Enantioselective Synthesis and Pharmacological Evaluation of a New Type of Verapamil Analog with Hypotensive and Calcium Antagonist Activities

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Purpose. The syntheses and evaluation for cardiovascular activity in the rat of both enantiomers of a verapamil analog in which the cyano group has been replaced by hydroxyl.

Methods. (+)- and (−)-α-[3-[[2-(3,4-Dimethoxyphenyl)ethyl]methylamino]propyl]-3,4-dimethoxy-α-(1-methyl ethyl)benzyl alcohol were prepared from chiral sulfoxides produced by microbial biotransformations using *Mortierella isabellina* ATCC 42613 or *Helminthsporium* species NRRL 4671, and were examined for hypotensive and calcium antagonist activity using anaesthetized normotensive rats and isolated rat aorta and atria.

Results. The analogs showed a pharmacological profile similar to that exhibited by verapamil, possessing a remarkable hypotensive activity, accompanied by a significant bradycardia, in anaesthetized normotensive rats. *In vitro*, these analogs displayed clear inhibitory effects: in isolated rat aorta they inhibited, in a concentration-dependent fashion, the contractions and $^{45}Ca^{2+}$ uptake induced by norepinephrine and high KCl, and in isolated rat atria the analogs considerably decreased the rate of contraction (negative chronotropic effects). No significant differences between the quantitative cardiovascular effects produced by the two enantiomers of the verapamil analogs were observed.

Conclusions. The results suggest that, like that of verapamil, the cardiovascular activity exhibited by the new compounds seems to be due, at least in part, to a blockage of transmembrane calcium channels present in vascular smooth muscle cells.

KEY WORDS: biotransformation; calcium antagonist; hypotensive; *Mortierella isabellina; Helminthosporium*; verapamil.

INTRODUCTION

The development of the calcium slow channel antagonist verapamil as a coronary vasodilator with antiarrhythmic and antihypertensive properties (1) has led to the investigation of several compounds with verapamil-like activity (2,3), and to the stereospecific synthesis of both enantiomers of verapamil (4). Structure-activity relationships in the verapamil series have identified the substituent patterns necessary for calcium antagonist activity (2), but the synthesis and pharmacological activity of a simple analog in which the benzylic cyano group had been replaced by hydroxyl has not been reported. We have prepared such an analog, α-[3-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]propyl]-3,4-dimethoxy-α-(1-methylethyl)benzyl alcohol (**5**, Fig. 1) in both enantiomeric forms, and describe herein their preparation in a short sequence of reactions starting from chiral sulfoxides, the product of microbial biotransformation reactions. We also describe in detail the pharmacological activities on the rat cardiovascular system of racemic and both enantiomeric forms of **5**.

MATERIALS AND METHODS

Synthesis

Melting points were determined on a Kofler hot stage and are uncorrected. The ^1H NMR spectra were recorded on a Bruker Avance series 300 spectrometer in $CDCl_3$ using residual $CHCl_3$ as the internal standard; chemical shifts are reported in parts per million (δ) and the signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). The ^{13}C NMR spectra were recorded at 75 mHz in $CDCl_3$ solution on the above spectrometer. Mass spectra were obtained in EI mode (unless otherwise stated) using a Kratos 1S spectrometer. IR spectra were obtained using a Mattson Research Series FTIR spectrometer. Optical rotations were recorded at ambient temperature in the stated solvent using a Rudolph Autopol 3 polarimeter. Optical purity (enantiomeric excess, e.e.) was determined by ^1H NMR analysis in the presence of (*S*)-(+)-α-methoxyphenylacetic acid or (*R*)-(−)-N-(3,5-dinitrobenzoyl)-α-methylbenzylamine as described (5,6). TLC was performed on Merck silica gel F_{254} plates, 0.2 mm, and column chromatography used Merck silica gel, 230–400 mesh and dichloromethane:methanol 10:1 as eluant. Elemental analyses were performed by Guelph Chemical Laboratories Ltd., Guelph ON. *Mortierella isabellina* ATCC 42613 and *Helminthosporium* species NRRL 4671 were maintained on malt agar slopes, grown at 27°C and stored at 4°C as described (5,7).

(*R*)-(+)-3-Chloropropyl 4-Methoxyphenyl Sulfoxide (1)

This was prepared by oxidation of 3-chloropropyl 4-methoxyphenyl sulfide using *Mortierella isabellina* ATCC 42613 as described (7). The title compound was obtained in 52% isolated yield, $[\alpha]_D$ +149.1 (c 2.1, $CHCl_3$), e.e. 82%. A single crystallization from ether-hexane gave material with mp 57–58°C, $[\alpha]_D$ +174.2 (c 1.1, $CHCl_3$), e.e. >98%. IR (KBr) ν_{max} 2946, 1594, 1496, 1173, 1088 cm^{-1}; ^1H NMR 2.01–2.15 (1H, m), 2.18–2.31 (1H, m), 2.83–2.92 (1H, m), 2.94–3.04 (1H, m), 3.57–3.71 (2H, m), 3.87 (3H, s) and 7.05/7.57 (4H, ABq); ^{13}C NMR 25.6, 43.9, 54.5, 55.9, 115.3, 126.2, 134.5, and 162.4; MS m/z M^+ 232.0334 (calcd. For $C_{10}H_{13}{}^{35}ClO_2S$, M^+ 232.0325). Anal. Calcd. For $C_{10}H_{13}ClO_2S$, C 51.61, H 5.63%, found C 52.0, H 5.76%

