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Synthesis of 1-alkyl-3-dialkylaminoalkylamine[1]benzothieno[2,3-*b*]pyrazin-2(1*H*)-ones and their ability to antagonize KCl-induced contractions

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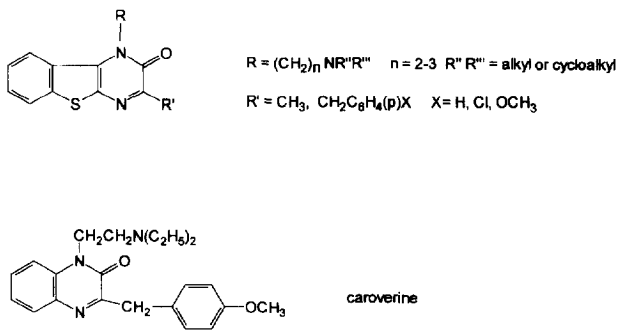
Summary — A series of 1-alkyl-3-dialkylaminoalkylamino[1]benzothieno[2,3-*b*]pyrazin-2(1*H*)-ones **5–35** was synthesized. Some of these compounds showed appreciable inhibition towards KCl-induced contractions in isolated rat aortic rings. The results obtained showed that, although this activity appears at higher concentrations than with caroverine, used as reference standard, this new tricyclic system could be considered as a nucleus with potential calcium antagonistic activity.

[1]benzothieno[2,3-*b*]pyrazine / Ca^{2+}-entry blocker / caroverine

Introduction

In a previous study [1], the authors described the synthesis of some 1-3 substituted benzothienopyrazin-2-ones structurally correlated to caroverine(1-diethyl aminoethyl-3-(*p*-methoxybenzyl)-1,2-dihydro quinoxaline-2-one, scheme 1). Caroverine is a molecule that has been described in the literature [2] as having antispasmodic, broncholytic and hypotensive properties. Further works [3–5] have demonstrated that these pharmacological properties can be attributed to a calcium-mediated mechanism. The main conclusion drawn here is that caroverine substituents in the benzothienopyrazinone series appear as having the weakest effect in inhibiting KCl-induced contractions in isolated rat aortic rings. Furthermore, the substituents in the benzothienopyrazinone series that have a greater effect, even if it is still weaker than that of caroverine, led to compounds that were much less active when transferred to the bicyclic (quinoxalinone and thienopyrazinone) series [6].

This work sets out to describe both the pharmacological activity of derivatives **5–19**, previously synthesized by the authors [7], and the synthesis and pharmacological activity of derivatives **20–35**, with the aim of accurately evaluating the influence of the benzothienopyrazinone system. The derivatives **5–34** are characterized by the presence of a hydrogen or an alkyl group (CH_3, C_3H_7 or C_5H_{11}) in position 1 of the pyrazinone nucleus and by a basic substituent in position 3. This is true for all compounds except compound **35**, which has a basic substituent in both positions. The basic nature of the substituents can be traced back to the observations of other authors [8] who have divided Ca^{2+} antagonists into two main



Scheme 1.

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groups, the first being the dihydropyridine group, the second including all compounds with a basic side chain (verapamil, diltiazem, bepridil, flunarizine, lidoflazine and prenylamine).

