH); 5.62-5.67 (m, 1H, butene CH); 6.19 (d, J=9.0 Hz, 1H, azepinone CH); 6.34 (d, J=9.0 Hz, 1H, azepinone CH); 6.69-6.72 (m, 3H, aromatics); 6.77-6.79 (m, 2H, aromatics) ppm. **^{13}C NMR** ($CDCl_3$, δ, APT): 13.82 (CH_3), 36.86 (CH_3), 39.23 (CH), 43.35 (CH_2), 44.64 (CH_2), 50.31 (CH_2), 56.02 (OCH_3), 56.06 (OCH_3), 56.13 (OCH_3), 60.15 (CH), 109.78 (CH), 111.39 (CH), 111.45 (CH), 112.71 (CH), 117.33 (CH), 121.25 (CH), 124.87 ((quat. C), 126.57 (quat. C), 126.97 (CH), 127.82 (CH), 132.07 (CH), 133.20 (quat. C), 147.46 (quat. C), 148.25 (quat. C), 148.94 (quat. C), 150.12 (quat. C), 167.65 (CO) ppm. $[\alpha]^{25}_{Na}$ +11.4° (c=0.5; $CHCl_3$)

With the same procedure, starting from **1b** and (R)-[3-(3,4-dimethoxyphenyl)-2-propyl]methylamine, ***(R)*-12** was obtained in 41% yields and transformed into the hydrochloride salt. NMR spectra were identical to those of its enantiomer

Example 14:

synthesis of **3-((Z)-4-{[2-(3,4-dimethoxyphenyl)propyl]methylamino}buten-2-yl)-7,8-dimethoxy-1,3-dihydro-benzo[d]azepin-2-one [(R)-13 e (S)-13]**

A solution of ***(R)*-5** (0.06 mmol; 30 mg), formic acid (17 eq), 37% aqueous formaldehyde (5 eq) in absolute ethanol (4 mL) was heated under reflux for 4 hours. The solvent was then removed by rotary evaporation and the residue was treated with $NaHCO_3$ (saturated solution) and extracted with dichloromethane (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and the solvent was removed under vacuum to give a residue that was purified by flash chromatography. Eluting with dichloromethane-methanol (96:4) afforded ***(R)*-13** as a clear oil in 92% yields.

^{1}H NMR ($CDCl_3$, δ): 1.24 (d, J=7.2 Hz, 3H, CCH_3); 2.22 (s, 3H, NCH_3); 2.40 (dd, J = 12.2 Hz, 7.6 Hz, 1H) and 2.49 (dd, J = 12.2 Hz, 7.6 Hz, 1H) ($ArCCH_2$); 2.86-2.91 (m, 1H, CHAr); 3.02-3.12 (m, 2H, C=CCH_2N); 3.44 (s, 2H, CH_2CO); 3.85 (s, 3H, OCH_3); 3.87 (s, 3H, OCH_3); 3.88 (s, 3H, OCH_3); 3.89 (s, 3H, OCH_3); 4.15-4.26 (m, 2H, CH_2NCO); 5.30-5.47 (m, 1H, butene CH); 5.60-5.66 (m, 1H, butene CH); 6.15 (d, J=9.2 Hz, 1H, azepinone CH); 6.31 (d, J=9.2 Hz, 1H, azepinone CH); 6.72-6.80 (m, 5H, aromatici) ppm. **^{13}C NMR**

($CDCl_3$, δ, APT): 20.41 (CH_3), 37.66 (CH), 42.64 (CH), 43.16 (CH_2), 44.48 (CH_2), 54.64 (CH_2), 55.81 (OCH_3), 55.84 (OCH_3), 55.95 (OCH_3), 65.07 (CH_2), 109.49 (CH), 110.49 (CH), 111.19 (CH), 117.08 (CH), 118.88 (CH), 124.65 (quat. C), 126.37 (quat. C), 127.18 (CH), 127.66 (CH), 131.03 (CH), 138.79 (quat. C), 147.29 (quat. C), 148.00 (quat. C), 148.79 (quat. C), 149.86 (quat. C), 167.46 (CO) ppm. $[\alpha]^{20}_D$ = -8.84° (c=1; $CHCl_3$).

Following the procedure described for ***(R)*-13**, starting from ***(S)*-5** (0.13 mmol; 60 mg), ***(S)*-13** was obtained as a clear oil. Yield: 34%. The ^{1}H NMR spectrum was identical to that of ***(R)*-5**. $[\alpha]^{20}_D$ = +8.00° (c=1; $CHCl_3$).

Example 15:

synthesis of *trans* **3-(3-{[2-(3,4-dimethoxyphenyl)ethyl]methylamino}cyclohexyl)-7,8-dimethoxy-1,3,4,5-tetrahydro-benzo[*d*]azepin-2-one 17**

Thionyl chloride (5.54 mmol; 0.4 mL) was added dropwise to a solution of a 4:1 cis-trans mixture of 3-(7,8-dimethoxy-2-oxo-4,5-dihydro-1H-benzo[d]azepin-3-yl)cyclohexanecarboxylic acid [E. Cerretini, 1999, thesis in CTF], (2 mmol; 0.8 g) in chloroform (50 mL). The reaction mixture was heated at 60 °C for 3 hours under nitrogen atmosphere, then the solvent was removed under vacuum leaving a residue that was washed with cyclohexane (2 × 30 mL) and dried under vacuum. Anhydrous acetone (10 mL) was poured into the flask followed by a saturated aqueous solution of NaN_3 (2 mL). After stirring for 10 minutes, a small excess of water was added to the solution. Acetone was evaporated under vacuum and the aqueous layer was extracted with dichloromethane (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum to give the corresponding acylazide as a gummy yellow solid. IR: ν= 2100 cm^{-1} (CON_3).

This compound was dissolved in toluene and heated under reflux overnight to allow the formation of isocyanate. IR: ν 2250 cm^{-1} (NCO). The toluene was removed under vacuum to give a residue that was dissolved in THF (3 mL) and treated with 2N HCl (3 mL). The resulting solution was stirred at room temperature overnight, then the organic solvent was evaporated under vacuum and the aqueous layer was washed with diethyl ether, basified with 2.5M NaOH and extracted with dichloromethane. The organic layers were collected, dried on Na_2SO_4 and removed under vacuum. The residue was purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 90:10:0.5) obtaining *trans*-

3-(3-aminocyclohexyl)-7,8-dimethoxy-4,5-dihydro-1H-benzo[d]azepin-2(3H)-one **16a** as a clear oil (50 mg, 8% yield) and 0.5 g of a mixture of **16a** and its *cis* isomer **16b**.

^{1}H NMR ($CDCl_3$, δ): 1.32-1.47 (m, 2H), 1.53-1.78 (m, 6H) (cyclohexane); 2.04 (bs, 2H; NH_2); 2.95-3.00 (m, 2H; 5-CH_2), 3.42 (s, 1H, 3'-H); 3.63-3.67 (m, 2H; 4-CH_2); 3.78 (s, 2H; 1-CH_2), 3.80 (s, 3H; OCH_3), 3.81 (s, 3H; OCH_3), 4.75-4.85 (m, 1H; 1'-H), 6.50 (s, 1H) and 6.58 (s, 1H) (aromatics) ppm. **^{13}C NMR** ($CDCl_3$, δ, APT): 19.40 (CH_2), 30.65 (CH_2), 31.61 (C_4), 33.84 (CH_2), 37.41 (C_5), 40.31 (CH_2), 42.98 (C_1), 46.58 ($C_{3'}$), 47.12 ($C_{1'}$), 55.80 (OCH_3), 55.92 (OCH_3), 113.08 (C_6 or C_9), 113.95 (C_9 or C_6), 123.38 (C_{5a} or C_{9a}), 127.06 (C_{9a} or C_{5a}), 147.00 (C_7 or C_8), 147.76 (C_8 or C_7), 172.04 (C_2).

A solution of **16a** (0.2 mmol, 0.085 g), anhydrous triethylamine (1 eq) and 3,4-dimethoxyphenethyl bromide (1 eq) in dry DMF (2 mL) was heated at 60 °C overnight under nitrogen atmosphere. The mixture was cooled to room temperature and then the solvent was evaporated under vacuum to give a residue that was treated with 2N HCl (3 mL) and washed with diethyl ether (2 × 15 mL). The acidic aqueous layer was basified with a sodium carbonate saturated aqueous solution and extracted with dichloromethane (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum to afford a residue that was purified by column chromatography. Eluting with dichloromethane-methanol-ammonia (90:10:0.5) gave *trans* 3-{3-[2-(3,4-dimethoxyphenyl)ethylamino]cyclohexyl}-7,8-dimethoxy-1,3,4,5-tetrahydrobenzo[*d*]azepin-2-one in 20% yield.

