

Design and synthesis of substituted compounds containing the 1,4-benzodioxin subunit. New potential calcium antagonists

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Abstract – New compounds possessing 1,4-benzodioxin or its saturated analogous heterocyclic system were synthesized and tested for calcium antagonist activity. Biological differences were seen between the different modifications applied. These compounds have been shown to be representative of a novel series of calcium channel antagonists. © 2000 Éditions scientifiques et médicales Elsevier SAS

calcium channel antagonists / 2,3-dihydro-1,4-benzodioxin / 1,4-benzodioxin / calmodulin antagonists

1. Introduction

In the last 10 years extensive research of calcium antagonists has been performed. The development of drugs that inhibit the entry of calcium ions into cells by blocking calcium channels are one of the most important achievements in the therapy of cardiovascular disorders [1]. The efficacy of some compounds as calcium antagonists and calcium entry blockers has been shown with several clinical studies, but adverse effects described led us to develop new anticalcium molecules with potential efficacy and reduced toxic effects.

These calcium antagonist agents are a group of structurally varied compounds acting on several types of calcium channels or as calmodulin inhibitors. As far as L-type voltage-operated channels (VOC) are concerned, preponderantly three distinct classes of selective, potent blockers are recognized [2, 3], which are represented by verapamil (**I**), nifedipine (**II**) and diltiazem (**III**) (*figure 1*). Some of them are also known to possess a calmodulin antagonistic property. This suggests that there is some structural similarity in the drug binding sites of the calcium channel and calmodulin. Moreover, little infor-

mation is available concerning SARs in these calcium antagonist agents. In general, the different results obtained with calcium antagonist agents seem to provide further support for the hypothesis that the pharmacological activity of these structures could be due to multiple different mechanisms of action which are differently affected by structural changes.

In continuing our interest on the development of new anticalcium agents we have prepared a series of compounds containing the 1,4-benzodioxin nucleus and an alkyl chain with a tertiary or secondary amine function. The 1,4-benzodioxin system is considered homologous to the 1,4-benzodioxepin, substructure of the known calcium antagonist HP-406 [4], and bioisostere of the 1,4-benzoxazine [5], both nuclei of interest as anticalcium agents. The selected amines are known substructures of intracellular calcium antagonists possessing high activity. 1,4-Benzoxazines possessing this chain at the 2 position were of particular interest. Moreover, compounds of this type have not been explored as new drugs with potential anticalcium activity that interact with calcium channels and/or calmodulin. This work has allowed us to study, among other things, the influence of

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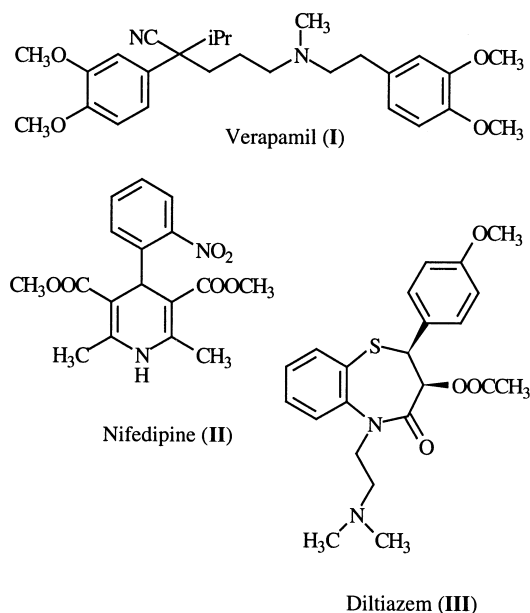


Figure 1. Structures of the most representative calcium channel blockers.

distinct amines and the effect of the 2,3-double bond of the heterocyclic system.

2. Chemistry

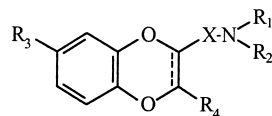
We have recently reported the synthesis and the anti-calcium activity of some 1,4-benzoxazine derivatives [5].

Now, as a continuation of that work, we have synthesized 12 new compounds (**1–12**) containing 2,3-dihydro-1,4-benzodioxin or its unsaturated analogue as a sub-structure (table I).

The synthetic routes used for preparing the compounds of this series were based on the synthesis of the functionalized heterocyclic systems followed by alkylation of the appropriate amine with the corresponding alkylating reagent. The synthesis of intermediates **35**, **36**, **37** and **38** was carried out by the Riom-CERM Laboratory, following their own method, whereas compound **39** was purchased as a commercial product (Aldrich) (figure 2).

Tertiary amines **1**, **2**, **3**, **4**, **5** and **6** were prepared as shown in figure 3, starting from esters **13a**, **13b** and **14** [6–9]. Treatment of **13a** with aqueous methylamine followed by reduction of the intermediate carboxamide **15**, gave the secondary amine **16**. This intermediate was then alkylated, according to standard procedures, with the halo derivatives **37** or **38** to provide the amines **2** and **1** (43 and 53%), respectively. The reduction of esters **13a**, **13b** and **14** gave the key hydroxy derivatives **17–19**, which were transformed into the appropriate alkylating intermediates. In this way, alcohols **17** and **19** were treated with *N*-bromosuccinimide (NBS)/triphenylphosphine (TPP) and provided the respective bromo compounds **21** and **22** in good yield. However, several attempts to obtain the corresponding halo derivative of alcohol **18** failed, probably due to its lack of stability, so we prepared the tosyl derivative **20** [10] as an alternative. Finally, the appropriate secondary amines **35** and **39** were alkylated with the alkyl halides described before, in the

Table I. Compounds containing the 1,4-benzodioxin sub-structure.



| Compound | X | R ₁ | R ₂ | R ₃ | R ₄ | C ₂ –C ₃ |
|-----------|---------------------------------|-----------------|--------------------------------------|------------------|-----------------|--------------------------------|
| 1 | CH ₂ | CH ₃ | C | H | H | sat. |
| 2 | CH ₂ | CH ₃ | B | H | H | sat. |
| 3 | CH ₂ | CH ₃ | A | H | H | unsat. |
| 4 | CH ₂ | CH ₃ | A | H | H | sat. |
| 5 | CH ₂ | CH ₃ | A | OCH ₃ | H | sat. |
| 6 | CH ₂ | D | D | H | H | sat. |
| 7 | CH ₂ | H | A | H | H | unsat. |
| 8 | CH ₂ | H | A | H | H | sat. |
| 9 | CO | CH ₃ | A | H | H | sat. |
| 10 | (CH ₂) ₂ | H | A | H | H | sat. |
| 11 | CH ₂ | CH ₃ | (CH ₂) ₂ –O–A | H | H | sat. |
| 12 | CH ₂ | CH ₃ | A | H | CH ₃ | unsat. |

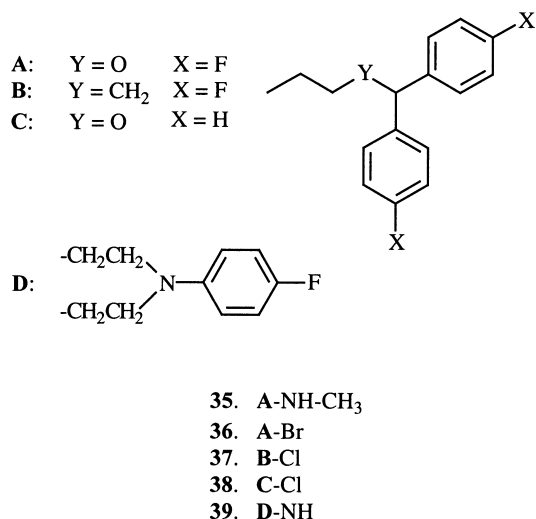


Figure 2. Structure of intermediates 35–39.

presence of a base (K_2CO_3) to give the tertiary amines **3**, **4**, **5** and **6** in variable yields.

Figure 4 depicts the synthetic procedure used to obtain the secondary amines **7** and **8**, which were prepared by alkylation of the corresponding amine intermediates **25** and **26**, respectively, with the halo derivative **36**. Both primary amines were prepared by reduction of cyano derivative **23** [11], but in the case of amine **25**, the previous dehydrogenation of the positions 2–3 was required [12]. The lower yield found for preparing **7** was attributed to the double bond of the heterocyclic system.

The 2-substituted-1,4-benzodioxin derivatives **9**, **10** and **11** were synthesized starting from the ester **13a** according to the synthetic route described in figure 5. Carboxamide **9** was directly obtained by treatment of the ester **13a** with the amine **35**. Compound **10** was prepared by alkylation of the amine intermediate **28** with the bromoalkane **36**. The transformation of the ester **13a** into the corresponding bromo derivative by reduction and treatment with NBS, followed by treatment with NaCN and reduction of the cyano derivative **27**, gave the primary amine **28** in four steps. The synthetic pathway for preparing the aminoether **11** was based on the alkylation of the aminoalcohol **30** following the Williamson reaction. This key intermediate **29** was obtained in moderate yield by treatment of the amine **16** with bromoethanol in basic media.

The 2,3-disubstituted-1,4-benzodioxin derivative **12** was prepared from the ester **13b** as outlined in figure 6. The initial step was the basic hydrolysis of the ester under

standard conditions obtaining the acid **30**. The key intermediate 3-methylsubstituted **31** was obtained by treatment of the carboxylic acid **30** with LDA [13], followed by the addition of iodomethane in tetrahydrofuran. The esterification followed by reduction of **31** gave the alcohol **33** in good yield. Finally, the compound **33** was converted in its respective tosyl derivative **34** to provide the compound **12** by alkylation of the amine **35**. Compounds **3**, **7** and **12**, which have the unsaturated 2–3 positions, are of interest to determine whether there are differences in the biological activities by comparison with the saturated analogues. All compounds prepared were obtained as racemic mixtures. The modest anticalcium activity manifested by these compounds did not prompt us to invest time in the corresponding enantioselective synthesis.

