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1,4-Dihydropyridines Bearing a Pharmacophoric Fragment of Lidoflazine

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Abstract—A series of 1,4-dihydropyridines bearing a pharmacophoric fragment of lidoflazine was synthesized. The compounds were evaluated for inotropic, chronotropic, and calcium antagonist activities. All compounds behave as inotropic and chronotropic agents, except for compounds **4b**, **5a**, and **5b**, which exibit a rather weak calcium antagonism in vascular smooth muscle (like aorta). Compound **5b** is about twofold more potent in decreasing both chronotropy and inotropy, while compound **5c** is about fivefold more potent in decreasing inotropy than nifedipine. Moreover, compound **5b** is the most potent calcium antagonist derivative of the series. Copyright © 1996 Elsevier Science Ltd

Introduction

The introduction of 4-aryl-1,4-dihydropyridines (DHPs) with highly potent calcium-channel blocking activity led to a new direction in cardiovascular therapy. Calcium-channel modulators are now well established in the treatment of angina pectoris, hypertension, certain cardiac arrhythmias, and peripheral vascular disorders.[1–5] The 1,4-dihydropyridine calcium-channel blockers inhibit the influx of extracellular Ca^{2+} via L-type potential dependent calcium channels and reduce vascular resistance. A great number of 4-aryl-1,4-dihydropyridine-3,5-dicarboxylates have been synthesized and evaluated biologically for cardiovascular activity allowing delineation of well-defined structure–activity relationships (SAR).[6–8]

The lidoflazine[9] **2** is a calcium antagonist and a vasorelaxing agent that may block an active saturable re-uptake process that transports adenosine into some cells, including heart cells and erythrocytes. Adenosine is a powerful vasodilator, and block of its re-uptake results in being more available to produce coronary dilatation.

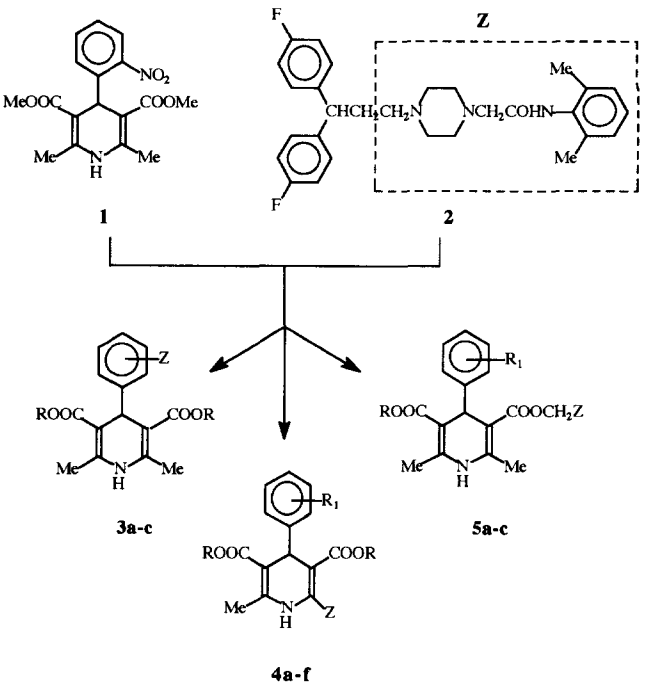
The 1,4-dihydropyridine-type calcium antagonists, in spite of the extensive SAR studies, further deserve the interest of the medicinal chemists. In the search for new and better nifedipine **1** analogues, we report the synthesis and the pharmacological profile of a series of 1,4-dihydropyridines in which a peculiar fragment of lidoflazine **2** was introduced, according to the so-called medicinal hybridation conception[10] as shown in Scheme 1.

In fact, compounds bearing peculiar fragments of vasorelaxing drugs acting with different mechanisms might show enhanced therapeutic efficacy.

The asymmetric compounds were tested as racemates. Attempts to resolve some of the most potent derivatives are in progress.

