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New optimized piperamide analogues with potent in vivo hypotensive properties

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Abstract

We describe herein the structural optimization of new piperamide analogues, designed from two natural prototypes, piperine **1** and piperdardine **2**, obtained from *Piper tuberculatum* Jacq. (Piperaceae). Molecular modeling studies using semiempirical AM1 method were made in order to establish rational modifications to optimize them by molecular simplification. The targeted compounds (**10**) and (**11**) were respectively obtained using benzaldehyde (**12**) and *para*-anisaldehyde (**13**) as starting materials. ^{1}H NMR spectra showed that the target compounds were diastereoselectively obtained as the (*E*)-isomer, the same geometry of the natural prototypes. These new synthetic amides presented significant hypotensive effects in cardiovascular essays using in vivo methodologies. Compound **11** (*N*-[5-(4′-methoxyphenyl)-2(*E*)-pentenoyl]thiomorpholine) showed a potency 10,000 times greater than its prototype **5**, evidencing an optimization of the molecular architecture for this class of hypotensive drug candidates.

Keywords: Piper tuberculatum; Piperamide analogues; SAR; Cardiovascular activity

1. Introduction

In a previous work, we described the isolation of a mixture of amides from *Piper tuberculatum* Jacq. (Piperaceae), i.e. piperine **1** and piperdardine **2** (Fig. 1), which showed, in mixture, an important hypotensive activity in rats (Araújo et al., 1997,1998). Exploring the molecular architecture of these natural bioactive compounds we described, in an attempt to improve their pharmacological properties, the synthesis (Araújo et al., 1999) and pharmacological evaluation (Cunha et al., 1998) of new analogues **3**–**7**, rationally designed by molecular hybridization of both natural prototypes **1** and **2** (Fig. 1). In addition, we recently described the total synthesis of **2** and a new bioactive isoster **8** (Araújo et al., 2001) using natural safrole **9** (*Ocotea pretiosa* and *Piper hispidinervum*) as starting material (Barreiro and Fraga, 1999) (Fig. 1). The evaluation of the cardiovascular properties of the pure natural products **1** and **2** and their synthetic analogues showed that these derivatives presented a significant ability to lower both blood pressure and heart rate in normotensive rats.

In this context, the present work describes with basis on the investigation of the structure–activity relationships related to the series of compounds, i.e. **3**–**7**, previously synthesized and pharmacologically evaluated by our research group, the design, aided by molecular modeling, of new bioactive piperamide analogues **10** and **11**. We also describe herein the

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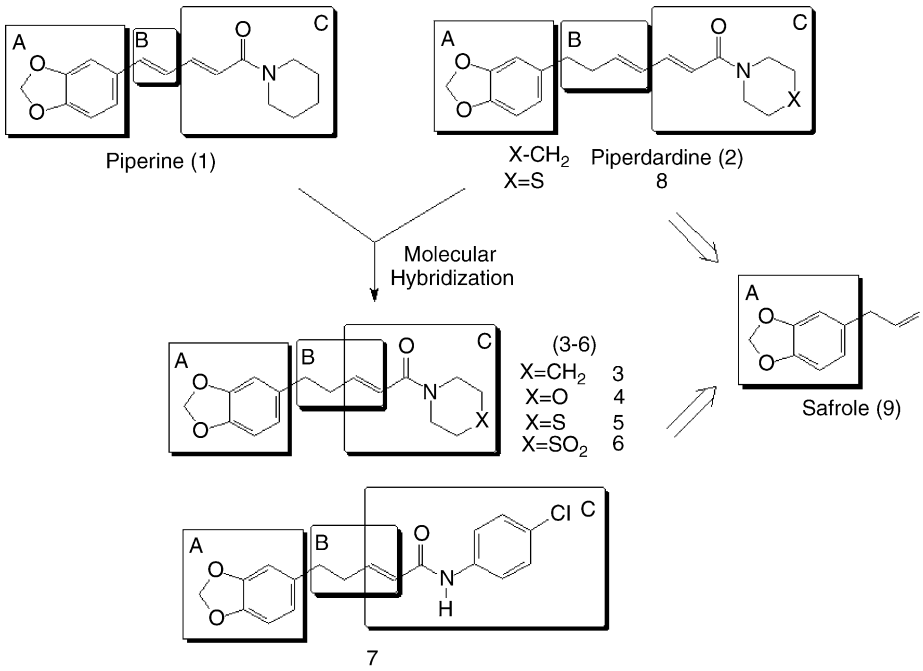

Fig. 1. Design concept of the piperamide analogues **3**–**7**.

synthetic approach to reach the new target compounds and their pharmacological evaluation as modulators of the arterial pressure.