^{1}H NMR ($CDCl_3$, δ): 1.34-1.93 (m, 9H, NH + cyclohexane); 2.29-3.04 (m, 6H, 5-CH_2 + CH_2CH_2); 3.21 (bs, 1H; 3'-H); 3.69 (t, J=5.8 Hz, 2H; 4-CH_2), 3.80-3.88 (m, 14H; 1-CH_2 + 4OCH_3); 4.81 (t, J=12 Hz, 1H; 1'-H), 6.51 (s, 1H; 6-CH), 6.64 (s, 1H; 9-CH), 6.73-6.77 (m, 3H; aromatics) ppm.

^{13}C NMR ($CDCl_3$, δ, APT): 19.73 (CH_2), 28.75 (CH_2), 30.41 (CH_2), 33.86 (CH_2), 40.57 (CH_2), 43.05 (CH_2), 47.65 (CH), 48.59 (CH_2), 53.40 (CH), 55.86 (OCH_3), 55.93 (OCH_3), 56.08 (OCH_3), 111.26 (CH), 112.05 (CH), 113.13 (CH), 114.08 (CH), 120.52 (CH), 123.33 (C), 127.03 (C), 147.12 (C), 147.42 (C), 172.24 (CO) ppm.

A solution of the above compound (0.05 mmol; 0.025 g), formic acid (17 eq) and 37% aqueous formaldehyde (5 eq) in absolute ethanol (2 mL) was heated at reflux for 15 hours. The solvent was then removed under vacuum and the residue (yellow solid) treated with $NaHCO_3$ (saturated solution) and extracted with dichloromethane (3 × 15 mL). The

organic layers were collected, dried (Na_2SO_4) and removed under vacuum to give a brown oil that was purified by gravity column chromatography. Eluting with dichloromethane-methanol-ammonia (95:5:0.5) afforded 17 as oil (65.9% yield).

^{1}H NMR ($CDCl_3$, δ): 1.22-1.35 (m), 1.46-1.55 (m), 1.60-1.65 (m), 1.71-1.80 (m) and 1.91-2.02 (m) (8H, cicloesano); 2.35 (s, 3H; NCH_3); 2.60-2.64 (m, 1H; 3'-H), 2.71 (s, 4H, CH_2CH_2); 3.01 (t, J=5.6 Hz, 2H; 5-CH_2), 3.69 (t, J=5.8 Hz, 2H; 4-CH_2), 3.84 (s, 2H; 1-CH_2), 3.86 (s, 3H; OCH_3), 3.87 (s, 3H; OCH_3), 3.88 (s, 3H; OCH_3), 3.90 (s, 3H; OCH_3), 4.86 (t, J=12 Hz, 1H; 1'-H), 6.54 (s, 1H; 6-CH), 6.61 (s, 1H; 9-CH), 6.70-6.78 (m, 3H; aromatics) ppm.

Example 15a:

synthesis of *cis* 3-{3-[(3,4-dimethoxybenzyl)methylamino]cyclohexyl}-7,8-dimethoxy-1,3,4,5-tetrahydro-benzo[*d*]azepin-2-one **18** and analogues **19** and **20**

cis 3-(3-Amino-cyclohexyl)-7,8-dimethoxy-1,3,4,5-tetrahydro-benzo[*d*]azepin-2-one **16b**, prepared as reported in (S. Betti, graduation thesis in Pharmaceutical Chemistry and Technology, 1998; E. Cerretini, graduation thesis in Pharmaceutical Chemistry and Technology, 1999) was treated with 3,4-dimethoxybenzyl chloride and then methylated according to the procedure reported for **17**. Compound **18** was obtained. **^{1}H NMR** ($CDCl_3$, δ): 1.20-1.52 (m), 1.65-1.72 (m), 1.82-1.99 (m), (8H, cyclohexane); 2.20 (s, 3H; NCH_3); 2.65 (t, 1H; 3'-H), 2.99-3.02 (m, 2H; 5-CH_2), 3.50-3.58 (m, 2H, CH_2N); 3.67-3.73 (m, 2H; 4-CH_2), 3.81 (s, 2H; 1-CH_2), 3.83 (s, 3H; OCH_3), 3.84 (s, 3H; OCH_3), 3.86 (s, 3H; OCH_3), 3.87 (s, 3H; OCH_3), 4.46 (t, J=12 Hz, 1H; 1'-H), 6.53 (s, 1H; CH), 6.60 (s, 1H; CH), 6.77-6.82 (m), 6.90 (s) (3H; aromatics) ppm.

With analogue procedure also *cis* 3-(3-{[3-(3,4-dimethoxyphenyl)propyl]methylamino}cyclohexyl)-7,8-dimethoxy-1,3,4,5-tetrahydro-benzo[*d*]azepin-2-one **19** and *cis* 3-(3-{[2-(3,4-dimethoxyphenoxy)-ethyl]methylamino}cyclohexyl)-7,8-dimethoxy-1,3,4,5-tetrahydro-benzo[*d*]azepin-2-one **20** were prepared reacting **16b** with 4-(3-iodopropyl)-1,2-dimethoxybenzene and 4-(2-bromoethoxy)-1,2-dimethoxybenzene respectively.

Example 16

Patch clamp experiments in HEK293 cells stably expressing HCN1, HCN2 o HCN4 channel isoforms.

Culture of HEK 293 cells and isolation

Human embryonic kidney cells (HEK293 cells DSMZ, Braunschweig, Germany), transfected with mHCN1, mHCN2 and hHCN4 cDNA (provided by Prof. M. Biel, University of Munchen), were cultured in DMEM medium (Gibco, DMEM + GlutaMax™-I x1) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 µg/ml streptomycin, 200 µg/ml geneticin (G418-Gibco) in T25 flasks and incubated at 37 °C with 5% CO_2. When confluent (3-5 days after plating), cells were detached using an enzymatic dissociation with trypsin-EDTA,. Digestion was stopped by adding medium and the sedimented cells were either re-plated or used for electrophysiological measurements.

Electrophysiological recordings

To measure I_f, dissociated HEK293 cells were incubated in normal Tyrode's solution.Patch-clamp recordings were performed using the whole-cell configuration of the patch-clamp technique.

The cells were placed in an experimental bath on the platform of an inverted microscope (Nikon Diaphot TMD and TMS, Japan) and superfused by means of a temperature-controlled microsuperfusor, allowing rapid changes of the solution bathing the cell. Patch-clamp pipettes had a resistance of 3.5-5.0 MΩ when filled with the internal solution. Cell membrane capacitance (C_m) was measured by applying a ± 20mV pulse from holding potential of -40mV.

The I_f current was evoked from a holding potential of -20mV to more negative voltages in a range of -40 to -150 and -60 to -150mV for HEK cells and SAN cells respectively, in 10mV increments. To investigate the use-dependence of the block I_f current was evoked by a series of 30 consecutive hyperpolarizing steps (-120mV) at 1Hz. Current amplitudes were calculated as the difference between the less negative value and the value at steady-state and normalized to C_m. Dose-effect curves were fitted by using the Hill equation: $y=E_{max}[x^n/(k^n+x^n)]$ where E_{max} is the maximum effect; k is EC_{50} (concentration at which 50% of E_{max} was obtained), x was drug's concentration and n was the Hill's coefficient.

Solutions

The composition of solutions used was the following (in mmol): *Solution A*: D-(+)-glucose 5.5, NaCl 140, KCl 5.4, $MgCl_2$1, $CaCl_2$ 1.8, HEPES-NaOH 5.0, (pH 7.4). *Solution B*: D-(+)-glucose 5.5, NaCl 140, KCl 5.4, $MgCl_2$ 0.5, KH_2PO_4 1.2, Taurine 50, HEPES-NaOH 5.0, (pH 6.9). *Solution C*: Taurine 20, D-(+)-glucose 10, glutammic acid 50, HEPES-KOH 10, EGTA 0.5, KCl 40,KH_2PO_4 20, $MgCl_2$ 3, (pH 7.2). *Tyrode solution*: D-(+)-glucose 10, NaCl 140, KCl 5.4, $MgCl_2$ 1.2, $CaCl_2$ 1.8, HEPES-NaOH 5.0, (pH 7.3). To measure the I_f current in SAN cells, Tyrode's solution was modified by adding $BaCl_2$ (2), $MnCl_2$ (2), 4-aminopyridine (0.5), and increasing KCl to25 mmol; this solution allowed for the reduction of interference from other currents, that is, L-type calcium current, inward rectifier-like current and transient outward potassium current. To measure the I_f current in HEK cells, Tyrode's solution increasing KCl to 25 mmol to amplify I_f current. Pipette solution: K-aspartate 130; Na_2-ATP 5, $MgCl_2$ 2, $CaCl_2$ 5, EGTA 11, HEPES-KOH 10 (pH 7.2; pCa 7.0).

Drugs solution were obtained from stock solutions (10^{-2}M) in water and diluted in the different Tyrode's solution (for HEK cells or SAN cells) to reach the final concentration (range 0.3 -30 μM).

Example 17

In observation of the 3Rs (replacing, refining and reducing the use of animals in research), we limited use of animals (rabbits and guinea-pigs) to the confirmation of f-channel blockade and negative inotropic effect of relevant compounds. This investigation conforms with the Guide for the Care and Use of Laboratory Animals (Directive U.E. n#86/609/EC, European Community Regulations on the Care and Use of Laboratory Animals, and/or any further implement). The compounds were tested on I_f (recorded from single SAN cells) in order to verify effectiveness on native f-current, as detailed below.