(*S*)-(−)-3-Mesyloxypropyl 4-Methoxyphenyl Sulfoxide (2) (Fig. 2)

(*S*)-Methyl 4-methoxyphenyl sulfoxide was obtained by oxidation of methyl 4-methoxyphenyl sulfide using *Helminthosporium* species NRRL 4671 as described (5). The title

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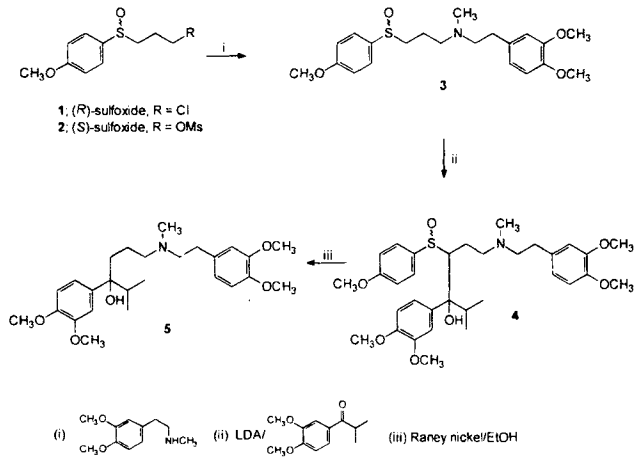
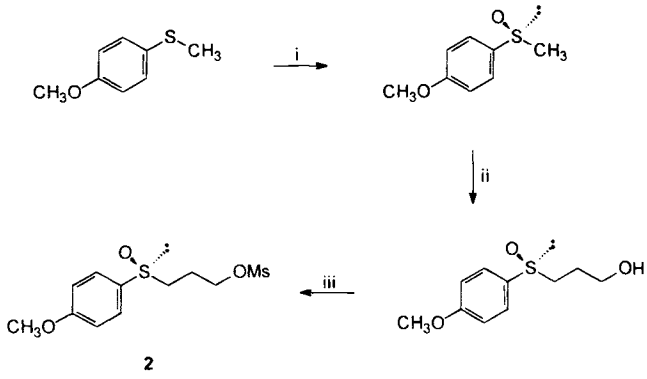


Fig. 1. Synthesis of the verapamil analogs **5**.

compound was obtained in 91% isolated yield, $[\alpha]_D$ −162.4 (c 1.2, $CHCl_3$), e.e. 96%. A single crystallization from ether-hexane gave material with mp 31–32°C, $[\alpha]_D$ −167.8 (c 1.2, $CHCl_3$), e.e. >98%, with spectral data as described (5). A solution of this sulfoxide (1.2 g, 7 mmol) in dry THF (8 ml) was added dropwise to a solution of LDA (prepared from n.butyl lithium (9.4 ml, 1.2 M in hexane) and di-isopropyl amine (1.6 ml)) in dry THF (20 ml) at −78°C. The resulting mixture was allowed to warm to −30°C over 1h, then re-cooled to −60°C. Ethylene oxide was bubbled through the mixture for 5 min, which was then allowed to warm to −20°C over 1.5 h. The reaction was quenched by the addition of saturated aqueous ammonium chloride solution, and the mixture extracted with dichloromethane. The extract was washed (satd. NaCl, water), dried (anhyd. $MgSO_4$) and evaporated, and the residue chromatographed to give (*S*)-(−)-3-hydroxypropyl 4-methoxyphenyl sulfoxide (1.09 g, 72%) as a colorless oil, $[\alpha]_D$ −190.2 (c 1.9, $CHCl_3$), e.e. >98%, IR (film) ν_{max} 3385, 2930, 1018 cm^{-1}; ^1H NMR 1.81–1.94 (2H, m), 2.83–3.01 (2H, m), 3.58–3.73 (2H, m), 3.84 (3H, s), and 7.01/7.54 (4H, ABq); ^{13}C NMR 26.4, 54.9, 55.9, 61.2, 115.2, 126.5, 134.0 and 162.4; MS m/z M^+ 214.0673 (calcd. For $C_{10}H_{14}O_3S$, M^+ 214.0664). Anal. Calcd. For $C_{10}H_{14}O_3S$, C 56.05, H 6.58%, found C 55.92, H 6.89%. A solution of this alcohol (0.642 g, 3 mmol) in dry dichloromethane (15 ml) was cooled to 0°C and treated with triethylamine (0.59 ml, 4.2 mmol) followed by methanesulfonyl chloride (0.412 g, 3.6 mmol). The mixture was then stirred at 0°C for 1 h, washed with 5% HCl, dried and evaporated. The resulting product **2** was used directly in the next step.



(i) *Helminthsporium* sp. NRRL 4671; (ii) LDA, ethylene oxide; (iii) MsCl, Et_3N

Fig. 2. Synthesis of (*S*)-3-mesyloxypropyl 4-methoxyphenyl sulfoxide (**2**).