3. Biological results and discussion

The therapeutic activity attributed to calcium antagonism was measured in terms of the inhibition of the contraction induced by modification of the concentration of free calcium. As we previously described, compounds with extracellular activity act on the calcium channel receptors [2, 14–17], whilst those with intracellular action inhibit the effect of calmodulin [18–20]. The compounds synthesized in this study were subjected to three pharmacological tests: caffeine (CAF), phenylephrine (PE) and potassium (K^+). Thus, responses to high concentrations of K^+ are mediated by influx of calcium into the cell, via L-type slow calcium channels. Since part of the concentration to PE is due to the mobilization of calcium from intracellular storage sites, any compound capable of inhibiting PE responses may have an intracellular locus of actions. Table II shows the results obtained in the three tests indicated for compounds **1**–**12**.

In general, the percentages of inhibition of contractions induced by caffeine range from 0–100% for an approximate dose of 30 μ M. The most notable compounds in this test were **3** and **12** with a benzhydryl ether as a substituent, containing, moreover, a tertiary amine in its structure. However, the influence of the substituent of amine is not marked for the response to the caffeine test and the saturated or unsaturated C_2 – C_3 was more important. The unsaturated compound **3** is interesting in the caffeine and K^+ tests.

Compound **6** showed greater activity according to the results of the PE test. This compound has a piperazinyl group as an *N*-substituent, whereas all other compounds have a substituted benzhydryl group.

The influence of the amine substituent (A, B, C and D) is marked for the response to the K^+ test (comparing

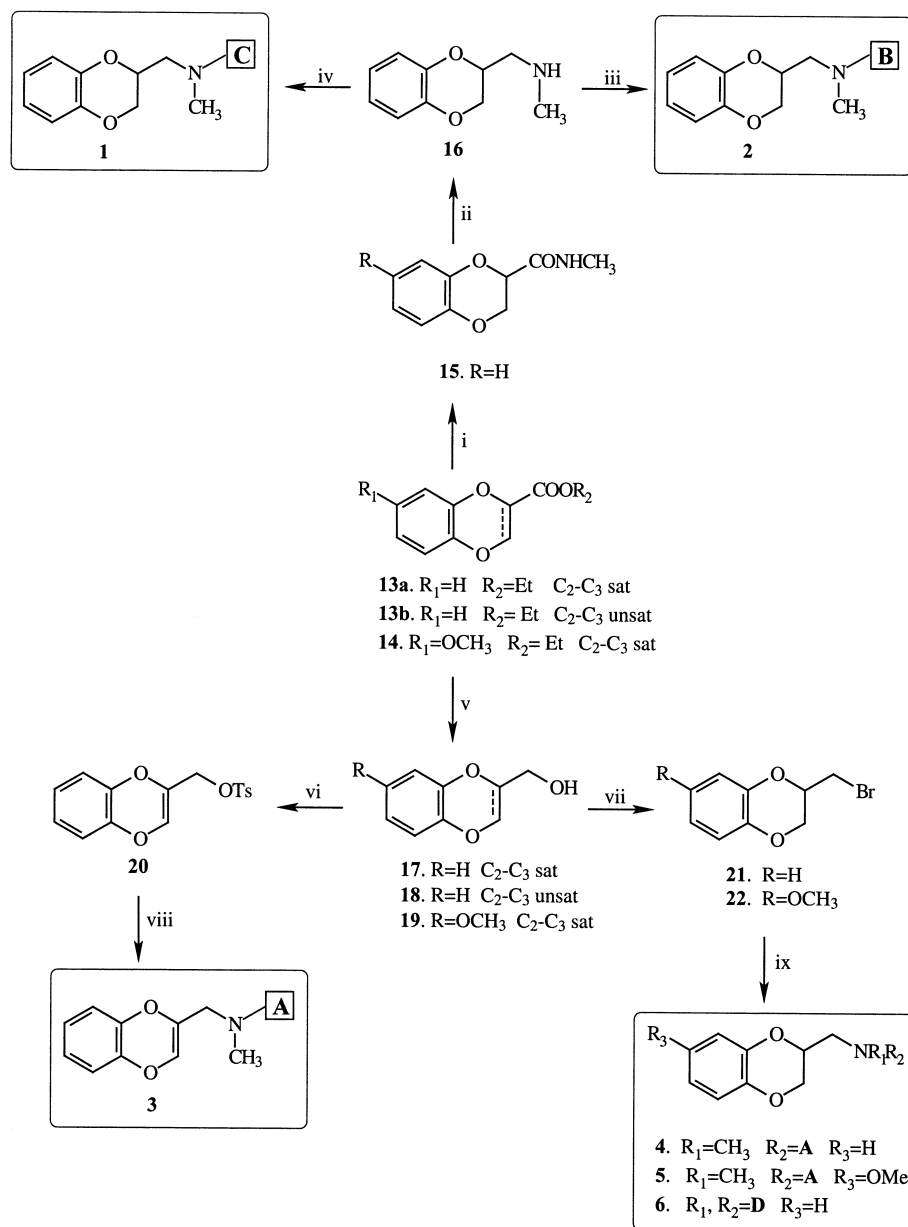


Figure 3. i. CH₃NH₂/toluene; ii. LiAlH₄/THF; iii. B-Cl/TEA/KI/DMF; iv. C-Cl/K₂CO₃/KI/DMF; v. LiAlH₄/THF; vi. TsCl/TEA/CH₂Cl₂; vii. NBS/TPP/DMF; viii. A-NH-(CH₃)/K₂CO₃/KI/DMF; ix. D-NH or A-NH-(CH₃)/K₂CO₃/KI/DMF (A, B, C, and D have been indicated in figure 2).

compounds **1**, **2** and **4** with **6**). It is important to point out that there is an inversion of activities in the tests of K⁺ and PE depending on the amine considered. While benzhydryl derivatives showed a slightly higher response for the contractions caused by a high concentration of K⁺ (compounds **1**, **2** and **4**), comparing with those induced

by PE, compound **6** with a 1-(4-fluorophenyl)piperazine group showed the opposite. As expected from the results, the amine radical indicated by **A** is the best substituent in these series of compounds for the inhibition of contractions induced by a high concentration of K⁺ (compound **4**, IC₅₀(K⁺) = 0.28 ± 0.09 μM).

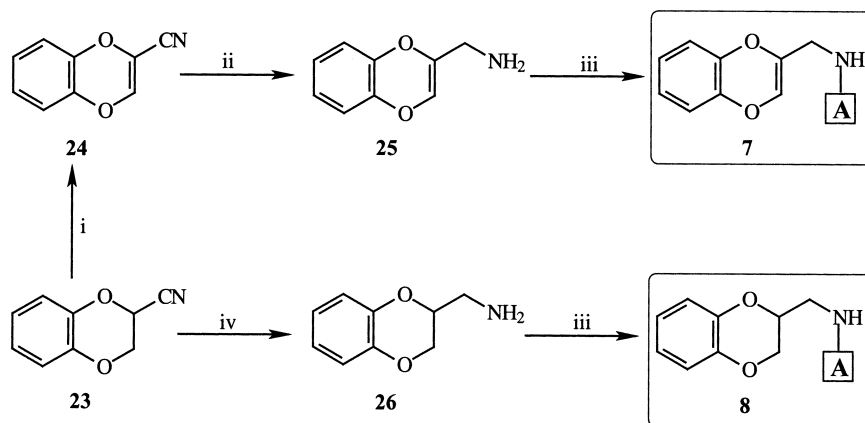


Figure 4. i. 1.NBS/ CCl_4 ; 2. NaI/acetone; ii. LiAlH_4 /THF; iii. $\text{A-Br}/\text{K}_2\text{CO}_3/\text{KI}/\text{DMF}$; iv. $\text{H}_2/\text{Pd-C}$ (**A** has been indicated in figure 2).

By comparison of the secondary (compounds **7**, **8** and **10**) and the tertiary amines (compounds **1**, **2**, **3**, **4**, **5** and **6**), the tertiary amine is preferable to the secondary amine in any of the tests studied.

The influence of the methoxy group at C_7 decreased the activity according to the results of the PE test and the K^+ test (comparing compound **5** with compound **4**).

The distance between the heterocyclic nucleus and the amine of the side chain (compound **10** compared with compound **8**) reveals an activity of the same order. Passing from $\text{X} = \text{CH}_2$ to $\text{X} = \text{CO}$ has great influence in the anticalcium activity. In general the activity decreased (comparing compound **9** with compound **4**).

4. Conclusions

Although an exact relationship between structure and calcium antagonistic activity was not seen, in general, the kind of substituent on the amino group and the saturation degree of $\text{C}_2\text{--C}_3$ have significant influence. While the 1-(4-fluorophenyl)piperazine shows a higher response for the contractions caused by PE, in this series (compound **6** $\text{IC}_{50} = 5.9 \mu\text{M}$), the benzhydryl group entity confers the ability to inhibit contractions induced by K^+ (compound **2** $\text{IC}_{50} = 1.5 \mu\text{M}$).

