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Preliminary communication

Negative inotropic activity of *para*-substituted diethyl benzylphosphonates related to fostedil *

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Summary — Several *p*-substituted diethyl benzylphosphonates related to the calcium antagonist diethyl 4-(2-benzothiazolyl)benzylphosphonate (fostedil) have been studied. They produce a dose-dependent negative inotropic effect on left atrial muscle isolated from guinea pig heart. Some of the compounds are equipotent or slightly more potent than fostedil and diltiazem taken as reference drugs. Structure—activity relationships are discussed.

Résumé — **Activité inotrope négative de diéthylbenzylphosphonates substitués en *para* apparentés au fostédil.** *Quelques diéthylbenzylphosphonates* para-*substitués apparentés à un antagoniste du calcium, le diéthyl (benzo-2-thiazolyl)-4 benzylphosphonate (fostedil), ont été étudiés. Ils produisent un effet inotrope négatif dose-dépendant sur le muscle atrial gauche isolé du cœur du cobaye. Quelques-uns des composés sont aussi actifs ou légèrement plus actifs que le fostedil et le diltiazem pris comme références. Des relations structure—activité sont discutées.*

calcium antagonists / *p*-substituted diethyl benzylphosphonates / cardiodepressant activity

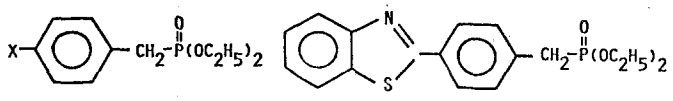
Introduction

A new compound, diethyl 4-(2-benzothiazolyl)benzylphosphonate (fostedil), which is currently under investigation as a new drug candidate, has recently been introduced into the heterogeneous family of calcium antagonists [1, 2]. Its structure is fairly different from that of other calcium antagonists and interestingly suggests an action on the enzymes responsible for phosphorylation and dephosphorylation of the calcium channel protein [3].

In our recent approach to the study of structure—activity relationships (SAR) in the field of calcium antagonists [4, 5], we were intrigued by the presence of the unusual phosphonate function and we tried to insert it into other carriers without any appreciable success.

In particular, the lack of activity of diethyl 3,4-dimethoxybenzylphosphonate was quite surprising and allowed us a better understanding of the relationships between structure and activity in this kind of compound.

In this paper, we present the results obtained testing the negative inotropic action of a series of compounds in which the benzylphosphonate moiety of fostedil was preserved while the benzothiazol nucleus was substituted by a number of groups with different lipophilic and electronic properties.



1—22 fostedil

X = H, CH_3, NO_2, Br, F, CN, C_6H_5, COOH, $COOC_2H_5$, NH_2, $NHCOCH_3$, NH—COC_6H_5, OH, OCH_3, SCH_3, $SOCH_3$, SO_2CH_3, $CH(CH_3)_2$, $C(CH_3)_3$, *n*-C_6H_{13}

Scheme 1.

Chemistry

Compounds **1** and **10** are commercially available (Aldrich). Compounds **2** [6], **3** [7], **4** [8], **5** [9], **6** [10], **7** [9], **8** [11], **9** [10], **13** [12], **14** [13] have been previously described. These and the remaining substances were prepared by standard procedures, mainly through the Arbuzov reaction of benzylbromides with triethylphosphite [14] or according to Scheme 2.