2. Experimental section

2.1. Molecular modeling studies

The protocols used for minimization, conformational search, superimposition, and volume calculations have already been described (Stewart, 1990). Briefly, three-dimensional models for compounds **3**–**7**, **10**, and **11** were constructed using MOLDEN software (Schaftenaar, 1997). Each structure was then submitted to MOPAC 6.0 semi-empirical software (Stewart, 1990) for geometry optimization and conformational search. The optimization was done without any geometrical restriction using the following keywords AM1, PRECISE, EF, HESS = 1, MMOK; in conformational search were used: AM1, STEP = 30, POINT = 12, GNORM = 1.0, MMOK. The obtained minimum energy conformation was geometry optimized under AM1 semi-empirical calculations. These calculations were carried out on a Pentium III 800 MHz under Linux Mandrake 8.0 operational system. The Search_Compare module of Insight II software (Search_Compare 95.0, 1995) was used for superimposition and volume calculations under a *Sillicon Graphics* O_2 R5000 workstation under IRIX 6.3 operational system. The molecules were superimposed using the atoms included in the α,β-unsaturated amides for compounds **3**–**7**, **10**, and **11**. After that, we submitted this superimposition to steric-electrostatic alignment using the similarity function (Search_Compare 95.0, 1995) and subsequently to volume operations.

2.2. Chemistry

Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated F245 Merck plates) and products visualized with iodine or UV-lighting (254 and 365 nm). ^{1}H and ^{13}C NMR spectra were determined in $CDCl_3$ solutions with a Bruker AC-200 spectrometer. Peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard, and J values are given in Hz. IR spectra were obtained with a Nicolet Magna IR 760 spectrometer. The samples were examined either as liquid films or potassium bromide disks. Microanalysis data were obtained with a Perkin-Elmer 240 analyzer, using Perkin-Elmer AD-4 balance. Melting points were determined on Quimis instrument and are uncorrected. Column chromatography purifications were performed using Merck 230–400 mesh silica gel. All products reported showed ^{1}H and ^{13}C NMR spectra in agreement with the assigned structures. Organic solutions were dried over anhydrous sodium sulfate and organic solvents were removed under reduced pressure by rotatory evaporator.

*2.2.1. General procedure for the preparation of compounds **14**, **15**, **20**, and **21***

To a stirred solution of the corresponding aldehyde (1.3 mmol) in pyridine (15 mL) and piperidine (1.5 mL), 0.3 g (2.86 mmol) of malonic acid was added. The resulting mixture was submitted to reflux for 1 h, when TLC analyses indicated the disappearance of the starting material. After

cooling to ambient temperature, the reaction mixture was neutralized with 1N hydrochloric acid under ice bath, observing crystallization of a white solid, which was filtered in a Büchner funnel and washed with cooled water. Recrystallization from aqueous ethanol (1:1) afforded the corresponding α,β-unsaturated acids in 94–95% yield.

*3-Phenyl-2(E)-propenoic acid (**14**)*. White solid, yield 95%, 1.23 mmol; mp 134 °C (lit. 131–136 °C; Heilbron and Bunbury, 1953); ^{1}H NMR ($CDCl_3$) δ: 6.47 (d, 1H, J = 16.1 Hz), 7.2–7.4 (m, 5H), 7.81 (d, 1H, J = 16.1 Hz), 11.02 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 117.4 (C-2); 128.4 (C-2′,6′); 128.9 (C-3′,5′); 130.7 (C-4′); 134.1 (C-1′); 147,1 (C-3); 172.8 (C=O). IR ν_{max} (KBr disk) (cm^{-1}): 3027, 2696, 1686, 1631, 1224, 982, 769, 712.

