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Research Article

Synthesis of Ranolazine Derivatives Containing the (1*S*,4*S*)-2,5-Diazabicyclo[2.2.1]Heptane Moiety and Their Evaluation as Vasodilating Agents

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Two diazabicyclic analogues of ranolazine, (*S*,*S*,*S*)-5 and (*S*,*S*,*R*)-5, and their epimeric mixture were synthesized. Furthermore, their vasomotor effects on rat aorta rings precontracted with phenylephrine were analyzed. These compounds showed vasodilating effects significantly greater than ranolazine. The vasodilating activities of these analogues have two components, one that depends on the endothelium, due to the release of NO, and another one due to a direct effect on the vascular smooth muscle. The compounds [(*S*,*S*,*S*)(*S*,*S*,*R*)]-5 and (*S*,*S*,*R*)-5 induce, in a manner similar to ranolazine, the release of a prostanoid from the cyclooxygenase pathway, whose vasoconstrictor effect is masked by the predominant vasodilation induced by these compounds.

Key words: chemical structure, chirality relationship to biology, drug discovery, ranolazine

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Ranolazine (Figure 2A) is a compound employed in the treatment for chronic stable angina pectoris. It was approved by the FDA in 2006 and was the first drug authorized over 20 years in USA for this disease (1). The mechanism of action of this drug involves the inhibition of the entrance of Na^+ through the Na^+ slow channels, thus avoiding the intracellular overload of Ca^{2+} and avoiding damage to the myocardial cells, dysfunction, and instability of the electrical activity (2). Recently, Paredes-Carbajal and co-workers (3) reported that this drug has a vasodilating effect on rat aorta rings, which is mostly endothelium independent.

The (1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptane (DBH) system, described for the first time in 1966 (4), is structurally equivalent to a piperazine, but it has a conformationally restricted equilibrium. This system has been incorporated to some compounds of medicinal character, that is, benzothiazoles A and B, with antianginal activity (Figure 1; 5).

In the search for improving the quality of life of patients with cardiovascular diseases, the development of more efficient pharmacological treatments takes a greatly important role due to the need of new and more active cardiovascular drugs with fewer side-effects.

In WO 01/62744 A2, Zablocki *et al.* (6) described the preparation of a wide number of ranolazine analogues, including some bicyclic compounds such as **(*S*,*S*,*S*)-5**, although they do not include any physical or spectroscopic data, nor the pharmacological assessment. Our research group, which has found interesting results in the substitution of the piperazine ring on diverse drugs by the diazabicyclic moiety (7), synthesized and evaluated the pharmacological activity, as antianginal agents, of the epimeric mixture of the ranolazine analogue in which the piperazine ring is replaced by the (1*S*,4*S*)-2,5-diazabicyclo [2.2.1]heptane moiety (Figure 2B), and of both epimers of this compound (Figure 2C).

Experimental Section

Synthesis

General experimental conditions

All starting materials were purchased from Aldrich, while the solvents used were purified by distillation prior to use. Solvent mixtures employed in chromatography were reported as volume-to-volume ratios.

Melting points were determined in open capillaries in a Buchi SMP 20 melting point apparatus. The products were characterized by ^1H and ^{13}C NMR in a 300-MHz JEOL Eclipse equipment using tetramethylsilane as an internal standard and $CDCl_3$ as a solvent; the chemical shifts (δ) are reported in parts per million (ppm) from tetramethylsilane; the signals were assigned according to the following

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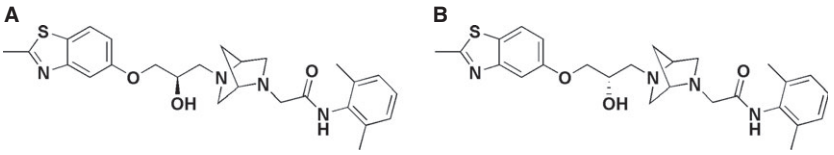


Figure 1: Chemical structures of diazabicyclic benzothiazoles A and B.

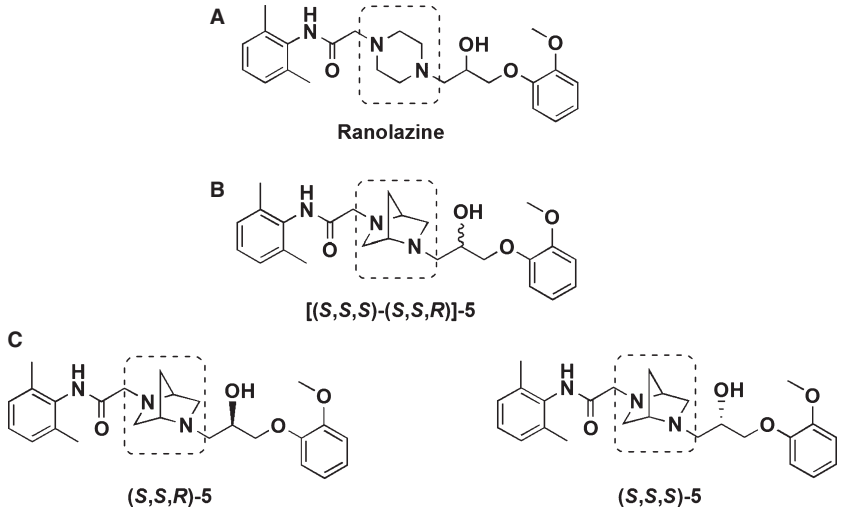


Figure 2: (A) Ranolazine, (B) epimeric mixture of the ranolazine analogue **5,** and (C) epimers of ranolazine analogue.

abbreviations: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet, br = broad singlet.

High-resolution mass spectra (HRMS) were determined in a MStation JMS-700 equipment. The infrared spectra were obtained in an infrared Bruker tensor 27 spectrometer. Optical rotations were determined in a Perkin-Elmer 341 polarimeter using a 1-dm cell length. Measurements were carried out using the sodium D-line (589 nm), at a sample compartment temperature of 20 °C and a concentration of 1% m/v. The specific rotations are reported along with the solvent used.

2-Bromo-*N*-(2,6-dimethylphenyl)acetamide (2)

In a 500-mL round-bottom flask equipped with magnetic stirrer, 19.2 g (158 mmol) of **1** was dissolved in 200 mL of $CHCl_3$ and 25.2 mL (16 g, 158 mmol) of Et_3N was added. The reaction mixture was cooled to −10 °C, stirred for 15 min and then 14.1 mL (32 g, 158 mmol) of bromoacetyl bromide, previously dissolved in 80 mL of $CHCl_3$, was added slowly (for 2 h). Stirring was continued for 15 min, and the mixture was washed with water (3 × 50 mL) and with 1N HCl (2 × 20 mL) until acidic pH in the aqueous phase. The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure on a rotary evaporator. A solid product weighing 39 g was obtained and crystallized in five parts of MeOH to give 33.8 g (88% yield) of **2** as white crystals. Mp: 158–160 °C; IR (KBr per cm) *v*: 3212, 3041, 2973, 1637, 1527, 1469, 1213, 1119, 758, 512; ^{1}H RMN (300 MHz, $CDCl_3$, δ, ppm): 2.22 (s, 6H), 4.03 (s, 2H), 7.05–7.13 (m, 3H), 7.78 (br, 1H); ^{13}C RMN (75 MHz, $CDCl_3$, δ, ppm): 18.2, 29.0, 127.8, 128.3, 132.9, 135.3, 164.0.