Isolations of single SAN myocytes

This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the European Community (86/609/CEE). Male guinea pigs (Pampaloni, Itay) were anesthetized with ether and killed. The heart was rapidly removed, put in solution A and the SAN tissue is surgically isolated and cut into 5–6 stripes. Cells have been isolated

by means of enzymatic dissociation procedure performed in solution B, containing collagenase, elastase, and protease to degrade intercellular matrix and loosen cell-to-cell adhesion in order to ease the mechanical cell dispersion procedure (see Solutions). Isolated single cells were collected in solution C and after 1h stored in Tyrode's solution; calcium from 0.2 to 0.8 mmol was added, and the cells were maintained in this solution for the day of the experiment. Electrophysiological recordings and solutions for patch-clamp were similar to those described in the previous example.

RESULTS

All compounds were tested at concentrations between 0.3 and 30 μM.

Figure 4 shows as an example a typical experiment performed for the aim of the present invention. This example concerns the effect of **EC32**, tested at 10 μM concentration, on a HEK293 cell transfected with the HCN1 channel isoform. Traces of f-current recorded in control conditions and after the application of EC32 10 μM are reported in panels A and B, respectively. In panel C the I_f activation curve is shown, which represents the conductance values normalized to membrane capacity calculated at the test voltages, and are calculated from traces recorded in control condition (black) and after the application of the compound (gray). Values are calculated as previously described. Finally, panel D shows the f-current amplitude, evoked by a series of 30 consecutive hyperpolarizing steps at -120 mV (at this potential the current is largely expressed and physiologically relevant) in the presence of increasing doses (1-30 μM) of **EC32**. Under these conditions the I_f amplitude is progressively reduced, demonstrating that the effect is use and dose-dependent. This property has been demonstrated for all tested compounds.

Similar experiments have been performed in HEK293 cells expressing the other isoforms, for all the compounds illustrative of the present invention. In figure 5 the mean activation curves are reported, calculated in the three isoforms for compound **EC4** at a 10μM concentration. We can see that this compound reduces the I_f amplitude in all the three isoforms, and this effect is more pronounced on HCN1 (at -120mV: CTR: 0.95±0.01 pS/pF, n=5; EC4 10μM: 0.29±0.1 pS/pF, n=3) and HCN4 (at -120mV: CTR: 0.89±0.03 pS/pF, n=8; EC4 10μM: 0.5±0.08 pS/pF, n=7) than on HCN2 (at -120mV: CTR: 0.83±0.03 pS/pF, n=8; EC4 10μM: 0.55±0.07 pS/pF, n=8). This compound therefore does not show isoform selectivity.

Compound **EC18** reduces f-current density with different efficacy in the three channel isoforms, as illustrated in figure 6, where the mean activation curves are reported. The current reduction on HCN1 is about 7% at -120 mV (CTR 0.96±0.02 pS/pF, n=5; EC18 10μM: 0.89 ±0.02 pS/pF, n=4) as shown from the activation curve (fig. 6A) It is evident that this compound show a more pronounced effect on HEK293 cells expressing the HCN4 isoform, where, at - 120 mV, the reduction is about 70% (CTR 0.92±0.05 pS/pF, n=4; EC18 10μM: 0.3±0.06 ps/pF, n=4), while on HCN2, the reduction is about 30%. We can conclude that **EC18** shows a isoform-specific blockade, being significantly more effective on HCN4 with respect to HCN1 and HCN2.

In figure 7 the effect of **EC32** is reported. From the mean activation curves it is possible to conclude that this compound behaves similarly to EC4, since the reduction of current density is not very different among the various isoforms.

Compound **MEL57A** represents another example of isoform-specific blocker of f-current, since it reduces in a remarkable and statistically significant way the f-current carried through the HCN1 channel isoform (fig. 8A), while its blocking activity drastically decreases going to the other isoforms. On HCN1 it reduces the current by 80% (CTR 0.94±0.02 pS/pF, n=5; MEL57A 10μM 0.2±0.1pS/pF, n=3) while on HCN2 by 40% (CTR 0.82±0.05 pS/pF, n=4; MEL57A 10μM 0.6±0.1pS/pF, n=4) and on HCN4 by only 7% (CTR 0.92±0.09 pS/pF, n=6; MEL57A 10μM 0.86±0.1pS/pF, n=5).

Table 1 reports the EC_{50} values (μM), calculated through Hill equation (see Methods) for the three HCN channel isoforms for representative compounds. Ivabradine and cilobradine (DK-AH-269) are used as reference substances: they are known I_f blocker but they do not show isoform selectivity.

Table 1: EC_{50} values for the I_f- blocking activity of representative compounds in the different isoforms

COMPOUND	EC_{50} (μM) HCN1	EC_{50} (μM) HCN2	EC_{50} (μM) HCN4
EC4	2.31 ± 0.37	17.22 ± 1.74	7.23 ±2.6
EC18	30.82 ± 0.95	87.15 ± 25.61	5.19 ±0.63
EC32	5.60 ± 0.26	24.58 ± 4.89	6.81 ± 0.37
MEL57A	0.6 ±0.07	18.3 ±0.14	103.78 ±29.8
MEL55A	9.41±0.25	2.3±0.6	24.94±0.1
DKAH269	4.66 ± 1.44	20.47 ± 4.77	12.01 ± 3.78
Ivabradine	4.50 ± 0.44	4.52 ± 2.82	4.28 ± 0.4

In order to easily compare the activity of the compounds on the three isoforms, we graphically reported (figure 9) the ratios between the EC_{50} of the compounds reported in table 1 on the three isoforms. We arbitrary selected the value 5 as threshold for selectivity, considering isoform-selective a compound showing a ratio higher than 5. For EC18 the calculated ratios HCN4 vs HCN2 and HCN4 vs HCN1 exceed this threshold, and therefore the If blocking activity on HCN4 is more that five times higher than on HCN1 and HCN2. According to this criterion, EC18 and MEL57A can be considered isoform-selective. In particular, for compound EC18 the selectivity ratios for HCN4 vs HCN2 and HCN4 vs HCN1 were, respectively, 17 and 6, and then it was selective for the isoform HCN4 of the channel. EC4 does not discriminate between HCN4 and the other two isoforms: this compound is more potent on HCN1 than on HCN2 (ratio = 7.5) although this is less interesting. EC32 is not selective, while MEL57A is the most potent and selective compounds among those tested so far, since its activity was 170-fold and 30-fold higher on HCN1 than, respectively, on HCN4 and HCN2. The reference compounds, DKAH269 (Cilobradine) and ivabradine (Iva), did not show isoform selectivity. Although EC4 and EC32 do not show isoform selectivity, they were the first to be synthesized and allowed us to design and synthesize EC18 and MEL57A, which turned out to be isoform selective in the f-current blockade.

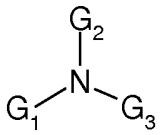
Effect on native I_f recorded on isolated SAN cells

Figure 10 shows an illustrative example of the experiment performed on isolated guinea pig SAN cells. In panel A a typical trace of I_f current recorded in control condition (black) and in presence of EC32 10 μM (gray) is reported. The corresponding activation curves, calculated as described in methods are reported in panel B. The hystogram in panel C summarized the current reduction for the three tested compounds at 10 μM concentration. **EC4** caused a 63% reduction of I_f at -120 mV (CTR 0.9±0.03 pS/pF, n=9; EC4 10μM: 0.32±0.05 pS/pF, n=3), **EC18** about 88% (CTR 0.9±0.03 pS/pF n=9; EC18 10μM: 0.1±0.01, n=3) e **EC32** about 46% (CTR 0.9±0.03 pS/pF, n=9; EC32 10μM: 0.49±0.07 pS/pF, n=6).

The effects of compounds EC4 and EC32 on single SAN cells of rabbit were shown in figure 11. In panel A the activation curve of I_f current calculated in control condition (black) and in presence of EC32 10 μM (grey) is shown. The histogram in panel B summarized the current reduction for EC4 (black) and EC32 (grey) at 10 μM of concentration. EC4 reduced the current density by about 51% (CTR: 0.74±0.07 pS/pF, n=7; EC4 10μM: 0.4±0.03 pS/pF, n=3) and EC32 by 70% (CTR: 0.74±0.07 pS/pF, n=7; EC32 10μM: 0.2±0.11 pS/pF, n=4).

