Ligands of Diltiazem Binding Site: An Overview of Some Chemotypes

R. Budriesi[1,*], P. Ioan[1], E. Carosati[2], G. Cruciani[2], B. S. Zhorov,[3] and A. Chiarini[1]

[1]*Dipartimento di Scienze Farmaceutiche, Università degli Studi di Bologna, Via Belmeloro 6, 40126 Bologna, Italia;*
[2]*Dipartimento di Chimica, Università di Perugia, via Elce di Sotto 8, 06123 Perugia, Italia*
[3]*Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario L8N 3Z5, Canada*

Abstract: The diltiazem binding site of L-type calcium channels is the least characterized to date. In this paper, we present some of the available chemotypes that bind to the benzothiazepine binding site: natural compounds, compounds synthesized by varying the benzothiazepine scaffold, and compounds discovered by means of computational approaches.

Key Words: (+)-*cis*-Diltiazem, L-type calcium channels, benzothiazepine, chemotype, computational approaches, voltage gated calcium channel, calcium entry blockers, structural and functional analogs.

1. INTRODUCTION

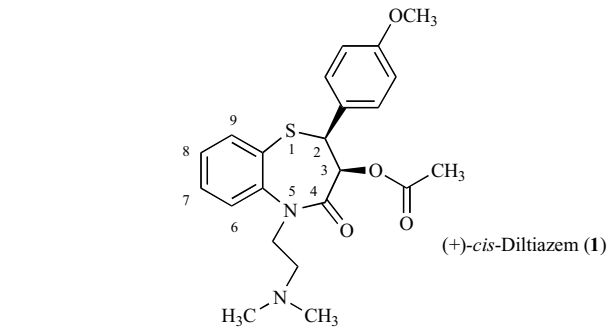
Voltage-gated calcium channels (VGCCs) mediate calcium influx through the cell surface membrane in response to the membrane potential changes. These channels are involved in key physiological processes such as muscle contraction, hormone secretion, neurotransmission, and gene expression in many different cell types [1].

Based on their physiological and electrophysiological properties, the VGCCs have been classified into several subtypes: L- ($Ca_v1.1$-1.4), P/Q- ($Ca_v2.1$), N- ($Ca_v2.2$), R- ($Ca_v2.3$) and T-type ($Ca_v3.1$-3.3) [1]. $Ca_v1.2$ channels are widely expressed in the cardiovascular system, and are found as the two splicing variants $Ca_v1.2a$ and $Ca_v1.2c$ in the heart and vascular smooth muscle respectively.

L-type calcium channels (LTCCs) belong to the Ca_v1 subfamily of Ca^{2+} channels. They are distinguished from other VGCC types by their high sensitivity to organic Ca^{2+} activators and blockers. LTCCs blockers are different groups of chemically unrelated compounds that are widely used for the treatment of cardiovascular diseases due to their cardiodepressant and vasodilator activities [2]. (+)-*cis*-Diltiazem (**1**), Nifedipine (**2**), and Verapamil (**3**) are prototype ligands of three chemically unrelated classes of LTCCs blockers (Fig. (**1**)): benzothiazepines (BTZ), 1,4-dihydropyridines (1,4-DHP), and phenylalkylamines (PAA) respectively.

(+)-*cis*-Diltiazem (**1**) (Fig. (**1**)), is the only clinically available member of the benzothiazepine class, and is most widely used as LTCC blockers [3]. It is used in the treatment of angina pectoris, supraventricular arrhythmias, and in the moderation of systemic hypertension due to its potent vasodilating effect without reflex tachycardia and its ability to suppress sinoatrial node stimulation with only mild negative inotropic effects. A detailed review on (+)-*cis*-diltiazem pharmacological properties and therapeutic uses was reported in literature [4,5].

*Address correspondence to this author at the Dipartimento di Scienze Farmaceutiche, Università degli Studi di Bologna, Via Belmeloro 6, 40126 Bologna, Italia; Tel: +39-051-2099737; Fax: +39-051-2099721;
E-mail: roberta.budriesi@unibo.it



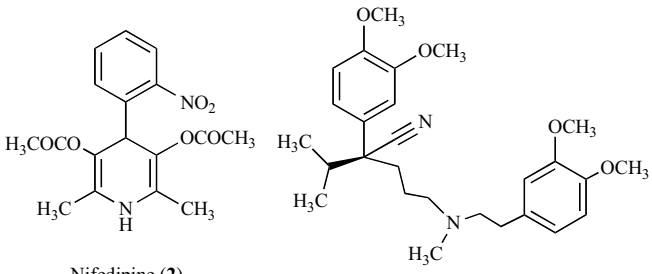


Fig. (1). Chemical structure of (+)-*cis*-Diltiazem (**1**) (BTZ), Nifedipine (**2**) (1,4-DHP), and (*S*)-Verapamil (**3**) (PAA).

The BTZ binding site is located in the α_1-subunit of LTCCs, but specific details of this site remain to be experimentally elucidated. In the absence of the crystallographic structure of calcium channels, results of site-mutagenesis studies, which are currently available, do not provide enough data for the comprehensive mapping of the BTZ binding site [6,7].

Earlier studies suggested that the BTZ binding site is coupled to the 1,4-DHP- and PAA-binding sites *via* non-competitive mechanisms and distinct binding sites for PAAs and BTZs were proposed [2,8]. Later studies however, support a common, or at least significantly overlapping binding sites for PAAs and BTZs [6,9].

The molecular architecture of LTCCs is described in the literature [6,9,10]. The pore-forming α_1 subunit consists of

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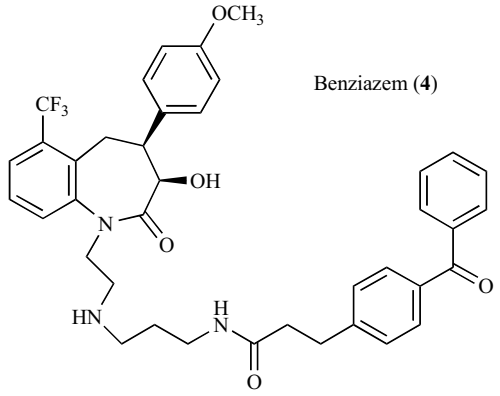


Fig. (2). Chemical structure of Benziazem (**4**).

four homologous repeats I-IV, each containing six putative transmembrane segments (S1-S6) and a membrane re-entrant P-loop. The BTZ-sensing residues are mostly located in the transmembrane segments IIIS6 and IVS6. Studies from the Glossmann and Striessnig group at the University of Innsbruck indicate that the BTZ and PAA receptors have common, but not identical, molecular determinants in the transmembrane segment IVS6 [11]. Experiments with a photoreactive (+)-*cis*-diltiazem derivative, [^{3}H]benziazem (**4**) (Fig. (**2**)), suggested additional (+)-*cis*-diltiazem interaction sites in IIIS6.

To date, several different and unrelated chemotypes were shown to bind to the (+)-*cis*-diltiazem binding site in the α_1-subunit of LTCC, and nowadays medicinal chemists are involved in the discovery, design and synthesis of new chemotypes structurally related to (+)-*cis*-diltiazem. In this review, we will describe some currently available chemotypes which interact with the BTZ binding site, together with the structure-activity relationships of (+)-*cis*-diltiazem analogs. We will highlight the different origin of the chemotypes and discuss substances, which are (i) extracted from natural compounds, (ii) synthesized by rational design starting from well-known scaffolds, and (iii) discovered *via* computational approaches such as virtual screening.