(*R*)-(+)-3-[Methyl-2-(3,4-dimethoxyphenyl)ethyl]amino 4-Methoxyphenyl Sulfoxide ((*R*)-3)

(*R*)-(+)-3-Chloropropyl 4-methoxyphenyl sulfoxide (**1**, 0.522 g, 2.24 mmol) and N-methyl homoveratrylamine (0.7 g, 3.59 mmol) were mixed and heated to 115°C in an argon atmosphere for 45 min. The mixture was then cooled, aqueous NaOH (1 M, 10 ml) and dichloromethane (10 ml) were added, and the mixture stirred for 5 min. The organic layer was removed, the aqueous phase extracted with further dichloromethane, the combined extracts washed (satd. NaCl), dried and evaporated and the residue was purified by chromatography to give the title compound as a pale yellow oil, $[\alpha]_D$ +61.5 (c 1.5, $CHCl_3$), e.e. >98%; IR (film) ν_{max} 2938, 1590, 1045 cm^{-1}; ^1H NMR 1.75–1.91 (2H, m), 2.23 (3H, s), 2.42–2.51 (2H, m), 2.53–2.58 (2H, m), 2.62–2.69 (2H, m), 2.74–2.80 (2H, m), 3.83 (6H, s), 3.84 (3H, s), 6.68–6.78 (3H, m), and 7.01/7.52 (4H, ABq); ^{13}C NMR 20.4, 33.7, 42.3, 55.4, 55.9, 56.2, 59.4, 111.6, 112.3, 115.1, 120.9, 126.3, 133.4, 135.1, 147.6, 149.1, and 162.2, MS(FAB) m/z M^+ 392 (M + 1).

(*S*)-(−)-3-[Methyl-2-(3,4-dimethoxyphenyl)ethyl]amino 4-Methoxyphenyl Sulfoxide ((*S*)-3)

A solution of (*S*)-(−)-3-mesyloxypropyl 4-methoxyphenyl sulfoxide (**2**) prepared from the corresponding alcohol (0.642 g, 3 mmol) as described above, in dry THF (5 ml), was treated dropwise with a suspension of the sodium salt of N-methyl homoveratrylamine (prepared by the addition of sodium hydride (180 mg, 7.5 mmol) to N-methyl homoveratrylamine (1.462 g, 7.5 mmol) in dry THF (5 ml) and reflux of the resulting mixture for 2 h). The mixture was then maintained under argon at 60–65°C overnight, and worked up by the addition of 1 M NaOH/dichloromethane. The organic extract was subjected to chromatography and gave the title compound (0.68 g, 58%) as a pale yellow oil, $[\alpha]_D$ −61.95 (c 1.5, $CHCl_3$), e.e. >98%; spectral data identical with those reported above for (*R*)-(+)-**3**.

(+)-1-[1-(3,4-Dimethoxyphenyl)-1-hydroxy-2-methylpropyl]-3-[methyl-2-(3,4-dimethoxyphenyl)ethyl]amino 4-Methoxyphenyl Sulfoxide ((+)-4)

A solution of (*R*)-(+)-**3** (0.765 g, 1.96 mmol) in dry THF (4 ml) was added dropwise to a solution of LDA (prepared from n.butyl lithium (2 ml, 1.2 M in hexane) and di-isopropylamine (0.35 ml, 2.4 mmol) in THF (8 ml)) at −78°C, and the mixture stirred at this temperature for 1 h. A solution of 3,4-dimethoxyphenyl isopropyl ketone (0.53 g, 2.55 mmol) in dry THF (2.5 ml) was then added dropwise, and the mixture allowed to warm to −45°C over 45 min. The reaction was then quenched with satd. ammonium chloride, extracted with dichloromethane and the extract washed (NaCl), dried and evaporated. The residue was subjected to chromatography to give (+)-**4** (0.834 g, 71%) as a colorless oil, $[\alpha]_D$ +56.4 (c 1.85,

$CHCl_3$); IR (film) ν_{max} 3418, 2952, 1614, 1590, 1028 cm^{-1}; ^1H NMR 0.82 (3H, d, J 6.3 Hz), 1.07 (3H, d, J 6.3 Hz), 1.59–1.71 (3H, m), 1.72 (3H, s), 1.92–2.24 (3H, m), 2.25–2.32 (2H, m), 2.78–2.82 (1H, m), 3.08–3.09 (1H, m), 3.77 (3H, s), 3.79 (3H, s), 3.82 (3H, s), 3.84 (3H, s), 3.86 (3H, s), 4.84 (1H, br.s), 6.51–6.56 (2H, m), 6.73–6.82 (3H, m), 7.0–7.08 (3H, m), and 7.53 (2H, d, J 8.7 Hz); ^{13}C NMR 17.7, 18.6, 19.2, 33.3, 37.5, 41.7, 55.9, 56.16, 56.24, 56.29, 56.34, 56.6, 59.4, 66.6, 81.6, 110.3, 111.1, 111.5, 112.3, 115.3, 119.0, 120.7, 126.4, 132.6, 133.1, 134.3, 147.6, 148.2, 148.5, 149.1, and 162.2 . MS(FAB) m/z M^+ 600 (M + 1). Anal. Calcd. For $C_{33}H_{45}NO_7S$, C 66.08, H 7.56, N 2.33%, found C 66.04, H 7.94, N 2.14%.

(−)-1-[1-(3,4-Dimethoxyphenyl)-1-hydroxy-2-methylpropyl]-3-[methyl-2-(3,4-dimethoxyphenyl)ethyl]amino 4-methoxyphenyl sulfoxide ((−)-4)

This was prepared from (*S*)-(−)-**3** as described above to give material with $[\alpha]_D$ −57.7 (c 3.2, $CHCl_3$) and spectral data identical with those reported above for (+)-**4**.