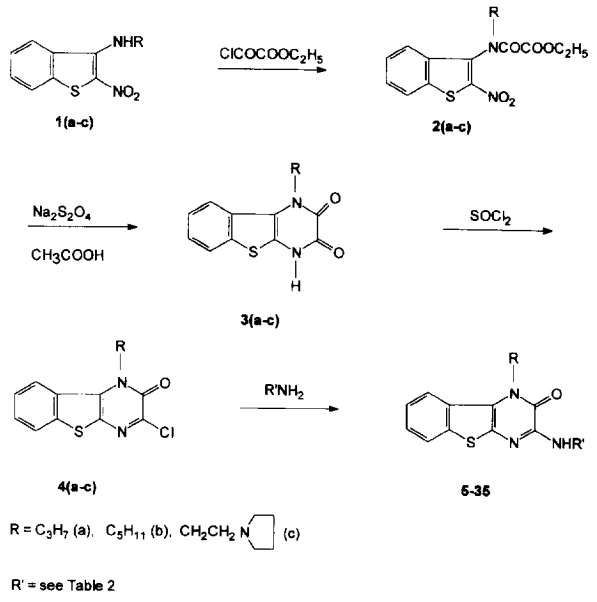
Chemistry

New derivatives **20–35** were synthesized following the procedure described for compounds **5–19** [7] as summarized in scheme 2. Compound **28** needed further steps, as described in scheme 3 and in the *Experimental protocols*. *N*-Acetyl derivatives **29** and **30** were prepared from amines **7** and **13** respectively, by refluxing in acetic anhydride.

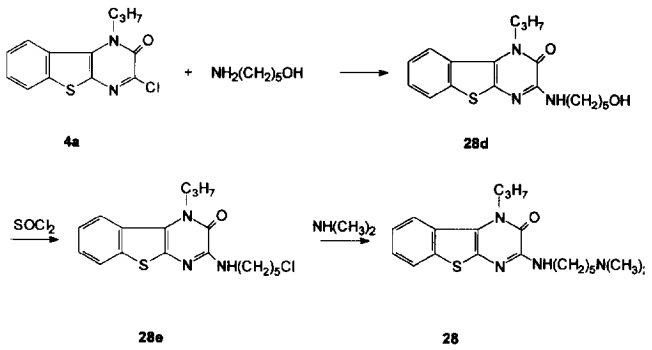
The structures were confirmed both by analytical data and IR and ^{1}H NMR spectra listed in table II with mp and yields.

Pharmacology

Calcium antagonism was examined in vitro by studying the inhibition of KCl-induced contractions in isolated rat aortic rings. These results are given in tables I and III, expressed as the percentage inhibition at a dose of 10^{-5} mol, a concentration that for most of active compounds produced the maximal effect. Only those compounds which halved KCl-induced contractions were then processed for the calculation of their IC_{50} values. The antagonistic effect of caroverine was



Scheme 2.



Scheme 3.

determined for comparison at 10^{-6} mol dose as described in the literature [5].

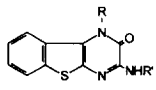
Results and discussion

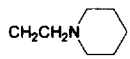
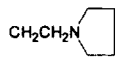
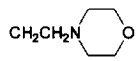
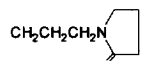
In this work we began testing derivatives **5–19** [7]. Many of these compounds (**7**, **10**, **12** and **13**) showed a high percentage of inhibition, reaching 89.9% in the case of compound **7** (table I). The most obvious general rule was that, no matter what type of amine was present in position 3, increased action was related to the substituent in position 1 as follows: H < CH_3 < C_3H_7. In the 1-propyl derivatives activity continued when position 3 (R', table I) was replaced by a piperidinylethyl or pyrrolidinylethyl group (**10** and **13**), whilst it diminished when the same position was replaced by a morpholinylethyl or 2-*oxo*-pyrrolidinylpropyl group (**16** and **19**). This is probably due to the reduction in hydrophobic properties in these two compounds.

Out of compounds **20–35**, **20** and **21**, where an aliphatic alkylene group was still present, were of greater interest than **22–26**, where an aromatic alkylene substituent had been introduced. In compounds **27** and **28** a longer alkylene chain, positioned between the two nitrogen atoms, caused a reduction in activity compared to compound **7**. For compounds **7** and **13**, acetylation brought about a more noticeable reduction in activity (see compounds **29** and **30**), thus confirming the importance of a free NH group. The introduction of a pentyl instead of a propyl radical in position 1 (compounds **31** and **34**) also brought about a reduction in activity compared to the 1-propyl derivatives (**7**, **12**, **16** and **21**). Lastly compound **35**, characterized by the presence of basic substituents in both positions of the pyrazinone nucleus, showed the sharpest reduction in activity.