2-[5-Benzyl-(1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptan-2-yl]-*N*-(2,6-dimethylphenyl) acetamide [(*S*,*S*)-3]

In a 250-mL round-bottom flask equipped with magnetic stirring were dissolved 5 g (21 mmol) of **2**, 3.9 g (21 mmol) of 2-benzyl-DBH, and 3.3 mL (2.5 g, 21 mmol) of Et_3N in 100 mL of CH_2Cl_2; the mixture was then stirred for 24 h. It was rinsed with water (3 × 50 mL); the organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure to give 7.8 g of crude product which was heated under reflux, with stirring, in hexane (40 mL) for 1 h, allowed to cool to room temperature and filtered to give 6.5 g (90% yield) of **(*S*,*S*)-3** as white crystals. Mp: 126–128 °C; $[\alpha]_D^{20}$ = +2.6 (c 1, MeOH); IR (KBr, cm^{-1}) ν_{max}: 3240, 2972, 2844, 1662, 1496, 762, 721, 696, 501; ^{1}H RMN (300 MHz, $CDCl_3$, δ, ppm): 1.73 (d, 1H, J = 9.9 Hz), 1.86 (d, 1H, J = 9.9 Hz), 2.23 (s, 6H), 2.73–2.78 (m, 2H), 2.81 (d, 1H, J = 2.4 Hz), 3.05 (d, 1H, J = 9.6 Hz), 3.33 (d, 1H, J = 16.8 Hz), 3.4 (s, 2H), 3.45 (d, 1H, J = 16.8 Hz), 3.72 (d, 1H, J = 2.4 Hz), 7.05–7.10 (m, 3H), 7.21–7.37 (m, 5H), 8.72 (br, 1H); ^{13}C RMN (75 MHz, $CDCl_3$, δ, ppm): 18.5, 33.9, 57.7, 58.1, 58.4, 59.4, 61.5, 63.8, 126.9, 127.1, 128.1, 128.3, 128.4, 133.7, 135.1, 139.4, 169.4; HRMS (FAB^+): m/z calculated for $C_{22}H_{28}N_3O$ $[M+H]^+$: 350.2232, found: 350.2216.

2-[(1*S*,4*S*)-5-H-2,5-diazabicyclo[2.2.1]heptan-2-yl] -*N*-(2,6-dimethylphenyl) acetamide [(*S*,*S*)-4]

In a 500-mL hydrogenation flask, 6.5 g (19 mmol) of **(*S*,*S*)-3** was dissolved in 38 mL (38 mmol) of 1N HCl; then 62 mL of H_2O, 100 mL of MeOH and 650 mg of 10% Pd/C were added. Hydrogenation at 60 psi was carried out for 2 h. MeOH was evaporated under vacuum and 38 mL (38 mmol)

of 1N NaOH was added; the mixture was extracted with CH_2Cl_2 (3 × 25 mL); the organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure to obtain 4.8 g (99% yield) of **(*S,S*)-4** as white crystals. Mp: 131–132 °C; $[\alpha]_D^{20}$ = +25.1 (c 1, MeOH); IR (KBr per cm) ν_{max}: 3208, 2962, 2856, 1658, 1492, 767, 518; ^{1}H RMN (300 MHz, $CDCl_3$, δ, ppm): 1.68 (d, 1H, J = 9.9 Hz), 1.83 (d, 1H, J = 9.9 Hz), 2.22 (s, 6H), 2.76 (d, 1H, J = 10.2 Hz), 2.84 (br, 1H), 2.96 (dd, 1H, J_2 = 10.5 Hz, J_1 = 2.1 Hz), 3.01 (dd, 1H, J_2 = 9.9 Hz, J_1 = 2.4 Hz), 3.13 (dd, 1H, J_2 = 10.5 Hz, J_1 = 0.6 Hz), 3.31 (d, 1H, J = 16.8 Hz), 3.42 (d, 1H, J = 16.8 Hz), 3.47 (s, 1H), 3.65 (s, 1H), 7.05–7.09 (m, 3H), 8.65 (br, 1H); ^{13}C RMN (75 MHz, $CDCl_3$, δ, ppm): 18.5, 36.0, 49.7, 57.1, 59.2, 62.7, 63.2, 127.1, 128.1, 133.6, 135.0, 169.2; HRMS (FAB$^+$): m/z calculated for $C_{15}H_{22}N_3O$ $[M+H]^+$: 260.1763, found: 260.1773.

General procedure for the synthesis of compounds 7

In a round-bottom flask, equipped with a magnetic stirrer, a 0.7 M solution of **6** in epichlorohydrin was stirred at room temperature for 24 h. The excess epichlorohydrin was distilled off (30 °C/0.005 mmHg); the residue was suspended in AcOEt and rinsed with water. The organic phase was dried over anhydrous sodium sulfate and concentrated on a rotary evaporator, affording the crude product which was purified by column chromatography (20 parts of flash silica, hexane/AcOEt 9:1).

(±)-2-[(2-Methoxyphenoxy)methyl]oxirane (*rac*-7). According to the general procedure, from 30 mL of 0.7 M solution of **6** (21 mmol) in (±)-epichlorohydrin was obtained 3.5 g of ***rac*-7**, as a light yellow solid (95% yield). Mp: 38 °C, [Lit. (9) Mp: 38–40 °C]; ^{1}H RMN (300 MHz, $CDCl_3$, δ, ppm): 2.73 (dd, 1H, J_2 = 5.1 Hz, J_1 = 2.4 Hz), 2.86–2.89 (m, 1H), 3.35–3.40 (m, 1H), 3.86 (s, 3H), 4.03 (dd, 1H, J_2 = 11.4 Hz, J_1 = 5.4 Hz), 4.23 (dd, 1H, J_2 = 11.4 Hz, J_1 = 3.6 Hz), 6.88–6.95 (m, 4H); ^{13}C RMN (75 MHz, $CDCl_3$, δ, ppm): 44.8, 50.1, 55.8, 70.1, 112.0, 114.3, 120.8, 121.8, 148.0, 149.6.

(*S*)-2-[(2-Methoxyphenoxy)methyl]oxirane [(*S*)-7]. According to the general procedure, 4.9 mL of 0.7 M solution of **6** (3.4 mmol) in (*R*)-epichlorohydrin afforded 530 mg of **(*S*)-7**, as a slightly yellow solid (86% yield). Mp: 55–56 °C; $[\alpha]_D^{20}$ = +15.9 (c 1, MeOH), {Lit. (8) Mp: 59–60 °C; $[\alpha]_D^{20}$ = +13.0 (c 0.61, EtOH)}; ^{1}H RMN (300 MHz, $CDCl_3$, δ, ppm): 2.72 (dd, 1H, J_2 = 4.9 Hz, J_1 = 2.6 Hz), 2.87 (dd, 1H, J_2 = 4.9 Hz, J_1 = 4.1 Hz), 3.35–3.40 (m, 1H), 3.86 (s, 3H), 4.03 (dd, 1H, J_2 = 11.4 Hz, J_1 = 5.5 Hz), 4.23 (dd, 1H, J_2 = 11.4 Hz, J_1 = 3.6 Hz), 6.85–6.98 (m, 4H); ^{13}C RMN (75 MHz, $CDCl_3$, δ, ppm): 44.8, 50.1, 55.8, 70.4, 112.0, 114.4, 120.8, 121.9, 148.0, 149.7.

(*R*)-2-[(2-Methoxyphenoxy)methyl]oxirane [(*R*)-7]. According to the general procedure, from 4.9 mL of 0.7 M solution of **6** (3.4 mmol) in (*S*)-epichlorohydrin there was obtained 555 mg of **(*S*)-7**, as a light yellow solid (90% yield). Mp: 56–57 °C; $[\alpha]_D^{20}$ = −14.2 (c 1, MeOH), {Lit. (8) Mp: 59–60 °C; $[\alpha]_D^{20}$ = −12.9 (c 0.53, EtOH)}; ^{1}H RMN (300 MHz, $CDCl_3$, δ, ppm): 2.72 (dd, 1H, J_2 = 4.9 Hz, J_1 = 2.6 Hz), 2.87 (dd, 1H, J_2 = 4.9 Hz, J_1 = 4.3 Hz), 3.35-3.40 (m, 1H), 3.86 (s, 3H), 4.03 (dd, 1H, J_2 = 11.4 Hz, J_1 = 5.5 Hz), 4.23 (dd, 1H, J_2 = 11.4 Hz, J_1 = 3.6 Hz), 6.85–6.98 (m, 4H); ^{13}C RMN (75 MHz, $CDCl_3$, δ, ppm): 44.8, 50.2, 55.8, 70.2, 112.0, 114.4, 120.8, 121.9, 148.0, 149.7.