(−)-α-[3-[[2-(3,4-dimethoxyphenyl)ethyl] methylamino]propyl]-3,4-dimethoxy-α-(1-methylethyl)-benzyl alcohol ((−)-5)

A mixture of (+)-**4** (0.7 g, 1.17 mmol) and Raney nickel (Aldrich) (7 g) in absolute ethanol (10 ml) was stirred at room temperature for 24 h. The mixture was then filtered through celite, and the filter bed and nickel residue washed thoroughly with methanol/chloroform (50%). The combined filtrate was evaporated and subjected to chromatography to give (−)-**5** (0.35 g) as a colorless oil, $[\alpha]_D$ −18.5 (c 1.75, $CHCl_3$); IR (film) ν_{max} 3068, 2923, 1598, 1515 cm^{-1}; ^1H NMR 0.72 (3H, d, J 6.6 Hz), 0.96 (3H, d, J 6.6 Hz), 1.38–1.44 (2H, m), 1.80–1.89 (2H, m), 2.13 (3H, s), 2.24–2.31 (2H, m), 2.42–2.62 (4H, m), 2.75–2.8 (2H, t, J 6.4 Hz), 3.85 (3H, s), 3.87 (9H, s), 6.68–6.7 (2H, m), 6.77–6.88 (3H, m), and 7.09 (1H, d, J 0.9 Hz); ^{13}C NMR 17.5, 18.4, 22.5, 33.1, 39.1, 40.0, 42.2, 56.16, 56.24, 56.31, 58.6, 60.0, 77.4, 110.7, 110.8, 111.7, 112.4, 118.9, 120.9, 132.7, 140.5, 147.2, 147.8, 148.5, and 149.3; MS m/z M^+ 445.2786 (calcd. For $C_{29}H_{39}NO_5$, M^+ 445.2828). Anal. Calcd. For $C_{29}H_{39}NO_5$, C 70.24, H 8.61, N 3.15%, found C 70.03, H 8.90, N 2.97%.

(+)-α-[3-[[2-(3,4-dimethoxyphenyl) ethyl] methylamino]propyl]-3,4-dimethoxy-α-(1-methylethyl)-benzyl alcohol ((+)-5)

This was prepared from (−)-**4** as described above to give material with $[\alpha]_D$ +18.6 (c 1.55, $CHCl_3$) and spectral data identical to those reported for (−)-**5**.

(±)-α-[3-[[2-(3,4-dimethoxyphenyl)ethyl] methylamino]propyl]-3,4-dimethoxy-α-(1-methylethyl)-benzyl alcohol ((±)-5)

This was prepared by the route shown in Fig. 1 starting with (±)-3-chloropropyl 4-methoxyphenyl sulfoxide. The intermediates and product possessed spectral data identical with those reported above for the chiral series.

Drugs, Chemicals, and Radioisotopes

The drugs and radioisotopes used in the experiments were: compounds **5**, heparin sodium salt (Analema), urethane, (−)-NE bitartrate, acetylcholine chloride, caffeine, (±)verapamil hydrochloride (Sigma Chemical Co), and $^{45}Ca^{2+}$ (New England Nuclear). Verapamil was considered as standard reference as a voltage-dependent calcium channel blocker in all experiments. Urethane and heparin were dissolved in saline to make several solutions of 25 g $(100\ ml)^{-1}$ and 12,000 iu $(100\ ml)^{-1}$, kept at 4°C. Caffeine was prepared daily in Ca^{2+}-free medium. The appropriate dilutions of the following drugs were prepared daily from concentrated stock solutions kept at −20°C: (−)-NE bitartrate from 100 mM stock solutions in de-ionized water (sodium bisulfite (0.2%) was added to the NE stock solutions to prevent oxidation); acetylcholine chloride (to test the absence of endothelium) from a stock solution (10 mM) in de-ionized water; verapamil and **5** from DMSO (Sigma) stock solutions (100 mM) in saline (for endovenous administration) or de-ionized water (for *in vitro* experiments).

Animals

Normotensive male Wistar-Kyoto rats (WKY rats, Iffa-Credo), purchased from Criffa (Barcelona, Spain), were used throughout this study, which adhered to the "Principles of Laboratory Animal Care" (NIH publication no. 85-23, 1985). They were housed in groups of five to a macrolon cage (Panlab, Barcelona, Spain) on poplar shaving bedding (B&K Universal, G. Jordi, Barcelona, Spain) in a standard experimental animal room, illuminated from 08:00 to 20:00 h (12 h light: 12 h dark cycle) and maintained at a temperature of 22–24°C. The animals had free access to food pellets (B&K Universal, G. Jordi, Barcelona, Spain) and drinking fluid (tap water), and were allowed to acclimatize for one week before the experiments.

In Vivo Studies with Anaesthetized Normotensive Rats

Normotensive male WKY rats weighing 250–300 g were anaesthetized by intraperitoneal injection of urethane (1.25 g/10 ml, 10 ml/kg) and cannulated as described (8). Upon completion of the surgical procedures, the preparations were allowed to equilibrate, generally for 30 min. After blood pressure and heart rate had stabilized, the vehicle (appropriate DMSO dilutions in saline; 1 ml/kg for control group), and solutions of (±)-verapamil or **5** (in DMSO/saline, 1 ml/kg, for treated groups) were injected intravenously via the left femoral vein in order to observe the effects on blood pressure and heart rate.

In Vitro Experiments: Rat Aortic Rings Preparation

Vascular rings were prepared from the aortae of male WKY rats weighing 230–270 g as described (8).