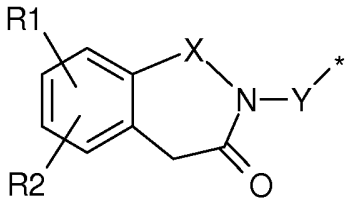
CLAIMS

1. Compounds of formula (I):

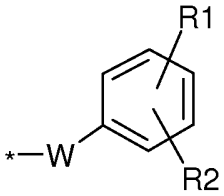
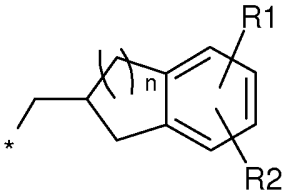


(I)

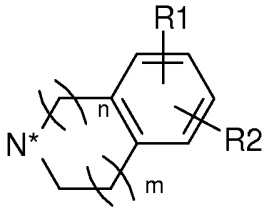
where

G1 =  ;

G2 = G1 or Ak_{1-4};

G3 =  o  ;

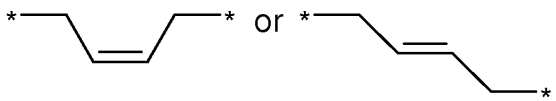
or G2 and G3 together with the bound nitrogen atom are included in a cyclic moiety such as:



and where

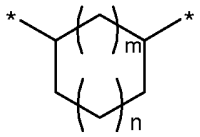
X = CH=CH, CH_2CH_2;

Y = but-2-en-1,4-di-yl of formula

 or ;

or

Y is a cycloalkanediyl such as:



;

W = Ak_{1-9}, $Ak_{1-4}OAk_{0-4}$, $Ak_{1-4}C(=O)Ak_{0-4}$, $Ak_{1-4}SAk_{0-4}$, $Ak_{1-4}N(R1)Ak_{0-4}$, under the condition that, when Y is but-2-en-1,4-di-yl and G2 = Ak_{1-4}, at least one Ak of W is branched and contain a stereogenic center;

R1 and R2 independently from each other can be H, halogen, -CN, -OH, $-CF_3$, $-Ak_{1-3}$, $-OAk_{1-3}$, $-SAk_{1-3}$, $-Ph-OAk_{1-3}$;

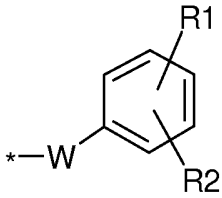
n = 0, 1, 2; m = 0, 1, 2;

under the condition that G2 = Ak_{1-4} when Y is a cycloakanediyl as described above;

with the exclusion of compound *cis* 3-(3-{[2-(3,4-dimethoxyphenyl)ethyl]methylamino}cyclohexyl)-7,8-dimethoxy-1,3,4,5-tetrahydro-benzo[*d*]azepin-2-one.

2. Compounds according to claim 1 where

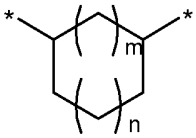
Y = but-2-en-1,4-di-yl;

G3 = 

;

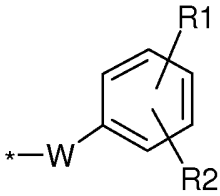
W = Ak_{1-9} branched and containing at least a stereogenic center.

3. Compounds according to claim 1 where

Y = 

;

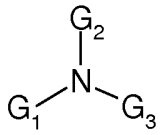
G2 = Ak_{1-4};

G3 = 

.

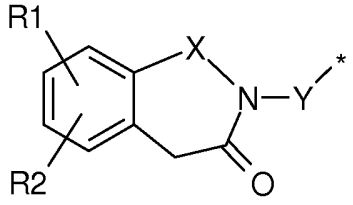
4. Compounds according to anyone of claims from 1-3 for use as medicaments.

5. Pharmaceutical composition including one compound of formula (I) according to anyone of claims 1-3, and at least another acceptable pharmaceutical ingredient.
6. Use of the compounds of formula (I) according to anyone of claims from 1-3 for the preparation of pharmaceutical compositions for the treatment of angina, arrhythmia, cardiovascular disorders, neurological diseases such as epilepsy including febrile seizure, neuropathic pain, cognitive dysfunctions.
7. Use of compounds of formula (I):

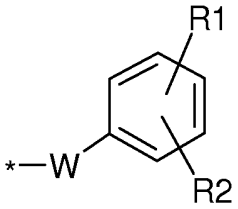
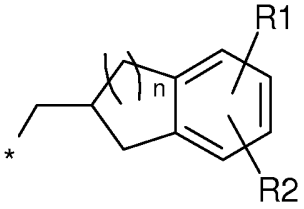


(I)

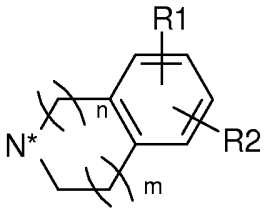
where

G1 =  ;

G2 = G1 o Ak_{1-4};

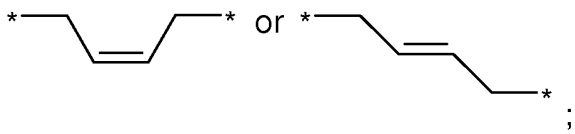
G3 =  or  ;

or G2 and G3 together with the bound nitrogen atom are included in a cyclic moiety such as:



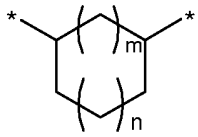
X = CH=CH, CH_2CH_2;

Y = but-2-en-1,4-di-yl of formula



or

Y is a cycloalkan-di-yl such as



;

W = Ak_{1-9}, $Ak_{1-4}OAk_{0-4}$, $Ak_{1-4}C(=O)Ak_{0-4}$, $Ak_{1-4}SAk_{0-4}$, $Ak_{1-4}N(R1)Ak_{0-4}$, under the condition that, when Y is but-2-en-1,4-di-yl and G2 = Ak_{1-4}, at least one Ak of W is branched and containing at least one stereogenic center;

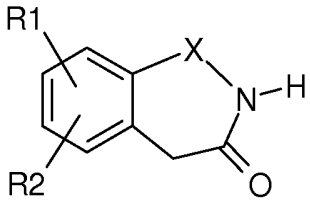
R1 and R2 independently from each other can be H, halogen, -CN, -OH, $-CF_3$, $-Ak_{1-3}$, $-OAk_{1-3}$, $-SAk_{1-3}$, $-Ph-OAk_{1-3}$;

n = 0, 1, 2; m = 0, 1, 2;

with the condition that G2 = Ak_{1-4} when Y = cycloalkcanediyl;

for the preparation of pharmaceutical compositions for the treatment of pathologies that can be alleviated or prevented by means of the selective inhibition of one HCN channel isoform.

8. Use according to claim 7 where the compounds of formula (I) are defined in any of the claims 2-3
9. Use of the compounds of formula (I) according to anyone of claims 7-8 for the preparation of a kit to be used as pharmacological tools to study the structure and the function of HCN channels and the physiological and/or pathological processes in which they are involved.
10. Process for the preparation of compounds of formula (I) according to anyone of claims 1- 3, said process including as intermediate a compound of formula (V)



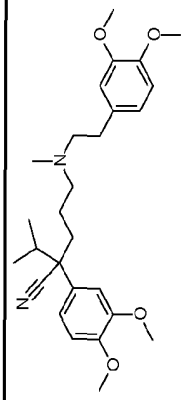
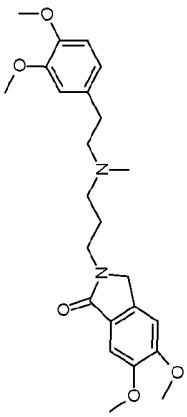
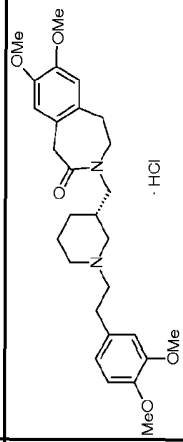
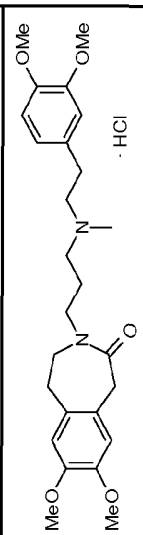
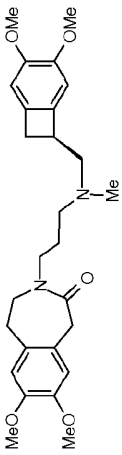
(V)

wherein R1 and R2 independently from each other can be H, halogen, -CN, -OH, -CF_3, -Ak_{1-3}, -OAk_{1-3}, -SAk_{1-3}, -Ph-OAk_{1-3};

X = CH=CH, CH_2CH_2.