2. DILTIAZEM BINDING SITE

Tikhonov and Zhorov [12] recently built a homology model of the pore-forming subunit (α_1) of $Ca_v1.2$ and employed ligand-docking computational experiments to elaborate a 3D model of the BTZ binding site with one of the most potent BTZ derivatives, SQ32910 (**5**) (Fig. (**3**)). This com-

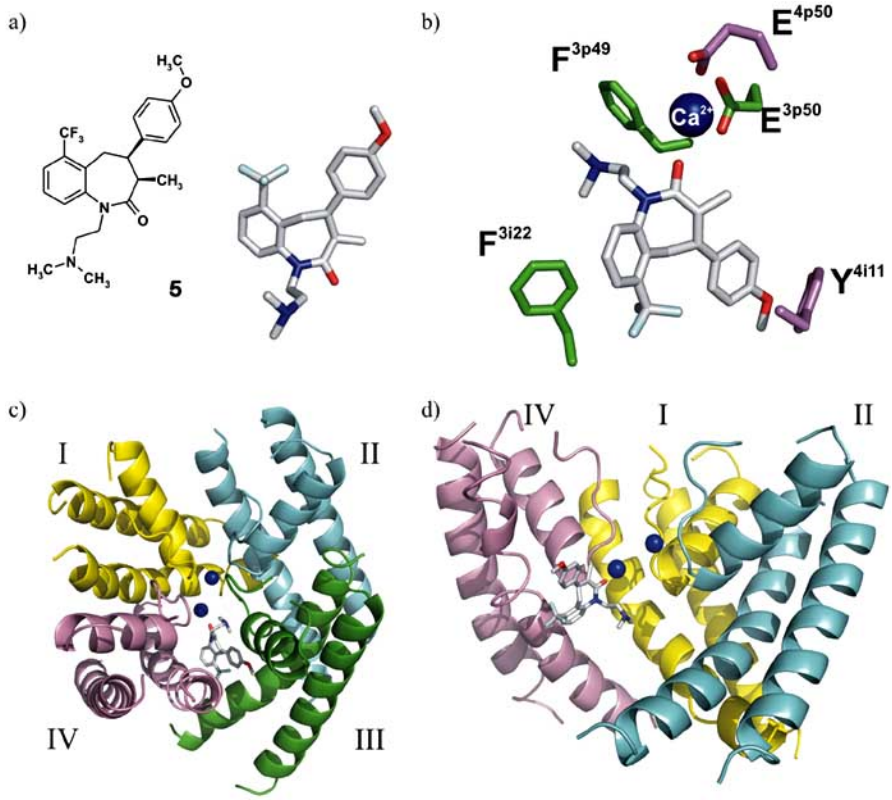


Fig. (3). Visualization of the BTZ-LTCC model by Tikhonov and Zhorov [12]. (a) 2D and 3D chemical structures of SQ32910 (**5**). (b) Some of the residues, which contribute to the BTZ binding site, are designated with labels used by Tikhonov and Zhorov [12]. Compound **5** is shown in the "interface binding mode" with the carbonyl oxygen of the ligand bound to the Ca^{2+} ion, which is chelated by the selectivity-filter glutamates E^{3p50} and E^{4p50}. The methoxy oxygen forms a hydrogen bond with the side chain of tyrosine Y^{4i11}. The fused aromatic ring of the ligand interacts with phenylalanine F^{3i22}, and the BTZ ammonium group is involved in cation-pi interactions with phenylalanine F^{3p49}. (c and d). Two different views of the ternary complex formed between the channel, Ca^{2+} ion, and the BTZ ligand. Ca^{2+} ions in the selectivity-filter region are shown by blue spheres. (c) When viewed from the extracellular side, repeats I-IV are arranged clockwise around the pore axis. (d) Side view of the complex with the front repeat removed for clarity. The figure was prepared with PyMol [l6].

pound was studied experimentally by Kimball and co-authors along with other (+)-*cis*-diltiazem derivatives. These will be discussed in the next section [13,14].

The L-type Ca^{2+} channel model by Tikhonov and Zhorov [12] consists of four repeats (I-IV) and each repeat contains the P-loop and two transmembrane segments, S5 and S6. This is the first published model in which BTZ-LTCC interactions are presented at the atomic level and which rationalizes various experimental observations reported in literature [7,15].

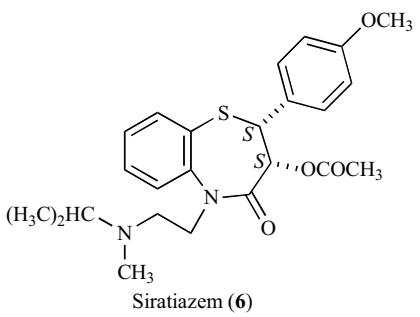
The model proposes two binding modes for BTZs, depending on the number of Ca^{2+} ions in the selectivity-filter region. In one mode, which would prevail when the negative charges at the selectivity-filter glutamates in the outer pore are not compensated by Ca^{2+} ions, the ammonium group of BTZ is attracted to the outer pore. In the other mode, which would prevail when the negative charges in the selectivity-filter glutamates are neutralized by Ca^{2+} ions, the carbonyl oxygen of BTZ directly interacts with a Ca^{2+} ion chelated by selectivity-filter glutamates. This second model is shown in Fig. (**3**).

This model is expected to provide a better understanding of the ligand-channel interactions and could help in the computer-aided design of novel BTZ-like LTCC blockers.

3. STRUCTURAL ANALOGS OF DILTIAZEM

The active pharmacological form of diltiazem as a calcium entry blocker is (+)-*cis*-diltiazem (**1**), the prototype of the BTZ class. (+)-*cis*-Diltiazem gives its name to the corresponding binding site located in the pore-forming α_1-subunit of LTCCs.

A recent review [17] considers various structural analogs of (+)-*cis*-diltiazem and their SARs. In particular, hydrogen atoms in the condensed aromatic ring were substituted with halogens, methyl or polar groups; the C-8 chlorine derivative, clentiazem, is one of the most potent compounds of this series [18]. In addition, the aminoalkyl group at N-5 was modified *via* quaternalization of the terminal amino group or transformation into a primary amino group, but these modifications led to inactive compounds. Replacing one of the N-bonded methyl groups with an isopropyl group yielded compounds such as in Siratiazem (**6**) (Fig. (**4**)), with a potency similar to that of (+)-*cis*-diltiazem in coronary vascular smooth muscle [19], confirming that aminoalkyl group at N-5 is essential for biological activity.



Siratiazem (**6**)

Fig. (4). Chemical structure of Siratiazem (**6**).

The acetoxy group at C-3 was replaced with diverse substituents such as hydroxy, alkoxy, ariloxy or aliphatic chains. Those changes affected the activity and the pharmacokinetics of diltiazem analogs in different ways since the acetoxy group is easily metabolized *in vivo* [20]. The replacement of the *p*-methoxyl group by a methyl group was tolerated as well [17].

In summary, the following three pharmacophore groups are essential because their removal causes the loss of the LTCC-blocking activity: (i) a basic aminoethyl group at N-5, (ii) a 4'-methoxyphenyl substituent at C-2 and (iii) a carbonyl oxygen at C-2. These groups are seen in diltiazem and all its active analogs and possible roles of these groups in BTZ-LTCC interactions were proposed in ref. [10]. Interesting structural modifications performed in the BTZ series aimed at changing the size of the thiazepine heterocycle ring. Modifications included the seven-membered ring contraction to the five-membered benzothiazole ring [21] or the six-membered benzothiazine ring [22], as well as the seven-membered ring expansion to the eight-membered naphthothiazocinone ring **7** [23] (Fig. (**5**)).

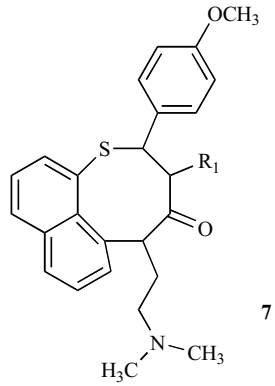


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Fig. (5). Chemical structures of naphthothiazocinone series (**7**).