Contraction Studies: General Procedure

Aorta rings were immediately transferred to an organ bath containing 20 ml of normal Krebs bicarbonate solution, thermoregulated at 37°C and bubbled with carbogen. Two stainless steel pins were inserted through the lumen of each arterial segment: one pin was fixed to the organ bath and the other was connected to a Letica TRI 201 force-displacement transducers

coupled to a Letica AMP 016/2 amplifiers, to record isometric tension on an Intel 486 DX2/66 computer, using a Letica Proto 5 software. Before initiating specific experimental protocols, aortae were equilibrated at a resting tension of 2 g for at least 1 h during which the physiological solution was replaced every 10 min.

The absence of acetylcholine (1 μM) vasorelaxant action in precontracted rings with NE (1 μM) and a simple hematoxylin-eosine staining technique were used to verify the removal of endothelial cells and the integrity of underlying smooth muscle (8).

Vasorelaxant Activity in Pre-Contracted Rat Aortic Rings

After a new equilibration period of at least 1 h, isometric contractions induced by NE (1 μM) or high K^+ (60 mM), instead of the equivalent amount of NaCl in order to maintain the osmolarity constant, were obtained. When the contraction of the tissue in response to the corresponding vasoconstrictor agent had stabilized (approximately after 10–15 min for NE and 15–20 min for KCl), increasing cumulative concentrations of the compound under study were added to the bath at approximately 10–15 min intervals (the time necessary to obtain a steady-state relaxation). Control tissues were simultaneously subjected to the same procedures, but omitting the drug and adding the vehicle (appropriate DMSO dilutions).

Studies in Ca^{2+}-Free Medium

These were carried out as previously described (8). The vasoconstrictor drugs used in these experiments were NE (1 μM) and caffeine (10 mM). In all the contraction studies, since (±)-verapamil and **5** had prolonged effects after washing, only one compound was tested in each experiment (per aortic ring).

$^{45}Ca^{2+}$ Uptake

The experimental protocol followed was that described (8). The vasoconstrictor agents used were NE (1 μM) or high K^+ (60 mM). To investigate the actions of DMSO (for control) or (±)-verapamil and (+)-, (−)-, and (±)-**5** (for treated groups) on basal and induced $^{45}Ca^{2+}$ uptake, the arteries were exposed to them 20 min before and during the incubation period with $^{45}Ca^{2+}$.

Isolated Rat Atria

Atria were dissected and prepared from male WKY rats (250–300 g), according to the general procedure described (8). The effects of (±)-verapamil and (+)-, (−)-, and (±)-**5** on the rate of contraction were studied following the protocol indicated in the above reference.

Data Presentation and Statistical Analysis

Unless otherwise specified, results shown in the tables are expressed as means ± SEM. Significant differences between two means ($P < 0.05$ or $P < 0.01$) were determined by Student's two-tailed t test for paired or unpaired data.

Hypotensive activity and effects on heart rate in anaesthetized rats are expressed as ED_{30}, the dosage of (±)-verapamil or **5** required to reduce DAP or heart rate by 30%, which was calculated by the least squares linear regression of log dosage (in mg/kg i.v.) on maximum pharmacological response (% reduction in DAP or in heart rate). In contraction studies using normal Krebs solution, concentration-response curves for the vasorelaxant effects of the compounds under study were analyzed using a sigmoidal curve-fitting analysis program (Origin 4.1) and the corresponding IC_{50} values of these drugs were calculated. In the experiments with radioactive calcium, $^{45}Ca^{2+}$ vascular tissue (rat aortic rings) uptake was calculated from the formula: $^{45}Ca^{2+}$ uptake [nmol/kg wet tissue] = [c.p.m. in tissue/kg wet tissue] × [nmol $^{45}Ca^{2+}$ in 1 liter solution/c.p.m. in 1 liter solution. The numerator of the second factor in this expression is the concentration of $^{45}Ca^{2+}$, not the total Ca^{2+} concentration. In these experiments, Ca^{2+} antagonist activity is expressed as IC_{50}, the concentration of the new compounds required to reduce NE- or high K^+ (60 mM)-induced $^{45}Ca^{2+}$ uptake by 50%, which was calculated by the least squares linear regression, using specific software, of log concentration (in μM) on maximum pharmacological response [% inhibition in NE- or high K^+ (60 mM)-induced $^{45}Ca^{2+}$ uptake]. In contraction studies using a Ca^{2+}-free medium, contractile responses induced by the different vasoconstrictor agents before and after treatment with (±)-verapamil and/or **5** are expressed in absolute tension values (mg).

In isolated rat aorta, the rate of contraction is expressed in beats/min. The IC_{50} values for the negative chronotropic effects of the tested compounds, i.e., the concentration of **5** required to reduce the rate of contraction by 50%, were calculated by the least squares linear regression of log concentration (in μM) vs. maximum pharmacological response (% inhibition of the basal rate of contraction).

RESULTS AND DISCUSSION

The syntheses of the enantiomeric forms of **5** are outline in Fig. 1. The key step in this route is the addition of an anion derived from a chiral sulfoxide (**3**) to 3,4-dimethoxyphenyl isopropyl ketone, a reaction that proceeded in high yield to give a single diastereomer of the alcohol **4**. The enantiomeric forms of the key intermediate (**3**) were obtained in high yield by the addition of 3,4-dimethoxyhomoveratrylamine to a suitably functionalized sulfoxide, either (*R*)-**1** or (*S*)-**2**. The former was obtained directly in moderate yield and >98% enantiomeric purity by biotransformation of the corresponding sulfide using *Mortierella isabellina* ATCC 42613 (6), and the latter by chemical modification of (*S*)-4-methoxyphenyl methyl sulfoxide, prepared in >98% enantiomeric purity by biotransformation of the corresponding sulfide using *Helminthosporium* species NRRL 4671 (5) as outlined in Fig. 2.