* *Third part in the series; for part II see ref.* [4].
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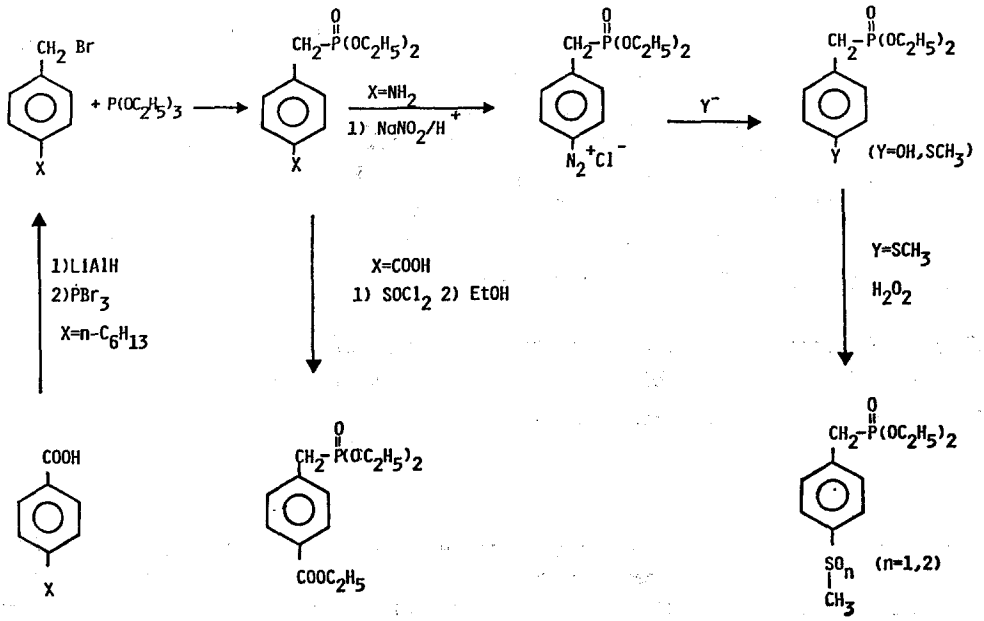


Scheme 2.

Pharmacology

Experiments on atrial muscle isolated from guinea pig hearts (see Experimental protocols) were designed to determine whether exposure of myocardium to the compounds would induce dose-dependent negative inotropic and/or chronotropic effects.

All the compounds were first analyzed at increasing doses to evaluate the dose-dependent decrease in developed tension. The compounds whose potencies were close to that of fostedil, used as the reference drug, were further tested on the same preparation to evaluate the ED_{50}'s from log concentration—response curves. Compounds **2** and **7** were also tested on precontracted aortic strips (KCl depolarization) to evaluate their calcium antagonistic activities.

Results and Discussion

All compounds showed a dose-dependent negative inotropic effect on the isolated guinea pig left atrium. In spontaneously-beating right atrium, they did not induce changes in frequency. Table I reports the decrease in the developed tension at 10^{-5} M, a concentration that for almost all compounds produces a maximal effect.

The same Table reports the R_f's on reverse—phase TLC of the compounds studied, as well as the values of the lipophilic and electronic constants of the *para*-substituents [15].

Table II reports the ED_{50}'s of the most potent compounds compared to fostedil and diltiazem.

In preliminary tests, compounds **2** and **7** significantly reduced (> 50%) the maximum force of contraction induced by KCl on guinea pig aortic strips at a concentration (5×10^{-5} M) which is some 100-times higher than that effective on isolated left atrium.

The R_f values reported in Table I are related to the lipophilicity of the molecules and together with π and σ constants should give us information on the molecular requirements for high negative inotropic activity in this class of compounds.

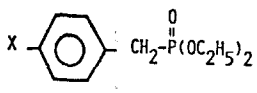
A quantitative analysis of the data is beyond the scope of this paper and has not been attempted. However, it is fairly apparent from a qualitative inspection of the data given in Table I that high potency is related to the lipophilic contribution of the *para*-substituent.

However, this is not the only requirement, and the electronic properties of the substituents also seem to play a role in the sense that an electron withdrawing action is detrimental to high potency. For instance, compound **4**, on which the bromine atom confers good lipophilicity but electron-withdrawing properties, is actually a positive inotropic agent.

As a matter of fact, the most interesting compounds (Table II) have lipophilic and slightly electron releasing groups. The optimum π and σ combination seems to be that of compound **23** which is the most active one. Other compounds with higher lipophilicity and similar electronic properties are almost equiactive. A surprising exception is compound **1** which is much less lipophilic than the other ones but is almost as potent.