General procedure for the synthesis of analogues 5

In a round-bottom flask were dissolved **(*S,S*)-4** and the corresponding oxirane **7** in MeOH. The reaction mixture was stirred under reflux for 12 h, it was concentrated under reduced pressure on the rotary evaporator, one equivalent of 1N HCl was added, and the aqueous solution was washed with AcOEt. One equivalent of 1N NaOH was added to the aqueous solution and it was extracted with AcOEt; the organic phase was dried over anhydrous sodium sulfate and concentrated on the rotary evaporator to obtain the crude product, which was recrystallized from AcOEt or purified by column chromatography (20 parts of flash silica, CH_2Cl_2/MeOH 95:5) to afford the pure product.

2-{(1*S*,4*S*)-5-[3-(2-methoxyphenoxy)-2-hydroxypropyl]-2,5-diazabicyclo[2.2.1] heptan-2-yl}-*N*-(2,6-dimethyl phenyl)acetamide {[(*S,S,S*)(*S,S,R*)]-5}. According to the general procedure, 6.1 g (13.9 mmol) of **[(*S,S,S*)(*S,S,R*)]-5** was obtained from 4.7 g (18 mmol) of **(*S,S*)-4** and 3.2 g (18 mmol) of ***rac*-7**, as a white powder (77% yield). The reaction was purified by recrystallization with AcOEt. Mp: 135-136 °C; IR (KBr, cm^{-1}) ν_{max}: 3253, 2864, 1633, 1504, 1253, 1230, 1025, 769, 734; ^{1}H RMN (300 MHz, $CDCl_3$, δ, ppm): 1.76-1.82 (m, 2H), 2.23 (s, 6H), 2.61-2.89 (m, 4H), 2.93 (dd, 1H, J_2 = 9.9 Hz, J_1 = 2.4 Hz), 3.01 (dd, 1H, J_2 = 10.2 Hz, J_1 = 4.5 Hz), 3.31 (d, 1H, J = 16.5 Hz), 3.41 (s, 2H), 3.42 (d, 1H, J = 16.5 Hz), 3.59 (br, 1H), 3.83 (s, 3H), 3.93–4.07 (m, 3H), 6.87–6.95 (m, 4H), 7.05-7.09 (m, 3H), 8.64 (br, 1H); ^{13}C RMN (75 MHz, $CDCl_3$, δ, ppm): 18.5, 34.3, 34.9, 55.8, 57.3, 57.5, 58.0, 58.6, 58.7, 59.4, 63.0, 63.2, 63.6, 67.7, 72.0, 72.1, 111.9, 114.7, 120.9, 121.8, 127.1, 128.2, 133.7, 135.0, 148.2, 149.8, 169.1.

2-{(1*S*,4*S*)-5-[(2*S*)-3-(2-methoxyphenoxy)-2-hydroxypropyl]-2,5-diazabicyclo [2.2.1]heptan-2-yl}-*N*-(2,6-dimethylphenyl)acetamide [(*S,S,S*)-5]. According to the general procedure, 748 mg (1.7 mmol) of **(*S,S,S*)-5** was obtained from 548 mg (2.1 mmol) of **(*S,S*)-4** and 378 mg (2.1 mmol) of **(*S*)-7**, as a white powder (81% yield). The reaction was purified by column chromatography. Mp: 142-143 °C; $[\alpha]_D^{20}$ = +8.57 (c 1, MeOH); IR (KBr, cm^{-1}) ν_{max}: 3388, 3254, 2865, 2805, 1634, 1505, 1253, 1026, 769, 734, 608, 457; ^{1}H RMN (300 MHz, $CDCl_3$, δ, ppm): 1.77 (d, 1H, J = 9 Hz), 1.82 (d, 1H, J = 9 Hz), 2.22 (s, 6H), 2.75-2.89 (m, 4H), 2.92 (d, 1H, J = 9 Hz), 3.03 (d, 1H, J = 9 Hz), 3.29 (d, 1H, J = 15 Hz), 3.42 (d, 1H, J = 15 Hz), 3.44 (d,

2H, J = 18 Hz), 3.83 (s, 3H), 3.97–4.03 (m, 3H), 4.18 (br, 1H), 6.86-6.97 (m, 4H), 7.08–7.09 (m, 3H), 8.66 (br, 1H); ^{13}C RMN (75 MHz, $CDCl_3$, δ, ppm): 18.6, 34.3, 55.9, 57.7, 58.5, 58.8, 59.4, 63.5, 63.6, 67.6, 72.0, 112.0, 114.6, 121.0, 121.9, 127.3, 128.3, 133.8, 135.2, 148.2, 149.8, 169.3; HRMS (FAB$^+$): m/z calculated for $C_{25}H_{34}N_3O_4$ $[M+H]^+$: 440.2549, found: 440.2539.

2-{(1*S*,4*S*)-5-[(2*S*)-3-(2-methoxyphenoxy)-2-hydroxypropyl]-2,5-diazabicyclo [2.2.1]heptan-2-yl}-*N*-(2,6-dimethylphenyl)acetamide [(*S*,*S*,*R*)-5]. According to the general procedure, 766 mg (1.7 mmol) of **(*S*,*S*,*R*)-5** was obtained from 548 mg (2.1 mmol) of **(*S*,*S*)-4** and 378 mg (2.1 mmol) of **(*R*)-7**, as a white powder (83% yield). The reaction was purified by column chromatography. Mp: 110–112 °C; $[\alpha]_D^{20}$ = +3.58 (c 1, MeOH); IR (KBr per cm) ν_{max}: 3257, 2990, 2861, 1645, 1504, 1252, 1228, 770, 731; ^{1}H RMN (300 MHz, $CDCl_3$, δ, ppm): 1.76 (d, 1H, J = 9 Hz), 1.80 (d, 1H, J = 9 Hz), 2.22 (s, 6H), 2.72–2.90 (m, 5H), 3.01 (d, 1H, J = 9 Hz), 3.30 (d, 1H, J = 15 Hz), 3.40-3.42 (m, 3H), 3.42 (d, 2H, J = 15 Hz), 3.84 (s, 3H), 3.97–4.04 (m, 3H), 6.87-6.97(m, 4H), 7.07–7.09 (m, 3H), 8.64 (br, 1H); ^{13}C RMN (75 MHz, $CDCl_3$, δ, ppm): 18.5, 34.3, 55.8, 57.6, 58.7, 58.8, 59.4, 63.3, 63.6, 67.8, 72.1, 112.0, 114.8, 120.8, 121.9, 127.1, 128.2, 133.7, 135.7, 148.3, 149.9, 169.2; HRMS (FAB$^+$): m/z calculated for $C_{25}H_{34}N_3O_4$ $[M+H]^+$: 440.2549, found: 440.2566.

Biological assays

General experimental conditions

Glucose was acquired from Merck (Darmstadt, Germany). Ranolazine was prepared according to a procedure described in the literature (9). All the other compounds were acquired from Sigma (St. Louis, MO, USA). Indomethacin was dissolved in a 4% sodium carbonate solution. Ranolazine, L-phenylephrine hydrochloride, carbachol (carbamoylcholine chloride), and the hydrochloride of Nω-nitro-L-arginate methyl were dissolved in deionized water.

The experiments were carried out on Wistar male adult rats from de strain. The animals were killed by cervical dislocation and immediate exsanguination. The thoracic aorta was extracted by means of a thoracotomy and taken to a dissection chamber that contained Tyrode solution, continuously aerated. The connective tissue that surrounded the vessel was eliminated under a microscope with extreme caution so as not to touch the innermost of the vessel in order to avoid damaging the endothelium. There were obtained small rings approximately 2 mm wide.

Two rings were selected (the endothelium was damaged mechanically in one of them), and they were mounted in a perfusion chamber between two stainless steel hooks; one of each pair of hooks was attached to the perfusion chamber, and the other was attached to an isometric force transducer (Grass FT03). Once the preparations were mounted in such device, basal tension of 2 g was applied and was allowed to stabilize for a 60-min period at a temperature of 37 °C. In this way, it was possible to maintain under the same experimental conditions both rings and simultaneously record the effects produced on the vascular smooth muscle and those in which the endothelium was involved.