Most of the tested compounds were shown to decrease the contraction developed in K^+-depolarized guinea pig ileum and to significantly decrease the blood pressure in spontaneously hypertensive (SH) rats after oral administration.

Bioisosteric replacement of the sulphur atom by a methylene bridge was reported in 1992 [24,25] for benzazepinones derivatives (**8**) (Fig. (**6**)), which are specific and reversible LTCC blockers. LTCC blocking activity of BTZ derivatives depends on the 4'-methoxyphenyl moiety at C-4 and the basic residue at N-1, whereas the methylene/sulphur exchange affects neither the activity nor the potency of compounds. It is also reported that substitutions in the fused ring and at C-3 might affect the access of the compound to the receptor site within the α_1-subunit of the channel.

4. FUNCTIONAL ANALOGS OF DILTIZEM

4.1. From Pyrrolobenzothiazepines to Thiazinooxadiazolones

Study of the functional activity of peripheral-type benzodiazepine receptor (PBR) ligands, such as antagonist PK 11195 (**9**) (Fig. (**7**)) and agonist Ro 5-4864 (**10**) (Fig. (**7**)) led

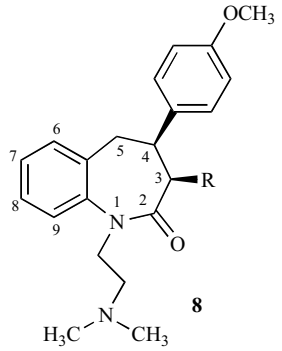


Fig. (6). General structure of benzazepinone derivatives (**8**).

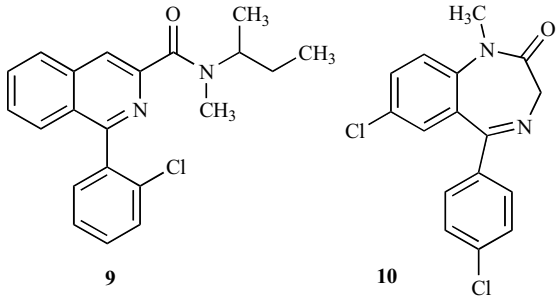


Fig. (7). Chemical structures of PK 11195 (**9**) and Ro 5-4864 (**10**)

to the hypothesis that PBRs could be functionally coupled to LTCCs. In particular, since compound **10** reduces the positive chronotropic activity, PBR ligands are hypothesised as a good starting point for the design and synthesis of new cardiovascular modulators [26,27].

In 1996, Campiani *et al*. [28] presented a novel selective ligand for PBR, which was used as the starting compound for the synthesis of new selective calcium entry blockers. The derived pyrrolo[2,1-*d*][1,5]benzothiazepines displace [^{3}H]nitrendipine from LTCC better than verapamil or (+)-*cis*-diltiazem. Compound **11** (Fig. (**8**)) exhibits the most potent negative inotropic effect in this series, with no affinity for PBR [28].

An alteration of the main scaffold of **11** led to the pyrrolo[2,1-*c*][1,4]benzothiazine system (compound **12**). A marked cardiac over vascular selectivity characterizes this series of compounds. [28-31].

The replacement of the pyrrole ring with a pyrrolidinone ring led to the lactam **13**, which demonstrates improved negative inotropic activity compared to **12** [29]. Instead, bioisosteric replacement of the sulphur atom with the oxygen (compound **14**) decreases the affinity [30].

In 2002 Budriesi *et al.* [32] described the cardiovascular activity of thiazinoxadiazolone derivatives (compounds **15**, **16**), a new chemotype that binds to the diltiazem site of LTCC. All the synthesized compounds have been made using the rational design scheme presented in Fig (**8**). The compounds show negative inotropic activity. A virtual receptor scheme derived from 3D Quantitative Structure-Activity Relationships (3D QSAR) study, describes the pharmacophoric requirements for a negative inotropic profile: a basic center, three lipophilic groups, and two hydrogen bond

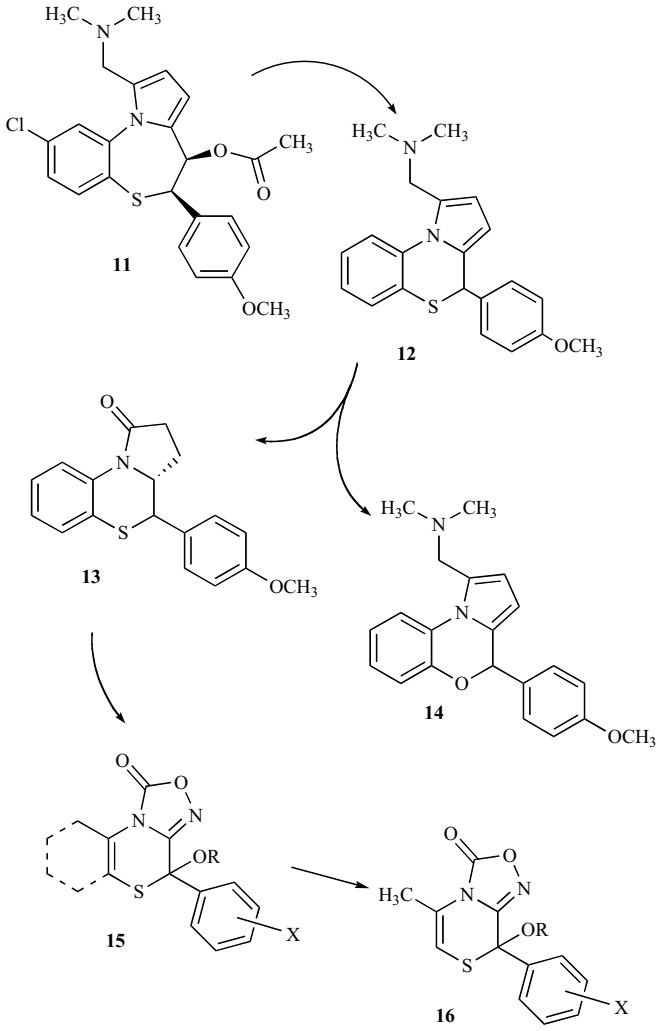


Fig. (8). Rational drug design of (+)-*cis*-diltiazem related compounds **11-16**.

(HB) acceptor groups. This 3D pharmacophore model could be used as an *in silico* filter to prioritize new synthetic work for the development of potent and selective compounds [33].

4.2. HOE 166

The reduction of the thiazepine ring of (+)-*cis*-diltiazem (**1**) or the enlargement of the thiazole moiety of SA 2572 (**17**) (a benzothiazoline with hypotensive activity [22]) lead to a novel class of LTCC blockers (Fig. (**9**)), the 1,4-benzothiazinones. The asymmetric center at C-2 in the benzothiazinone ring of Sesamodil is relevant, since the (*R)*-enantiomer (also known as Semotiadil) (**18**) is the most promising compound of the series described by Fujita *et al.* [22] that showed greater selectivity and potency at vascular smooth muscle assay than (+)-*cis*-diltiazem (**1**).