5. Experimental

5.1. Chemistry

All melting points were determined in capillary tubes on a Gallenkamp apparatus and are uncorrected. NMR

spectra were recorded either on a Varian Gemini-200 MHz and/or Varian XL-300 MHz spectrometer. Chemical shifts are reported as δ values in parts per million downfield from tetramethylsilane as the internal standard in CDCl_3 . The following abbreviations are used to denote signal patterns: s: singlet; d: doublet; dd: double doublet; t: triplet; q: quadruplet; ba: broad absorption; m: multiplet; cs: complex signal. IR spectra were recorded in an FTIR Perkin Elmer 1600 spectrometer. Reported analytical data are within $\pm 0.4\%$ of the theoretical values. Merck 60 (40–60 microns) and Merck 60 F_{254} silica gel were used for column chromatography and thin layer chromatography, respectively. The organic extracts were dried over Na_2SO_4 . All air and moisture sensitive reactions were carried out in an atmosphere of inert gas (argon). Yields were not optimized. All reagents were of commercial quality or were purified before use. Organic solvents were purified by standard procedures.

5.1.1. 2-(*N*-Methylcarbamoyl)-2,3-dihydro-1,4-benzodioxin **15**

Compound **13a** (10 g; 48.07 mmol) and 40% methylamine (23 mL; 721 mmol) were dissolved in toluene (20 mL). The resulting solution was stirred at 35°C for 40 h. After removing the solvent and methylamine, the suspension obtained was extracted with CH_2Cl_2 , dried, filtered and concentrated under vacuum. The residue was purified by recrystallization from hexane/AcOEt to give 7 g (75%) of **15** as white crystals. M.p.: $112\text{--}113^\circ\text{C}$. $^1\text{H-NMR}$ (CDCl_3 , 200 MHz) δ (ppm): 2.87 and 2.90 (s, 3H, CH_3N); 4.17 (dd, $J_1 = 11 \text{ Hz}$, $J_2 = 7 \text{ Hz}$, 1H, C_3H); 4.53 (dd, $J_1 = 11 \text{ Hz}$, $J_2 = 3 \text{ Hz}$, 1H, C_3H); 4.68 (dd, $J_1 = 7 \text{ Hz}$, $J_2 = 3 \text{ Hz}$, 1H, C_2H); 6.65 (ba, 1H, NH); 6.91 (m,

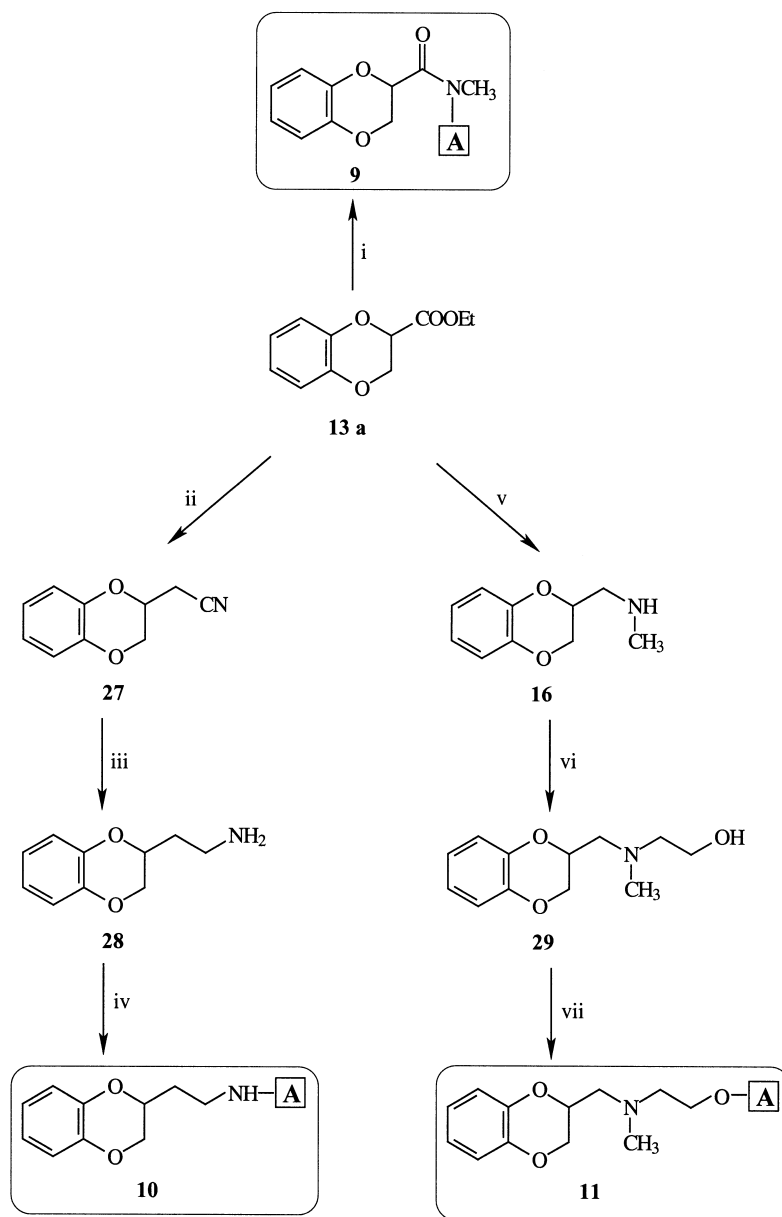


Figure 5. i. $\text{A-NH-(CH}_3\text{)}/\text{K}_2\text{CO}_3/\text{DCC}/\text{toluene}$; ii. $\text{LiAlH}_4/\text{THF}$, 2. $\text{NBS}/\text{TPP}/\text{DMF}$, 3. NaCN/DMSO ; iii. $\text{H}_2/\text{Pd-C}$; iv. $\text{A-Br}/\text{K}_2\text{CO}_3/\text{KI}/\text{DMF}$; v. 1. $\text{CH}_3\text{NH}_2/\text{toluene}$, 2. $\text{LiAlH}_4/\text{THF}$; vi. $\text{BrCH}_2\text{CH}_2\text{OH}/\text{K}_2\text{CO}_3/\text{DMF}$; vii. $\text{A-Br}/\text{NaH}/\text{THF}$ (**A** has been indicated in figure 2).

4H , C_5H , C_6H , C_7H , C_8H). $^{13}\text{C-NMR}$ (CDCl_3 , 50.4 MHz) δ (ppm): 25.7 (CH_3 , CH_3N); 65.3 (CH_2 , C_3); 73.3 (CH , C_2); 117.1 and 117.8 (CH , C_5 , C_8); 122.0 and 122.5 (CH , C_6 , C_7); 141.7 and 143.4 (C , C_{4a} , C_{8a}); 167.9 (C , CONH).

5.1.2. 2-(N-Methylaminomethyl)-2,3-dihydro-1,4-benzodioxin **16**

A solution of **15** (4 g; 20.7 mmol) in anhydrous THF (20 mL) was slowly added to a suspension of LiAlH_4

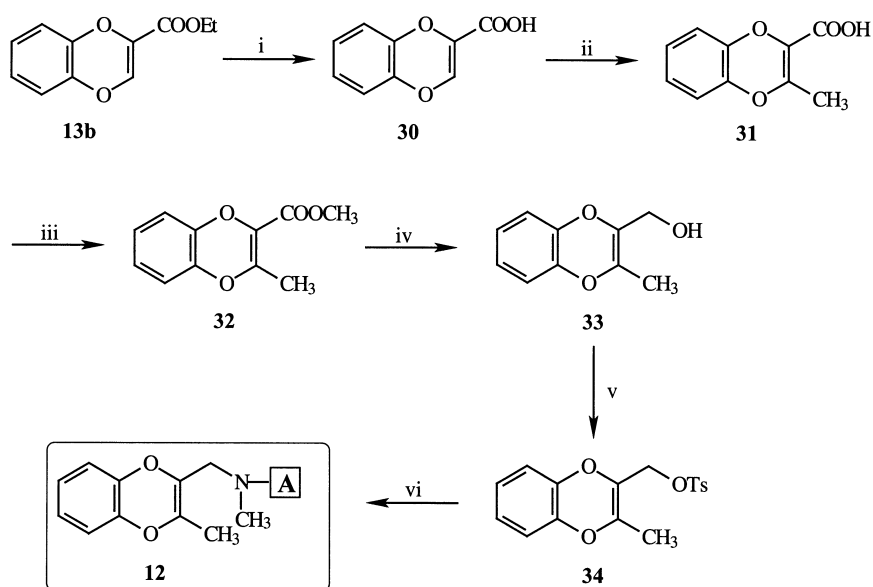


Figure 6. i. 1. NaOH 8%, 2. HCl 37%; ii. 1. LDA/THF, 2. CH₃I/THF; iii. NaHCO₃/CH₃I/DMA; iv. LiAlH₄/THF; v. TsCl/TEA/CH₂Cl₂; vi. A–NH–(CH₃)/K₂CO₃/KI/DMF (A has been indicated in figure 2).