*3-(4′-Methoxyphenyl)-2(E)-propenoic acid (**15**)*. White solid, yield 94%, 1.22 mmol; mp 173–175 °C (lit. 170–173 °C; Heilbron and Bunbury, 1953); ^{1}H NMR ($CDCl_3$) δ: 3.81 (s, 3H), 6.41 (d, 1H, J = 16.0 Hz), 6.98 (d, 2H, J = 8.5 Hz), 7.58 (d, 1H, J = 16.0 Hz), 7.65 (d, 2H, J = 8.5 Hz), 12.01 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 55.2 ($O\underline{C}H_3$); 114.3 (C-3′,5′); 116.5 (C-2); 126.8 (C-1′); 129.9 (C-2′,6′); 143.7 (C-1′); 160.9 (C-4′); 167.8 (C=O). IR ν_{max} (KBr disk) (cm^{-1}): 3031, 2589, 1689, 1601, 1514, 1259, 1176, 826.

*5-Phenyl-2(E)-pentenoic acid (**20**)*. White solid, yield 94%, 1.22 mmol; mp 105 °C (lit. 104 °C; Cristau and Taillefer, 1998); ^{1}H NMR ($CDCl_3$) δ: 2.42 (q, 2H, J = 7.2 Hz), 2.72 (t, 2H, J = 7.2 Hz), 5.84 (d, 1H, J = 15.3 Hz), 7.06 (d, 1H, J = 15.3 Hz), 7.1–7.3 (m, 5H), 11.03 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 30.8 (C-4); 33.6 (C-5); 121.4 (C-2); 126.3 (C-4′); 128.4 (C-2′,6′); 141.1 (C-1′); 149.9 (C-3); 171.7 (C=O). IR ν_{max} (KBr disk) (cm^{-1}): 3029, 2695, 1684, 1632, 1225, 984, 771, 710.

*5-(4′-Methoxyphenyl)-2(E)-pentenoic acid (**21**)*. White solid, yield 95%, 1.23 mmol; mp 137 °C; ^{1}H NMR ($CDCl_3$) δ: 2.44 (q, 2H, J = 7.3 Hz), 2.75 (t, 2H, J = 7.3 Hz), 3.77 (s, 3H), 6.02 (d, 1H, J = 15.6 Hz), 6.91 (d, 2H, J = 8.7 Hz), 6.98 (d, 1H, J = 8.7 Hz), 7.07 (d, 2H, J = 15.6 Hz), 12.03 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 28.3 (C-4); 33.2 (C-5); 55.1 ($O\underline{C}H_3$); 113.3 (C-3′,5′); 116.5 (C-2); 121.6 (C-1); 129.9 (C-2′,6′); 133.5 (C-1′); 149.9 (C-2); 158.2 (C-4′); 171.2 (C=O). IR ν_{max} (KBr disk) (cm^{-1}): 3030, 2586, 1685, 1603, 1618, 1257, 1174, 825.

2.2.2. General procedure for the preparation of compounds **16** and **17**

To a suspension of lithium aluminum hydride (0.114 g, 3 mmol) in THF (10 mL), under nitrogen atmosphere, a solution of the corresponding acid (1.0 mmol) in THF (10 mL) was gently added, under ice bath. The resulting mixture was stirred until it reached ambient temperature and then it was submitted to reflux for 4 h, when TLC analyses indicated the disappearance of the starting material. After cooling to ambient temperature, 10 mL of methanol and then 10 mL of water were added to the reaction mixture, which was concentrated under reduced pressure. The crude mixture was neutralized with 1N hydrochloric acid and extracted with ethyl acetate (3 × 30 mL). The organic layers were dried under anhydrous sodium sulfate and the solvent was totally removed under reduced pressure. The resulting yellow oil was purified by column chromatography using hexane:ethyl acetate (7:3) as eluent.

*3-Phenyl-1-propanol (**16**)*. Yellow oil, yield 94%, 0.94 mmol; ^{1}H NMR ($CDCl_3$) δ: 1.92 (quintet, 2H, J = 4.7 Hz), 2.73 (t, 2H, J = 4.7 Hz), 3.68 (t, 2H, J = 4.7 Hz), 7.2–7.4 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 32.2 (C-2), 34.3 (C-3), 62.2 (C-1), 126.0 (C-4′), 128.5 (C-3′,5′), 128.6 (C-2′,6′), 141.3 (C-1′). IR ν_{max} (film) (cm^{-1}): 3458, 2929, 1738, 1036, 701.