Chemistry

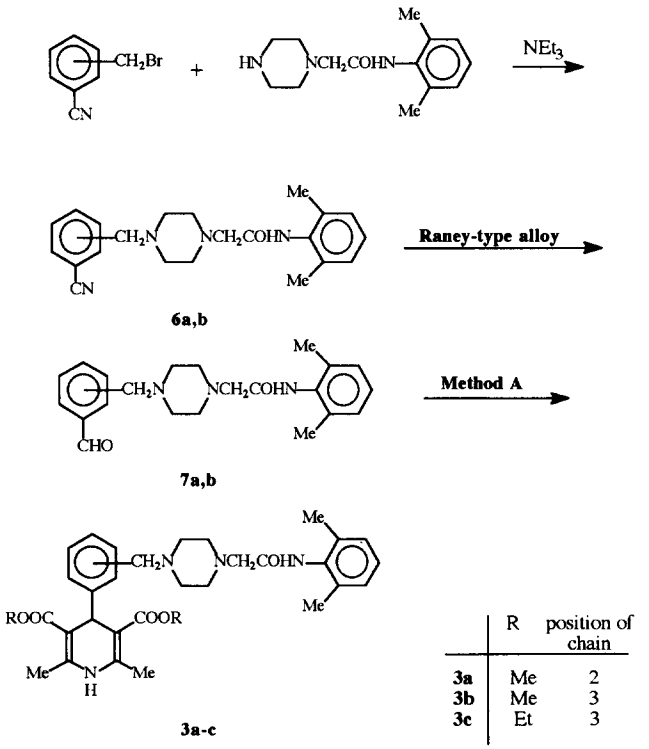
The studied compounds were prepared as shown in Schemes 2–4. In Scheme 2 the synthesis of compounds **3a–c** is reported. The *o*- and *m*-cyanobenzyl bromides[11,12] were condensed with *N*-(2,6-dimethylphenyl)-2-piperazin-1-ylacetamide[13] in presence of triethylamine to yield *N*-(2,6-dimethylphenyl)-2-[4-(2-cyanobenzyl)-piperazin-1-yl]-acetamide **6a** and *N*-(2,6-dimethylphenyl)-2-[4-(3-cyanobenzyl)-piperazin-1-yl]-acetamide (**6b**), respectively. These compounds, treated with Raney-type alloy and formic acid, afforded the corresponding aldehydes **7a** and **7b**, which were used in the classical Hantzsch reaction[14] to yield **3a–c** (Method A).



Scheme 1.

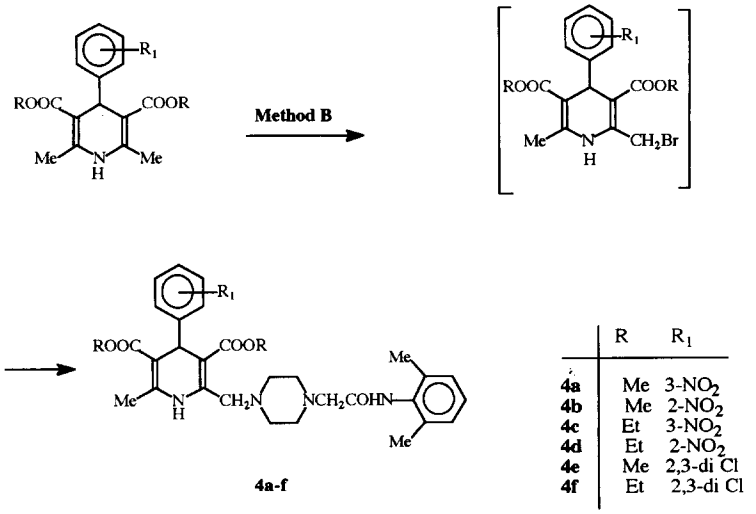
In Scheme 3 the preparation of compounds **4a–f** is reported. The selected aldehydes were treated with methyl (or ethyl) acetylacetate and ammonia to yield the corresponding dihydropyridines.[15,16] These compounds were treated with pyridinium bromide perbromide to afford the 2-bromomethyl derivatives (not isolable), which were condensed with *N*-(2,6-dimethylphenyl)-2-piperazin-1-ylacetamide to yield **4a–f** (Method B).

The preparation of compounds **5a–c** is reported in Scheme 4. The selected aldehydes were treated with methyl 3-aminocrotonate and cyanoethyl acetylacetate to yield methyl, cyanoethyl ester of 1,4-dihydro-2,6-dimethyl-4-(R_1-phenyl)-pyridine-3,5-dicarboxylic acids.[17,18] These esters were hydrolysed with LiOH[19] to yield the corresponding monoesters **10a–c** that were condensed with *N*-(2,6-dimethylphenyl)-2-[4-(2-hydroxyethylpiperazin-1-yl)]acetamide[20] in presence of 1,1′-carbonyldiimidazole (CDI) or 1,3-dicyclohexylcarbodiimide (DCC) to afford **5a–c** (Methods C and D).



	R	position of chain
3a	Me	2
3b	Me	3
3c	Et	3

Scheme 2.



	R	R_1
4a	Me	3-NO_2
4b	Me	2-NO_2
4c	Et	3-NO_2
4d	Et	2-NO_2
4e	Me	2,3-di Cl
4f	Et	2,3-di Cl

Scheme 3.