Table II. Pharmacological activity of compounds 1–12.

| Compound | IC ₅₀ ^a (CAF) (μM) (rabbit renal artery) | IC ₅₀ (PE) (μM) (rabbit aortic tissue) | IC ₅₀ (K ⁺) (μM) (rabbit aortic tissue) |
|-------------|---|--|---|
| 1 | >30 (0 %) | >10 (39 %) | 2.2 ± 0.3 |
| 2 | >30 (1 %) | >10 (38 %) | 1.5 ± 0.1 |
| 3 | >30 (59 %) | – | 7.9 |
| 4 | >30 (0 %) | >10 (37 %) | 0.28 ± 0.09 |
| 5 | >30 (10 %) | – | 11.2 |
| 6 | >30 (0 %) | 5.9 | 152 |
| 7 | >30 (7 %) | – | >100 (7 %) |
| 8 | >30 (14 %) | >10 (3 %) | >100 (2 %) |
| 9 | >30 (0 %) | – | >100 (59 %) |
| 10 | >30 (0 %) | – | >100 (38 %) |
| 11 | >30 (7 %) | – | >100 (33 %) |
| 12 | >30 (60 %) | – | – |
| Bepridil | 36.8 ± 3.5 | 18.5 ± 15 | 1.52 ± 0.36 |
| Diltiazem | <100 (10 %) | 64 | 0.64 |
| Fendiline | 6.7 ± 0.6 | 4.2 | 1.6 |
| Flunarizine | >30 (5 %) | 18 | 1.5 |
| Nifedipine | >10 (4 %) | >10 (0 %) | 0.0086 |
| Prazosin | >30 (23 %) | 0.0023 | >10 (0 %) |
| Rianodine | 0.039 ± 0.001 | >10 (0 %) | >10 (0 %) |
| Verapamil | >100 (32 %) | 4.4 ± 0.61 | 0.24 ± 0.06 |

^a IC₅₀ is defined as the concentration (10^{–6} M) of the tested compounds that inhibited 50% of contraction induced by caffeine, phenylephrine or K⁺. Each result was obtained from five to nine preparations.

(2.4 g; 62.1 mmol) in anhydrous THF (50 mL) and stirred at 50 °C for 24 h. Then the reaction mixture was poured into ice-water. The precipitates were separated from the solution by filtration. After removing the THF, the residue was extracted with ether (3 × 40 mL). The organic layers were washed with water, dried, filtered and concentrated in vacuo, obtaining compound **16** as an unstable oil (2.8 g) that was used in the subsequent reaction without purification. ¹H-NMR (CDCl₃, 200 MHz) δ (ppm): 2.52 (s, 3H, CH₃N); 2.87 (m, 2H, CH₂N); 4.02 (dd, *J*₁ = 12 Hz, *J*₂ = 8 Hz, 1H, C₃H); 4.28 (ba, 2H, C₂H, C₃H); 6.86 (m, 4H, C₅H, C₆H, C₇H, C₈H). ¹³C-NMR (CDCl₃, 50.4 MHz) δ (ppm): 36.5 (CH₃, CH₃N); 51.9 (CH₂, CH₂N); 66.5 (CH₂, C₃); 72.2 (CH, C₂); 117.2 and 117.4 (CH, C₅, C₈); 121.5 and 121.6 (CH, C₆, C₇); 140.9 and 141.1 (C, C_{4a}, C_{8a}).

5.1.3. 2-Aminomethyl-1,4-benzodioxin **25**

The cyano derivative **24** (2 g; 12.6 mmol) was slowly added, under an argon atmosphere, to a suspension of LiAlH₄ (2.4 g; 63 mmol) in anhydrous THF (50 mL) and the mixture was stirred at room temperature for 12 h. After hydrolysis of LiAlH₄ with small amounts of water, the suspension was filtered and the THF removed. The residue was diluted with water and extracted with ether (3 × 30 mL). The organic layers were dried, filtered and concentrated to give a dark, unstable oil whose instability did not allow us to obtain the pure amine.

5.1.4. 2-Aminomethyl-2,3-dihydro-1,4-benzodioxin **26**

Platinum oxide (IV) (124 mg; 0.54 mmol) and HCl (37%) (2 mL) were added to a solution of **23** (4 g; 24.8 mmol) in dry methanol (100 mL). The resulting suspension was carried out to hydrogenation (atmospheric pressure and room temperature; 36 h). After separation of the catalyst by filtration, the solvent was removed. The resulting solid was dissolved in water and Na₂CO₃ was added until basic pH. The aqueous layer was extracted with CH₂Cl₂, dried, filtered and concentrated under reduced pressure. The residue obtained was purified by silica gel column chromatography eluting with CH₂Cl₂/MeOH (90:10) to give 3.4 g (83%) of **26** as a colourless oil. ¹H-NMR (CDCl₃, 200 MHz) δ (ppm): 2.33 (ba, 2H, NH₂); 2.96 (d, *J* = 6 Hz, 2H, CH₂N); 4.11 (m, 3H, C₂H, C₃H); 6.85 (m, 4H, C₅H, C₆H, C₇H, C₈H).

5.1.5. 2-Cyanomethyl-2,3-dihydro-1,4-benzodioxin **27**

Bromo derivative **21** (3.4 g; 14.8 mmol), obtained from the ester **13a** by reduction with LiAlH₄ in tetrahydrofuran, was dissolved in 10 mL of DMSO and NaCN (725 mg; 14.8 mmol) was slowly added to that solution. After stirring at 65 °C for 3 days, the mixture was poured into ice-water (50 mL) and extracted with ether. The

organic layers were washed several times with water, dried, filtered and concentrated under reduced pressure. The residue obtained was subjected to silica gel column chromatography to give 1.55 g (60%) of a white solid (compound **27**) when hexane/AcOEt (70:30) was used as eluent. M.p.: 67–69 °C. ¹H-NMR (CDCl₃, 200 MHz) δ (ppm): 2.77 (s, 2H, CH₂CN); 4.07 (dd, *J*₁ = 11 Hz, *J*₂ = 6 Hz, 1H, C₃H); 4.31 (dd, *J*₁ = 11 Hz, *J*₂ = 2 Hz, 1H, C₃H); 4.49 (m, 1H, C₂H); 6.89 (m, C₅H, C₆H, C₇H, C₈H).

5.1.6. 2-Aminoethyl-2,3-dihydro-1,4-benzodioxin **28**

A small amount of palladium-carbon (10%) and 37% HCl (1 mL) were added to a solution of compound **27** (860 mg; 4.9 mmol) in 30 mL of methanol and the suspension obtained was carried out to hydrogenation (atmospheric pressure and room temperature). When the consumption of H₂ was finished, the catalyst was filtered and the solvent removed. The solid obtained was dissolved in water and extracted with ether (3 × 20 mL). The aqueous layer was basified with a solution of 1 N NaOH and extracted again with CH₂Cl₂ (3 × 20 mL). The organic layers were dried, filtered and concentrated in vacuo obtaining **28** as a colourless oil with enough purity to be directly employed in the next reaction (800 mg; 92%). ¹H-NMR (CDCl₃, CD₃OD, 200 MHz) δ (ppm): 1.71 (m, 2H, CH₂CH₂N); 2.70 (ba, 2H, NH₂); 2.93 (m, 2H, CH₂N); 3.88 (dd, *J*₁ = 11 Hz, *J*₂ = 8 Hz, 1H, C₃H); 4.17 (m, 2H, C₂H, C₃H); 6.83 (m, 4H, C₅H, C₆H, C₇H, C₈H). ¹³C-NMR (CDCl₃, CD₃OD, 50.4 MHz) δ (ppm): 33.7 (CH₂, CH₂CH₂N); 37.6 (CH₂, CH₂N); 67.6 (CH₂, C₃); 71.0 (CH, C₂); 116.9 and 117.1 (CH, C₅, C₈); 121.2 and 121.4 (CH, C₆, C₇); 143.1 (C, C_{4a}, C_{8a}).

5.1.7. 2-(*N*-Hydroxyethyl-*N*-methylaminomethyl)-2,3-dihydro-1,4-benzodioxin **29**

2-Bromoethanol (140 mg; 1.12 mmol) and anhydrous K₂CO₃ (232 mg; 1.68 mmol) were added to a solution of the methylamine **16** (100 mg; 0.56 mmol) in distilled DMF (15 mL) and the mixture was stirred at 80 °C for 48 h. After removing the solvent, the residue obtained was diluted with water and extracted with ether (3 × 15 mL). The organic layers were dried, filtered and concentrated under vacuum and the crude product was subjected to silica gel column chromatography eluting with hexane/AcOEt (50:50). The corresponding amino-alcohol **29** (45 mg; 50%) was obtained as a yellow oil. ¹H-NMR (CDCl₃, 300 MHz) δ (ppm): 2.38 (s, 3H, CH₃N); 2.70 (m, 4H, CH₂N); 3.00 (ba, 1H, OH); 3.60 (t, *J* = 5 Hz, 2H, CH₂OH); 3.98 (dd, *J*₁ = 11 Hz, *J*₂ = 7 Hz, 1H, C₃H); 4.25 (m, 2H, C₂H, C₃H); 6.86 (s, 4H, C₅H, C₆H, C₇H, C₈H).

5.1.8. (3-Methyl-1,4-benzodioxin-2-yl)carboxylic acid **31**

A solution of **30** (2 g; 11.23 mmol) in 10 mL of anhydrous THF was cooled at -78°C . Then, a solution of LDA (2 M in hexane) (22.5 mL; 44.92 mmol of base) was slowly added, under an argon atmosphere, and the mixture was stirred at -78°C for 2.5 h. After, iodomethane was added (7 mL; 112.3 mmol) and the mixture was stirred until room temperature was reached. Then, a saturated solution of NH_4Cl was added (20 mL) and the suspension obtained was extracted with ether (3×30 mL). The organic layers were dried and filtered. After removing the solvent, a yellow oil (1.5 g; 70%) was obtained but the compound of methylation was not purified because of its high polarity.