Overall we can say that compounds **7** and **13** showed the greatest capacity for inhibiting KCl-

Table I. Calcium-blocking activity on KCl (100 mmol)-stimulated rat aortic strips at 10^{-5} mol for derivatives **5–19** ($n = 8$) and 10^{-6} mol for caroverine [5].



Compound	*R*	*R'*	*% Inhibition*	IC_{50}
5	H	$CH_2CH_2N(CH_3)_2$	5 ± 0.25	
6	CH_3	"	20 ± 0.9	
7	C_3H_7	"	89.9 ± 0.38	3.5×10^{-6}
8	H		62.6 ± 4.81	1.7×10^{-5}
9	CH_3	"	66.6 ± 8.2	1.8×10^{-5}
10	C_3H_7	"	70 ± 1.2	1.4×10^{-5}
11	H		20 ± 0.57	
12	CH_3	"	71.33 ± 2.02	2×10^{-5}
13	C_3H_7	"	80.9 ± 3.13	4.3×10^{-6}
14	H		40 ± 1.15	
15	CH_3	"	45.3 ± 2.1	
16	C_3H_7	"	55.1 ± 1.2	4.3×10^{-5}
17	H		18 ± 0.3	
18	CH_3	"	25 ± 0.88	
19	C_3H_7	"	48 ± 1.73	
Caroverine			70 ± 3.1	2.19×10^{-7}

induced contractions (relative to the maximum effect), whilst compounds **20** and **21** were also of interest statistically.

To conclude, although previously-synthesized compounds [1] were structurally closer to caroverine, the most active compounds contain these new benzothienopyrazinone nucleus derivatives, even though activity occurs at concentrations ten times stronger than for our reference drug (caroverine).

Experimental protocols

Chemistry

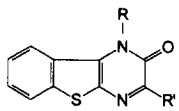
Melting points were determined on a Büchi 510 apparatus and are uncorrected. Elemental analyses were performed on a C Erba Model 1106 elemental analyser, and the data of C, H, N are within ±0.4% of calculated values. Thin layer chromatography (TLC) was used to monitor reactions. IR spectra were recorded as KBr pellets using a Perkin-Elmer 281 spectrophotometer. ^1H NMR spectra were obtained on a Brucker WP 80 spectrometer in $CDCl_3$ and have been expressed as δ units (ppm) compared with TMS as internal standard.

The synthesis of the compounds described below was achieved following the methods we have used previously [7, 9]; this section reports the chemical and physical data for the new compounds and a description of the mode of synthesis where modification was necessary.

2-Nitro-N-pentylbenzo[b]thiophene ***1b***. Yield 80%; crystallization solvent: ethanol; mp 129–131 °C, IR 3200, 1550, 1340 cm^{-1}.

N-Pentyl-N(2-nitrobenzo[b]thien-3-yl)oxalinate ***2b***. Yield 72.5%; crystallization solvent: ethanol; mp 95–97 °C, IR 1765, 1680, 1540, 1340 cm^{-1}.

Table II. Physical data of derivatives **20–35**.