Pharmacology

The biological profile in functional experiments of the synthesized compounds was assessed on isolated guinea pig left and right atria to evaluate negative inotropic, chronotropic effects and on guinea pig aortic strips to test vasorelaxing activity, which is a measure of calcium antagonism. The potency of the compounds was expressed as ED_{50} values accordingly to the different functional experiments or as percent inhibition at a particular concentration if no ED_{50} has been measured.

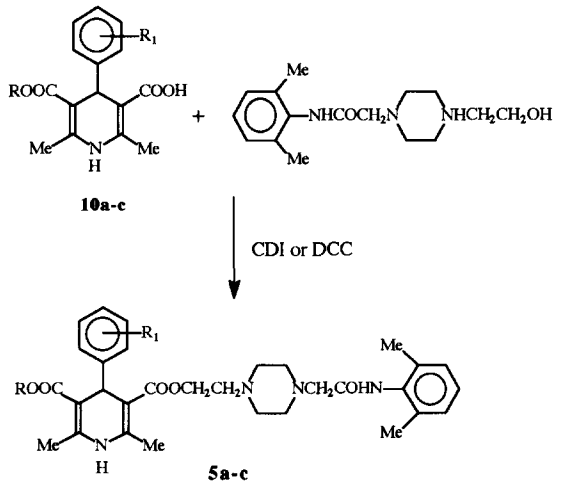
Results and Discussion

The pharmacological results at the left driven atria, at the spontaneously beating right atria and at the smooth muscle aortic vessel, are reported in Table 1, together with those of the standard compounds nifedipine and lidoflazine. A first inspection of the biological data clearly indicates that all compounds, except **4b**, **5a**, and **5b**, exibit a rather weak calcium antagonism in vascular smooth muscle (like aorta) but almost all behave as negative inotropic and chronotropic agents, **5b** and **5c** being more active than nifedipine.

As regards to the cardiodepressant activity, compounds **5a–c**, possessing the lidoflazine fragment inserted in the ester function at the 3-position of the 1,4-DHP ring and *ortho-/meta*-electron-withdrawing groups (NO_2, Cl) in the 4-phenyl ring, elicit high efficacy and potency both in decreasing the developed tension of the driven

left atrium and in lowering the frequency of the right atrium, therefore, cardiac versus vascular activity and selectivity are improved.

Taking a closer look at Table 1 it emerges that compound **5b** is about twofold more potent in decreasing both chronotropy and inotropy, while compound **5c** is about fivefold more potent in decreasing inotropy than nifedipine. Moreover compound **5b** is the most potent ($IC_{50}=0.32$ μM) calcium antagonist derivative of the series.



	R	R_1
5a	Me	3-NO_2
5b	Me	2,3-di Cl
5c	Me	2-NO_2

Scheme 4.

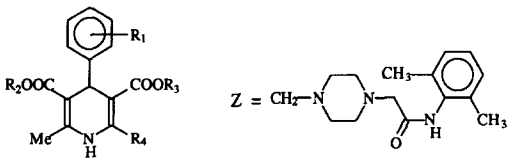
A detailed analysis of the results reveals that the derivatives bearing lidoflazine fragment at the 2-position of the 1,4-DHP ring and *ortho-/meta*-electron-withdrawing groups (NO_2, Cl) in the 4-phenyl ring, compounds **4a–f**, are slightly less potent than the 3-substituted compounds, as far as negative inotropic and chronotropic properties are concerned.

In particular the *o*-nitro derivatives (**4b**, **4d**, and **5c**) are more effective and more potent than the *m*-nitro ones (**4a**, **4c**, and **5a**).

The ester function at the 3,5-positions of the 1,4-DHP ring elicits variable effects on myocardial depressant action: when a NO_2 group is present the diethyl ester (compound **4d**) shows a better activity than the dimethyl one (compound **4b**); on the contrary in presence of the 2,3-dichlorine groups the methyl ester (compound **4e**) shows a better activity than the ethyl one (compound **4f**).