The effects of the compounds studied are similar to those obtained by decreasing the Ca^{2+} concentration. Since a calcium antagonistic action has been claimed for

Table I. Physicochemical characteristics and negative inotropic activity of compounds **1**—**22**.



Compound	X	σ_x[a]	π_x[a]	R_f[b]	Negative inotropic activity[c]
17	SO_2CH_3	0.72	— 1.63	0.78	45 ± 5.5
16	$SOCH_3$	0.49	— 1.58	0.75	54 ± 5.7
13	OH	— 0.37	— 0.67	0.74	51 ± 5.6
8	COOH	0.45	— 0.32	0.74	54 ± 4.7
11	$NHCOCH_3$	0.00	— 0.97	0.73	53 ± 5.4
6	CN	0.66	— 0.57	0.72	17 ± 3.5
10	NH_2	— 0.66	— 1.23	0.71	27 ± 5.3
3	NO_2	0.78	— 0.28	0.68	43 ± 2.6
12	$NHCOC_6H_5$	— 0.19	0.49	0.68	52 ± 7.2
1	H	0.00	0.00	0.67	68 ± 6.3
14	OCH_3	— 0.27	— 0.02	0.65	41 ± 6.7
5	F	0.06	0.14	0.65	45 ± 5.6
2	CH_3	— 0.17	0.56	0.56	73 ± 1.9
9	COOEt	0.45	0.51	0.55	48 ± 6.3
4	Br	0.23	0.86	0.54	118 ± 2.6[d]
15	SCH_3	0.00	0.61	0.53	62 ± 8.4
7	C_6H_5	— 0.01	1.96	0.44	75 ± 2.3
20	$CH(CH_3)_2$	— 0.15	1.53	0.40	73 ± 4.0
21	$C(CH_3)_3$	— 0.20	1.98	0.40	82 ± 3.6
22	n-C_6H_{13}	— 0.15	3.17	0.18	82 ± 2.6
Fostedil		—	—	0.30	79 ± 3.0
Diltiazem		—	—	0.13	77 ± 3.2

[a] See reference [15].
[b] R_f on TLC plates RP8 F254 (Merck) eluted with a 9:1 MeOH/H_2O mixture.
[c] Decrease in the developed tension at 10^{-5} M expressed as % changes from control ± SEM. The atria were driven at 1 Hz. The 10^{-5} M concentration gives the maximum effect for most compounds.
[d] The compound shows an increase in developed tension.

Table II. Cardiodepressant potency on isolated guinea pig left atrium[a].

Compound	ED_{50}(mol/l)	(95% confidence limits)[b]
		($\times 10^{-7}$)
1	6.3	(3.9 — 8.1)
2	5.0	(4.7 — 5.3)
7	6.3	(5.2 — 7.5)
15	9.1	(6.0 — 13)
20	4.0	(1.1 — 14)
21	4.6	(2.9 — 7.2)
22	5.0	(3.1 — 7.9)
Fostedil	4.0	(2.0 — 7.9)
Diltiazem	8.9	(7.1 — 11)

[a] Driven at 1 Hz.
[b] Calculated from log concentration curves through linear regression analysis with $n = 5$ or 6.

the closely related fostedil [16], the negative inotropic action of our compounds might be due to some kind of calcium antagonism.

This hypothesis is partially supported by the ability of compounds **2** and **7** to reduce the contraction induced by KCl on guinea pig aortic strips. However, the concentrations active in this test are much higher than those active on left atria. This result could be explained with tissue selectivity but could also imply that the main mechanism of action of this class of compounds is not that of calcium antagonism. Therefore, more work is needed to establish the mechanism of action of this series of compounds. Nevertheless, it is quite clear from these preliminary results that the benzothiazolyl moiety of fostedil does not seem necessary for potent negative inotropic activity, which seems to be strictly connected with the benzylphosphonate function. Its role on the calcium antagonistic activity of fostedil seems to be more important, but remains to be established on sounder bases.