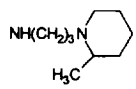
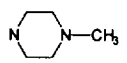
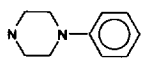
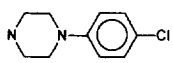
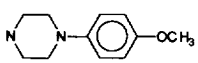
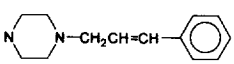
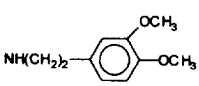
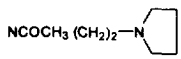
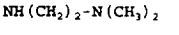
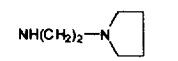
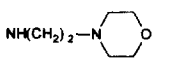
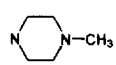
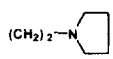
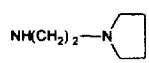
Compound	*R*	*R'*	*Mp (cryst solv)*	*Yield (%)*	*IR (KBr) (cm^{-1})*	*^{1}H NMR (DMSO) (δ, ppm)*
20	nC_3H_7		122–124 (A)	58	3320 1630	0.91 (d, 3H, C*H*$_3$ pip); 1.13 (t, 3H, C*H*$_3$ C*H*$_3$ prop); 1.99 (m, 2H, CC*H*$_2$C); 1.25–2.90 (m, 13H, C*H*$_2$ chain and pip); 3.55 (dt, 2H, J = 6.8 and 6.6 Hz, NHC*H*$_2$); 4.51 (m, 2H, NC*H*$_2$); 7.18 (t, 1H, J = 6.6 Hz, N*H*); 7.38–7.86 (m, 5H, arom)
21	"		118–120 (A)	60	2980 2760 1645	1.13 (t, 3H, C*H*$_3$ prop); 1.94 (sex, 2H, CC*H*$_2$C); 2.34 (s, 3H, NC*H*$_3$); 2.57 (t, 4H, piperaz); 3.98 (t, 4H, piperaz); 4.48 (m, 2H, NC*H*$_2$); 7.31–8.19 (m, 4H, arom)
22	"		105 dec (A)	58	3040 1650	1.14 (t, 3H, C*H*$_3$ prop); 1.96 (sex, 2H, CC*H*$_2$C); 3.33 (t, 4H, piperaz); 4.13 (t, 4H, piperaz); 4.50 (m, 2H, NC*H*$_2$); 6.88–7.92 (m, 9H, arom)
23	"		176–178 (A)	40	3030 1650	1.14 (t, 3H, C*H*$_3$ prop); 1.96 (sex, 2H, CC*H*$_2$C); 3.29 (t, 4H, piperaz); 4.12 (t, 4H, piperaz); 4.51 (m, 2H, NC*H*$_2$); 6.81–7.93 (m, 8H, arom)
24	"		146–148 (A)	50	3030 1645	1.14 (t, 3H, C*H*$_3$ prop); 1.96 (sex, 2H, CC*H*$_2$C); 3.22 (t, 4H, piperaz); 3.77 (s, 3H, OC*H*$_3$); 4.13 (t, 4H, piperaz); 4.50 (m, 2H, NC*H*$_2$); 6.78–7.92 (m, 8H, arom)
25	"		190 dec (A)	40	3020 1635	1.13 (t, 3H, C*H*$_3$ prop); 1.96 (sex, 2H, CC*H*$_2$C); 2.92 (t, 4H, piperaz); 3.46 (d, 2H, J = 6.3 Hz, C*H*$_2$CH=CH); 4.19 (t, 4H, piperaz); 4.49 (m, 2H, NC*H*$_2$); 6.37 (dt, 1H, J = 14.3 and 6.3 Hz, CH$_2$-C*H*=CH); 6.68 (d, 1H, J = 14.3 Hz, CH$_2$CH=C*H*); 7.26–7.93 (m, 9H, arom)
26	"		173–175 (A)	83	3315 1630	1.11 (t, 3H, C*H*$_3$ prop); 1.95 (sex, 2H, CC*H*$_2$C); 2.93 (t, 2H, J = 6.6 Hz, C*H*$_2$C$_6$H$_5$); 3.74 (dt, J = 6.6 and 6.0 Hz, 2H, NHC*H*$_2$); 3.86 (s, 3H, OC*H*$_3$); 3.89 (s, 3H, OC*H*$_3$); 4.52 (m, 2H, NC*H*$_2$); 6.55 (t, 1H, J = 6.0 Hz, N*H*); 6.81–7.84 (m, 7H, arom)
27	"	$NH(CH_2)_3N(CH_3)_2$	145–147 (A)	60	3320 1630	1.12 (t, 3H, C*H*$_3$ prop); 1.76–2.09 (m, 4H, C*H*$_2$ prop); 2.28 (s, 3H, N(C*H*$_3$)$_2$); 2.40 (dt, 2H, J = 6.8 and 6.6 Hz C*H*$_2$N); 3.59 (dt, 2H, J = 6.6 and 6.0 Hz, NHC*H*$_2$); 4.52 (m, 2H, NC*H*$_2$); 7.18 (t, 1H, J = 6.0 Hz, N*H*); 7.32–7.86 (m, 5H, arom)