Regarding the compounds with the lidoflazine fragment at the *ortho-* or *meta*-position of the 4-phenyl ring, compounds **3a–c**, the cardiodepressant properties are maintained (except compound **3b** for negative inotropic activity), with the meta derivative being slightly more potent than the ortho one. For these compounds the 3,5-ethyl ester (compound **3c**), in

Table 1. Inotropic, chronotropic, and calcium antagonist activities



Compd	R_1	R_2	R_3	R_4	ED_{50} ino.	95% Conf. Lim.	ED_{50} chrono.	95% Conf. Lim.	ED_{50} CCB	95% Conf. Lim.
3a	2-Z	Me	Me	Me	1.65	1.37–2.15	2.03	1.85–2.31	27±1.3%	@10 μM
3b	3-Z	Me	Me	Me	40±2.3%	@100 μM	4.78	4.55–5.15	19± 1.1%	@50 μM
3c	3-Z	Et	Et	Me	1.05	0.89–1.13	0.86	0.82–0.91	32±1.3%	@50 μM
4a	3-NO_2	Me	Me	Z	45±1.8%	@50 μM	41±3.1%	@10 μM	23±1.4%	@50 μM
4b	2-NO_2	Me	Me	Z	0.72	0.67–0.79	1.01	0.78–1.30	0.76	0.69–0.85
4c	3-NO_2	Et	Et	Z	32±1.5%	@50 μM	1.45	1.37–1.54	39±2.3%	@50 μM
4d	2-NO_2	Et	Et	Z	0.45	0.40–0.52	0.24	0.21–0.32	46±2.2%	@50 μM
4e	2,3-di Cl	Me	Me	Z	0.71	0.62–0.81	2.11	1.64–2.73	44±3.4%	@50 μM
4f	2,3-di Cl	Et	Et	Z	47±0.8%	@50 μM	41±3.1%	@50 μM	21±1.3%	@10 μM
5a	3-NO_2	Me	CH_2-Z	Me	1.36	1.18–1.65	0.27	0.19–0.39	0.62	0.42–0.85
5b	2,3-di Cl	Me	CH_2-Z	Me	0.16	0.12–0.21	0.031	0.022–0.043	0.32	0.26–0.40
5c	2-NO_2	Me	CH_2-Z	Me	0.058	0.052–0.07	0.16	0.11–0.23	7±0.2%	@50 μM
Nifedip.					0.26	0.19–0.36	0.039	0.031–0.048	0.009	0.003–0.02
Lidofla.					7.71	6.82–8.84	38±1.5%	@10 μM	42±1.7%	@10 μM

ED_{50} values with 95% conf. lim. are calculated from log-concentration response curves (probit analysis by Litchfield and Wilcoxon).[22] Each compound was tested at least five times. ED_{50} ino. shows the ED_{50} values for the negative inotropic potency of tested compounds on stimulated guinea pig left atrium in μM or as a percent inhibition [mean±SEM] at a particular concentration if no ED_{50} has been measured. Similarly the ED_{50} chrono. in spontaneously beating right atrium, and ED_{50} CCB in K^+ depolarized guinea pig aortic strips, reflect the negative chronotropic activity and calcium-channel blocking activity.

comparison with 3,5-methyl esters (compounds **3a** and **3b**), improves negative inotropic and chronotropic potency.

As far as calcium antagonism on smooth muscle (like aorta) is concerned an inspection of the results of Table 1 seems to indicate that the essential structural features to improve vasorelaxing activity in this series of compounds are: the contemporary presence in the molecule of the lidoflazine fragment at the 3-position (at the 2-position for compound **4b**) of the 1,4-DHP ring and the *ortho*- or *meta*-NO_2 group in the 4-phenyl ring. In fact, the calcium antagonist potency (IC_{50}) is spread over the range 0.30–0.75 μM, the most potent derivative being about 30-fold less active than nifedipine. On the other hand these results are in keeping with the previous studies on 1,4-DHPs SAR.[21]

In short, these preliminary results show that in this series of compounds the negative inotropic and chronotropic activities increase with regard to nifedipine when the lidoflazine fragment is inserted at the 3-position of 1,4-DHP ring (compounds **5b** and **5c**), while all the synthesized compounds exhibit a rather weak calcium antagonism in vascular smooth muscle (like aorta). This interesting result prompts us to continue the work in this area with the synthesis and SAR studies of new derivatives directed at gaining a better understanding of the structural requirements for improving cardiac and vascular selectivity.

Experimental

Chemistry

Melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were obtained for $CDCl_3$ solutions on a Gemini 300 spectrometer. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Purification by gravity column chromatography on Merck silica-gel 60, 70–230 mesh and by flash chromatography on 230–400 mesh were carried out using the slurry method for column packing. Elemental analyses were within ±0.4% of the theoretical values. Compounds were named following IUPAC rules as applied by AUTONOM, a PC software for systematic names in organic chemistry, Beilstein-Institut and Springer.