The absolute configurations at the benzylic carbinol centers of **4** and **5** derived from (*R*)- or (*S*)-**3** could not be assigned unambiguously, as both compounds are non-crystalline and we have been unable to obtain a salt of either compound suitable for X-ray crystallographic analysis. The conversion of **3** to **4**, based on a 6-membered cyclic chair-conformation transition-state involving lithium-coordinated oxygens and a strongly stabilizing π-π interaction between aromatic rings (9), is shown in Fig. 3 for the addition of (*S*)-**3** to 3,4-dimethoxyphenyl isopropyl ketone, and would result in the formation of $(S)_C$-**4**, thence (*S*)-**5** (assuming retention of configuration during the

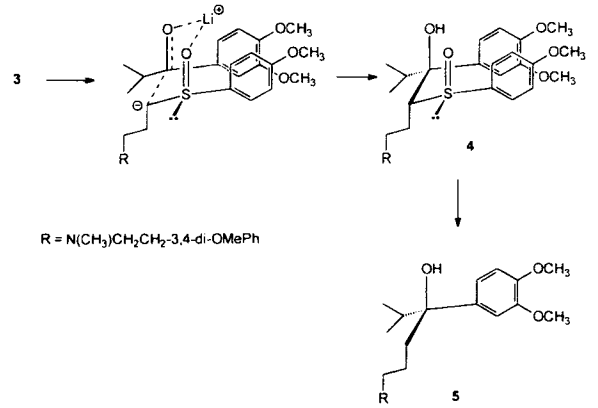


Fig. 3. Transition state for the conversion of **3** to **4** and possible stereochemical relationships for the conversion of **3** to **5**.

conversion of **4** to **5**. The (*R*)-sulfoxide (**3**) may produce $(R)_C$-**4** and thence (*R*)-**5** in an analogous way, but these stereochemical relationships should be regarded as tentative in the absence of experimental confirmation of the configurations of **4** and **5**. Conversion of the sulfoxides **4** to the verapamil analogs **5** was achieved by Raney-nickel-catalyzed desulfurization. The analogs **5**, in both enantiomeric and racemic forms, were examined in parallel with verapamil for their activity on the rat cardiovascular system. *In vivo*, using urethane-anaesthetized normotensive rats, acute administration of verapamil (0.8–2 mg/kg i.v.) and **5** (5–8 mg/kg i.v.) produced a rapid and significant reduction in normal diastolic arterial blood pressure (DAP; basal values of 80 ± 4 mmHg, n = 20), accompanied by a significant decrease in heart rate (basal values of 364 ± 8, n = 20). Verapamil was more effective than **5** in reducing both DAP and heart rate (Table I), and there were no significant differences between the *in vivo* cardiovascular effects produced by the racemic and enantiomeric forms of **5**. The enantiomers of **5** are configurationally stable in neutral solution, so the observed effect cannot be attributed to racemization of **5** under the conditions of the assay. The hypotensive activity of (*S*)-(−)-verapamil has been reported to be slightly greater than that of (*R*)-(+)-verapamil in the anaesthetized rat (10).

The hypotensive effect produced by verapamil and **5** was, however, not accompanied by the reflex tachycardia typical after treatment with a number of hypotensive agents (11). This may be due to the fact that urethane inhibits the cardiovascular reflexes responsible for hypotensive drug-induced reflex tachycardia in conscious normotensive rats (11). For this reason, we consider that our observations are attributable to a direct effect of **5** on the heart of anaesthetized normotensive rats.

We have also examined the pharmacological effects of **5** on endothelium-denuded rat aorta and rat atria, in order to ascertain the correlation with the cardiovascular activity observed *in vivo*, and to investigate the selectivity of the *in vitro* action of these compounds. High K^+ concentrations have been reported to cause contractions in vascular smooth muscle by depolarizing cell membranes and by increasing the influx of Ca^{2+} through L and T voltage-dependent channels which, in turn, may induce CICR (Ca^{2+}-induced Ca^{2+} release) from intracellular stores (12). It has also been shown that the activation of several postsynaptic α_1-adrenoceptors by norepinephrine (NE) in rat aorta induces a biphasic contraction (13): an initial transient contraction produced by IP_3-mediated release of Ca^{2+} from intracellular stores (via activation of inositol 1,4,5-triphosphate (IP_3) specific receptors), and a slow, sustained contraction, caused by Ca^{2+} influx through the controversial so-called receptor-operated Ca^{2+} channels (for a review see ref. 12).

In the present work, both (±)-verapamil and **5** relaxed, in a concentration-dependent fashion, the contractions induced by NE and high K^+ (60 mM) in rat aortic rings (Table II). The IC_{50} values exhibited by **5** against the contractions induced by NE and KCl were significantly higher than those found for (±)-verapamil. These results suggest a reduced potency for the former as vasodilators in rat aorta. Again, there were no significant differences between the quantitative vascular effects produced by the stereoisomers of **5** in rat aorta (Table II, $P > 0.05$, n = 5).

On the other hand, both **5** and verapamil produced greater reduction in the KCl than in the NE-induced contraction ($P < 0.01$, n = 5), indicating that the NE- and high KCl-induced contractions may involve different mechanisms (see above). This supports previous studies with verapamil (14). It should be noted, however, that contradictory results have been previously reported for the vasorelaxant effects of nifedipine and other classical calcium antagonists on NE- and high KCl-induced contractions in vascular smooth muscle (15).