*3-(4′-Methoxyphenyl)-1-propanol (**17**)*. Yellow oil, yield 96%, 0.96 mmol; ^{1}H NMR ($CDCl_3$) δ: 1.87 (quintet, 2H, J = 3.5 Hz), 2.65 (t, 2H, J = 3.5 Hz), 3.67 (t, 2H, J = 3.5 Hz), 3.80 (s, 3H), 6.84 (d, 2H, J = 8.6 Hz), 7.13 (d, 2H, J = 8.6 Hz). ^{13}C NMR ($CDCl_3$) δ: 31.3 (C-2), 34.6 (C-3), 55.5 ($O\underline{C}H_3$), 62.5 (C-1), 114.0 (C-3′,5′), 129.5 (C-2′,6′), 134.1 (C-1′), 158.0 (C-4′). IR ν_{max} (film) (cm^{-1}): 3364, 2936, 1513, 1037, 832.

2.2.3. General procedure for the preparation of compounds **18** and **19**

To a mixture of pyridinium chlorochromate (PCC) (0.323 g, 1.5 mmol) and dichloromethane (50 mL), 1.0 mmol of the corresponding alcohol was added. The resulting mixture was stirred for 1 h under ambient temperature, when TLC analyses indicated the disappearance of the starting material. The mixture was filtered over silica gel and magnesium sulfate, under vacuum, washing with dichloromethane. The solvent was totally removed under reduced pressure, resulting in a yellow oil, which was purified by column chromatography using hexane:ethyl acetate (8:2) as eluent.

*3-Phenyl-1-propanaldehyde (**18**)*. Yellow oil, yield 93%, 0.93 mmol; ^{1}H NMR ($CDCl_3$) δ: 2.82 (t, 2H, J = 5.9 Hz), 2.99 (t, 2H, J = 5.9 Hz), 7.2–7.4 (m, 5H), 9.8 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 27.5 (C-2), 44.7 (C-3), 126.4 (C-4′), 128.4 (C-3′,5′), 128.7 (C-2′,6′), 139.7 (C-1′), 201.2 (C=O). IR ν_{max} (film) (cm^{-1}): 2928, 1739, 1032, 701.

*3-(4′-Methoxyphenyl)-1-propanaldehyde (**19**)*. Yellow oil, yield 96%, 0.96 mmol; ^{1}H NMR ($CDCl_3$) δ: 2.67 (t, 2H, J = 6.0 Hz), 2.85 (t, 2H, J = 6.0 Hz), 3.73 (s, 3H), 6.77 (d, 2H, J = 10.0 Hz), 7.07 (d, 2H, J = 10.0 Hz), 9.75 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 27.4 (C-3), 45.6 (C-2), 55.4 ($O\underline{C}H_3$), 114.4 (C-3′,5′), 129.3 (C-2′,6′), 132.5 (C-1′), 158.2 (C-4′), 201.9 (C=O). IR ν_{max} (film) (cm^{-1}): 2933, 1513, 1036, 831.

2.2.4. General procedure for the preparation of compounds **10** and **11**

To the corresponding acid (1.0 mmol), thionyl chloride (7.2 mL, 100 mmol) was added, under inert atmosphere. The resulting mixture was refluxed for 1 h. The solvent was completely removed under vacuum and the crude material was dissolved in dichloromethane (8 mL). Then, thiomorpholine (0.289 g, 2.8 mmol) was added, and the resulting reacting mixture was stirred at ambient temperature for 30 min, when TLC analyses indicated the disappearance of the starting material. Water (30 mL) was added and the mixture

was extracted with dichloromethane (3 × 30 mL). The organic layers were dryed under anhydrous sodium sulfate and solvent was totally removed under reduced pressure. The resulting light brownish oil was purified by column chromatography using hexane:ethyl acetate (7:3) as eluent.

*N-[5-Phenyl-2(E)-pentenoyl]thiomorpholine (**10**)*. Light brownish oil, yield 91%, 0.91 mmol; ^{1}H NMR ($CDCl_3$) δ: 2.68–2.85 (m, 4H), 2.99 (t, 2H, J = 7.2 Hz), 3.18 (t, 2H, J = 7.2 Hz), 3.97 (s, 4H), 6.33 (d, 1H, J = 15.2 Hz), 7.05 (dt, 1H, J = 7.2 Hz and 15.2 Hz), 7.36–7.59 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 27.5 (C-3), 27.8 (C-5), 34.3 (C-3′), 34.7 (C-4′), 44.5 (C-2), 48.4 (C-6), 121.1 (C-1′), 126.2 (C-4″), 128.6 (C-3″,5″), 128.7 (C-2″,6″), 141.2 (C-1″), 145.3 (C-2′), 165.9 (C=O). IR ν_{max} (film) (cm^{-1}): 2916, 2859, 1651, 1251, 1190. Anal. Calcd. for $C_{15}H_{19}NOS$: C, 68.93; H, 7.33; N, 5.36; O, 6.12; S, 12.27. Found: C, 68.84; H, 7.26; N, 5.31.