HOE 166 (**19**), which closely resembles Sesamodil (**18**), shows calcium antagonist properties being a potent inhibitor of K^+-induced contraction in the pulmonary artery [34]. HOE 166 is suggested [34] to bind to a new site of LTCC, where it seemingly interacts in a negative heterotropic manner with the receptor site of DHPs and PAAs. Moreover, its binding mode could be considered moderately stereoselective, since

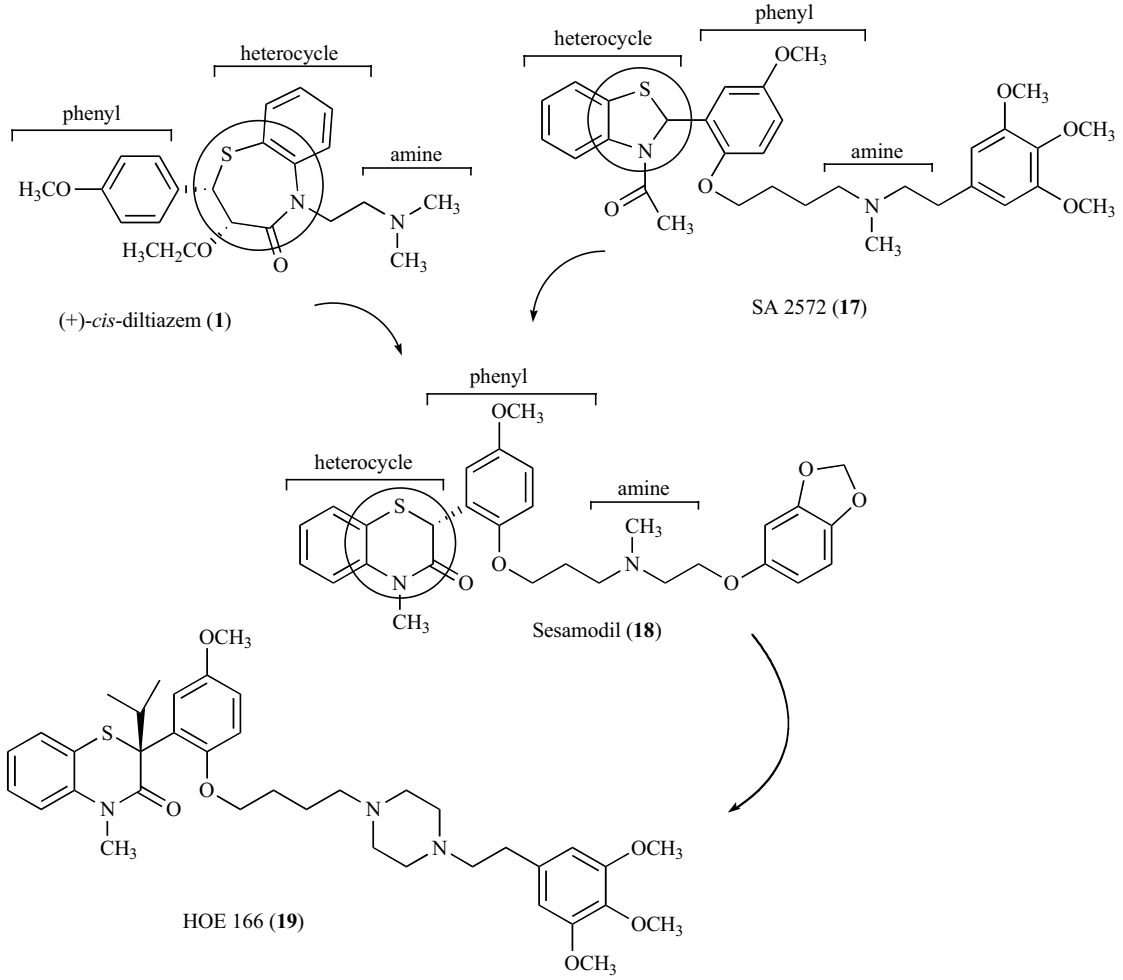


Fig. (9). Design of (R)-Sesamodil (**18**) and chemical structure of HOE 166 (**19**).

the (*S*)-enantiomer is about 10-fold less potent than the (*R*)-enantiomer.

4.3. Fostedil and Belfosdil

A series of benzothiazolylphosphonic esters, which are structurally related to previously described benzothiazolines, were studied by Morita *et al*. [35,36] KB 944 (fostedil) (**20**) (Fig. (**10**)), a phosphonate derivative, was found to relax dog coronary vessels in a manner similar to that of diltiazem.

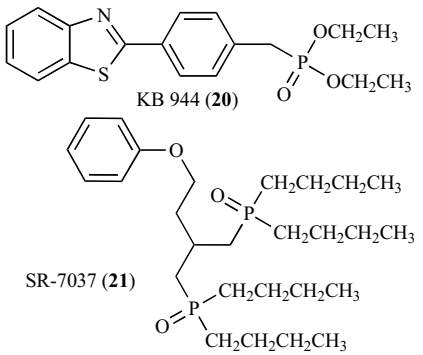


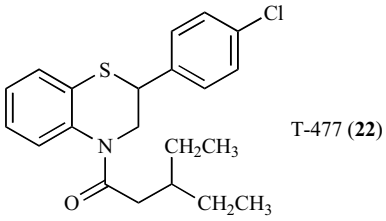
Fig. (10). Chemical structures of Fostedil (KB 944) (**20**) and Belfosdil (SR-7037) (**21**).

Intensive SARs studies on this class of compounds suggest that the diethoxyphosphinyl group may improve potency of compounds with vasorelaxant activity. KB 944 stimulates [^{3}H]nimodipine binding to the DHP site, as does (+)-*cis*-diltiazem [35,36]. Some authors argue against the hypothesis that binding to the BTZ site is a prerequisite to upregulate DHPs labeling [37,38].

Many structural modifications of the LTCC blocker **20** were made with the goal to improve the coronary vasodilator activity. Some of the resulting compounds were found to be more potent than fostedil and (+)-*cis*-diltiazem [39]. In particular, SR-7037 (belfosdil) (**21**) (Fig. (**10**)), a 1,3-diphosphonate derivative, inhibits labelling by [^{3}H]diltiazem, DHP [^{3}H]PN200-100 and PAA [^{3}H]D888. Thus, **21** appears to interact with the binding sites of the three main classes of LTCC blockers, suggesting that the inhibition of LTCC plays an important role in the smooth muscle relaxation and hypotensive action of diphosphonates [40].

4.4. T-477

The BTZ derivative T-477 (**22**) (Fig. (**11**)) has a different biological profile because it exerts neuroprotective effects with a weak activity on smooth muscle contraction [41]; this different behaviour could be related to its ability to inhibit



T-477 (**22**)

Fig. (11). Chemical structure of T-477 (**22**).

various VGCCs, including the sodium channel. The blocking potency of T-477 does not vary significantly in different types of calcium channels (cardiac L-type IC_{50} = 52 μM) [42], and T-477 also blocks Na^+ influx induced by veratridine, a well known Na^+ channel site 2 agonist [43].

4.5. Thiosalycilamides

On the basis of the pharmacophores of ligands of the (+)-*cis*-diltiazem site in LTCCs suggested by Barrish *et al*. [44] and the receptor binding mode proposed by Kimball *et al.*, [14] Mehanna and coworkers patented in 2003 a series of compounds as calcium channels blockers [45]. Despite these new thiosalicylamides derivatives (**23**) lack the important benzothiazepine ring (Fig. (**12**)), they show weak to moderate potency in the inhibition of the contraction induced in K^+-depolarizated rat aortic strips. Unfortunately, no data on [^{3}H]diltiazem displacement was reported for these compounds [46].

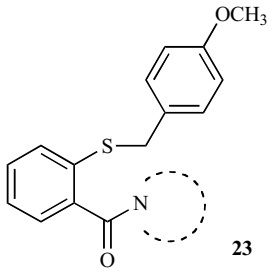


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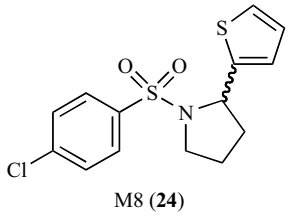
Fig. (12). General structure of thiosalycilamides series (**23**).

These findings suggest that the presence of a heterocyclic ring system is not essential for diltiazem-like calcium channel blocking activity, but its absence could result in a considerable decrease of activity.