As a model to study the release of NO, the relaxation effect of carbachol (10^{-5} M) was utilized in the rings with the intact endothelium and precontracted with phenylephrine (10^{-5} M), which when contrasted with the response to carbachol of rings without endothelium is a reliable physiological indicator of the release of NO.

Perfusion system

A peristaltic pump with polyethylene tubing was employed to conduct the Tyrode and test solutions to the perfusion chamber. The perfusion solutions continuously aerated with carbogen entered through the bottom of the chamber whose volume was 0.5 mL and were drained to the outside by overflow, through fluting on the upper edge of the chamber, and provided with a cellulose wick which avoided sudden changes in volume.

Temperature control

The polyethylene tubes that conducted the perfusion solution passed, forming a spiral, through a thermostatic system adjusted to bring the temperature of the solution in the perfusion chamber to the desired value.

Recording system

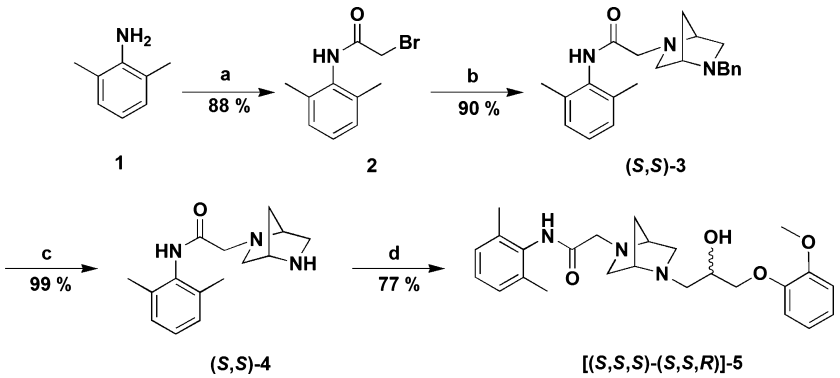
The tension developed by each ring was picked up by an isometric force transducer (Grass FT03), and the signal was taken to a polygraph Grass model 79.

Results and Discussion

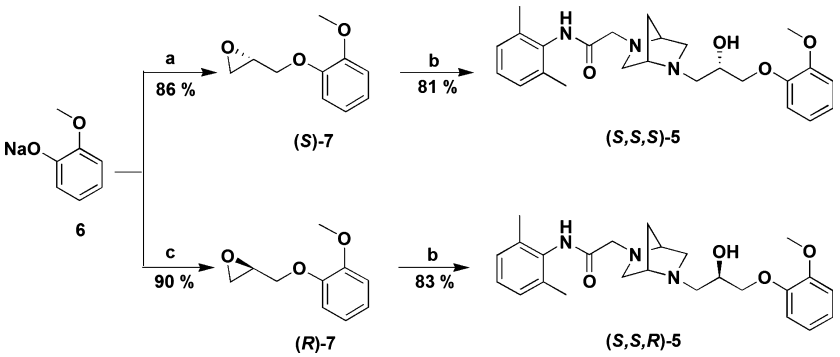
Chemistry

The synthesis of the epimeric mixture of the target diazabicyclic analogue is described in Scheme 1. Acetamide **(*S*,*S*)-4** was prepared from the aniline **1**, which in the presence of bromoacetyl bromide yielded the bromoacetamide **2**; this upon reaction with 2-benzyl-DBH [prepared according to Melgar-Fernandez *et al.* (10)] afforded the protected diamine **(*S*,*S*)-3,** which produced, by palladium-catalyzed hydrogenolysis, the intermediate **(S,S)-4,** which reacted with racemic epoxide **7** to give the epimeric mixture of **(*S*,*S*,*S*)-5** and **(*S*,*S*,*R*)-5**.

The synthesis of pure diastereoisomers **(*S*,*S*,*S*)-5** and **(*S*,*S*,*R*)-5** is described in Scheme 2. The epoxides **(*S*)-7** and **(*R*)-7** were obtained by the reaction of (*R*)-epichlorohydrin and (*S*)-epichlorohydrin, respectively, with sodium 2-methoxyphenolate **(6)**. The condensation of **(*S*,*S*)-4** with **(*S*)-7**



Scheme 1: Reagents: (A) bromoacetyl bromide, Et_3N, CH_2Cl_2; (B) 2-benzyl-DBH, Et_3N, CH_2Cl_2; (C) H_2, Pd/C, MeOH; ***rac*-7**, MeOH.



Scheme 2: Reagents: (A) (*R*)-epichlorohydrin; (B) **(*S*,*S*)-4**, MeOH; (C) (*S*)-epichlorohydrin.

and **(*R*)-7** afforded the respective pure epimers **(*S*,*S*,*S*)-5** and **(*S*,*S*,*R*)-5**.

Pharmacology

The pharmacological evaluation of compounds **[(*S*,*S*,*S*)-(*S*,*S*,*R*)]-5**, **(*S*,*S*,*S*)-5** and **(*S*,*S*,*R*)-5** was carried out on aorta rings from adult male Wistar rats. For each experiment, two 2-mm rings were used, one with endothelium and another without endothelium, which was removed by manual friction.

Both rings were suspended in an miniature organ bath (vol. 0.5 mL), between a pair of stainless steel hooks, one attached to the wall of the chamber and the other attached to an isometric force transducer (Grass, model FT03). The rings were continuously perfused (1 mL/min) with an oxygenated Tyrode solution (95% O_2, 5% CO_2); the pH was adjusted to 7.2, and the temperature was maintained at 37 °C.

At the start of each experiment, the response to phenylephrine (10^{-5} M) and carbachol (10^{-5} M) was evaluated. The relaxation induced by carbachol on the precontracted rings with phenylephrine was taken as evidence that those rings have the endothelium preserved, while the absence of relaxation confirmed the absence of a functional endothelium.

To investigate a possible relaxant action of the epimeric compound **5** and the mixture of them, the effects of these compounds at cumulatively increasing concentrations (10^{-6}–10^{-4} M) on phenylephrine (10^{-5} M)-precontracted rings were analyzed. These effects were analyzed in the absence and in the presence of either indomethacin (10^{-6} M) or L-NAME (300 μM).

Data analysis: The relaxations induced by these compounds are expressed as the percent of the maximal tension induced by phenylephrine (10^{-5} M). IC_{50} (−log of the mean molar concentration of the compound producing 50% of the maximal response) was determined with the software package GRAPH PAD PRISM (v.5; San Diego, CA, USA). Data are expressed as mean ± DS for tension development and as mean ± SE for IC_{50} values.

Comparisons of means were made by one-way analysis of variance (ANOVA), and differences between the groups were evaluated using Student–Newman–Keuls method [Graph Pad Prism (v.5) software; St. Louis MO, USA)]; p value of 0.05 or less was considered significant.

Effects of compound [(*S*,*S*,*S*)-(*S*,*S*,*R*)]-5

In precontracted rings with phenylephrine (10^{-5} M), at successively increasing concentrations (10^{-6}–10^{-4} M) of **[(*S*,*S*,*S*)-(*S*,*S*,*R*)]-5**, a relaxation effect was produced that depended on the concentration and partially on the presence of the endothelium.