5.1.9. 3-Methyl-2-methoxycarbonyl-1,4-benzodioxin **32**

The oil containing the carboxylic acid obtained in the previous reaction was dissolved in 15 mL of dimethylacetamide and NaHCO_3 (3.35 g; 39.3 mmol) and methyl iodide (2.2 mL; 33.7 mmol) were added. The mixture was stirred at room temperature for 36 h, then it was washed with brine and extracted with CH_2Cl_2 (3×25 mL). The organic layers were dried, filtered and concentrated under reduced pressure. The crude product was distilled (20 mm Hg; 60°C) in order to remove the excess of DMA, giving yellow crystals of a high degree of purity (2.3 g; 100%). M.p.: $74\text{--}76^{\circ}\text{C}$. IR (ν , cm^{-1}): 1700, 1650, 1250. $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ (ppm): 2.31 (s, 3H, CH_3C); 3.85 (s, 3H, CH_3O); 6.61 (m, 2H, C_5H , C_8H); 6.77 (m, 2H, C_6H , C_7H). $^{13}\text{C-NMR}$ (CDCl_3 , 50.4 MHz) δ (ppm): 16.2 (CH_3 , CH_3C); 51.8 (CH_3 , CH_3O); 115.8 and 116.1 (CH , C_5 , C_8); 123.9 and 125.0 (CH , C_6H , C_7H); 137.4 (C , C_2); 141.2 and 142.1 (C , C_{4a} , C_{8a}); 148.2 (C , C_3); 168.9 (C , COOMe).

5.1.10. Reduction of esters to alcohols.

General procedure

A solution of the suitable ester (1 mmol) in anhydrous THF (10 mL) was slowly added, under an argon atmosphere, to a suspension of LiAlH_4 (2 mmol) in anhydrous THF (50 mL). The mixture was stirred at room temperature for 30 min. After hydrolysis with small amounts of water, the suspension obtained was filtered and the THF removed. The residue was diluted with water and extracted with ether. The organic layers were dried, filtered and concentrated to give white crystals pure enough to be employed in the next reaction.

5.1.10.1. 2-Hydroxymethyl-2,3-dihydro-1,4-benzodioxin **17**

Compound **17** (98% yield) was obtained from ester **13a** following the general procedure for reduction of esters to alcohols.

5.1.10.2. 2-Hydroxymethyl-1,4-benzodioxin **18**

Compound **18** was obtained from the ester **13b** following the general method for reduction of esters (91% yield).

5.1.10.3. 2-Hydroxymethyl-7-methoxy-2,3-dihydro-1,4-benzodioxin **19**

Compound **19** was synthesized from the ester **14** via the general procedure for reduction of esters (85% yield). $^1\text{H-NMR}$ (CDCl_3 , 200 MHz) δ (ppm): 2.40 (s, 1H, OH); 3.72 (s, 3H, CH_3O); 3.85 (d, $J = 4$ Hz, 2H, CH_2OH); 4.05 (dd, $J_1 = 12$ Hz, $J_2 = 6$ Hz, 1H, C_3H); 4.21 (m, 2H, C_2H , C_3H); 6.45 (m, 2H, C_6H , C_8H); 6.80 (d, $J = 8$ Hz, 1H, C_5H).

5.1.10.4. 2-Hydroxymethyl-3-methyl-1,4-benzodioxin **33**

Alcohol **33** was synthesized from the ester **32** in 84% yield following the general procedure for reduction of esters to alcohols. M.p.: $90\text{--}92^{\circ}\text{C}$. IR (ν , cm^{-1}): 3500–2900, 1710, 1270. $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ (ppm): 1.72 (t, 1H, OH); 1.84 (s, 3H, CH_3C); 4.15 (d, $J = 6$ Hz, 2H, CH_2OH); 6.70 (m, 4H, C_5H , C_6H , C_7H , C_8H). $^{13}\text{C-NMR}$ (CDCl_3 , 50.4 MHz) δ (ppm): 13.7 (CH_3 , CH_3C); 56.6 (CH_2 , CH_2OH); 115.9 (CH , C_5 , C_8); 123.5 (CH , C_6 , C_7); 131.1 and 132.6 (C , C_2 , C_3); 142.0 (C , C_{4a} , C_{8a}).

5.1.11. Bromo derivatives from alcohols.

General procedure

N-bromosuccinimide (2 mmol) and triphenylphosphine (2 mmol) were added to a solution of the appropriate alcohol (1 mmol) in 10 mL of dry DMF. The mixture was stirred at 40°C for 6 h. After removing the excess of DMF, the suspension obtained was poured into water and extracted with ether. The organic layers were dried, filtered and concentrated under reduced pressure. Purification of the crude mixture by silica gel column chromatography, eluting with hexane/AcOEt (70:30) gave the corresponding bromide compound as a colourless oil.

5.1.11.1. 2-Bromomethyl-2,3-dihydro-1,4-benzodioxin **21**

Compound **21** was obtained from the alcohol **17** via the general procedure for bromination of alcohols (96% yield). $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ (ppm): 3.53 (dd, $J_1 = 6$ Hz, $J_2 = 5$ Hz, 2H, CH_2Br); 4.17 (dd, $J_1 = 11$ Hz, $J_2 = 6$ Hz, 1H, C_3H); 4.32 (m, 2H, C_2H , C_3H); 6.87 (m, 4H, C_5H , C_6H , C_7H , C_8H). $^{13}\text{C-NMR}$ (CDCl_3 , 75.5 MHz) δ (ppm): 28.8 (CH_2 , CH_2Br); 65.5 (CH_2 , C_3); 71.9 (CH , C_2); 117.1 and 117.2 (CH , C_5 , C_8); 121.6 and 121.8 (CH , C_6 , C_7); 142.3 and 142.8 (C , C_{4a} , C_{8a}).

5.1.11.2. 2-Bromomethyl-7-methoxy-2,3-dihydro-1,4-benzodioxin **22**

The bromo derivative **22** was prepared from the alcohol **19** following the general procedure of bromination (70% yield). $^1\text{H-NMR}$ (CDCl_3 , 200 MHz) δ (ppm): 3.52 (d, $J = 5$ Hz, 2H, CH_2Br); 3.73 (s, 3H, CH_3O); 4.13 (m, 1H, C_3H); 4.27 (m, 2H, C_2H , C_3H); 6.45 (m, 2H, C_6H , C_8H); 6.79 (d, $J = 9$ Hz, 1H, C_5H).

5.1.12. Tosyl derivatives from alcohols.

General procedure

A solution of the corresponding alcohol (1 mmol) in 5 mL of dry CH_2Cl_2 was cooled at 0°C and tosyl chloride (1.3 mmol) and triethylamine (1.3 mmol) were added. The mixture was stirred at 0°C for 10 min. Next, the reaction continued, keeping the mixture at -20°C for 12 h without stirring. When the room temperature was recovered, the mixture was acidified with a solution of 1 N HCl and extracted with CH_2Cl_2 . The organic layer was dried, filtered and the solvent removed to give a crude product which was used in the next reaction without purification because of its instability.

5.1.12.1. Tosyl derivative of 2-hydroxymethyl-1,4-benzodioxin **20**

Compound **20** was prepared from the alcohol **18** following the Sharpless procedure.

5.1.12.2. Tosyl derivative of 2-hydroxymethyl-3-methyl-1,4-benzodioxin **34**

Tosylate **34** is obtained from hydroxyderivative **33** via the general procedure.

5.1.13. Alkylation of the corresponding amines with the suitable alkylating agent. General procedures

Method A:

Distilled TEA and a catalytic amount of KI were added to a solution of the corresponding amine and the alkylating agent in 15 mL of dry DMF. The mixture was stirred, under argon atmosphere, at 80°C for 48 h. After removing the solvent, the residue was diluted with water and extracted with ether. The organic layers were dried, filtered and concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography, eluting with hexane/AcOEt (70:30) allowed us to obtain the corresponding alkylated compound.

Method B:

The general procedure B was carried out in the same way as described in method A, but using anhydrous K_2CO_3 as a base and keeping the mixture reaction stirring at room temperature under argon atmosphere for 5 days.

5.1.13.1. 2-(*N*-Diphenylmethoxyethyl-*N*-methylaminomethyl)-2,3-dihydro-1,4-benzodioxin **1**

Compound **1** (53% yield) was prepared via the general procedure B, starting from diphenylmethoxyethyl chloride **38** (1.65 g; 6.7 mmol); the amine **16** (600 mg; 3.35 mmol) and K_2CO_3 (1.4 g; 10.05 mmol). Anal. calcd. for $\text{C}_{25}\text{H}_{27}\text{NO}_3$: C, 77.09% H, 6.99% N, 3.59%. Found: C, 77.14% H, 6.97% N, 3.59%. IR (ν , cm^{-1}): 3 100, 2 868, 1 465, 1 269, 1 098. $^1\text{H-NMR}$ (CDCl_3 , 200 MHz) δ (ppm): 2.35 (s, 3H, CH_3N); 2.69 (m, 4H, CH_2NCH_2); 3.55 (t, $J = 5$ Hz, 2H, CH_2O); 3.86 (dd, $J_1 = 11$ Hz, $J_2 = 7$ Hz, 1H, C_3H); 4.18 (m, 2H, C_2H , C_3H); 5.30 (s, 1H, CHAr); 6.84 (m, 4H, C_5H , C_6H , C_7H , C_8H); 7.31 (m, 10H, C_2H , C_3H , C_4H , C_5H , C_6H). $^{13}\text{C-NMR}$ (CDCl_3 , 50.4 MHz) δ (ppm): 43.6 (CH_3 , CH_3N); 57.4 and 57.6 (CH_2 , CH_2N); 66.4 and 67.1 (CH_2 , CH_2O , C_3); 71.3 (CH, C_2); 83.8 (CH, CHAr); 116.9 and 117.3 (CH, C_5 , C_8); 121.1 and 121.3 (CH, C_6 , C_7); 126.8 (CH, C_2 , C_6'); 127.3 (CH, C_4'); 128.3 (CH, C_3' , C_5'); 142.2 (C, C_{4a} , C_{8a}); 143.2 (C, $\text{C}_{1'}$).