FIGURE 1

Compounds known at the state of the art

Name	Structure
verapamil	
falipamil	
cilobradine	
zatebradine	
ivabradina	

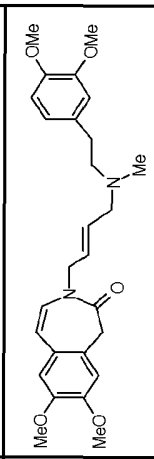
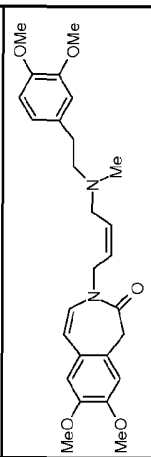
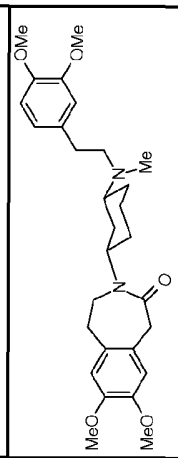
Name	Structure
EC4	
EC32	
EC18	

Figure 2

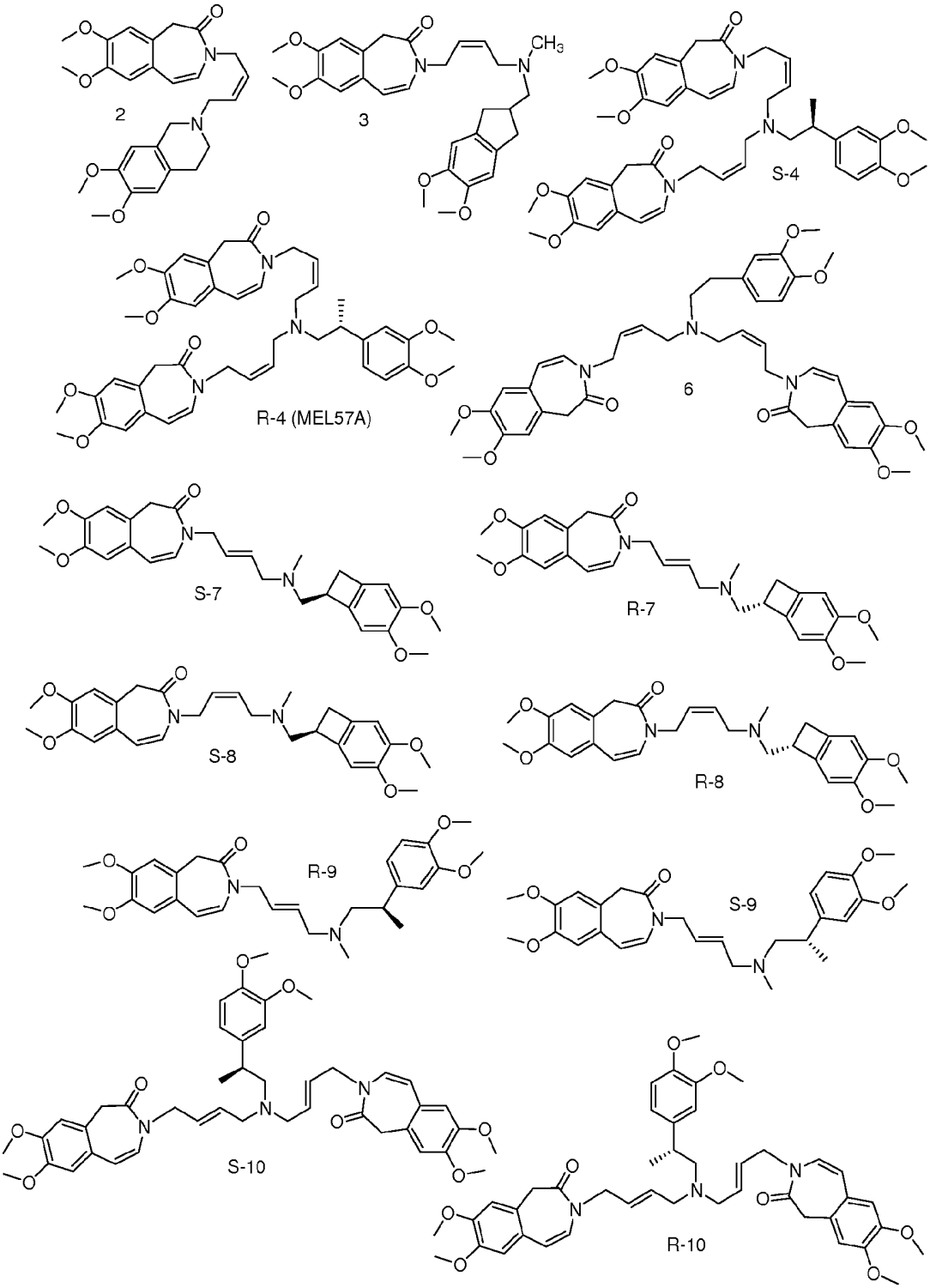
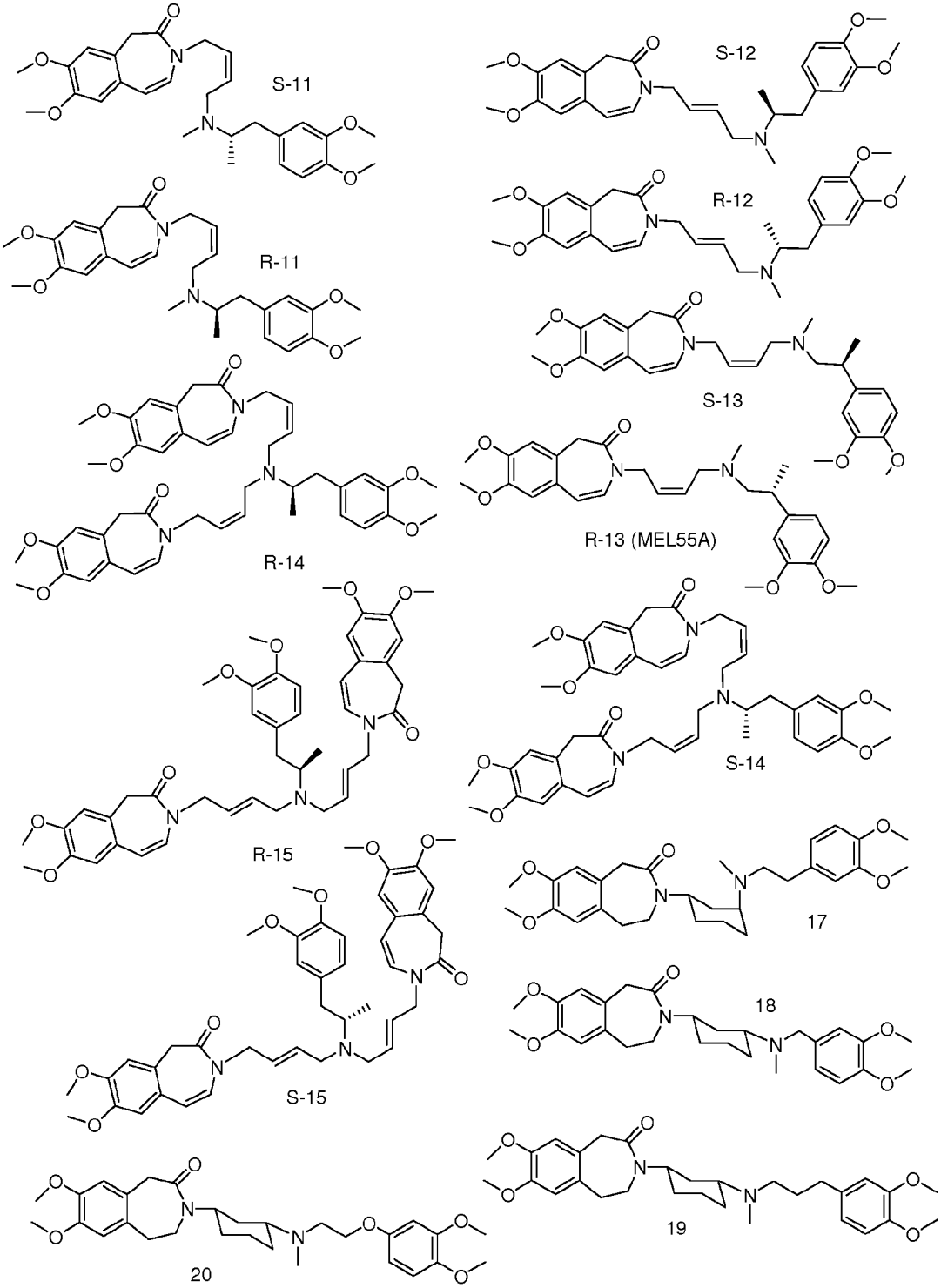