The maximum relaxation response was observed with 10^{-4} M (78.61 ± 5.79% in the rings with endothelium and

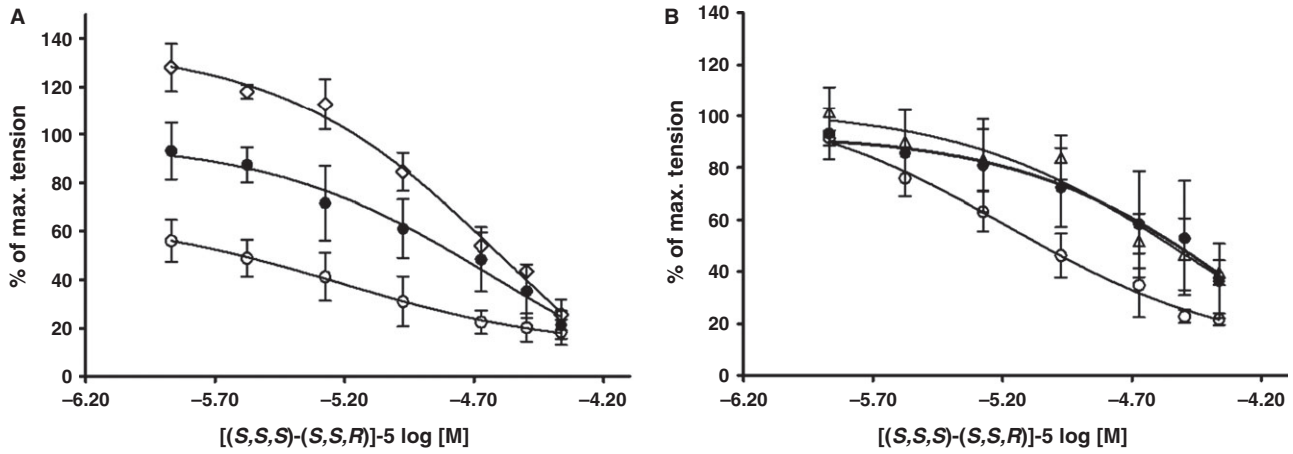


Figure 3: Concentration–response curve at successively increasing concentrations to **[(*S,S,S*)-(*S,S,R*)]-5** in aorta rings with endothelium (A) and without endothelium (B). The curves represent the relaxation response of the precontracted aorta rings with phenylephrine (10^{-5} M), to **[(S,S,S)-(S,S,R)]-5** (10^{-6}–10^{-4} M) (●), in the presence of indomethacin (10^{-6} M) (o), and in the presence of L-NAME (300 μM) (□). The data are expressed as the mean ± DS, n = 5.

Table 1: IC_{50} and maximum tension in rat aorta rings induced by ranolazine, **[(*S,S,S*)-(*S,S,R*)]-5**, **(*S,S,S*)-5** and **(*S,S,R*)-5** in the presence and absence of indomethacin and L-NAME

		With endothelium		Without endothelium	
Experiment	Compounds	IC_{50} (log[M])	Max. Tension (%)	IC_{50} (log[M])	Max. Tension (%)
1	Ranolazine	4.61 ± 0.05	56.78 ± 6.81	4.27 ± 0.02	47.88 ± 4.70
2	Ranolazine + A	5.39 ± 0.17[a]	66.05 ± 10.70	4.61 ± 0.04[a]	66.25 ± 4.45[a]
3	Ranolazine + B	3.98 ± 0.09[a,b]	34.94 ± 9.88[a,b]	4.42 ± 0. 03[a,b]	52.38 ± 9.76
4	**[(*S,S,S*)-(*S,S,R*)]-5**	4.78 ± 0.03*	78.61 ± 5.79*	4.55 ± 0.03*	63.59 ± 14.40
5	**[(*S,S,S*)-(*S,S,R*)]-5** + A	5.47 ± 0.09[a]	81.53 ± 5.28	5.00 ± 0.02[a]	78.39 ± 2.22
6	**[(*S,S,S*)-(*S,S,R*)]-5** + B	4.53 ± 0.04[a,b]	74.47 ± 6.33	4.53 ± 0.049[b,*]	60.35 ± 4.76[b]
7	**(*S,S,S*)-5**	4.85 ± 0.03*	76.65 ± 3.42*	4.55 ± 0.05	64.42 ± 12.15
8	**(*S,S,S*)-5** + A	4.75 ± 0.04[a,*]	74.14 ± 9.67	4.46 ± 0.09	66.82 ± 8.57
9	**(*S,S,S*)-5** + B	3.96 ± 0.09[a,b]	33.81 ± 15.14[a,b]	4.09 ± 0.12[a,b]	45.72 ± 12.37[b]
10	**(*S,S,R*)-5**	4.47 ± 0.07	60.69 ± 3.42	4.25 ± 0.02	43.53 ± 11.64
11	**(*S,S,R*)-5** + A	5.09 ± 0.12[a]	72.66 ± 8.28	4.69 ± 0.02[a]	70.85 ± 4.50[a]
12	**(*S,S,R*)-5** + B	4.21 ± 0.05[a,b,*]	46.53 ± 3.08[a,b]	4.17 ± 0.03[a,b,*]	43.38 ± 7.53[b]

IC_{50}: log of the mean molar concentration that causes 50% of the relaxation induced by ranolazine, **[(*S,S,S*)-(*S,S,R*)]-5**, **(*S,S,S*)-5,** and **(*S,S,R*)-5** (10^{-6}–10^{-4} M). Maximum tension developed in response to phenylephrine (10^{-5} M). A: indomethacin (10^{-6} M). B: L-NAME (300 μM). The percentage data from the maximum relaxation are presented as the mean ± DS. The IC_{50} data are presented as the mean ± ES [a,b]denotes significant differences between the same group ([a] versus. in absence of A or B, [b] versus A) ($p < 0.05$). *Denotes significant differences between the groups in their respective conditions ($p < 0.05$). n = 5 for all of the groups.

63.59 ± 14.40% in the rings without endothelium; these values were not significantly different; Figures 3A,B, experiments 4–6, Table 1). Indomethacin (10^{-6} M) displaced the concentration–response curve to the left (experiment 5, Table 1).

In the rings with endothelium, in the presence of L-NAME (300 μM), when the concentrations were between 10^{-6} and 10^{-5} M of **[(*S,S,S*)-(*S,S,R*)]-5**, an additional increase in the tension induced by phenylephrine was observed (experiment 6, Table 1). These results show that **[(*S,S,S*)-(*S,S,R*)]-5** has primordially a direct relaxation effect over the vascular smooth muscle and also acts on the endothelium releasing NO as well as a vasoconstrictor prostanoid.

When comparing the concentration–response curve of **[(*S,S,S*)-(*S,S,R*)]-5** with that of ranolazine, it was found that the one from compound **[(*S,S,S*)-(*S,S,R*)]-5** is displaced to the left (IC_{50} 4.78 ± 0.03 versus 4.61 ± 0.05, respectively, experiments 1 and 4, Table 1). In the aortic rings with endothelium, **[(*S,S,S*)-(*S,S,R*)]-5** produced a maximum relaxation of greater magnitude than ranolazine (78.61 ± 6.33% versus 56.78 ± 6.81%, respectively, Figure 4A, experiments 1 and 4, Table 1).

In the presence of indomethacin (10^{-6} M), the relaxation responses induced by this compound on the aortic rings with or without endothelium were not significantly different to the ones from ranolazine in similar experimental

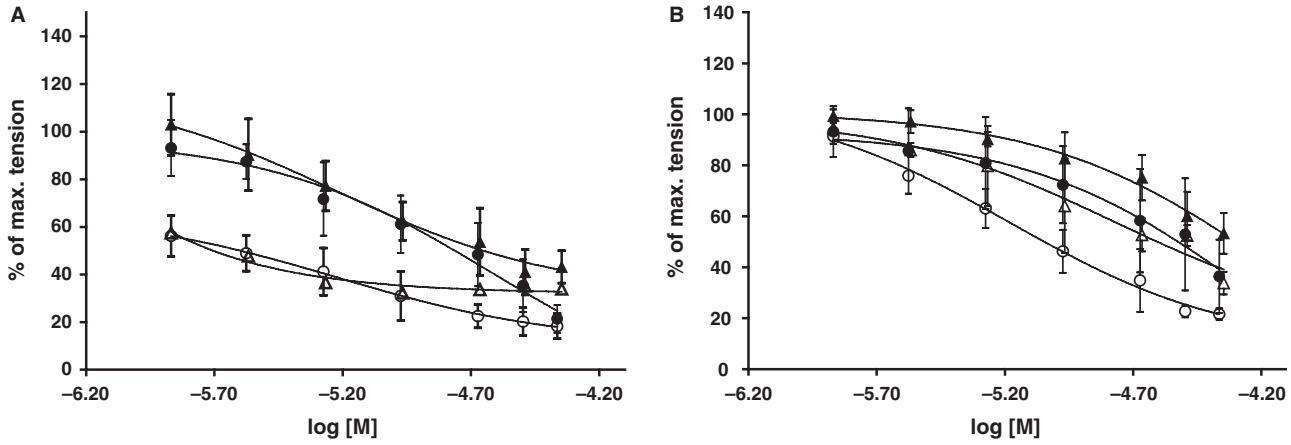


Figure 4: Concentration–response curve to **[(*S,S,S*)-(*S,S,R*)]-5** and ranolazine at successively increasing concentrations, in aorta rings with endothelium (A) and without endothelium (B). The curves represent the relaxation response of the aortic rings precontracted with phenylephrine (10^{-5} M) to **[(*S,S,S*)-(*S,S,R*)]-5** (●) and ranolazine (10^{-6}–10^{-4} M) (▲), in the absence (closed symbols) and in the presence of indomethacin (10^{-6} M) (open symbols). The data are expressed as the mean ± DS, $n = 5$.