5.1.13.2. 2-[*N*-[4,4-Bis(*p*-fluorophenyl)butyl]-*N*-methylaminomethyl]-2,3-dihydro-1,4-benzodioxin **2**

Compound **2** (43% yield) was obtained following the general method A, from the amine **16** (2 g; 11.17 mmol); 4,4-bis(*p*-fluorophenyl)butylchloride **37** (3.4 g; 12.3 mmol) and TEA (4.6 mL; 33.51 mmol). Anal. calcd. for $\text{C}_{26}\text{H}_{27}\text{F}_2\text{NO}_2$: C, 73.74% H, 6.42% N, 3.31%. Found: C, 73.75% H, 6.44% N 3.29%. IR (ν , cm^{-1}): 2 943, 2 803, 1 506, 1 265, 1 158. $^1\text{H-NMR}$ (CDCl_3 , 200 MHz) δ (ppm): 1.42 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2$); 2.00 (q, $J = 8$ Hz, 2H, $\text{CH}_2\text{CH}_2\text{CH}$); 2.31 (s, 3H, CH_3N); 2.50 (t, $J = 7$ Hz, 2H, CH_2N); 2.62 (t, $J = 6$ Hz, 2H, CH_2N); 3.85 (t, $J = 8$ Hz, 1H, CHAr); 3.94 (dd, $J_1 = 12$ Hz, $J_2 = 7$ Hz, 1H, C_3H); 4.25 (m, 2H, C_2H , C_3H); 6.84 (s, 4H, C_5H , C_6H , C_7H , C_8H); 6.95 (m, 4H, C_3H , C_5H); 7.15 (m, 4H, C_2H , C_6H). $^{13}\text{C-NMR}$ (CDCl_3 , 50.4 MHz) δ (ppm): 25.2 (CH_2 , $\text{CH}_2\text{CH}_2\text{CH}_2$); 33.3 (CH_2 , CH_2CH); 42.8 (CH_3 , CH_3N); 49.5 (CH, CHAr); 57.4 and 57.9 (CH_2 , CH_2NCH_2); 66.6 (CH_2 , C_3); 71.1 (CH, C_2); 115.2 and 115.6 (CH, $J = 21$ Hz, $\text{C}_{3''}$, $\text{C}_{5''}$); 117.2 and 117.5 (CH, C_5 , C_8); 121.5 and 121.7 (CH, C_6 , C_7); 129.1 and 129.2 (CH, $J = 8$ Hz, $\text{C}_{2''}$, $\text{C}_{6''}$); 140.5 (C, C_{4a} , C_{8a}); 143.4 (C, $\text{C}_{1''}$); 159.1 and 164.0 (C, $J = 246$ Hz, $\text{C}_{4''}$).

5.1.13.3. 2-[*N*-Bis(*p*-fluorophenyl)methoxyethyl-*N*-methylaminomethyl]-1,4-benzodioxin **3**

The preparation of compound **3** (10% global yield) was carried out by the same procedure as described in method B, taking as starting compounds the impurified tosyl derivative **20** (7.6 mmol theoretically); *N*-methyl-bis(*p*-fluorophenyl)methoxyethylamine **35** (4.12 g; 14.9 mmol)

and K_2CO_3 (2.05 g; 14.88 mmol). M.p.: 100–102 °C. Anal. calcd. for $C_{25}H_{23}F_2NO_3$: C, 70.91% H, 5.47% N, 3.31%. Found: C, 70.87% H, 5.42% N, 3.28%. IR (ν , cm^{-1}): 3 000, 2 863, 1 510, 1 264, 1 153. 1H -NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.40 (s, 3H, CH_3N); 2.78 (m, 4H, CH_2NCH_2); 3.58 (m, 2H, CH_2O); 5.32 (s, 1H, CHAr); 5.49 (s, 1H, C_3H); 6.88 (m, 8H, C_5H , C_6H , C_7H , C_8H , $C_3'H$, $C_5'H$); 7.30 (m, 4H, $C_2'H$, $C_6'H$). ^{13}C -NMR ($CDCl_3$, 50.4 MHz) δ (ppm): 43.3 (CH_3 , CH_3N); 55.9 (CH_2 , CH_2N); 57.7 (CH_2 , CH_2N); 66.0 (CH_2 , CH_2O); 82.7 (CH, CHAr); 92.7 (CH, C_3); 115.0 and 115.4 (CH, $J = 22$ Hz, C_3' , C_5'); 116.7 and 117.7 (CH, C_5 , C_8); 121.6 and 121.8 (CH, C_6 , C_7); 128.2 and 128.4 (CH, $J = 8$ Hz, C_2' , C_6'); 137.9 (C, C_2 , C_1'); 141.0 (C, C_{4a} , C_{8a}); 159.6 and 164.5 (C, $J = 247$ Hz, C_4').

5.1.13.4. 2-[N-Bis(p-fluorophenyl)methoxyethyl-N-methylaminomethyl]-2,3-dihydro-1,4-benzodioxin 4

The preparation of compound **4** (26% yield) was carried out by the procedure of method A, starting from the halo derivative **21** (2.2 g; 9.6 mmol); *N*-methyl-bis(p-fluorophenyl)methoxyethylamine **35** (2.9 g; 10.56 mmol) and TEA (3 g; 28.8 mmol). Anal. calcd. for $C_{25}H_{25}F_2NO_3$: C, 70.57% H, 5.92% N, 3.29%. Found: C, 70.52% H, 5.90% N, 3.32%. IR (ν , cm^{-1}): 2 900, 2 867, 1 494, 1 265, 1 093. 1H -NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.41 (s, 3H, CH_3N); 2.78 (m, 4H, CH_2NCH_2); 3.54 (t, $J = 6$ Hz, 2H, CH_2O); 3.98 (dd, $J_1 = 10$ Hz, $J_2 = 6$ Hz, 1H, C_3H); 4.25 (m, 2H, C_2H , C_3H); 5.31 (m, 1H, CHAr); 6.84 (m, 4H, C_5H , C_6H , C_7H , C_8H); 7.03 (m, 4H, $C_3'H$, $C_5'H$); 7.28 (m, 4H, $C_2'H$, $C_6'H$). ^{13}C -NMR ($CDCl_3$, 50.4 MHz) δ (ppm): 43.2 (CH_3 , CH_3N); 56.9 and 57.3 (CH_2 , CH_2NCH_2); 66.0 and 66.7 (CH_2 , CH_2O , C_3); 70.9 (CH, C_2); 82.0 (CH, CHAr); 114.6 and 115.0 (CH, $J = 21$ Hz, C_3' , C_5'); 116.6 and 116.9 (CH, C_5 , C_8); 120.8 and 121.0 (CH, C_6 , C_7); 127.9 and 128.1 (CH, $J = 8$ Hz, C_2' , C_6'); 137.4 (C, C_1'); 144.0 (C, C_{4a} , C_{8a}); 159.8 and 164.2 (C, $J = 235$ Hz, C_4').

5.1.13.5. 2-[N-Bis(p-fluorophenyl)methoxyethyl-N-methylaminomethyl]-7-methoxy-2,3-dihydro-1,4-benzodioxin 5

Compound **5** (50% yield) was obtained via the general method B, taking as starting compounds the amine **35** (1.07 g; 3.86 mmol); the halo derivative **22** (0.5 g; 1.93 mmol) and K_2CO_3 (801 mg; 5.8 mmol). Anal. calcd. for $C_{26}H_{27}F_2NO_4$: C, 68.56% H, 5.97% N, 3.07%. Found: C, 68.57% H, 5.94% N, 3.05%. IR (ν , cm^{-1}): 2 950, 2 800, 1 450, 1 259, 1 158. 1H -NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.37 (s, 3H, CH_3N); 2.76 (m, 4H, CH_2N); 3.52 (t, $J = 4$ Hz, 2H, CH_2O); 3.71 (s, 3H, CH_3O); 3.90 (m, 2H, C_3H); 4.20 (m, 1H, C_2H); 5.31 (s, 1H, CHAr); 6.45 (m, 2H, C_6H , C_8H); 6.78 (d, $J = 9$ Hz,

1H, C_5H); 6.99 (m, 4H, $C_3'H$, $C_5'H$); 7.26 (m, 4H, $C_2'H$, $C_6'H$). ^{13}C -NMR ($CDCl_3$, 50.4 MHz) δ (ppm): 43.8 (CH_3 , CH_3N); 55.6 (CH_3 , CH_3O); 57.6 (CH_2 , CH_2N); 57.8 (CH_2 , CH_2N); 66.4 (CH_2 , CH_2O); 67.2 (CH_2 , C_3); 71.7 (CH, C_2); 82.6 (CH, CHAr); 102.6 (CH, C_8); 107.1 (CH, C_6); 115.0 and 115.4 (CH, $J = 21$ Hz, C_3' , C_5'); 117.1 (CH, C_5); 128.4 and 128.5 (CH, $J = 8$ Hz, C_2' , C_6'); 137.9 (C, C_7); 138.0 (C, C_1'); 143.0 (C_{4a} , C_{8a}); 159.9 and 164.7 (C, $J = 242$ Hz, C_4').