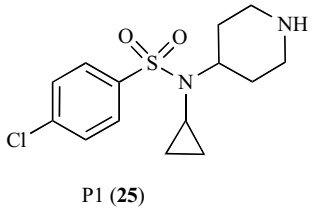
4.6. Benzosulfonamides Found by Virtual Screening

Novel cardioselective compounds, which bind to the BTZ site in $Ca_v1.2$ but lack structural resemblance with (+)-*cis*-diltiazem, were discovered in 2006 using a multidisciplinary approach based on a virtual screening procedure [47].

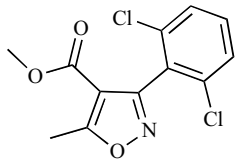
Starting from a database of purchasable compounds, 340,000 molecules were screened *in silico*, taking into account the pharmacophoric and stereo-chemical properties of a small library of previously described functional (+)-*cis*-diltiazem analogs [32,33]. Only twenty compounds out of these were selected for binding and functional assays. Three novel chemotypes were characterized as LTCC blockers: compounds **24** and **25** with different forms of the benzenesulfonamide scaffold; 3-phenyl-4-isoxazolecarboxylate (**26**); and 4-phenyl-thiomorpholine-3,5-dione (**27**) (Fig. (**13**)). The



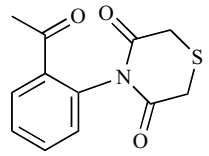
M8 (**24**)



P1 (**25**)



M2 (**26**)



M7 (**27**)

Fig. (13). Chemical structures of three novel chemotypes: benzenesulfonamides M8 (**24**) and P1 (**25**), isoxazolecarboxylate derivative M2 (**26**), and the thiomorpholine derivative M7 (**27**).

compound named M8, which is [(4-Chlorophenyl)sulfonyl]-2-(2-thienyl)pyrrolidine (**24**), is considered as one of the most interesting due to its biological profile (vasorelaxant properties and negative inotropic effect). Another interesting sulphonamide P1, 4-chloro-N-cyclopropyl-N-(piperidin-4-yl)benzenesulfonamide (**25**), is the only compound with basic nitrogen. This common moiety with **1** was hypothesized to be essential for interaction with LTCC. Compound **25** was also employed as reference compound in tandem with (+)-*cis*-Diltiazem (**1**), in a second virtual screening [48].

4.7. Dimethyl- and Diethylamino Derivatives from Virtual Screening

In 2008, using ligand-based virtual screening [48], other new chemotypes (Fig. (**14**)) were found to block LTCC by inhibiting the (+)-*cis*-diltiazem (**1**) binding. In the last stage of the procedure, similarity between **25** and **1** allowed to identify four compounds **28**-**31** (Fig. (**14**)) with moderate activity that competite with [^{3}H]diltiazem for the BTZ site. These dialkylaminoethyl derivatives with highly selective negative inotropic effect share some chemical features: a basic nitrogen atom, 2- or 3-methylene groups linker, and a large aromatic moiety such as quinoline, naphthamide or 2*H*-chromen-2-one.

4.8. Propranolol

In 1988 Weishaar *et al.*, with the aim of understanding the relationships between β-receptor antagonists and calcium channel blockers, explored how propranolol affects isolated cardiac and vascular muscles treated with calcium antagonists and found an interesting evidence for the interaction between propranolol and calcium channel blockers [49]. The pre-treatment with propranolol significantly affects the *in vitro* activity of the LTCC DHP blocker nifedipine, but not that of BTZ blocker (+)-*cis*-diltiazem. *L*-propranolol, *L*-betaxolol, timolol and carteolol were shown to inhibit binding of [^{3}H]diltiazem and [^{3}H]nitrendipine in rat cortical membranes [50]. More recently, Carosati *et al.* [48] have shown that (*R*)-(+)-propranolol (**32**) (Fig. (**15**)), which is reportedly 60-100 times less active β-blocker than the (*S*)-(-)

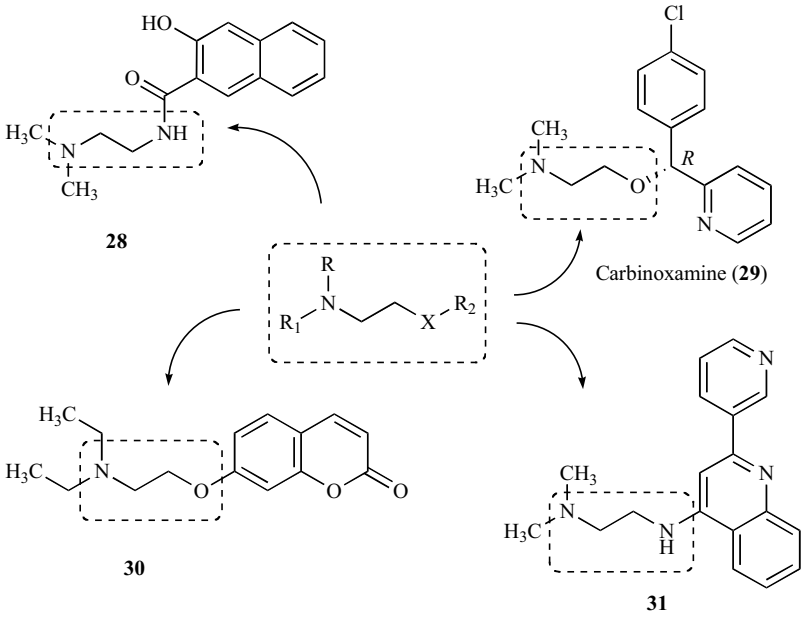
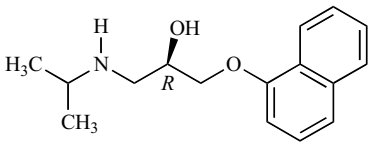


Fig. (14). Chemical structures of R415227 (**28**), C8278 (Carbinoxamine) (**29**), R735884 (**30**) and R407348 (**31**).

enantiomer [51], exhibits intrinsic activity in isolated guinea pig left atria, namely negative inotropic activity. Moreover, (*R*)-(+)-propranolol displaces [^{3}H]diltiazem from its binding site in rat cardiomyocytes [48].

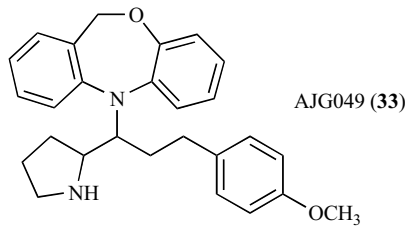


(*R*)-(+)-propranolol (**32**)

Fig. (15). Chemical structures of (*R*)-(+)-propranolol (**32**).

4.9. AJG049

Recently, a new chemotype was identified as an LTCC blocker acting at the (+)-*cis*-diltiazem binding site [52]. The dibenzooxazepine derivative AJG049 (**33**) (Fig. (**16**)) was reported as a potent inhibitor of LTCCs in guinea pig ileal, colonic and vascular smooth muscle cells, with a small selectivity for the gut. The selectivity of AJG049 and its different activity on different tissues agrees with the hypothesis that it binds differently to the isoforms of the LTCC α_{1C} subunit, which are expressed from different genes producing various splice variants. [53,54]. The pharmacological profile of **33** makes it useful in the treatment of abnormal motility in patients with irritable bowel syndrome [54].



AJG049 (**33**)

Fig. (16). Chemical structure of AJG049 (**33**).

4.10. Benzylisoquinolines

Benzylisoquinolines such as papaverine (**34**), and bis(benzylisoquinolines) such as tetrandrine (**35**) (Fig. (**17**)) are natural alkaloids previously described to block LTCCs through interaction at the BTZ binding site [55-58].