Experimental protocols

Chemistry

All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin—Elmer 337 spectrophotometer in nujol mull for solids and neat for liquids. ^1H NMR spectra were measured on a Varian EM 360 L spectrometer using Me_4Si or DSS (3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt) as the internal standards. Chromatographic separations were performed on a silica gel column (Kieselgel 40, 0.063—0.200 mm, Merck). Where analyses are indicated using symbols, the analytical results are within ± 0.4% of the theoretical values.

4-[(Diethoxyphosphinyl)methyl]benzoic acid chloride ***23***

1 g of **8** was refluxed for 2 h with an excess of $SOCl_2$. The $SOCl_2$ was removed under vacuum, anhydrous benzene added to the oily

residue and the solvent removed under vacuum. This procedure was repeated 3 times and the compound obtained was used as such for the following reactions. Yield 0.9 g.

IR (neat) ν = 1740 and 1780 (CO) cm^{-1}. NMR ($CDCl_3$) δ = 1.28 and 1.36 (tt, 6, OCH_2CH_3); 3.68 (d, 2, CH_2; J_{HP} = 23 cps); 4.18 (qq, 4, OCH_2CH_3); 7.50 (dd, 2, 2H and 6H); 8.08 (dd, 2, 3H and 5H) ppm.

Ethyl 4-[(diethoxyphosphinyl)methyl]benzoate ***9***

1.0 g of **18** was dissolved in anhydrous ethanol and left at room temperature overnight. The excess ethanol was removed under reduced pressure and the residue extracted with ether to give 0.9 g of an oil that was distilled under vacuum. bp 155—158°C/0.6 mm Hg (lit. [10] bp 184°C/1.5 mm Hg).

Diethyl p-*acetylaminobenzylphosphonate* ***11***

1 g of **10** was dissolved in acetic anhydride and left at room temperature for 2 h. The excess anhydride was destroyed with H_2O and the white solid obtained was crystallized from H_2O. Yield 90%. mp = 144—146°C.

IR (nujol) ν = 3320, 3270, 3200, 3120 (NH); 1680 (CO) cm^{-1}. NMR (DMSO) δ = 1.18 (t, 6, OCH_2CH_3); 2.02 (s, 3, $COCH_3$); 3.13 (d, 2, CH_2; J_{HP} = 22 cps); 3.95 (quint, 4, OCH_2CH_3); 7.20 (dd, 2, 2H and 6H); 7.55 (d, 2, 3H and 5H); 9.90 (s, 1, NH) ppm. Anal. ($C_{13}H_{20}NO_4P$) C, H, N.

Diethyl p-*benzoylaminobenzylphosphonate* ***12***

1 g of **10** was dissolved in anhydrous benzene (10 ml) and 0.3 g of $N(C_2H_5)_3$ and 0.45 g of benzoylchloride were added. After 2 h the white solid obtained was filtered and recrystallized from EtOH. Yield 80%. mp = 150—152°C.

IR (nujol) ν = 3180, 3300 (NH); 1690 (CO) cm^{-1}. NMR ($CDCl_3$) δ = 1.20 (t, 6, OCH_2CH_3); 3.10 (d, 2, CH_2; J_{HP} = 22 cps); 3.93 (quint, 4, OCH_2CH_3); 7.0—8.1 (m, 9, aromatics); 9.3 (s, 1, NH) ppm. Anal. ($C_{18}H_{22}NO_4P$) C, H, N.

Diethyl p-*hydroxybenzylphosphonate* ***13***

1 g of **10** was dissolved in 2 N HCl (10 ml) and cooled to — 10°C. A solution of 0.28 g of $NaNO_2$ in 5 ml of H_2O was added over 10 min and the solution was left at 0°C for 20 min. The mixture was then heated at 70°C until the evolution of N_2 ended and was extracted with ether. Evaporation of the solvent left 0.7 g of an oil that was purified from minor by-products by column chromatography (ethyl acetate) mp = 88—90°C from ethyl acetate (lit. [12] bp 89—91°C).