Figure 3



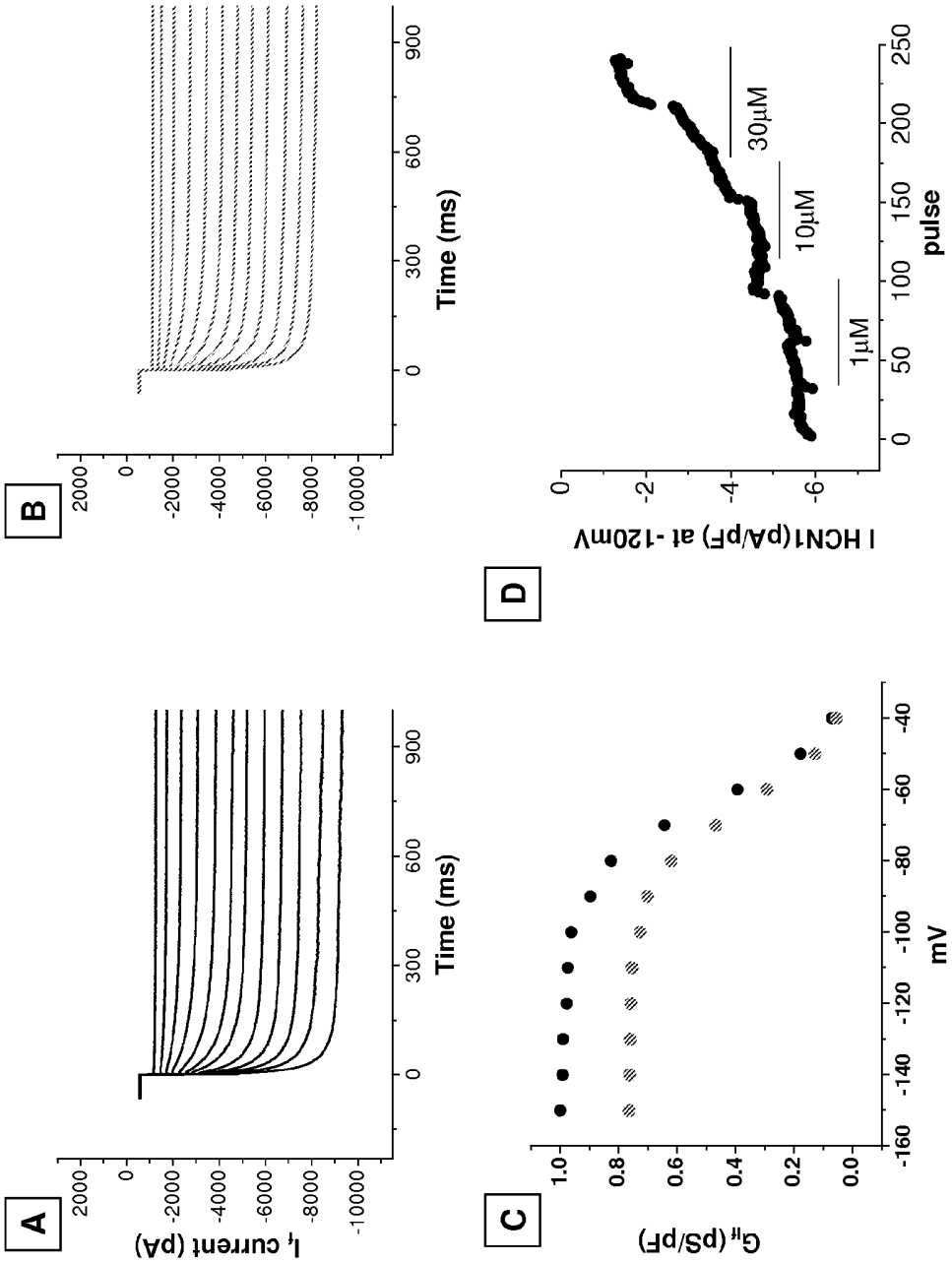


FIGURE 4
Typical experiment to study the new f-current blockers in the different channel isoforms.