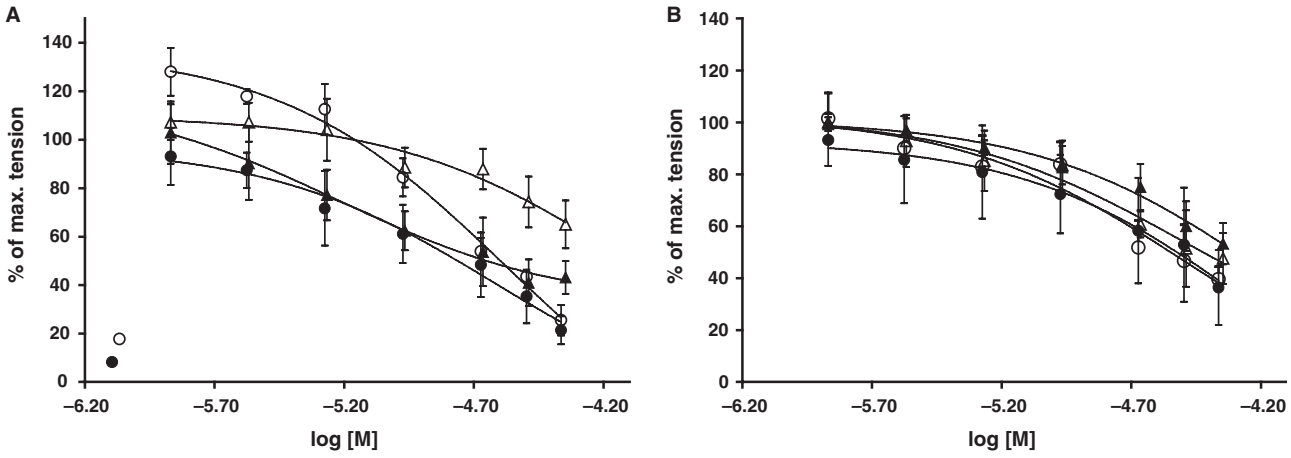


Figure 5: Concentration–response curve to **[(*S,S,S*)-(*S,S,R*)]-5** and ranolazine at successively increasing concentrations, in aorta rings with endothelium (A) and without endothelium (B). The curves represent the relaxation response to the aortic rings precontracted with phenylephrine (10^{-5} M) to **[(*S,S,S*)-(*S,S,R*)]-5** (●) and ranolazine (10^{-6}–10^{-4} M) (▲), in the absence (closed symbols) and in the presence of L-NAME (300 μM) (open symbols). The data are expressed as the mean ± DS, $n = 5$.

conditions (Figure 4A,B, experiments 2 and 5, Table 1). The inhibition of the synthesis of NO with L-NAME (300 μM) decreased the maximum relaxation induced by ranolazine, but not the one induced by **[(*S,S,S*)-(*S,S,R*)]-5**. These results show that the relaxation induced by this compound depends less on the synthesis of NO than the one induced by ranolazine, even though the relaxation effect is greater (Figure 5A,B, experiments 3 and 6, Table 1).

Effects of compound (*S,S,S*)-5

In phenylephrine-precontracted rings, **(*S,S,S*)-5** induced a relaxation dependent on the concentration and partially dependent on the presence of the endothelium (76.65 ± 3.42% in the rings with endothelium and 64.42 ± 12.15% in the rings without endothelium, Figure 6A,B, experiment 7, Table 1).

No effect whatsoever was observed when blocking cyclooxygenase with indomethacin (10^{-6} M; Figure 6A,B, experiment 8, Table 1). In the rings with endothelium, the maximum relaxation induced by this compound decreased significantly by the addition of L-NAME (300 μM; 76.65 ± 3.42% versus 33.81 ± 15.14%, respectively, Figure 7A,B experiment 9, Table 1). These results suggest that the relaxation induced by this compound is in part caused by the release of endothelial nitric oxide.

When comparing the effects of **(*S,S,S*)-5** with those of ranolazine, it was observed that the compound **(*S,S,S*)-5** induces on the aortic rings with endothelium a vasodilation

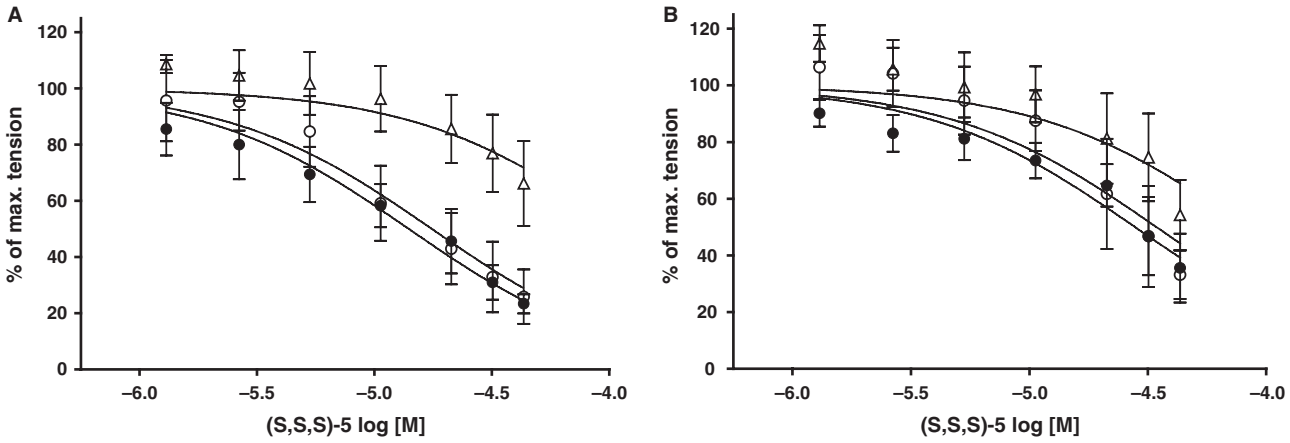


Figure 6: Concentration–response curve to **(*S*,*S*,*S*)-5** at successively increasing concentrations, in aorta rings with endothelium (A) and without endothelium (B). The curves represent the relaxation response of the aorta rings precontracted with phenylephrine (10^{-5} M), to **(*S*,*S*,*S*)-5** (10^{-6}–10^{-4} M) (•), in the presence of indomethacin (o) and in the presence of L-NAME (300 μM) (Δ). The data are expressed as the mean ± DS, n = 5.