*N-[5-(4′-Methoxyphenyl)-2(E)-pentenoyl]thiomorpholine (**11**)*. Light brownish oil, yield 89%, 0.89 mmol; ^{1}H NMR ($CDCl_3$) δ: 2.50–2.70 (m, 2H), 2.61 (s, 4H), 2.72–2.79 (m, 2H), 3.81 (s, 3H), 3.60–4.0 (m, 4H), 6.16 (d, 1H, J = 15.2 Hz), 6.85 (d, 2H, J = 7.2 Hz), 6.80–7.0 (m, 1H), 7.12 (d, 2H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$) δ: 27.8 (C-3,5), 33.8 (C-3′), 34.6 (C-4′), 44.6 (C-2), 48.6 (C-6), 55.4 (O$\underline{C}$H$_3$), 113.9 (C-3″,5″), 121.0 (C-1′), 129.5 (C-2″,6″), 133.1 (C-1″), 145.5 (C-2′), 158.1 (C-4″), 166.0 (C=O). IR ν_{max} (film) (cm^{-1}): 2919, 2836, 1651, 1248, 1188. Anal. Calcd. for $C_{16}H_{21}NO_2S$: C, 65.95; H, 7.26; N, 4.81; O, 10.98; S, 11.00. Found: C, 66.02; H, 7.31; N, 4.77.

2.3. Pharmacology

2.3.1. Materials

Normotensive male Wistar rats, weighting between 250 and 350 g, from Núcleo de Pesquisas em Produtos Naturais, Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba (João Pessoa, PB, Brazil), were kept under temperature control (21 ± 1 °C) and lighting (06:00 a.m. to 06:00 p.m.), with free access to food and tap water. Experimental protocols and procedures were approved by the Laboratório de Tecnologia Farmacêutica Animal Care and Use Committee. The following substances were used in this protocol:

- (*N*,*N*,*N*′,*N*′)-bis(beta-aminoethylether)ethyleneglycoltetracetic acid (EGTA), sodium nitroprusside (NPS) and cremophor, from Sigma;
- sodium bicarbonate ($NaHCO_3$), magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$), calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$), sodium chloride (NaCl), glucose, sodium dihydrogenophosphate monohydrate ($NaH_2PO_4{\cdot}H_2O$), disodium ethylenedinitrylotetracetate dihydrate (EDTA), all from Merck;
- potassium chloride (KCl), from Vetec (Brazil);
- sodium heparin, from Roche (Brazil);
- halothane, from Cristália (Brazil).

All substances above were dissolved in saline solution and kept under refrigeration. The test compounds **10** and **11** were solubilized in cremophor (final concentration of 5%). For arterial blood pressure registering in vivo, a pressure transducer (Stathan P23 ID, Gould) coupled with a two-channel physiograph (Beckman R-511A) was used. The vehicles (cremophor 5% or saline) given alone were completely devoid of effect on arterial blood pressure in non-anaesthetized rats.

2.3.2. Methods

The arterial blood pressure measurements were made directly (Cerutti et al., 1985) in normotensive male Wistar rats. Two days before the experiment, the animals were briefly anesthetized with halothane (2% in O_2) and polyethylene catheters (a PE-10 strained segment coupled to a PE-50 segment, of 0.58 mm inner diameter and 0.96 mm outer diameter) were inserted into the lower abdominal aorta and inferior cava vein, through the femoral artery and vein, respectively. The reduction of diameter of the PE-10 catheter segment minimized the risk of leg ischemia. After insertion and fixation, the catheters were filled with heparinized saline and emerged from an incision at the posterior cervical region (*scapulae*). The arterial catheter was connected to a pressure transductor (Stathan P23 ID), which was coupled to a two-channel physiograph (Beckman R-511 A), for the arterial blood pressure registering, and the venous catheter was used for the test compounds administration. On the day of experiment, after 30 min of cardiovascular parameters stabilization, sodium nitroprusside (8 μg/kg, i.v.) was administered to verify the effectiveness of the venous catheter implantation. Subsequently, a dose response curve with compounds **10** and **11** in crescent doses (0,087; 0,174; 0,348; 0,841; 1,70 and 3,48 μmol/kg, i.v.) was obtained. During the experiments, the animals were kept in a cage, where they were unrestrained and had free access to food and water. Doses were administered randomly with enough time intervals so that all the hemodynamic parameters returned to their basal values.