The results obtained in the contraction studies in rat aorta also suggest (admitting the classical mechanism of contraction of NE and K^+) that the vasorelaxant effects exhibited by both (±)-verapamil and **5** in rat aortic rings may be due to a non-selective vasodilator action, possibly related to either an intracellular activity, or to a non-selective effect of the new compounds on the cell membrane. The lack of intracellular effects

Table I. ED_{30} Values (mg/kg i.v.) for the Effects of (±)-verapamil and **5** on DAP and Heart Rate in Anaesthetized Normotensive Rats

Compound	DAP	Heart rate
DMSO dilutions	—	—
(±)-verapamil	1.16 ± 0.08	0.99 ± 0.07
(±)-**5**	6.6 ± 0.45*	7.0 ± 0.51*
(+)-**5**	6.3 ± 0.46*	6.6 ± 0.49*
(−)-**5**	6.8 ± 0.49*	7.2 ± 0.54*

Note: Level of statistical significance: *$P < 0.01$ with respect to the corresponding ED_{30} of (±)-verapamil. Each value represents the mean ± SEM from 5 experiments.

Table II. IC_{50} Values (μM) for (±)-verapamil and **5**-induced Vasorelaxation in Endothelium-Denuded Rat Aortic Rings Pre-Contracted with NE (1 μM) or KCl (60 mM)

Compound	NE	KCl
DMSO dilutions	—	—
(±)-verapamil	0.85 ± 0.054	0.24 ± 0.017
(±)-**5**	7.01 ± 0.51[a]	0.79 ± 0.053[b]
(+)-**5**	6.93 ± 0.48[a]	0.77 ± 0.055[b]
(−)-**5**	7.26 ± 0.53[a]	0.82 ± 0.052[b]

Note: Level of statistical significance: [a]$P < 0.01$ *versus* the IC_{50} of verapamil against NE-induced contractions; [b]$P < 0.01$ with respect to the IC_{50} of verapamil against KCl-induced contractions. Data represent the mean ± SEM from 5 experiments.

of **5** was apparent from experiments carried out in a Ca^{2+}-free medium. Under these conditions NE and caffeine induce, via different mechanisms, a contractile response in the vascular smooth muscle which is only due to the release of intracellular Ca^{2+} from the internal stores sensitive to the agonists (12). NE (1 μM) produces a characteristic contraction with two distinct components: an initial transient contraction (fast component) that relaxes to a sustained tension (slow component). Caffeine (10 mM), however, produces only an initial and fast transient contraction (Table III). The vehicle (appropriate dimethyl sulfoxide (DMSO) dilutions), verapamil and **5** had no effect on NE- and caffeine-induced contractions in calcium-free medium, which suggests that these compounds do not act intracellularly (12). Similar results have been previously described for a number of calcium antagonists (15).

The fact that verapamil and the analog **5** relaxed the NE- and high KCl-induced contractions would also tend to suggest the existence of a non-selective mechanism of action for both (±)-verapamil and **5** on the cell membrane, involving (among other mechanisms), the opening of K^+ channels and/or the blockage of Ca^{2+} influx through both voltage-dependent and receptor-operated Ca^{2+} channels. The effects of **5** on the smooth muscle cell membrane of rat aorta does not seem to be due to the opening of ATP-sensitive K^+ channels (K_{ATP}), since cromakalim and other K_{ATP} agonists do not inhibit contractions induced by KCl at concentrations greater than about 30 mM in vascular smooth muscle. This latter effect is due to the fact that, when the cell is depolarized by an increased extracellular KCl concentration (>30 mM), the membrane potential approaches the Nernst equilibrium potential for K^+. Consequently, the opening of K_{ATP} by cromakalim (or any other K^+ channel opener) will then have very little influence on membrane potential (16).

The action of **5** on transmembrane Ca^{2+} channels is demonstrated by experiments with radiolabeled calcium. Basal $^{45}Ca^{2+}$ uptake, the amount of Ca^{2+} entering by means of leak channels (12), did not change on the addition of verapamil or **5**, whereas the same compounds strongly inhibited the uptake of $^{45}Ca^{2+}$ induced by NE and K^+ (60 mM) (Tables IV and V). These results suggest that the compounds may inhibit, at least in part, the contractions induced by NE and high KCl in rat aorta by blocking transmembrane Ca^{2+} influx through voltage- and receptor-dependent Ca^{2+} channels. These results also show that both **5** and verapamil inhibit high KCl-induced $^{45}Ca^{2+}$ uptake at similar concentrations to those that are required to antagonize NE-induced uptake, as previously described for verapamil and other calcium antagonists (15,17).

Table III. Effects of DMSO (1.4 mM), (±)-verapamil (10 μM), and **5** (10 μM) on the Contractions (mg) Induced by NE (1 μM) or Caffeine (10 mM) in Rat Aortic Rings Deprived of Endothelium in a Ca^{2+}-Free Medium

Compound	NE (fast)	NE (slow)	Caffeine
Control	446 ± 24	138 ± 9	128 ± 10
DMSO	461 ± 27	141 ± 11	125 ± 8
(±)-verapamil	443 ± 26	147 ± 14	136 ± 11
(±)-**5**	462 ± 21	134 ± 10	122 ± 9
(+)-**5**	454 ± 25	145 ± 12	135 ± 12
(−)-**5**	438 ± 20	136 ± 11	131 ± 11

Note: Data are presented as means ± SEM of 5 experiments.