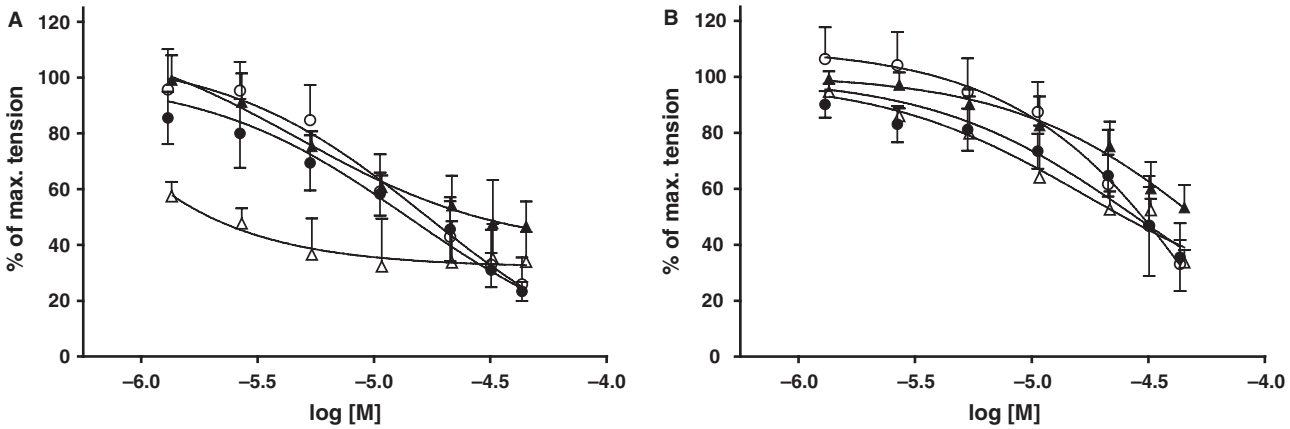


Figure 7: Concentration–response curve to **(*S*,*S*,*S*)-5** and ranolazine at successively increasing concentrations, in aorta rings with endothelium (A) and without endothelium (B). The curves represent the relaxation response of the aortic rings precontracted with phenylephrine (10^{-5} M), to **(*S*,*S*,*S*)-5** (•) and ranolazine (10^{-6}–10^{-4} M) (▲), in the absence (closed symbols) and in the presence of indomethacin (10^{-6} M) (open symbols). The data are expressed as the mean ± DS, n = 5.

of greater magnitude than the one from ranolazine (76.65 ± 3.42% versus 56.80 ± 6.81%, respectively, Figure 6A, experiments 1 and 7, Table 1). In the rings with endothelium, when cyclooxygenase was blocked with indomethacin (10^{-6} M), only the ranolazine curve was displaced to the left (Figure 7A,B, experiments 2 and 8, Table 1).

In the rings with endothelium, L-NAME (300 μM) decreased the maximum relaxation induced by **(*S*,*S*,*S*)-5** as well as the induced by ranolazine (Figure 8A, experiments 3 and 9, Table 1). These results suggest that compound **(*S*,*S*,*S*)-5** shares with ranolazine the effect of inducing the release of endothelial NO, but not the synthesis of some vasoconstricting prostanoid. On the other hand, these results show that in the relaxation induced by **(*S*,*S*,*S*)-5**, there is a greater participation of the endothelial NO synthesis/release than in the one induced by ranolazine.

Effects of compound (*S*,*S*,*R*)-5

In the aortic rings precontracted with phenylephrine, **(*S*,*S*,*R*)-5** produced a relaxation response dependent on the concentration and partially on the presence of endothelium (60.69 ± 3.42% in the rings with endothelium and 43.53 ± 11.64% in the rings without endothelium, Figure 9A,B, experiment 10, Table 1). The addition of indomethacin (10^{-6} M) produced a displacement of the concentration–response curve to the left (IC_{50}, 4.47 ± 0.07 versus 5.09 ± 0.02, in the rings with endothelium and 4.25 ± 0.02 versus 4.69 ± 0.02 in the rings without

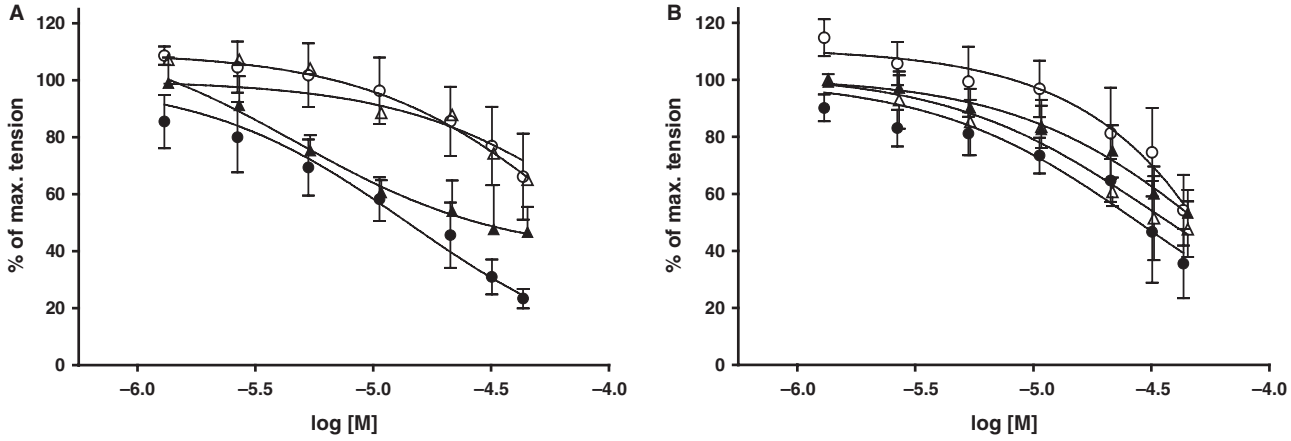


Figure 8: Concentration–response curve to **(*S,S,S*)-5** and ranolazine at successively increasing concentrations, in aorta rings with endothelium (A) and without endothelium (B). The curves represent the relaxation response of the aortic rings precontracted with phenylephrine (10^{-5} M), to **(*S,S,S*)-5** (●) and ranolazine (10^{-6}-10^{-4} M) (▲), in the absence (closed symbols) and in the presence of L-NAME (300 μM) (open symbols). The data are expressed as the mean ± DS, n = 5

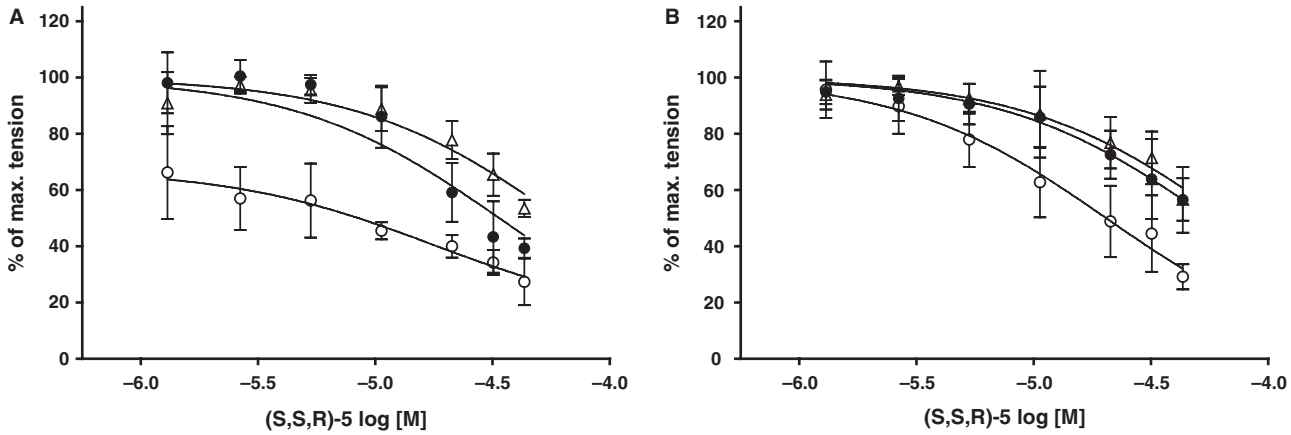


Figure 9: Concentration–response curve to **(*S,S,R*)-5** at successively increasing concentrations, in aorta rings with endothelium (A) and without endothelium (B). The curves represent the relaxation response to aortic rings precontracted with phenylephrine (10^{-5} M), to **(*S,S,R*)-5** (10^{-6}–10^{-4} M) (●), in the presence of indomethacin (10^{-6} M) (o) and in the presence of L-NAME (300 μM) (Δ). The data are expressed as the mean ± DS, n = 5.

endothelium) and an increase in the magnitude of the maximum relaxation (60.69 ± 3.42% versus 72.66 ± 8.28, in the rings with endothelium and 43.53 ± 11.64% versus 70.85 ± 5.50%, in the rings without endothelium, Figure 9A,B, experiment 11, Table 1).