3. Results and discussion

Table 1 shows the values of the effective dose to reach 50% of maximum effect (ED_{50}) measured from the pharmacological evaluation of the derivatives **3**–**7** previously described by Cunha et al. (1998).

With these biological results in hands, the next step to construct a probable pseudopharmacophoric model consisted in to evaluate the conformational behavior of these compounds (**3**–**7**) applying the semiempirical AM1 method (Stewart, 1990), followed by superimposition of their minimum energy conformer in order to explain qualitatively the in vivo profile of the inactive amide derivative **7**. It is important to note that the bioactive conformation may not be the prevailing one observed in the solid state, in solution, or in vacuum. The selection of the bioactive conformation between several "non-bioactive" ones can be based on its comparison with

Table 1
Pharmacological results for compounds **3**–**7** and **10**–**11**

Compound	*X*	*n*[a]	ED_{50}[b] (μmol/kg)
3	CH_2	6	1.86 ± 0.33
4	O	6	1.80 ± 0.31
5	S	6	0.92 ± 0.31
6	SO_2	6	1.29 ± 0.26
7	–	6	Inactive[c]
10	H	6	0.69 ± 0.19
11	OCH_3	6	<0.0001[d]

[a] Number of animals used in this experimental protocol.
[b] Values obtained from the dose-response curve of the average arterial pressure control protocol in awake unrestrained rats (Cunha et al., 1998).
[c] No variation in the arterial pressure was obtained until the maximal doses of 1.0 mg/kg (15.2 μmol/kg).
[d] The effect on arterial pressure was not evaluated at doses below 0.025 mg/kg (0.0001 μmol/kg).

other less flexible molecules acting at the same binding site (Gund, 1996). However, since the binding site for molecules **3**–**7** has not yet been identified, we decided to perform an initial procedure in vacuum, by means of semi-empirical methods, as the starting point to study the ligand-receptor complementarities, to be further refined with the identification of the biological target.

Our group has already described the use of volume descriptors to develop pharmacophore models (Verli et al., 2002), suggesting that a volume analysis can be indicative of the binding site shape. Compound **7** presents the amide bond conjugated with the aromatic *para*-chloro-phenyl group, which is in the same plane that double bond. This aromatic region appears to be *sterically unfavorable*, in complete agreement with the absence of hypotensive activity displayed by this compound (Fig. 2). The investigation of molecular volume of derivatives **3**–**7** shows all values at the same order of magnitude (232.4, 225.4, 236.3, 244.7, and 244.5 $Å^3$ for compounds **3**–**7**, respectively). Despite this fact, an additional volume of **7** is located in a region not occupied by compounds **3**–**6**, approximately in the same plane of the amide bond (Fig. 2), which agrees with the conformational considerations described above. Thus, this result shows an additional

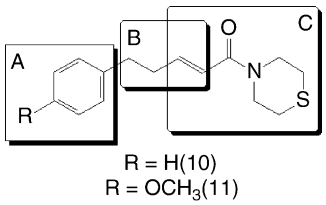

Fig. 3. Structure of the new designed piperamide analogues **10** and **11**.

volume of 10,641 $Å^3$ in a supposed steric-limited region of the active site.

We also identify a hydrophobic pocket at the neighborhood of the steric-limited site enunciated above, justifying the best hypotensive profile for compound **5** in a comparison with **3**, **4** and **6**. The presence of hydrophobic aromatic residues, e.g. tyrosine, could also explain the better activity showed by **6** in comparison with that evidenced for compounds **3** and **4**, since this residue is able to perform both hydrophobic interactions (through its phenyl ring) and hydrogen bond interactions (by its *para*-hydroxyl group).

The structure-activity profile of compounds **3**–**7** enables us to enumerate the contributions of the substituents at the amide bond level (**C**-region; Fig. 1). It would be suitable now to investigate how **A**- and **B**-regions contribute to the improvement of the hypotensive profile, in order to optimize the natural prototypes **1** and **2**. We had already modified the **B**-region in a previous work (Duarte et al., 1999), obtaining an inferior piperine (**2**) vinylogue series, which resulted in much less potent hypotensive compounds. On doing so, we are able to propose new analogues, such as compounds **10** and **11** (Fig. 3), keeping the thiomorpholine group, elected as the best substituent at **C**-region, as well as the C4-spacer between **A** and **C**, and modifying the benzo[1,3]dioxolyl group, in a way to investigate the electronic contributions of this biophore to this series of compounds. Also, conformational analysis of compounds **10** and **11** suggested that the modification of **A**-region does not induce relevant alterations on the shape of the molecules, so the ligand-receptor complementarities in regions **B** and **C** appear to be kept in compounds **10** and **11**.