Table II. *(Continued.)*

Compound	*R*	*R'*	*Mp (cryst solv)*	*Yield (%) (%)*	*IR (KBr) (cm^{-1})*	*^{1}H NMR (DMSO) (δ, ppm)*
28	"	$NH(CH_2)_5N(CH_3)_2$	198–200 (B)	50	3320 1635	1.12 (t, 3H, CH_3 prop); 1.90–2.09 (m, 8H, CH_2 propyl and pentyl); 2.81 (s, 6H, $N(CH_3)_2$); 2.79–3.13 (m, 2H, CH_2N); 3.55 (dt, 2H, J = 6.3 and 6.0 Hz, $NHCH_2$); 4.52 (m, 2H, NCH_2); 6.65 (t, 1H, J = 6.3 Hz, NH); 7.26 –7.87 (m, 4H, arom)
29	"	$NCOCH_3(CH_2)_2N(CH_3)_2$	112–114 (C)	60	1690 1650	1.16 (t, 3H, CH_3 prop); 1.96 (sex, 2H, CCH_2C); 2.04 (s, 6H, $N(CH_3)_2$); 2.10 (s, 3H, CH_3 acetyl); 2.49 (t, 2H, CH_2N); 4.05 (t, 2H, CH_2N acetyl); 4.60 (m, 2H, NCH_2); 7.43–8.15 (m, 4H, arom)
30	"		149–151 (A)	74	1695 1655	1.16 (t, 3H, CH_3 prop); 1.27–1.42 (m, 4H, CH_2 pyrr); 1.95 (sex, 2H, CCH_2C); 2.10 (s, 3H, CH_3 ac); 2.23–2.42 (m, 4H, CH_2 pyrr); 2.67 (t, 2H, CH_2N); 4.08 (t, 2H, CH_2Nac); 4.56 (m, 2H, NCH_2); 7.38–8.21 (m, 4H, ar)
31	n-C_5H_{11}		138–140 (A)	45	3320 1635	0.94 (t, 3H, CH_3 pent); 1.12–1.99 (m, 6H, CH_2 pent); 2.33 (s, 6H, $N(CH_3)_2$); 2.64 (t, 2H, J = 6.2 Hz, CH_2N); 3.61 (dt, 2H, J = 6.2 and 5.9 Hz); 4.56 (m, 2H, NCH_2); 6.92 (t, 1H, J = 5.9 Hz, NH); 7.98 (m, 5H, arom)
32	"		135–137 (A)	32	3320 1630	0.95 (t, 3H, CH_3 pent); 1.22–1.69 (m, 4H, CH_2 pent); 1.72–2.21 (m, 6H, CH_2 pent and pirr); 2.57–2.79 (m, 3H, CH_2 pyrr); 2.83 (t, 3H, J = 6.2 Hz, CH_2N); 3.67 (dt, 2H, J = 6.2 and 5.7 Hz, $NHCH_2$); 7.27 (t, 1H, J = 5.7 Hz, NH); 7.29–7.84 (m, 4H, arom)
33	"		132–134 (A)	45	3330 1630	0.94 (t, 3H, CH_3 pent); 1.18–2.32 (m, 6H, CH_2 pent); 2.66–2.92 (m, 4H, CH_2 morf); 2.88 (t, 2H, J = 6.3 Hz, CH_2N); 3.81 (dt, 2H, J = 6.3 and 5.8 Hz, $NHCH_2$); 3.78–3.96 (m, 4H, CH_2O); 4.54 (m, 2H, NCH_2); 7.01 (t, 1H, J = 5.8 Hz, NH); 7.37–7.98 (m, 4H, arom)
34			225 dec (D)	40	2980 2740 1650	0.95 (t, 3H, CH_3 pent); 1.15–2.29 (m, 6H, CH_2 pent); 2.87 (s, 3H, NCH_3); 2.82–3.61 (m, 8H, piperaz); 4.53 (m, 2H, NCH_2); 7.38–8.16 (m, 4H arom)
35			148–150 (C)	60	3320 1630	1.72–1.96 (m, 8H, CH_2 pyrr); 2.57–2.81 (m, 8H, CH_2N pyrr); 2.85 (t, 2H, J = 6.2 Hz, CH_2N[R']); 2.83–3.07 (m, 2H, CH_2N[R]); 3.67 (dt, 2H, J = 6.2 and 5.8 Hz, $NHCH_2$); 4.75 (m, 2H, NCH_2); 7.20 (t, 1H, J = 5.8 Hz, NH); 7.28–8.11 (m, 4H, arom)

A = Ethanol; B = cromatographic column, C = ligroin, D = acetonitrile.