IR (nujol) ν = 3200 cm^{-1} (OH). NMR ($CDCl_3$) δ = 1.22 (t, 6, OCH_2CH_3); 3.08 (d, 2, CH_2; J_{HP} = 21 cps); 4.01 (quint, 4, OCH_2CH_3); 6.6—7.3 (m, 4, aromatics); 8.1 (broad s, 1, OH) ppm. Anal. ($C_{11}H_{17}O_4P$) C, H.

The compound was also identified through phenylcarbamate **24** which melts at 110—112°C (from EtOH).

IR (nujol) ν = 3250, 3200, 3140 (NH); 1750 (CO) cm^{-1}. NMR ($CDCl_3$) δ = 1.28 (t, 6, OCH_2CH_3); 3.14 (d, 2, CH_2; J_{HP} = 22 cps); 4.04 (quint, 4, OCH_2CH_3); 6.8—7.8 (m, 9, aromatics); 8.1 (s, 1, NH) ppm. Anal. ($C_{18}H_{22}NO_5P$) C, H, N.

Diethyl p-*methoxybenzylphosphonate* ***14***

0.8 g of **13** in absolute ethanol (10 ml) was added to a solution of 0.075 g of Na in ethanol (5 ml) and the solution evaporated to dryness. The residue was dissolved in anhydrous dimethylformamide (DMF, 5 ml) to which 2 ml of CH_3I has been added and was left at room temperature overnight. 20 ml of H_2O were then added and the solution extracted with ether to give 0.9 g of an oil which was distilled through a short Vigreux mp = 110—112°C/0.4 mmHg (lit. 143°C/1 mmHg [13]). Yield 75%.

Diethyl p-*methylthiobenzylphosphonate* ***15***

0.77 g of **10** in 3 ml of 2 N HCl were cooled at — 10°C and a solution of 0.2 g of $NaNO_2$ in 5 ml of H_2O was added during 10 min. After 20 min at 0°C, the solution was added to 0.4 g of CH_3SNa in 10 ml of H_2O and left at room temperature for 1 h. The mixture was then extracted with ether to give 0.75 g of an oil which was purified through column chromatography (chloroform—methanol, 95:5). Yield 65%.

NMR ($CDCl_3$) δ = 1.23 (t, 6, OCH_2CH_3); 2.43 (s, 3, 5-CH_3); 3.10 (d, 2, CH_2; J_{HP} = 22 cps); 4.03 (quint, 4, OCH_2CH_3); 7.22 (s, 4, aromatics) ppm. MS 274 (M^+) *m/e*. Anal. ($C_{12}H_{19}O_3PS$) C, H.

Diethyl p-*methylsulfinylbenzylphosphonate* ***16***

1 g of **15** in glacial CH_3COOH (5 ml) were added to 2 ml of 30% H_2O_2 and left at room temperature for 30 min. The solution was made alkaline with 2.5 N NaOH and extracted with $CHCl_3$. Evaporation of the solvent gave 1.1 g of an oil which was purified from minor by-products through column chromatography ($CHCl_3$—MeOH, 95:05). Yield 90%. The compound solidified when cooled but melted at room temperature.

NMR ($CDCl_3$) δ = 1.25 (t, 6, OCH_2CH_3); 2.70 (s, 3, $SOCH_3$); 3.20 (d, 2, CH_2; J_{HP} = 22 cps); 4.05 (quint, 4, OCH_2CH_3); 7.3—7.8 (m, 4, aromatics) ppm. MS 290 (M^+) *z/e*. Anal. ($C_{12}H_{19}O_4PS$) C, H.

Diethyl p-*methylsulphonylbenzylphosphonate* ***17***

1 g of **15** (or **16**) was dissolved in glacial CH_3COOH (5 ml), to which 2 ml of 30% H_2O_2 had been added and was left at room temperature for 2 days. Working-up the solution as described above gave 1.1 g of an oil that was purified from minor by-products by column chromatography ($CHCl_3$—MeOH, 9:1).