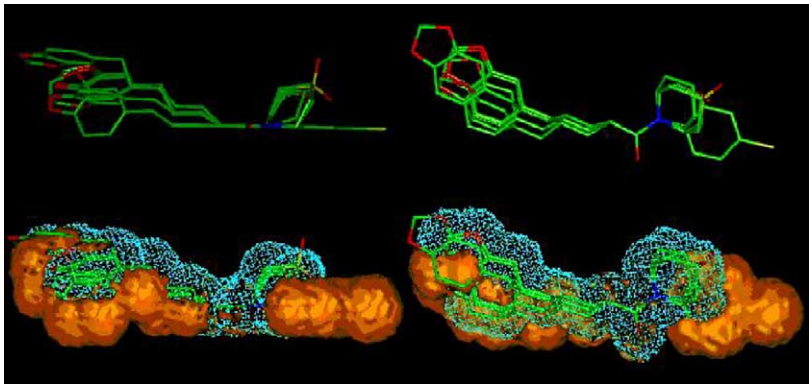

Fig. 2. Superimposition of the compounds **3**–**7**. The intersection volume of active compounds **3**–**6** can be seen in a line-delimited volume, while the exclusion volume of compound **7** can be seen in a solid-orange volume.

Considering the evidences for important contributions of a sterically limited region at the binding site to the activity of compounds **3**–**7**, summarized in Fig. 2, a new step of molecular modifications was initiated concerning the **A**-region (Fig. 3). So compound **10** was designed by complete removal of the 3,4- methylenedioxy group, which could imply in the loss of the ability of making hydrogen bonds, demonstrating how important is the interaction with hydrogen bond donating residues of the target interacting site. In contrast, in compound **11** we kept the *para*-position oxygenated, introducing a *para*-methoxy group. This strategy enables an improvement of the electronic donating effect of *para*-O atom at the phenyl ring, abolishing the electronic withdrawing effect of *meta*-O atom present in the benzo[1,3]dioxolyl system (Fig. 3).

Derivatives **10** and **11** were synthesized exploring useful classic methodologies depicted in Fig. 4. The first step consisted in the base catalyzed condensation of malonic acid with the respective aromatic aldehydes **12** and **13** (Duarte et al., 1999), furnishing the corresponding cinnamic acids **14** and **15**, which were next submitted to complete reduction with lithium aluminum hydride (Bode et al., 1996), giving the corresponding saturated alcohols **16** and **17**. They showed two triplets and one quintet in the ^{1}H NMR spectra, with a coupling constant ($^2J_{H,H}$) of 4 Hz approximately, corresponding to the saturated three-carbon system. The subsequent oxidation of alcohols **16** and **17** to the corresponding aldehydes **18** and **19** was achieved with pyridinium chlorochromate (PCC) as described by Corey and Suggs (1975). The 3-phenylpropionaldehydes **18** and **19** were submitted to the same conditions described for the first step (Duarte et al., 1999), furnishing the desired α,β-unsaturated acids **20** and **21** presenting an ethylene spacer. The analyses of ^{1}H NMR spectra of these derivatives allowed us to characterize them as the corresponding (*E*)-diastereomers due to the presence of a doublet and a double triplet between 6.0 and 7.0 ppm, both with $^2J_{H,H}$ = 15 Hz, a typical AB pattern of the desired configuration of the double bond. Finally, these acids were reacted with thionyl chloride and then the acyl chloride intermediate was *one-pot* reacted with thiomorpholine, as described by our group in the synthesis of piperdardine and its analogues (Araújo et al., 1999,2001), affording amides **10** and **11** in 71% and 65% overall yields, respectively.