Table III. Calcium blocking activity on KCl (100 mmol)-stimulated rat aortic strips at 10^{-5} mol (n = 8) for derivatives **20–35** and 10^{-6} mol for caroverine at [5].

Compound	*% Inhibition*	IC_{50}
20	72.5 ± 2.5	1.5×10^{-6}
21	75.28 ± 2.9	1.6×10^{-6}
22	62 ± 1.8	1.7×10^{-5}
23	61 ± 2.3	2.3×10^{-5}
24	55 ± 0.25	3.1×10^{-5}
25	45 ± 1.5	
26	58 ± 3.1	2.1×10^{-5}
27	60 ± 2.1	3.1×10^{-5}
28	52 ± 1.3	1.7×10^{-5}
29	63 ± 1.8	2.19×10^{-5}
30	45 ± 0.3	
31	49 ± 1.5	
32	52 ± 0.9	1.8×10^{-5}
33	49 ± 1.3	
34	40 ± 2.5	
35	38 ± 2.3	
Caroverine	70 ± 3.1	2.19×10^{-7}

N-Pyrrolidinoethyl-N-(2-nitrobenzo[b]thien-3-yl)oxalinate ***2c***. Yield 77.8%; crystallization solvent: cyclohexane; mp 107–109 °C, IR 2900–2700, 1750, 1710, 1540, 1340 cm^{-1}.

1-Pentyl-1,2,3,4-tetrahydro[1]benzothieno[2,3-b]pyrazine-2,3-dione ***3b***. Yield 78%; mp >260 °C, crystallization solvent: ethanol; IR 3100, 1710, 1660 cm^{-1}.

1-Pyrrolidinoethyl-1,2,3,4-tetrahydro[1]benzothieno[2,3-b]-pyrazine-2,3-dione ***3c***. Yield 55%; mp 230–232 °C, crystallization solvent: DMF; IR 3600–3300, 1695, 1675 cm^{-1}.

1-Pentyl-3-chloro[1]benzothieno[2,3-b]pyrazine-2(1H)-one ***4b***. Yield 67%; crystallization solvent: ethanol; mp 137–139 °C, IR 2980–2890, 1670 cm^{-1}.

1-Pyrrolidinoethyl-3-chloro[1]benzothieno-[2,3-b]pyrazine-2-(1H)-one ***4c***

This reaction was carried out with $POCl_3$ and was refluxed for 2 h. $POCl_3$ was then concentrated in vacuum and the residue dissolved in ethanol and treated with water and 5% NaOH until precipitation occurred. Yield 73%; crystallization solvent: cyclohexane; mp 132–135 °C, IR 2900–2700 cm^{-1}.

1-Propyl-3-dialkylamino-N-acetylamino[1]benzothieno[2,3-b]pyrazine-2(1H)-ones ***29 and 30***

A solution of derivative **7** or **13** 0.7 mmol was kept in acetic anhydride and refluxed for 1 h. The acetic anhydride having concentrated, the residue was dissolved in water and alkalinized with 5% NaOH; the solid which separated was filtered, washed, dried and crystallized as shown in table II.