Papaverine (**34**) is an opiate alkaloid with multiple activities. It acts as a cyclic nucleotide phosphodiesterase inhibitor, as a Ca^{2+} channel blocker *via* specific binding to the BTZ receptor site in the Ca^{2+} channel, and as an α-adrenoceptor antagonist [59-61]. Considering the ability of papaverine to displace [^{3}H]diltiazem from LTCC, its chemical structure can be taken as a model for the synthesis of new papaverine-like compounds with selectivity for the Ca^{2+} antagonist activity.

Tetrandine (**35**), an alkaloid derived from Chinese medicinal herb *Sthephania tetrandra*, is used in traditional Chinese medicine for the treatment of angina and hypertension [62-64]. Tetrandine has a conformationally rigid macrocyclic structure (Fig. (**17**)), which is a unique feature among compounds competing for the BTZ binding site. Despite pharmacological profile of tetrandrine *in vivo* is similar to that of verapamil, binding experiments clearly indicate that tetrandrine interacts with the BTZ binding site [58]. In addition, tetrandrine modulates binding of other blockers at the 1,4-DHP- and PAA-binding sites, as (+)-*cis*-diltiazem does [58]. An explanation for these observations was proposed by Tikhonov and Zhorov [12].

4.11. Trans-Diclofurime

In the end of the 80's, the unusual calcium antagonist *trans*-diclofurime (**36**) (Fig. (**18**)) was claimed to interact with the BTZ binding site. Compound **36** is characterized as

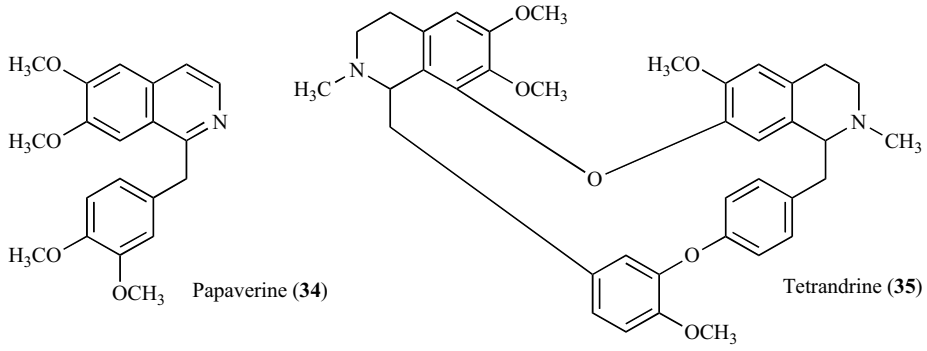


Fig. (17). Chemical structures of papaverine (**34**) and tetrandrine (**35**).

a strong calcium antagonist that displaces [^{3}H]diltiazem binding with greater potency than (+)-*cis*-diltiazem, yet without affecting [^{3}H]nitrendipine binding [65,66].

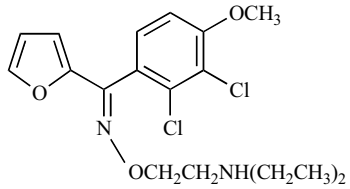


Fig. (18). Chemical structure of *trans*-diclofurime (**36**).

4.12. Verapamil-Like Derivatives

Recently, steric and electronic properties of verapamil-like compounds McN-6186 (**37**) and McN-5691 (**38**) (Fig. (**19**)) were characterized [66]. These unusual LTCC blockers belong to the PAA class of calcium entry blockers, but they competitively inhibit [^{3}H]diltiazem in binding experiments [67,68]. Schleifer and Tot investigated the pharmacophoric requirements common to diltiazem-like derivatives and proposed a model that suggests binding of these unusual calcium antagonists to the (+)-*cis*-diltiazem binding site [69].

4.13. Cyanopyridin-2(1*H*)-Ones Derivatives

In 2000 Manna *et al.* [70] described synthesis and biological activity for a series of new cyanopyridines related to (+)-*cis*-diltiazem and in preliminary pharmacological tests found compounds with calcium channel blocking activity similar or superior to that of **1**. Fig. (**20**) shows the chemical structure of the most interesting compound **39**.

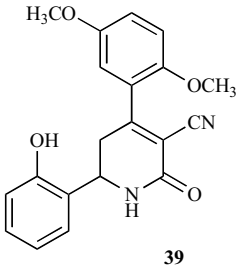


Fig. (20). Chemical structure Cyanopyridin-2(1*H*)-ones derivative **39**.

CONCLUSIONS

Since their discovery in the second half of the last century, the calcium channel blockers have drawn attention of

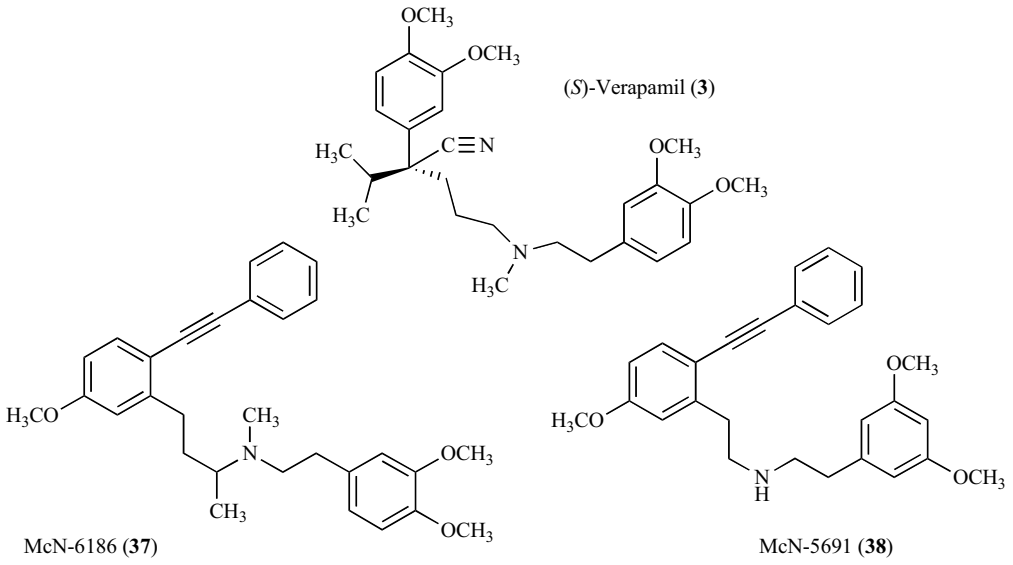


Fig. (19). Chemical structures of (*S*)-verapamil, McN-6186 (**37**) and McN-5691 (**38**).

medicinal chemists due to their potential therapeutic applications. Identification of new more potent and more selective compounds is a challenge. Of the three best characterized classes of ligands, namely DHPs, PAAs, and BTZs, the latter class is the least studied, and the corresponding binding site in the α_1-subunit of LTCC is the least characterized.

Recent progress in the applications of methods of molecular biology and computational chemistry and large contributions provided by medicinal chemists, raise hope for further characterization and better understanding of the BTZ binding site in future. The amino acid sequences and subunit composition of calcium channel subtypes determined by molecular biology provide the basis for the rational design of new ligands of the BTZ binding site. Future screening of virtual libraries of thousands of compounds and modelling the BTZ receptor site is likely to yield new lead compounds.