The compounds **10** and **11** were submitted to the pharmacological evaluation of their antihypertensive profile in the model of arterial blood pressure measurement in awake unrestrained normotensive rats. Test compounds were administered intravenously in crescent doses and the measurement of the arterial pressure was done as described by Cerutti et al. (1985). The resulting dose-response curve was used to calculate ED_{50} values for each compound (Table 1). Once compound **10** was more active than compound **5** it is reasonable to note that the benzo[1,3]dioxolyl group is not an essential motif to the higher molecular recognition by the supposed bioreceptor under the pharmacological essay performed. Moreover, the introduction of *para*-OCH_3 group increases greatly the showed activity of this series of compounds, indicating that the **A**-region can make important interactions, being probably essential to optimize the hypotensive profile.

It is also relevant to mention that the vasodilator effect of the compounds amide derivatives developed herein, i.e. **3**–**7**, **10**, and **11**, investigated through in vitro methodologies, shows that the probable mechanism of action does not depend on the vascular endothelium, but is directly related to the inhibition of intracellular calcium release from noradrenaline-sensitive stores (Araújo, 2001).

All observations above were summarized in a 2D model (Fig. 5), which shows the probable structural features associated with the hypotensive profile of this new series of α,β-unsaturated amide derivatives.

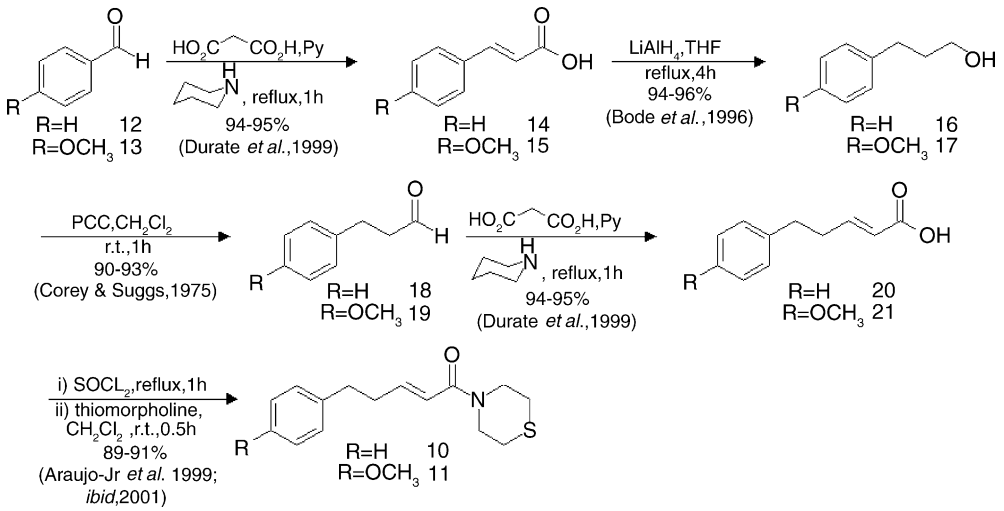

Fig. 4. Synthetic route for the preparation of α,β-unsaturated amides **10** and **11**.

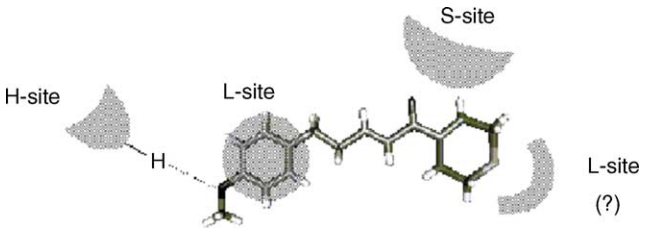

Fig. 5. Two-dimensional binding model for compound **11** (H: hidrogen bond donating; L: lipophilic; and S: steric).

4. Conclusions

We have examined some structural properties of five new amide derivatives (**3**–**7**) with hypotensive activity, using semi-empirical methods and molecular volume calculations, which suggested the presence of a *sterically limited* region in the resulting model (Fig. 1). Based on this consideration, two new compounds (**10** and **11**) emerged possessing a structural modification in the oxygenation pattern of the lead **5**. The results from the pharmacological essays for both new compounds suggest that hydrogen bond with oxygen atoms of benzo[1,3]dioxolyl group is not essential to the bioactivity of this series of compounds. Additionally, substitution at the aromatic ring can be related to other properties rather than hydrogen bonding, e.g. redox potential.

Finally, in spite of the potential toxicophoric character of the Michael accepting group (Ahn and Sok, 1996) present in the amide derivatives developed herein, we were able to identify the powerful hypotensive profile of the compound **11**, a new lead-compound of cardioactive agent.

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