Synthesis of 1-propyl-3-dimethylaminopentylamino[1]benzothieno[2,3-b]pyrazine-2(1H)-one ***28*** *(scheme 3)*

A solution of 0.4 g (1.4 mmol) **4a** [7] and 0.39 g (3.7 mmol) 5-amino-1-penthanol in 30 mL toluene was refluxed for 2 h. After cooling at room temperature, the solid that separated was filtered, washed, dried and crystallized with ethanol to give 0.4 g (81%) 1-propyl-3-(5-hydroxy-1-pentylamino) [1]benzothieno[2,3-*b*]pyrazine-2(1*H*)-one **28d**. Mp 176–178 °C; IR 3600–3300, 2940–2860, 1630 cm^{-1}.

$SOCl_2$ (10 mmol) was added to a solution of **28d** in 30 mL $CHCl_3$ and the mixture was refluxed for 1 h. The solvent was then removed in vacuo and the residue crystallized with ethanol to give 0.2 g (47.6%) 1-propyl-(5-chloro-1-pentylamino)-[1]benzothieno[2,3-*b*]pyrazine-2-one **28c**. Mp 103–105 °C, IR 3320, 2940–2860, 1635 cm^{-1}.

A mixture of 0.2 g of the above compound and 5 mL of *N,N*-dimethylamine was warmed at 90 °C for 20 h. On adding water, a solid separated and was filtered, washed, dried and purified in a chromatographic column using 0.063–0.200-mm silica gel and a mixture of $CHCl_3$/MeOH, 9.5:0.5 (v/v) as eluent. From this column we collected 0.1 g of derivative **28**. Analytical and spectroscopic data are given in table II.

Pharmacological evaluation

Rat aortic rings

These experiments were carried out on normal Wistar rats. Immediately after the animal's sacrifice, the thoracic aorta was carefully cleaned of adhering fat and connective tissue, removed and cut into 2–3-mm-wide rings. A maximum of four rings was obtained from each aorta. The endothelium was gently removed by rubbing the rings four or five times over steel wires, in order to avoid any endothelium-relaxing interference during the relaxation studies [10]. The rings were incubated in a 10-mL tissue bath containing an oxygenated (95% O_2 + 5% CO_2) physiological salt solution (PSS) made up of the following in mmol: NaCl 118; KCl 4.75; $CaCl_2 \cdot 2H_2O$ 2.25; KH_2PO_4 1.19; $MgSO_4 \cdot 7H_2O$ 1.19; $NaHCO_3$ 12.5; and glucose 10. The solution was thermostatically maintained at 37 °C (±0.25 °C) [11]. The rings were connected to a Letica TRI 110 isometric transducer and a Basile Gemini chart recorder by stainless steel hooks (45 μm diameter) and silk ligatures. Tissues were allowed to equilibrate at a 1 g resting tension for a period of 60 min, the PSS being replaced every 20 min. In order to be assured of endothelium removal, we first tested the response to acetylcholine on norepinephrine-induced contractions; only those rings showing less than 10% relaxation were used.

In order to obtain calcium concentration–response curves, preparations were pre-incubated for 30 min in Ca-free PSS and depolarized with a 100 mmol K-solution from which the $CaCl_2$ had been removed. The $CaCl_2$ was then added cumulatively, the tested $CaCl_2$ concentrations being as follows: 0.05, 0.1, 0.5, 1.25, 2.5, 5 and 10 mmol [12].

In relaxation experiments, caroverine chloridrate (Donau Pharmazie) or tested compounds were cumulatively added (half-$\log_{10}$ increasing stepwise concentration) when the plateau of contractions induced by 100 mmol KCl had been reached. In control experiments the contractions induced by 100 mmol KCl were followed for a length of time equal to that required for the completion of the concentration–response curves to vasorelaxants (approximately 60 min). The calculation of relaxation induced by assayed drugs could then be corrected for the time-dependent fade in tension of 100 mmol KCl.

Statistical evaluation
The results are presented as mean values ± se. All contractile responses are expressed as a percentage of the maximal increase in tension induced by 100 mmol KCl (E_{max} = 100%). Relaxation responses were calculated as a percentage of the induced tension existing at the start of a relaxant experiment. IC_{50} values were determined by a least-squares non linear regression analysis using a specific computer program (Origin, from MicroCal Software Inc), and were evaluated only for those compounds which exhibited a maximum percentage of inhibition >50%.

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