NMR (DMSO) δ = 1.28 (t, 6, OCH_2CH_3); 3.10 (s, 3, SO_2CH_3); 3.31 (d, 2, CH_2; J_{HP} = 21 cps); 4.08 (quint, 4, OCH_2CH_3); 7.55 (dd, 2, 2 and 6H); 7.94 (dd, 2, 3 and 5H) ppm. MS 306 (M^+) *z/e*. Anal. ($C_{12}H_{19}O_5PS$) C, H.

p-n-*Hexylbenzyl alcohol* ***18***

p-n-Hexylbenzoic acid (available from Fluka) (10 mM) was dissolved in dry ether and added to a suspension of $LiAlH_4$ (10 mM) in 100 ml of dry ether. The mixture was refluxed for 6 h and then ethyl acetate (15 ml) and H_2O (2 ml) were added. The solid was filtered away and the organic solution evaporated to leave an oil that was distilled through a short Vigreux. bp = 180—185°C/0.5 mm Hg. Yield 75%.

IR (neat) ν = 3350 cm^{-1} (OH). NMR ($CDCl_3$) δ = 0.9—1.9 (m, 13, *n*-hexane protons); 2.50 (t, 2, CH_2—C_6H_4—); 3.20 (s, 1, OH); 4.70 (s, 2, —C_6H_4—CH_2OH); 7.22 (s, 4, aromatics) ppm. Anal. ($C_{13}H_{20}O$) C, H.

p-n-*Hexylbenzyl bromide* ***19***

Compound **18** (5 mM) was mixed with PBr_3 (15 mM) and the solution heated for 10 h at 80°C. The mixture was then cooled and carefully added with water and ice. Extraction with ether gave an oil which was purified through column chromatography using ethyl acetate/cyclohexane (2:8) as the eluent. Yield 70%.

NMR ($CDCl_3$) δ = 1.0—2.1 (m, 13, *n*-hexane protons); 2.42 (t, 2, CH_2—C_6H_4—); 4.35 (s, 2, C_6H_4—CH_2Br); 7.13 (s, 4, aromatics) ppm. Anal. ($C_{13}H_{19}Br$) C, H.

Diethyl p-*alkylbenzylphosphonates* ***20—22***

The suitable benzylbromide was mixed with 2 mol of triethylphosphite and refluxed at 150°C for 6 h. The mixture was then distilled under reduced pressure to eliminate the excess phosphite and the oily residue purified by column chromatography using ethyl acetate as the eluent.

Diethyl p-*isopropylbenzylphosphonate* ***20*** was obtained in 75% yield, starting from *p*-isopropylbenzyl bromide [17].

NMR ($CDCl_3$) δ = 1.18 (t, 6, CH_3—CH_2); 1.22 (d, 6, $CH(CH_3)_2$); 2.85 (m, 1, $CH(CH_3)_2$); 3.08 (d, 2, CH_2P; J_{HP} = 22 Hz); 4.00 (quint, 4, CH_2—CH_3); 7.20 (s, 4, aromatics) ppm. Anal. ($C_{14}H_{23}PO_3$) C, H.

Diethyl p-t-*butylbenzylphosphonate* ***21*** was obtained in 83% yield, starting from *p-t*-butylbenzyl bromide [18].

NMR ($CDCl_3$) δ = 1.18 (t, 6, CH_2—CH_3); 1.30 (s, 9, $C(CH_3)_3$); 3.08 (d, 2, CH_2P; J_{HP} = 22 Hz); 3.95 (quint, 4, CH_2—CH_3); 7.28 (s, 4, aromatics) ppm. Anal. ($C_{15}H_{25}PO_3$) C, H.

Diethyl p-n-*hexylbenzylphosphonate* ***22*** was obtained in 90% yield, starting from **19**.