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ABBREVIATIONS

VGCCs	=	Voltage-Gated Calcium Channels
LTCC	=	L-Type Calcium Channel
BTZ	=	Benzothiazepines
1,4-DHP	=	1,4-Dihydropyridines
PAA	=	Phenylalkylamines
SARs	=	Structure-Activity Relationships
SH	=	Spontaneously Hypertensive
PBR	=	Peripheral-type Benzodiazepine Receptor
3D QSAR	=	3D Quantitative Structure-Activity Relationships
HB	=	Hydrogen Bonding

REFERENCES

[1] Catterall, W.A.; Perez-Reyes, E.; Snutch, T.P.; Striessnig, J. *Pharmacol. Rev.*, **2005**, *57*, 411-25.
[2] Triggle, D.J. *Biochem. Pharmacol.*, **2007**, *74*, 1-9.
[3] Pool, P.E. Diltiazem. In *Cardiovascular Drug Therapy*, 2nd ed., Messerli FH Ed.; Philadelphia: W. B. Saunders, **1996**; pp.931-71.
[4] Buckley, M.M.; Grant, S.M.; Goa, K.L.; Mc Tavish, D. Sorkin, E.M. *Drugs*, **1990**, *39*, 757-806.
[5] Striessnig, J. *Cell Physiol. Biochem.,* **1999**, *9*, 242-69.
[6] Kraus, R.L.; Hering, S.; Grabner, M.; Ostler, D.; Striessnig, J. *J Biol Chem.*, **1998**, *273*, 27205-12.
[7] Dilmac, N.; Hilliard, N.; Hockerman, G.H. *Mol. Pharmacol.,* **2003**, *64*, 491-501.
[8] Glossmann, H.; Striessnig, J. *Rev. Physiol. Biochem. Pharmacol.,* **1990**, *114*, 1-105.
[9] Striessnig, J.; Grabner, M.; Mitterdorfer, J.; Hering, S.; Sinnegger, M.J.; Glossmann, H. *Trends Pharmacol. Sci*., **1998**, *19*, 108-15.
[10] Kraus, R.; Reichl, B.; Kimball, S.D.; Grabner, M.; Murphy, B.J.; Catterall, W.A.; Striessnig, J. *J. Biol Chem.*, **1996**, *271*, 20113-8.
[11] Hering, S.; Aczél S.; Grabner, M.; Döring, F.; Berjukow, S.; Mitterdorfer, J.; Sinnegger, M.J.; Striessnig, J.; Degtiar, V.E.; Wang, Z.; Glossmann, H. *J. Biol. Chem.,* **1996**, *271*, 24471-5.
[12] Tikhonov, D.B.; Zhorov, B.S. *J. Biol. Chem.*, **2008**, *283*, 17594-604.
[13] Floyd, D.M.; Kimball, S.D.; Krapcho, J.; Das, J.; Turk, C.F.; Moquin, R.V.; Lago, M.W.; Duff, K.J.; Lee, V.G.; White, R.E.; Ridgewell, R.E.; Moreland, S.; Brittain, R.J.; Normandin, D.E.; Hedberg, S.A.; Cucinotta, G.G. *J. Med. Chem.*, **1992**, *35*, 756-72.
[14] Kimball, S.D.; Hunt, J.T.; Barrish, J.D.; Das, J.; Floyd, D.M.; Lago, M.W.; Lee, V.G.; Spergel, S.H.; Moreland, S.; Hedberg, S.A.; Gougoutas, J.Z.; Malley, M.F.; Lau, F. *Bioorg. Med. Chem.*, **1993**, *1*, 285-307.
[15] Hering, S.; Savchenko, A.; Strübing, C.; Lakitsch, M.; Striessnig, J. *Mol. Pharmacol.*, **1993**, *43*, 820-26.
[16] DeLano, W.L. The PyMOL Molecular Graphics System. **2008** DeLano Scientific LLC, Palo Alto, CA, USA. http://www.pymol.org
[17] Budriesi, R.; Cosimelli, B.; Ioan, P.; Carosati, E.; Ugenti, M.P.; Spisani, R. *Curr. Med. Chem.*, **2007**, *14*, 279-87.
[18] Innoe, M.; Konda, M.; Hashiyama, T.; Otsuka, H.; Takahashi, K.; Gaino, M.; Date, T.; Aoe, K.; Takeda, M.; Murata, S., Narita, H.; Nagao, T. *J. Med. Chem.*, **1991**, *34*, 675-87.
[19] Hagerty, M.; Panesar, M.; Wadsworth, R.; Kane, K. *Gen. Pharmacol.*, **1995**, *26*, 1349-54.
[20] Piepho, R.W.; Bloedow, D.C.; Lacz, J.P.; Runser, D.J.; Dimmit, D.C.; Browne, R.K. *Am. J. Cardiol.,* **1982**, *49*, 525-8.
[21] Yamamoto, K.; Fujita, M.; Tabashi, K.; Kawashima, Y.; Kato, E.; Oya, M.; Ito, S.; Iwao, J. *J. Med. Chem.*, **1988**, *31*, 919-30.
[22] Fujita, M.; Ito, S.; Ota, A.; Kato, N.; Yamamoto, K.; Kawashima, Y.; Yamauchi, H.; Iwao, J. *J. Med. Chem.*, **1990**, *33*, 1898-05.
[23] Mochacsi, E.; O'Brien, J.; Grove, C. U.S. Patent 4,640,930, 1987.
[24] Kimball, S.D.; Floyd, D.M.; Das, J.; Hunt, J.T.; Krapcho, J.; Rovnyak, G.; Duff, K.J.; Lee, V.G.; Moquin, R.V.; Turk, C.F.; Hedberg, S.A.; Moreland, S.; Brittain, R.J.; McMullen, D.M.; Normandin, D.E.; Cucinotta, G.G. *J. Med. Chem.*, **1992**, 35, 780-93.
[25] Das, J.; Floyd, D.M.; Kimball, S.D.; Duff, K.J.; Vu, T.C.; Moquin, R.V.; Gougoutas, J.Z.; Malley, M.F.; Moreland, S.; Brittain, R.J.; Hedberg, S.A.; Cucinotta, G.G. *J. Med. Chem.,* **1992**, *35*, 773-80.
[26] Gavish, M.; Katz, Y.; Bar-Ami, S.; Weizman, J. *Neurochemistry*, **1992**, *58*, 1589-601.
[27] Veenman, L.; Gavish, M. *Pharmacol. Ther.*, **2006**, *110*, 503-24.
[28] Campiani, G.; Fiorini, I.; De Filippis, M.P.; Ciani, M.S.; Garofalo, A.; Nacci, V.; Giorgi, G.; Sega, A.; Botta, M.; Chiarini, A.; Budriesi, R.; Bruni, G.; Romeo, M. R.; Manzoni, C.; Mennini, T. *J. Med. Chem.,* **1996**, *39*, 2922-38.
[29] Campiani, G.; Garofalo, A.; Fiorini, I.; Nacci, V.; Botta, M.; Tafi, A.; Chiarini, A.; Budriesi, R.; Bruni, G.; Romeo, M.R. *Bioorg. Med. Chem. Lett.,* **1994**, *4*, 1235-40.
[30] Campiani, G.; Garofalo, A.; Fiorini, I.; Botta, M.; Nacci, V.; Tafi, A.; Chiarini, A.; Budriesi, R.; Bruni, G.; Romeo, M. R. *J. Med. Chem.,* **1995**, *38*, 4393-410.
[31] Corelli, F.; Manetti, F.; Tafi, A.; Campiani, G.; Nacci, V.; Botta, M. *J. Med. Chem.*, **1997**, *40*, 125-31.
[32] Budriesi, R.; Cosimelli, B.; Ioan, P.; Lanza, C.Z.; Spinelli, D.; Chiarini, A. *J. Med. Chem.,* **2002**, *45*, 3475-81.
[33] Budriesi, R.; Carosati, E.; Chiarini, A.; Cosimelli, B.; Cruciani, G.; Ioan, P.; Spinelli, D.; Spisani, R. *J. Med. Chem.,* **2005**, *48*, 2445-56.
[34] Striessnig, J.; Meusburger, E.; Grabner, M.; Knaus, H.-G.; Glossmann, H.; Kaiser, J.; Sachölkens, B.; Becker, R.; Linz, W.; Henning, R. *Naunyn-Schmiedeberg' Arch. Pharmacol.*, **1988**, *377*, 331-40.
[35] Morita, T.; Fukuda, T.; Sukamoto, T.; Tajima, S.; Sato, I.; Ikeda, Y.; Kanazawa, T.; Hamada, Y.; Morimoto, Y.; Kitamura, H.; Kawakami, K.; Ito, K.; Nose T. *Arzneim.-Forsch./Drug Res.*, **1982**, *32*, 1060-8. b) Morita, T.; Ito, K.; Nose, T. *Arzneim.-Forsch./Drug Res.*, **1982**, *32*, 1053-9. c) Morita, T.; Kanazawa, T.; Ito, K.; Nose, T. *Arzneim.-Forsch./Drug Res.*, **1982**, *32*, 1047-52. d) Morita, T.; Kanazawa, T.; Ito, K.; Nose, T. *Arzneim.-Forsch./Drug Res.*, **1982**, *32*, 1043-6. e) Morita, T.; Yoshino, K.; Kanazawa, T.; Ito, K.; Nose, T. *Arzneim.-Forsch./Drug Res.*, **1982**, *32*, 1037-42.
[36] Yoshidomi, M.; Sukamoto, T.; Morita, T.; Ito, K.; Nose, T. *Arzneim.-Forsch./Drug Res.*, **1982**, *32*, 1056-59.
[37] Staudinger, R.; Knaus, H.G.; Glossmann, H. *J Biol Chem.*, **1991**, *266*, 10787-95.
[38] Linn, T.; Ferry, D.R.; Glossmann, H. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **1983**, *324*, R45.