5.1.13.6. 2-[4-(p-Fluorophenyl)-1-piperazinylmethyl]-2,3-dihydro-1,4-benzodioxin 6

Compound **6** (65% yield) was synthesized via the general procedure B, taking as depart products 4-(p-fluorophenyl)piperazine **39** (3 g; 16.25 mmol); the halo derivative **21** (1.5 g; 6.5 mmol) and K_2CO_3 (2.7 g; 19.5 mmol). M.p. (HCl): 228–230 °C. Anal. calcd. for $C_{19}H_{21}FN_2O_2$: C, 69.49% H, 6.44% N, 8.53%. Found: C, 69.53% H, 6.42% N, 8.54%. IR (ν , cm^{-1}): 2 948, 2 827, 1 500, 1 262. 1H -NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.65 (m, 6H, CH_2NCH_2 , CH_2N); 3.11 (m, 4H, CH_2N -Ar); 4.01 (dd, $J_1 = 11$ Hz, $J_2 = 7$ Hz, 1H, C_3H); 4.33 (m, 2H, C_2H , C_3H); 6.97 (m, 8H, C_5H , C_6H , C_7H , C_8H , $C_2'H$, $C_3'H$, $C_5'H$, $C_6'H$). ^{13}C -NMR ($CDCl_3$, 50.4 MHz) δ (ppm): 50.1 (CH_2 , CH_2N); 53.8 (CH_2 , CH_2N); 58.4 (CH_2 , CH_2N); 66.8 (CH_2 , C_3); 71.1 (CH, C_2); 115.2 and 115.7 (CH, $J = 22$ Hz, C_3'' , C_5''); 117.1 and 117.4 (CH, C_5 , C_8); 117.6 and 117.8 (CH, $J = 7$ Hz, C_2'' , C_6''); 121.4 and 121.5 (CH, C_6 , C_7); 142.5 and 142.8 (C, C_{4a} , C_{8a}); 148.9 (C, C_1''); 154.3 and 159.2 (C, $J = 245$ Hz, C_4'').

5.1.13.7. 2-[N-Bis(p-fluorophenyl)-methoxyethylaminomethyl]-1,4-benzodioxin 7

Compound **7** (4% overall yield) was synthesized via the general method (B), starting from the impure amine **25** (12.6 mmol theoretically); the halo derivative **36** (8.2 g; 25.2 mmol) and K_2CO_3 (5.2 g; 37.8 mmol). Anal. calcd. for $C_{24}H_{21}F_2NO_3$: C, 70.40% H, 5.17% N, 3.42%. Found: C, 70.35% H, 5.16% N, 3.45%. 1H -NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.88 (t, $J = 6$ Hz, 2H, $CH_2NCH_2CH_2$); 3.12 (s, 1H, NH); 3.50 (t, $J = 6$ Hz, 2H, CH_2O); 5.29 (s, 3H, CHAr, $C-CH_2NCH_2$); 5.80 (s, 1H, C_3H); 6.61 (m, 2H, C_5H , C_8H); 6.83 (m, 2H, C_6H , C_7H); 6.92 (m, 4H, $C_3'H$, $C_5'H$); 7.24 (m, 4H, $C_2'H$, $C_6'H$). ^{13}C -NMR ($CDCl_3$, 50.4 MHz) δ (ppm): 53.6 (CH_2 , CH_2N); 53.7 (CH_2 , CH_2N); 67.6 (CH_2 , CH_2O); 82.5 (CH, CHAr); 115.1 and 115.5 (CH, $J = 21$ Hz, C_3' , C_5'); 116.0 and 116.3 (CH, C_5 , C_8); 123.9; 124.0 and 124.6 (C_3 , C_6 , C_7); 128.5 and 128.7 (CH, $J = 8$ Hz, C_2' , C_6'); 135.1 (C, C_2); 137.9 (C, C_1'); 141.1 and 141.2 (C, C_{4a} , C_{8a}); 159.8 and 164.7 (C, $J = 245$ Hz, C_4').

5.1.13.8. 2-[N-Bis(p-fluorophenyl)methoxy-ethylaminomethyl]-2,3-dihydro-1,4-benzodioxin **8**

The preparation of compound **8** (52% yield) was carried out following the general procedure (B), starting from the amine **26** (450 mg; 2.72 mmol); the bromo derivative **36** (1.8 g; 5.44 mmol) and K_2CO_3 (1.1 g; 8 mmol). Anal. calcd. for $C_{24}H_{23}F_2NO_3$: C, 70.06% H, 5.63% N, 3.40%. Found: C, 70.02% H, 5.67% N, 3.37%. IR (ν , cm^{-1}): 3 068, 3 019, 2 866, 1 502, 1 262, 1 100. 1H -NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.87 (m, 4H, CH_2NCH_2); 3.46 (t, $J = 6$ Hz, 2H, CH_2O); 3.91 (dd, $J_1 = 11$ Hz, $J_2 = 7$ Hz, 1H, C_3H); 4.18 (m, 2H, C_3H , C_2H); 5.25 (s, 1H, CHAr); 6.83 (s, 4H, C_5H , C_6H , C_7H , C_8H); 7.00 (m, 4H, C_3H , C_5H); 7.23 (m, 4H, C_2H , C_6H). ^{13}C -NMR (CD_3OD , 50.4 MHz) δ (ppm): 56.7 and 56.9 (CH_2 , CH_2NCH_2); 67.6 and 68.9 (CH_2O , C_3); 73.5 (CH , C_2); 83.7 (CH , CHAr); 116.0 and 116.4 (CH , $J = 22$ Hz, C_3' , C_5'); 118.2 and 118.5 (CH , C_5 , C_8); 122.4 and 122.6 (CH , C_6 , C_7); 129.3 and 130.1 (CH , $J = 8$ Hz, C_2' , C_6'); 139.9 (C , C_1'); 144.1 and 144.8 (C , C_{4a} , C_{8a}); 161.4 and 166.3 (C , $J = 246$ Hz, C_4').

5.1.13.9. 2-[N-Bis(p-fluorophenyl)methoxyethyl-N-methylcarbamoyl]-2,3-dihydro-1,4-benzodioxin **9**

Anhydrous K_2CO_3 (1.1 g; 8.1 mmol) and a catalytic amount of DCC were added to a solution of the amine **35** (1.5 g; 5.4 mmol) and the ester **13a** (1.7 g; 8.1 mmol) in dry toluene (100 mL). The mixture was refluxed for 48 h, then the solvent was removed. The suspension obtained was poured into ice-water and extracted with CH_2Cl_2 (3×25 mL). The organic layers were dried, filtered and concentrated under reduced pressure to give a white solid (hexane/AcOEt) identified as the compound **9** (1.36 g; 58% yield). M.p.: 102–103 °C. Anal. calcd. for $C_{25}H_{23}F_2NO_4$: C, 68.33% H, 5.27% N, 3.19%. Found: C, 68.35% H, 5.24% N, 3.25%. 1H -NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.96 and 3.29 (s, 3H, CH_3N rotamers A and B); 3.61 (cs, 4H, CH_2N , CH_2O); 4.25 (dd, $J_1 = 11$ Hz, $J_2 = 8$ Hz, 1H, C_3H); 4.37 (dd, $J_1 = 11$ Hz, $J_2 = 2$ Hz, 1H, C_3H); 4.85 (dd, $J_1 = 8$ Hz, $J_2 = 2$ Hz, 1H, C_2H); 5.06 (dd, $J_1 = 8$ Hz, $J_2 = 3$ Hz, 1H, C_2H); 5.32 (s, 1H, CHAr); 6.89 (m, 4H, C_5H , C_6H , C_7H , C_8H); 6.96 (m, 4H, C_3H , C_5H); 7.25 (m, 4H, C_2H , C_6H). ^{13}C -NMR ($CDCl_3$, 50.4 MHz) δ (ppm): 33.8 and 36.8 (CH_3 , CH_3N rotamers A and B); 48.3 and 49.3 (CH_2 , CH_2N rotamers A and B); 64.9 and 65.1 (CH_2 , CH_2O rotamers A and B); 65.9 and 66.6 (CH_2 , C_3 rotamers A and B); 70.3 and 70.6 (CH , C_2 rotamers A and B); 82.4 and 82.9 (CH , CHAr rotamers A and B); 115.1 and 115.6 (CH , $J = 22$ Hz, C_3' , C_5' rotamer A); 115.3 and 115.6 (CH , $J = 22$ Hz, C_3' , C_5' rotamer B); 117.3 (CH , C_5 , C_8); 121.8 and 122.2 (CH , C_6 , C_7); 128.4 and 128.5 (CH , $J = 8$ Hz, C_2' , C_6'); 137.2 and 137.6 (C ,

C_1' , rotamers A and B); 142.8 and 143.3 (C , C_{4a} , C_{8a}); 159.8 and 159.9 (C , $J = 247$ Hz, C_4'); 166.6 and 167.4 (C , CONR, rotamers A and B).