NMR ($CDCl_3$) δ = 0.8—1.9 (m, 17, CH_2—CH_3 and *n*-hexane protons) 2.56 (t, 2, CH_2—C_6H_4); 3.10 (d, 2, CH_2—P, J_{HP} = 22 Hz); 4.02 (quint, 4, CH_2—CH_3); 7.19 (s, 4, aromatics) ppm. Anal. ($C_{17}H_{29}PO_3$) C, H.

Pharmacology

Isolated left and right atrial preparations

Guinea pigs (350—400 g, male and female) were sacrificed by cervical dislocation. After thoracotomy, the hearts were immediately removed and washed by perfusion through the aorta with an oxygenated Tyrode

solution of the following composition (mM): 136.9 NaCl; 5.4 KCl; 2.5 $CaCl_2$; 1.0 $MgCl_2$; 0.4 $Na_2HPO_4 \cdot 2\ H_2O$; 11.9 Na_2CO_3; 5.5 glucose. The solution was buffered to pH 7.4 by saturation with 95% O_2—5% CO_2 gas and the temperature was maintained at 35°C. Isolated guinea pig heart preparations were used: spontaneously-beating right atria and left atria driven at 1 Hz.

For each preparation, the entire left and right atria were dissected from the ventricles, cleaned of excess tissue, hung vertically in a 15 ml organ bath containing the Tyrode solution described above, continuously bubbled with 95% O_2—5% CO_2 gas at 35°C, pH 7.4. The contractile activity was recorded isometrically by means of a force transducer connected to a pen recorder (Battaglia—Rangoni KV 220). The left atria were stimulated by rectangular pulses of 0.6—0.8 ms duration and about 50% threshold-voltage through two platinum contact electrodes in the lower holding clamp (Grass S44 stimulator). After the tissue had been beating for several min, the length—tension curve was determined and the muscle length was maintained at the point which elicited 90% of maximum contractile force as observed at the optimal length. A stabilization period of 45—60 min was allowed before the atria were challenged with various agents. During this equilibration period, the bathing solution was changed every 15 min and the threshold voltage was ascertained for the left atria.

Atrial muscle preparations were used to examine the inotropic and chronotropic activities of the compounds (0.1, 0.5, 1, 5 and 10 μM), which were first dissolved in DMF, then diluted with the Tyrode solution described above. According to this procedure the concentration of DMF in the bathing solution never exceeded 0.30%, a concentration which did not produce any appreciable inotropic and/or chronotropic effects. During generation of cumulative dose—response curves, the next highest concentration of the compound was added only after the preparation reached a steady-state.

Guinea pig aortic strip preparations

The thoracic aorta was removed and placed in a Tyrode solution of the following composition (mM): 118 NaCl; 4.75 KCl; 2.54 $CaCl_2$; 1.20 $MgSO_4$; 1.19 KH_2PO_4; 25 $NaHCO_3$; 11 glucose; equilibrated with 95% O_2 and 5% CO_2 at pH 7.4.

The vessel was cleaned of extraneous connective tissue. Two helical strips (10 × 1 mm) were cut from each aorta begining from the end most proximal to the heart. Vascular strips were then tied with surgical thread (6—0) and suspended in a jacketed tissue bath (15 ml) containing aerated PSS (physiological salt solution) at 35°C. Strips were secured at one end to plexiglass hooks and connected *via* the surgical thread to a force transducer for monitoring changes in isometric force.

Aortic strips were subjected to a resting force of 1.0 g and washed every 20 min with fresh PSS for 1 h. After the equilibration period, guinea pig aortic strips were contracted by being washed in PSS containing 80 mM KCl (equimolar substitution of K^+ for Na^+). Subsequent to the contraction reaching a plateau (approximately 30 min) the compounds (**2** and **7**) were added cumulatively to the bath allowing for any relaxation to obtain an equilibrated level of force. Addition of the drug vehicle had no appreciable effect on the K^+-induced level of force (DMF for all compounds).

Statistical evaluation

Data were analyzed with Student's *t*-test and the *F*-test. The criterion for significance was a *P* value below 0.05. The ED_{50} values were calculated from log concentration—response curves (linear regression analysis, $n = 5$ or 6).

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