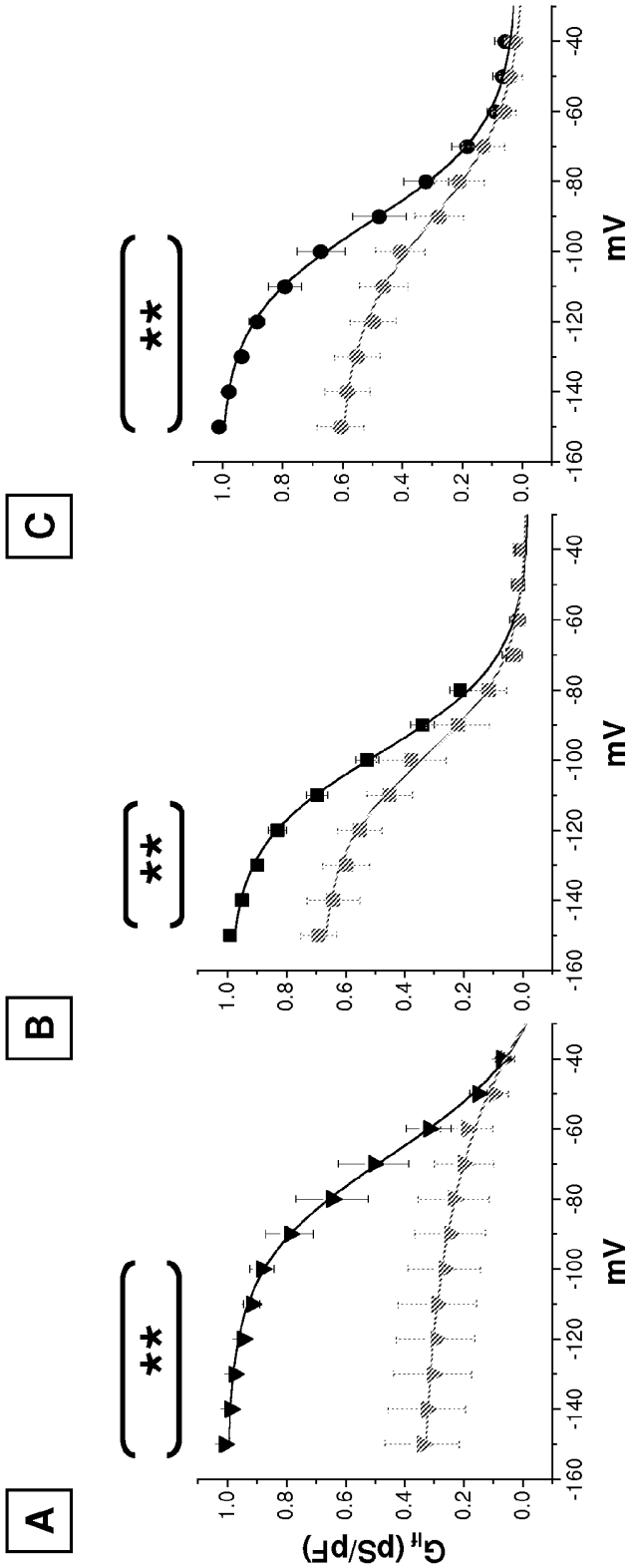


FIGURE 5
Effect of compound EC4 10µM

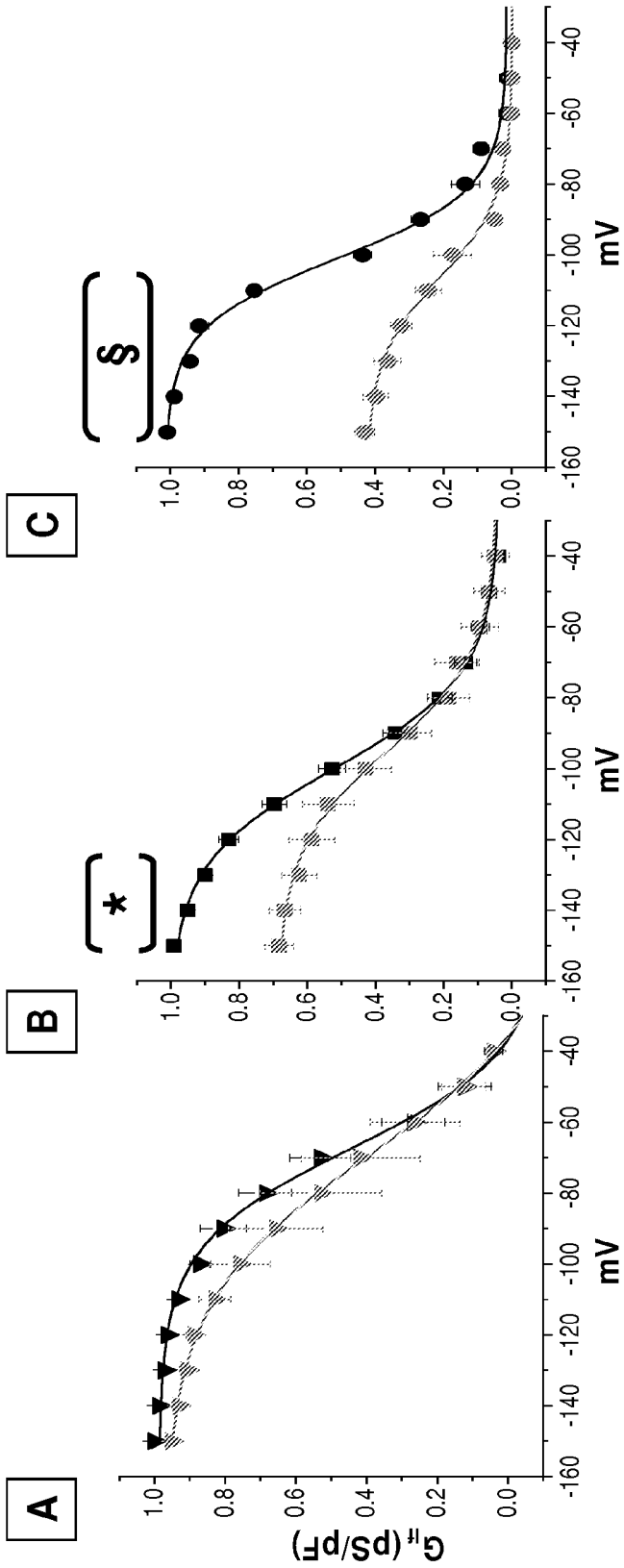


FIGURE 6
Effect of compound EC18 10µM

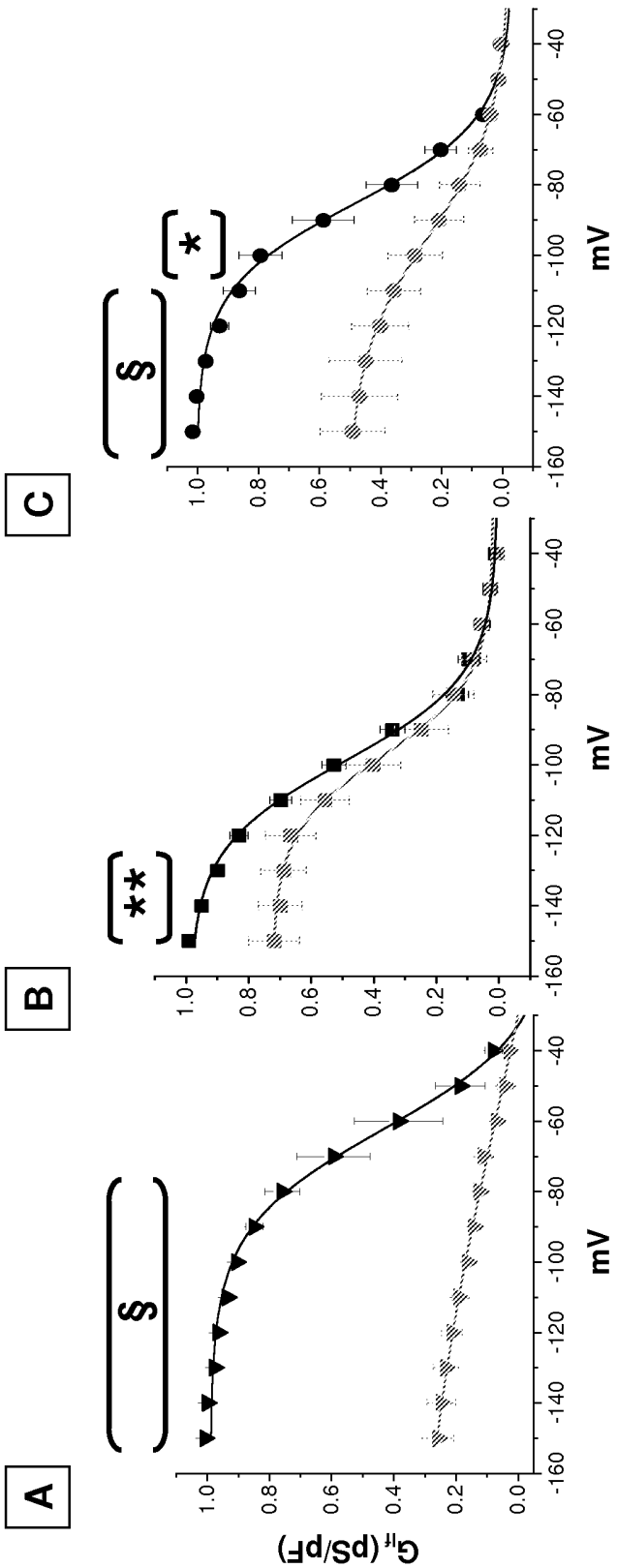


FIGURE 7
Effect of compound EC32 10µM

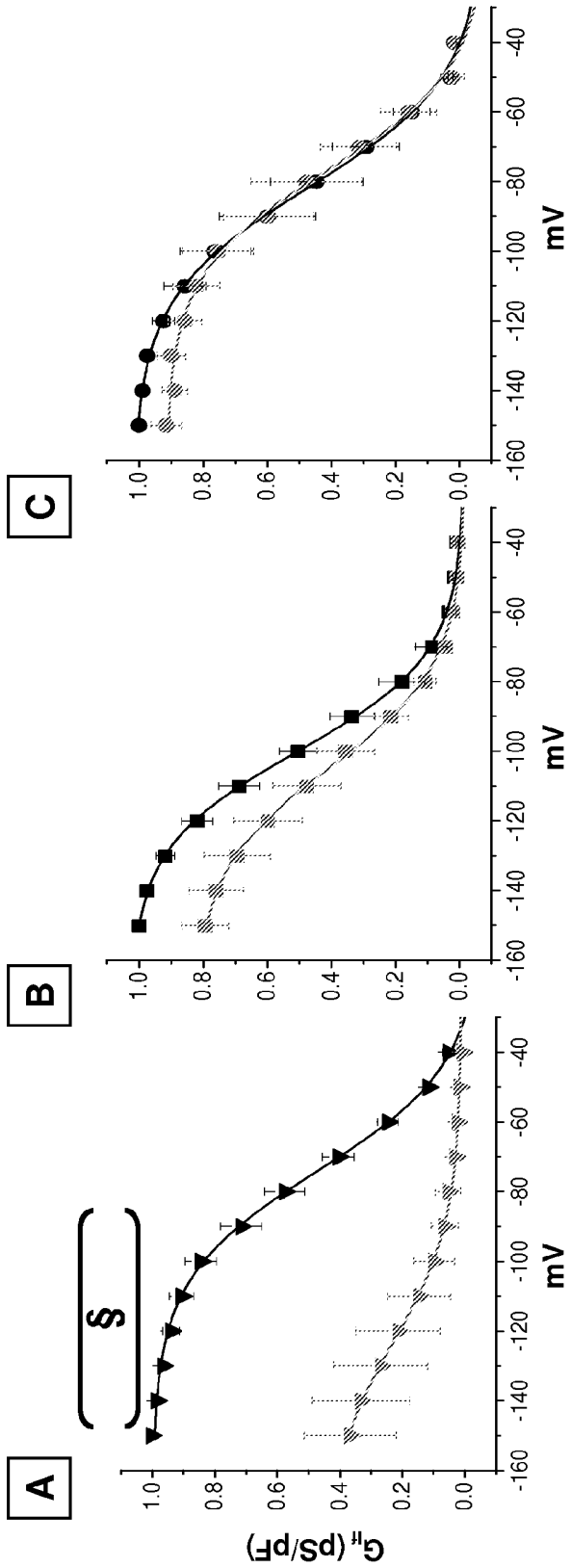
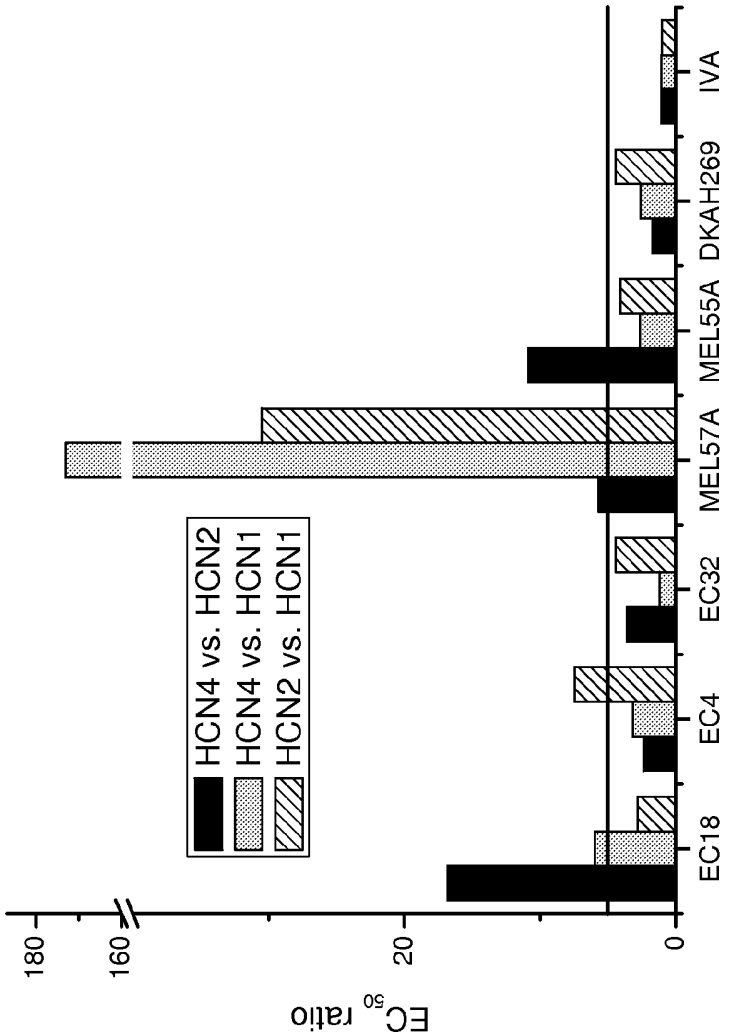