[39] Yoshino, K.; Goto, K.; Yoshiizumi, K.; Morita, T.; Tsukamoto, G. *J. Med. Chem.*, **1990**, *33*, 2192-6 and references therein.
[40] Rossier, J.R.; Cox, J.A.; Niesor, E.J.; Bentzen, C.L. *J. Biol. Chem.*, **1989**, *264*, 16598-607.
[41] Ishii, T.; Okamoto, M.; Kume, T.; Norita, H.; Kudo, Y.; Matsuoka, Y. *Jpn. J. Pharmacol.*, **1996**, *71*, 175P (abstr.).
[42] Kobayashi, T.; Strobeck, M.; Schwartz, A.; Mori, Y. *Eur. J. Pharmacol*., **1997**, *332*, 313-20.
[43] Okuyama, K.; Ciuchi, S.; Okamoto, M.; Marita, H.; Kudo, Y. *Eur. J. Pharmacol.*, **2000**, *398*, 209-16.
[44] Barrish, J.C.; Spergel, S.H.; Moreland, S.; Grover, G.; Hedberg, S.A.; Pudzianowski, A.T.; Gougoutas, J.Z.; Malley, M.F. *Bioorg. Med. Chem. Lett.*, **1993**, *1*, 309-25.
[45] Mehanna, A.S.; Kim, J.Y. U.S. Patent 6,541,479 B1, 2003.
[46] Mehanna, A.S.; Kim, J.Y. *Bioorg. Med. Chem.*, **2005**, *13*, 4323-31.
[47] Carosati, E.; Cruciani, G.; Chiarini, A.; Budriesi, R.; Ioan, P.; Spisani, R.; Spinelli, D.; Cosimelli, B.; Fusi, F.; Frosini, M.; Matucci, R.; Gasparrini, F.; Ciogli, A.; Stephens, P.J.; Devlin, F. J. *J. Med. Chem.*, **2006**, *49*, 5206-16.
[48] Carosati, E.; Budriesi, R.; Ioan, P.; Ugenti, M.P.; Frosini, M.; Fusi, F.; Corda, G.; Cosimelli, B.; Spinelli, D.; Chiarini, A.; Cruciani, G. *J. Med. Chem.*, **2008**, *51*, 5552-65.
[49] Weishaar, R.E.; Quade, M.M.; Pugsley, T.A.; Shih, Y.H.; Thaylor, D.G. Jr. *J. Mol. Cell. Cardiol.*, **1988**, *20*, 897-903.
[50] Melena, J.; Wood, J.P.M.; Osborne, N.N. *Eur. J. Pharmacol.*, **1999**, *378*, 317-22.
[51] Kalsner S. The effects of (+)- and (-)-propranolol on 3H-transmitter efflux in guinea-pig atria and the presynaptic beta-adrenoceptor hypothesis. *Br. J. Pharmacol*., **1980**, *70*, 491-8.
[52] Sakata, K.; Tsuji, T.; Tokumasu, M.; Takahashi, K.; Hirasawa, S.; Ezaki, J. European Patent, WO02096891 (A1) 20021205, 2002.
[53] Perez-Reyes, E.; Schneider, T. *Kidney Int.,* **1995**, *48*, 1111-24.
[54] Hashimoto, M.; Teramoto, N.; Zhu, H-L.; Takahashi, K.; Ito, I. *Br. J. Pharmacol.*, **2006**, *149*, 155-62.
[55] Chulia, S.; Ivorra, M.D.; Martinez, S.; Elorriaga, M.; Valiente, M.; Noguera, M.A.; Lugnier, C.; Advenier, C.; D'Ocon, P. *Br. J. Pharmacol*., **1997**, *122*, 409-16.
[56] Rampe, D.; Triggle D.J. *Trends Pharmacol. Sci.,* **1990**, *11*, 112-15.
[57] Felix, J.P.; King, V.F.; Shevell, J.L.; Garcia, M.L.; Kaczorowscki, G.J.; Bick, I.R.C.; Slaughter, R.S. *Biochemistry,* **1992**, *31*, 11793-800.
[58] King, V.F.; Garcia, M.L.; Himmel, D.; Reuben, J.P.; Lam, Y.K.; Pan, J.X.; Han, G.Q.; Kaczorowski, G.J. *J Biol Chem.*, **1988**, *263*, 2238-44.
[59] Lacroix, P.; Linee, P.; Forest, M.C. *Eur. J. Pharmacol.,* **1991**, *192*, 317-27.
[60] Ivorra, M.D.; Lugnier, C.; Schott, C.; Catret, M.; Noguera, M.A.; Anselmi, E.; D'Ocon, M.P. *Br. J. Pharmacol.,* **1992**, *106*, 387-94.
[61] Chuliá, S.; Ivorra, M.D.; Lugnier, C.; Vila, E.; Noguera, M.A.; D'Ocon, P. *Br. J. Pharmacol.,* **1994**, *113*, 1377-85.
[62] Gao, Y.; Chang, M.Y.; Mao, H.Y.; Chao, H.Y.; Chen, D.H. *Chin. J. Intern. Med.,* **1965**, *13*, 504-7.
[63] No authors listed. *Chin. Med. J.,* **1979**, *92*, 193-8.
[64] Fang, D.C.; Jiang, M.X. *Chin. Med. J.,* **1986**, *99*, 638-44.
[65] Spedding, M.; Gittos, M.; Mir, A. K. *J Cardiovasc Pharmacol.*, **1987**, *9*, 461-8.
[66] Mir, A.K.; Spedding, M. *J Cardiovasc Pharmacol.*, **1987**, *9*, 469-77.
[67] Rampe, D.; Skattebøl, A.; Triggle, D.J.; Brown, A.M. *J. Pharmacol. Exp. Ter.,* **1989**, *248*, 164-70.
[68] Flaim, S.F.; Gleason, M.M.; Hedberg, A.; Shank, R.P.; McCulloug, J.R.; Gill, A.; Damiano, B.P.; Brannan, M.D.; Carson, J.R. *J. Pharmacol. Exp. Ther.,* **1991**, *256*, 279-88.
[69] Schleifer, K.J.; Tot, E. *J. Comput. Aided Mol. Des.*, **2000**, *14*, 427-33.
[70] Manna, F.; Chimenti, F.; Bolasco, A.; Bizzarri, B.; Botta, M.; Tafi, A.; Filippelli, A.; Rossi, S. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 1883-5.

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