In the rings with endothelium, L-NAME (300 μM) decreased the maximum relaxation induced by this compound (60.69 ± 3.42% versus 46.53 ± 3.08%, respectively, Figure 9A, experiment 12, Table 1). These results suggest that the relaxation induced by **(*S,S,R*)-5** is attributable to a direct effect on the vascular smooth muscle as well as to the discharge of endothelial NO and that this compound induces, in addition, the release of some vasoconstricting prostanoid.

When comparing the effects of compound **(*S,S,R*)-5** with those of ranolazine, it was observed that **(*S,S,R*)-5** induces on the aortic rings a maximum vasodilation similar in magnitude to that of ranolazine (60.69 ± 3.42% versus 56.80 ± 6.81%, in the rings with endothelium and 43.53 ± 11.64% versus 46.67 ± 46.67 ± 8.01% in the rings without endothelium, experiments 1 and 10, Table 1).

When blocking cyclooxygenase with indomethacin (10^{-6} M), no significant differences were observed with regard to the concentration–relaxation curves of ranolazine in similar experimental conditions (Figure 10A,B, experiments 2 and 11, Table 1).

In the rings with endothelium, L-NAME (300 μM) partially inhibited the relaxation induced by **(*S,S,R*)-5** as well as the one induced by ranolazine (60.69 ± 3.42% versus 46.53 ± 3.08 and 56.80 ± 6.81% versus 34.94 ± 9.88%, Figure 11A, experiments 3 and 12, Table 1). These results

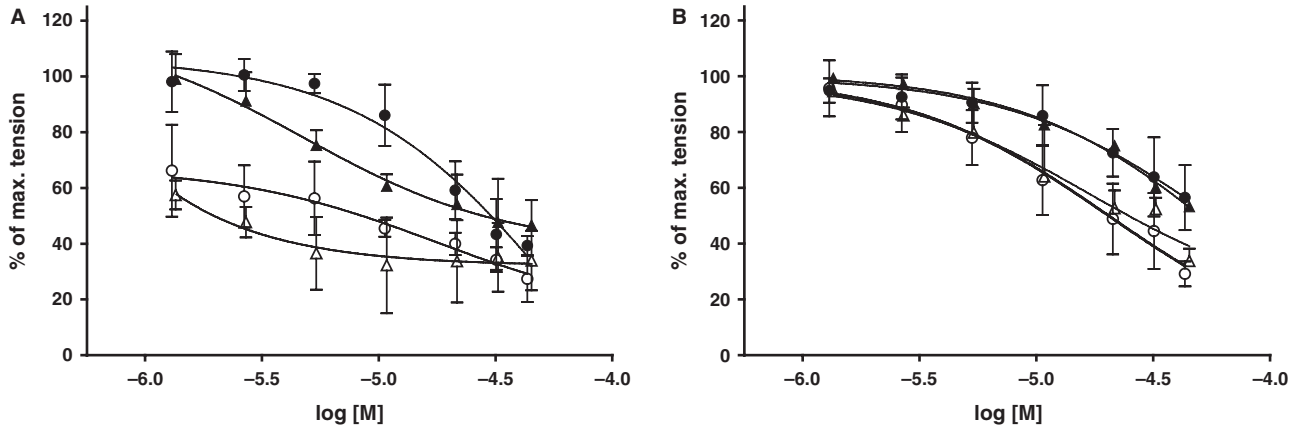


Figure 10: Concentration–response curve to **(*S,S,R*)-5** and ranolazine at successively increasing concentrations, in aorta rings with endothelium (A) and without endothelium (B). The curves represent the relaxation response of the aortic rings precontracted with phenylephrine (10^{-5} M), to **(*S,S,R*)-5** (●) and ranolazine (10^{-6}–10^{-4} M) (▲), in the absence (closed symbols) and in the presence of indomethacin (10^{-6} M) (open symbols). The data are expressed as the mean ± DS, $n = 5$.

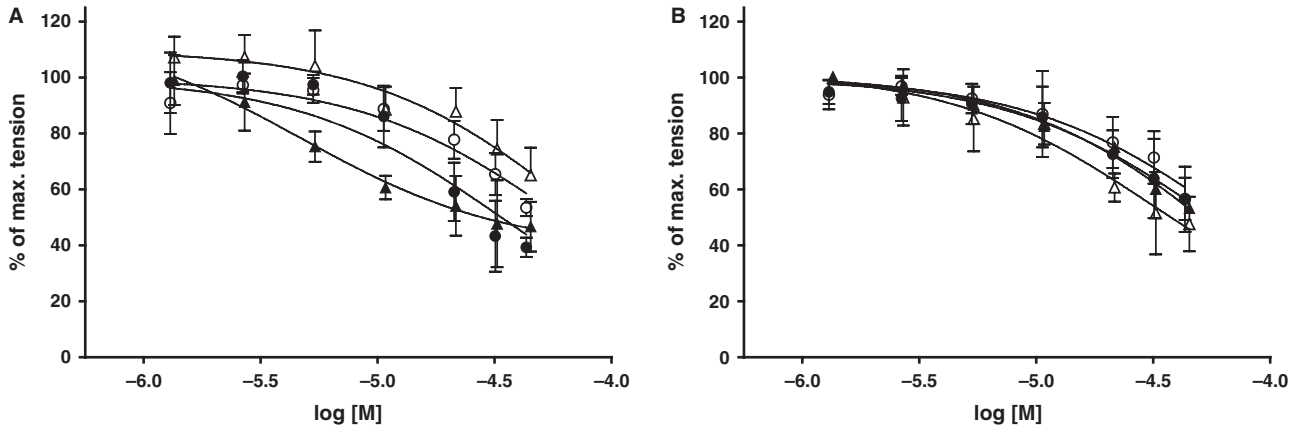


Figure 11: Concentration–response curve to **(*S,S,R*)-5** and ranolazine at successively increasing concentrations, in aorta rings with endothelium (A) and without endothelium (B). The curves represent the relaxation response of the aortic rings precontracted with phenylephrine (10^{-5} M), to **(*S,S,R*)-5** (●) and ranolazine (10^{-6}–10^{-4} M) (▲), in the absence (closed symbols) and in the presence of L-NAME (300 μM) (open symbols). The data are expressed as the mean ± DS, $n = 5$.

suggest that **(*S,S,R*)-5** shares with ranolazine the effect of inducing the release of endothelial NO.

Conclusions

The diazabicyclic analogues of ranolazine **[(*S,S,S*)(*S,S,R*)]-5**, **(*S,S,S*)-5**, and **(*S,S,R*)-5**, were synthesized from commercially available starting materials and applying simple synthetic strategies.

In view of the pharmacological results obtained, it can be stated that the epimeric mixture **[(*S,S,S*)(*S,S,R*)]-5** has a vasodilating effect induced by two components, one due to the release of endothelial NO and another due to a direct effect on the vascular smooth muscle in addition to inducing the release of some vasoconstricting prostanoid from the cyclooxygenase pathway, which is independent of the endothelium.

The compounds **(*S,S,S*)-5** and **(*S,S,R*)-5** have a vasodilating effect induced by two components, one due to the release of endothelial NO and another one due to a direct effect on the vascular smooth muscle. On the other hand, **(*S,S,R*)-5** induces the release of some vasoconstricting prostanoid from the cyclooxygenase pathway. Comparing these effects to those of ranolazine, it may be concluded that both the epimeric mixture **[(*S,S,S*)(*S,S,R*)]-5** and compound **(*S,S,S*)-5** have a higher vasodilating efficiency due to the participation of NO in the endothelium.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. MS, IR, 1H NMR and 13C NMR spectra of all synthetized compounds.