FIGURE 8
Effect of compound MEL57A 10µM

FIGURE 9

Ratio between the EC_{50} values on the three channel isoforms of selected compounds representative of the invention, in comparison with compounds known at the state of the art



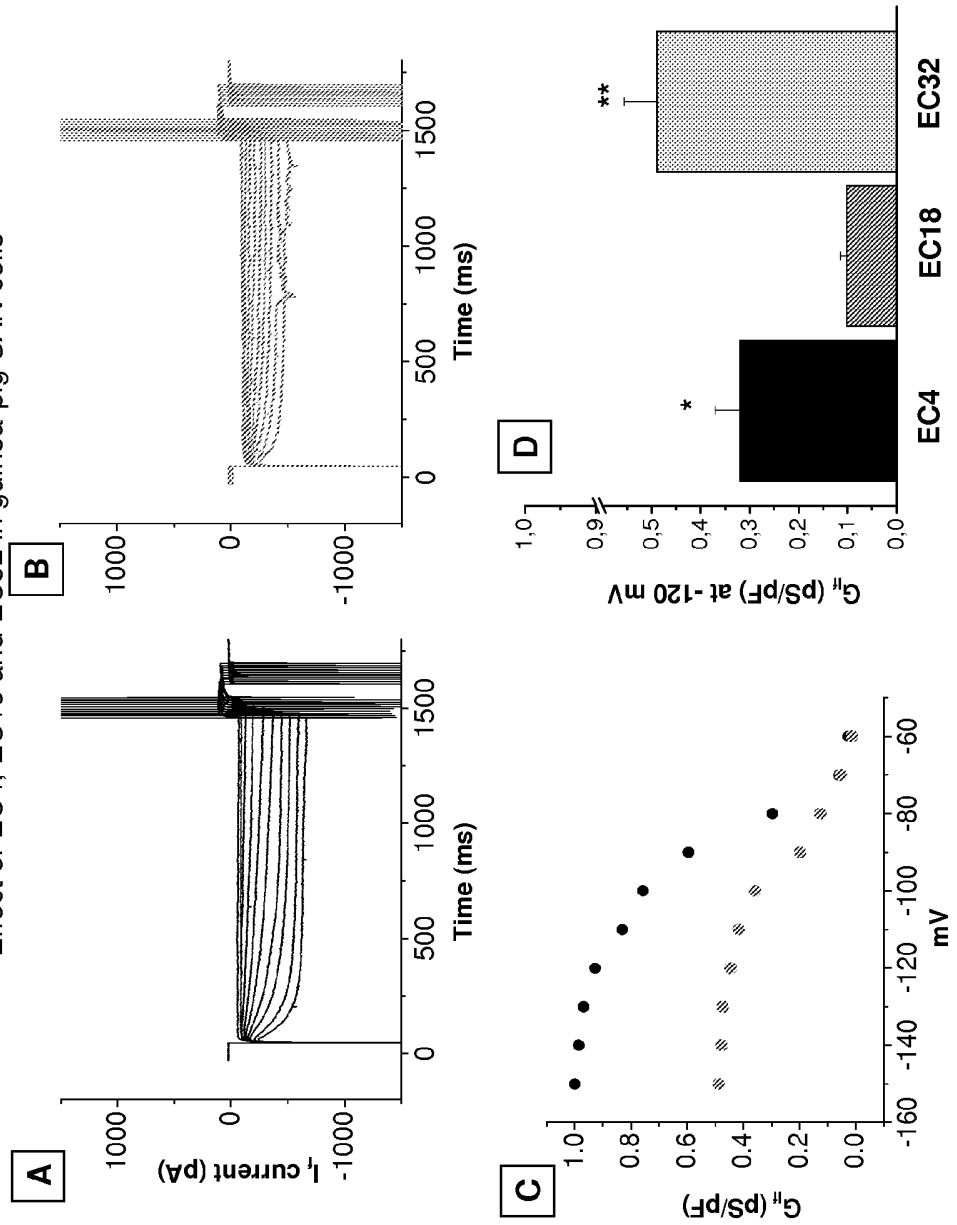


FIGURE 10
Effect of EC4, EC18 and EC32 in guinea-pig SAN cells

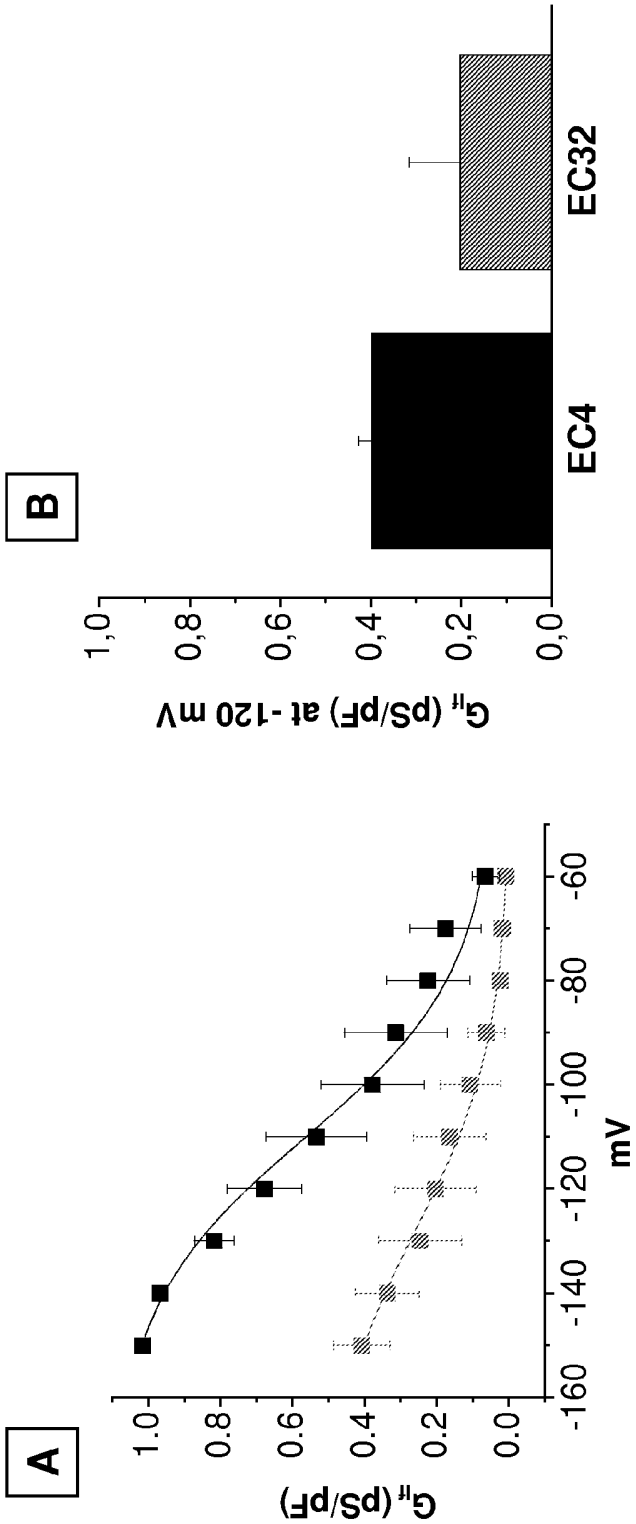


FIGURE 11
Effect of **EC4 e EC32** in rabbit SAN cells

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/059369

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D223/16 C07D401/06 A61K31/55

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NOVELLA ROMANELLI M ET AL: "Design, synthesis and preliminary biological evaluation of zatebradine analogues as potential blockers of the hyperpolarization-activated current" BIOORGANIC & MEDICINAL CHEMISTRY, ELSEVIER SCIENCE, OXFORD, GB, vol. 13, no. 4, 15 February 2005 (2005-02-15), pages 1211-1220, XP004776025 ISSN: 0968-0896 cited in the application Abstract; Chart 1.table 1; compounds 15,16 ----- -/--	1-10

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means
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Date of the actual completion of the international search	Date of mailing of the international search report
2 September 2010	14/09/2010
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Goss, Ilaria

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/059369

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YAN T. LEE, DMITRY V. VASILYEV ET AL.: "Novel pharmacological activity of loperamide and CP-339,818 on human HCN channels characterized with an automated electrophysiology assay" EUROPEAN JOURNAL OF PHARMACOLOGY,, vol. 581, 2008, pages 97-104, XP002566077 Abstract;figure 1; tables 1,2 -----	1-10
A	TARDIF J-C ET AL: "From coronary artery disease to heart failure: Potential benefits of ivabradine If inhibition: a new strategy to improve the management of coronary patients, Proceedings of a satellite symposium held during the World Congress of Cardiology, Barcelona, Spain, September 4, 2006" EUROPEAN HEART JOURNAL SUPPLEMENTS, SAUNDERS, US, vol. 8, no. D, 1 January 2006 (2006-01-01), pages D24-D29, XP002438445 ISSN: 1520-765X the whole document -----	1-10