5.1.13.10. 2-[N-Bis(p-fluorophenyl)methoxy-ethylaminoethyl]-2,3-dihydro-1,4-benzodioxin **10**

Compound **10** (32% yield) was obtained following the general method B, taking as starting compounds the amine **28** (540 mg; 3.01 mmol); the bromo derivative **36** (2 g; 6.02 mmol) and K_2CO_3 (1.24 g; 9 mmol). Anal. calcd. for $C_{25}H_{25}F_2NO_3$: C, 70.57% H, 5.92% N, 3.29%. Found: C, 70.61% H, 5.96% N, 3.31%. IR (ν , cm^{-1}): 2 928, 2 855, 1 507, 1 236, 1 158. 1H -NMR ($CDCl_3$, 200 MHz) δ (ppm): 1.62 (m, 2H, CH_2CH_2N); 2.69 (m, 4H, CH_2NCH_2); 3.73 (dd, $J_1 = 11$ Hz, $J_2 = 8$ Hz, 1H, C_3H); 3.82 (t, $J = 6$ Hz, 2H, CH_2O); 4.05 (m, 2H, C_2H , C_3H); 5.16 (s, 1H, CHAr); 6.76 (m, 4H, C_5H , C_6H , C_7H , C_8H); 6.86 (m, 4H, C_3H , C_5H); 7.11 (m, 4H, C_2H , C_6H). ^{13}C -NMR ($CDCl_3$, 50.4 MHz) δ (ppm): 24.9 (CH_2 , CH_2CH_2N); 45.3 (CH_2 , CH_2N); 54.3 (CH_2 , CH_2N); 67.7 (CH_2 , CH_2O , C_3); 71.5 (CH , C_2); 82.6 (CH , CHAr); 115.0 and 115.4 (C , $J = 22$ Hz, C_3' , C_5'); 117.0 and 117.2 (CH , C_5 , C_8); 121.2 and 121.3 (CH , C_6 , C_7); 128.4 and 128.5 (CH , $J = 8$ Hz, C_2' , C_6'); 138.2 (C , C_1'); 142.2 (C , C_{4a} , C_{8a}); 159.9 and 164.8 (C , $J = 247$ Hz, C_4').

5.1.13.11. 2-[N-7,7-Bis(p-fluorophenyl)-3,6-dioxahexyl]-N-methylaminomethyl]-2,3-dihydro-1,4-benzodioxin **11**

A solution of aminoalcohol **29** (800 mg; 3.6 mmol) and the halo derivative **36** (3 g; 8.9 mmol) in 20 mL of anhydrous THF were slowly added to a suspension of NaH (215 mg; 4.5 mmol; 50% wealth) and refluxed under argon. When the room temperature was recovered, the mixture was poured into water and extracted with ether (3×20 mL). The organic layers were dried, filtered and the solvent removed under reduced pressure. The aminoether **11** (300 mg; 18%) was separated from the residue obtained by silica gel column chromatography, using hexane/AcOEt (70:30) as an eluent. IR (ν , cm^{-1}): 3 100, 2 866, 1 508, 1 263, 1 232. Anal. calcd. for $C_{27}H_{29}F_2NO_4$: C, 69.07% H, 6.22% N, 2.98%. Found: C, 69.09% H, 6.25% N, 2.94%. 1H -NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.40 (s, CH_3N); 2.72 (m, 4H, CH_2NCH_2); 3.59 (m, 6H, CH_2O); 3.94 (dd, $J_1 = 12$ Hz, $J_2 = 7$ Hz, 1H, C_3H); 4.28 (m, 2H, C_2H , C_3H); 5.32 (s, 1H, CHAr); 6.85 (s, 4H, C_5H , C_6H , C_7H , C_8H); 6.99 (m, 4H, C_3H , C_5H); 7.27 (m, 4H, C_2H , C_6H). ^{13}C -NMR ($CDCl_3$, 50.4 MHz) δ (ppm): 43.8 (CH_3 , CH_3N); 57.4 and 57.8 (CH_2 , CH_2NCH_2); 66.6; 66.8; 68.5 and 70.4 (CH_2 , CH_2O); 71.4 (CH , C_2); 82.5 (CH , CHAr); 115.1 and 115.5 (CH , $J = 22$ Hz, C_3' , C_5'); 117.1 and 117.4 (CH , C_5 , C_8); 121.4 and 121.6 (CH , C_6 , C_7); 128.6 and 128.8 (CH , $J = 8$ Hz,

C_{2'}, C_{6'}); 137.8 (C, C_{1'}); 143.2 and 143.3 (C, C_{4a}, C_{8a}); 159.9 and 164.8 (C, *J* = 247 Hz, C_{4'}).

5.1.13.12. 2-[N-Bis(*p*-fluorophenyl)methoxyethyl-N-methylaminomethyl]-3-methyl-1,4-benzodioxin **12**

Compound **12** (6% global yield) was prepared following the general procedure (B), starting from the unpurified tosyl derivative **34** (3.8 mmol theoretically); the amine **35** (3.15 mmol; 11.4 mmol) and K₂CO₃ (1.5 g; 11.4 mmol). Anal. calcd. for C₂₆H₂₅F₂NO₃: C, 71.38% H, 5.76% N, 3.20%. Found: C, 71.35% H, 5.72% N, 3.24%. IR (ν, cm⁻¹): 2900, 2868, 1502, 1498, 1150. ¹H-NMR (CDCl₃, 200 MHz) δ (ppm): 1.49 (s, 3H, CH₃C); 2.38 (s, 3H, CH₃N); 2.76 (m, 4H, CH₂N₂CH); 3.51 (t, *J* = 7 Hz, 2H, CH₂O); 5.29 (s, 1H, CHAr); 6.80 and 7.01 (cs, 8H, C₅H, C₆H, C₇H, C₈H, C₃H, C₅H); 7.39 (m, 4H, C₂H, C₆H). ¹³C-NMR (CDCl₃, 50.4 MHz) δ (ppm): 24.1 (CH₃, CH₃C); 43.3 (CH₃, CH₃N); 56.8 and 57.8 (CH₂, CH₂NCH₂); 66.1 (CH₂, CH₂O); 82.8 (CH, CHAr); 115.1 and 115.5 (CH, *J* = 20 Hz, C₃, C₅); 116.7 and 117.2 (CH, C₅, C₈); 121.5 and 121.8 (CH, C₆, C₇); 128.6 and 128.4 (CH, *J* = 8 Hz, C₂, C₆); 131.2 and 132.8 (C, C₂, C₃); 137.6 (C, *J* = 3 Hz, C₁); 140.0 and 140.9 (C, C_{4a}, C_{8a}); 160.1 and 165.0 (C, *J* = 247 Hz, C₄).

5.2. Pharmacological methods

5.2.1. Assay method [21]

for phenylephrine or K⁺ induced contractions

5.2.1.1. Test medium

An aortic ring preparation is placed in an organ bath containing a Krebs solution of the following composition: NaCl (118.1 mmol/L); KCl (4.7 mmol/L); MgSO₄·7H₂O (0.6 mmol/L); KH₂PO₄ (1.8 mmol/L); D-glucose (11.1 mmol/L); NaHCO₃ (25.0 mmol/L); CaCl₂·2H₂O (25.0 mmol/L); Ultrapure water (Milli-Q quality) to 1 L. The pH is approximately 7.4 after 30 min gassing with 5% CO₂ in O₂.

5.2.1.2. Aortic ring preparation

A male rabbit (New Zealand white rabbits, weighting 2.2–3.5 kg) is stunned by a blow to the neck or by a pentobarbital sodium injection and exsanguinated. The thorax is opened and the descending aorta dissected free, put into a Petri dish and covered with gassed Krebs solution. The vessel is carefully cleaned of fat and connective tissue and then cut into rings of 3–5 mm width. Each ring is placed onto stainless steel hooks, care being taken to avoid damage to the intimal surface and then mounted in an organ bath, with one hook being attached to the bath and the other to the tension transducer. The organ bath is previously filled with gassed

Krebs solution and maintained at 37 °C. Each ring is set at an optimal pre-load of 10 g (determined from pre-load–response curves and the pre-calibrated pen recorder). The aortic ring is maintained under 10 g applied tension for 60–90 min with adjustments for any spontaneous relaxation. Following washing with fresh Krebs solution the preparation is contracted two or three times by addition of acetylcholine chloride (AChCl, 1 μmol/L). If AChCl produces more than 50% relaxation of the phenylephrine induced contraction the endothelium is considered functionally intact, otherwise the preparation is discarded and replaced.

5.2.1.3. Procedure

The acceptable aortic rings are contracted with either phenylephrine (0.3 μmol/L) or KCl (40 μmol/L) until stable tone is attained. The preparation is washed with fresh Krebs solution, allowed to relax to baseline values and again stimulated with the same agonist. The procedure is repeated until stable control responses are obtained. Following the last relaxation to baseline values the first concentration of test compound (usually 0.1 μmol/L) is added to the preparation and another five contractions are provoked by the previous agonist. Each reduction in contraction is expressed as a percentage of the control response. With an equilibration time of approximately 90 min this procedure is repeated with 1 μmol/L and 10 μmol/L dose levels of the test compound. Each test compound is tested in at least three different tissue preparations.

5.2.1.4. Evaluation of responses

The percentage inhibition of contractions is plotted against the logarithm of concentration of test compound. The concentration of test compound which inhibits contractions to phenylephrine or to high K⁺ by 50% is termed the IC₅₀.

5.2.2. Assay method [22]

for caffeine-induced contractions

The principle of the test is to assess intracellularly mediated vasodilation using caffeine-induced contraction in isolated renal arteries.

5.2.2.1. Procedure

Left renal artery rings, approximately 2.5–3 mm long were obtained from male New Zealand white rabbits and mounted between stainless steel hooks in an organ bath filled with Krebs solution at 37 °C gassed with 95% oxygen/5% CO₂. Each ring is set at an optimal pre-load of 3 g. Tissues are contracted with caffeine (10 mM) and test substances added 20 min before challenge with agonist.

5.2.3. IC_{50} determination

IC_{50} values defined the concentration (μM) of the test compounds that inhibited the 50% of contraction induced by potassium, phenylephrine or caffeine and were obtained using linear regression analysis.

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