Table IV. Effects of DMSO (0.14 mM), (±)-verapamil (0.3 μM), and **5** (1 μM) on Basal and NE (1 μM)- or KCl (60 mM)-Induced $^{45}Ca^{2+}$ Uptake (nmol/kg) in Rubbed Rat Aortic Rings

Compound	Basal	NE	KCl
Control	21.9 ± 1.58	37.9 ± 2.54*	44.8 ± 3.05*
DMSO (0.1 M)	21.6 ± 1.47	38.1 ± 2.33*	44.5 ± 3.16*
(±)-verapamil	22.1 ± 1.59	28.26 ± 1.89**	31.05 ± 2.21**
(±)-**5**	21.4 ± 1.50	27.51 ± 1.97**	30.4 ± 2.04**
(+)-**5**	22.3 ± 1.71	27.16 ± 1.70**	29.93 ± 2.12**
(−)-**5**	22.0 ± 1.68	28.14 ± 1.86**	31.14 ± 2.20**

Note: Level of statistical significance: *$P < 0.01$ with respect to the corresponding basal $^{45}Ca^{2+}$ uptake value; **$P < 0.01$ versus the corresponding NE- or high KCl-induced $^{45}Ca^{2+}$ uptake. Data are presented as means ± SEM of 5 experiments.

Finally, it should be pointed that the calcium antagonist activity shown by verapamil and **5** in rat aorta smooth muscle cells could explain, at least in part, the decrease in the automaticity of sinus node pacemaker cells (negative chronotropic effects) produced *in vitro* (isolated rat atria) by these compounds (Table VI). Similar inhibitory effects were previously described for verapamil in the same cardiac tissue (18). According to the IC_{50} values obtained, the inhibitory activities (negative chronotropic effects) of the stereochemical forms of **5** were similar, but lower than that of verapamil. In addition, a good correlation (in order of potency) between the effective doses of verapamil and **5** required to reduce heart rate by 30% *in vivo* (anaesthetized normotensive rats) and the IC_{50} values obtained in isolated rat atria was found. On the other hand, the IC_{50} values of verapamil and **5** necessary to reduce the rate of contraction in isolated rat atria were approximately 3- to 30-fold higher than the IC_{50} needed to relax the contractions induced by NE and high KCl in rat aortic rings (see Tables II and VI).

The verapamil analogs **5** have therefore been characterized as agents with a remarkable hypotensive activity in anaesthetized normotensive rat (accompanied by a significant bradycardia) and clear inhibitory effects (vasodilator and negative chronotropic effects) on rat aortic rings and isolated rat atria, effects probably due to transmembrane Ca^{2+}-antagonist properties on smooth muscle and sinus node packemaker cells. A good correlation was found between the results obtained *in vivo* (anaesthetized normotensive rats) and *in vitro* (isolated rat aorta

Table V. IC_{50} Values (μM) for the Effects of (±)-verapamil and **5** on NE (1 μM)- or KCl (60 mM)-Induced $^{45}Ca^{2+}$ Uptake in Endothelium-denuded Rat Aortic Rings

Compound	NE	KCl
DMSO dilutions	—	—
(±)-verapamil	0.24 ± 0.016	0.27 ± 0.019
(±)-**5**	0.79 ± 0.054*	0.81 ± 0.051**
(+)-**5**	0.77 ± 0.049*	0.80 ± 0.054**
(−)-**5**	0.80 ± 0.055*	0.84 ± 0.06**

Note: Level of statistical significance: *$P < 0.01$ versus the IC_{50} of verapamil against NE-induced uptake; **$P < 0.01$ with respect to the IC_{50} of verapamil against KCl-induced uptake. Data represent the mean ± SEM from 5 experiments.

Table VI. IC_{50} Values (μM) for the Negative Chronotropic Effects of (±)-verapamil and **5** in Isolated Rat Atria

Compound	IC_{50} values
DMSO dilutions	—
(±)-verapamil	3.05 ± 0.22
(±)-**5**	20.87 ± 1.49*
(+)-**5**	19.68 ± 1.32*
(−)-**5**	22.44 ± 1.53*

Note: Level of statistical significance: *$P < 0.01$ versus the IC_{50} of verapamil. Basal rate of contraction = 306 ± 4 beats/min (n = 20). Data represent the mean ± SEM from 5 experiments.

and rat atria), which suggest that: (a) if the analogs exhibit a similar behavior in resistance blood vessels, their hypotensive activity in anaesthetized normotensive rats may be due, at least in part, to a decrease in peripheral vascular resistance as a result of a direct vasodilator effect on vascular smooth muscle cells, and (b) the calcium antagonist activity noted in isolated rat atria seems to be responsible for the bradycardia observed *in vivo*.

The new compounds show a pharmacological profile similar to that exhibited by verapamil. Given that both verapamil and **5** display a *in vitro* calcium antagonist activity, we may conclude that, if the analogs **5** possess a favorable LD_{50}/DE_{50} ratio, they could have interesting therapeutic potential and a promising future as drugs active on the cardiovascular system. In addition, the enantiomers of **5** can be prepared by a synthetic sequence that is much shorter and more convenient than that currently employed for the enantiomers of verapamil (4), and which may be readily modified for the synthesis of further analogs for the study of structure-activity relationships to generate new, less toxic and more efficient cardio- and vasoactive agents in chiral form.

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