***N*-(2,6-Dimethylphenyl)-2-[4-(2-cyanobenzyl)piperazin-1-yl]acetamide (6a).** A solution of 2-cyanobenzyl bromide (1.96 g, 0.01 mol), *N*-(2,6-dimethylphenyl)-2-piperazin-1-yl-acetamide (2.53 g, 0.01 mol), and triethylamine (1.01 g, 0.01 mol) in toluene (100 mL) was refluxed for 3 h. The mixture was washed with water, dried over sodium sulfate, and evaporated to dryness. The residue was crystallized from ligroin to yield 3.25 g (90%) of **6a**, mp 158–162 °C; ^{1}H NMR: δ 2.2 (6, s), 2.6 (4, m), 2.75 (4, m), 3.2 (2, s), 3.75 (2, s), 7.1–7.7 (7, m), 8.7 (1, s). Anal. calcd ($C_{22}H_{26}N_4O$) C, H, N.

***N*-(2,6-Dimethylphenyl)-2-[4-(3-cyanobenzyl)piperazin-1-yl]acetamide (6b).** Using the precedent procedure and starting from 3-cyanobenzyl bromide (1.96 g, 0.01 mol), 3.1 g (85%) of **6b** mp 122–126 °C (ligroin), were obtained; ^{1}H NMR: δ 2.2 (6, s), 2.55 (4, m), 2.75 (4, m), 3.2 (2, s), 3.55 (2, s), 7.1–7.7 (7, m), 8.75 (1, s). Anal. calcd ($C_{22}H_{26}N_4O$) C, H, N.

***N*-(2,6-Dimethylphenyl)-2-[4-(2-formylbenzyl)piperazin-1-yl]acetamide (7a).** A mixture of **6a** (1.8 g, 0.005 mol) and Raney-type alloy (1.8 g) in formic acid (20 mL) was refluxed for 1 h. The reaction mixture was filtered, washed with boiling ethanol, diluted with water, alkalinized with potassium carbonate, and extracted with methylene chloride. The organic layer was washed with water, dried over sodium sulfate, and evaporated to dryness. The residue was crystallized from ligroin to give 1.45 g (80%) of **7a**, mp 124–127 °C; ^{1}H NMR: δ 2.2 (6, s), 2.6 (4, m), 2.75 (4, m), 3.2 (2, d), 3.8 (2, d), 7.05–7.85 (7, m), 8.65 (1, s), 10.4 (1, s). Anal. calcd ($C_{22}H_{27}N_3O_2$) C, H, N.

***N*-(2,6-Dimethylphenyl)-2-[4-(3-formylbenzyl)piperazin-1-yl]acetamide (7b).** Using the precedent procedure and starting from **6b** (1.8 g, 0.005 mol), 1.64 g (90%) of **7b** mp 114–116 °C (ligroin), were obtained; ^{1}H NMR: δ 2.2 (6, s), 2.55 (4, m), 2.7 (4, m), 3.2 (2, d), 3.6 (2, d), 7.1–7.85 (7, m), 8.7 (1, s), 10.05 (1, s). Anal. calcd ($C_{22}H_{27}N_3O_2$) C, H, N.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[2-[4-(2,6-dimethylphenylcarbamoylmethyl)piperazin-1-ylmethyl]phenyl]pyridine-3,5-dicarboxylate (3a). Method A. A solution of **7a** (1.1 g, 0.03 mol), methyl acetylacetate (0.7 g, 0.06 mol) and ammonia (3 mL) in isopropyl alcohol (30 mL) was refluxed for 30 h. The separated solid, on cooling, is filtered and crystallized from toluene to yield 0.25 g (15%) of **3a**, mp 218–222 °C; ^{1}H NMR: δ 2.2 (6, s), 2.3 (6, s), 2.7–2.8 (8, m), 3.25 (2, s), 3.6 (6, s), 4.0 (2, s), 5.2 (1, s), 5.6 (1, s), 7.1–7.4 (7, m), 8.8 (1, s). Anal. calcd ($C_{32}H_{40}N_4O_5$) C, H, N.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[3-[4-(2,6-dimethylphenylcarbamoylmethyl)piperazin-1-ylmethyl]phenyl]pyridine-3,5-dicarboxylate (3b). Using the precedent procedure and starting from **7b** (1.1 g, 0.03 mol), 0.168 g (10%) of **3b**, mp 238–240 °C (toluene) were obtained; ^{1}H NMR: δ 2.2 (6, s), 2.3 (6, s), 2.5–2.7 (8, m), 3.2 (2, s), 3.5 (2, s), 3.7 (6, s), 5.0 (1, s), 5.7 (1, s), 7.0–7.3 (7, m), 8.7 (1, s). Anal. calcd ($C_{32}H_{40}N_4O_5$) C, H, N.

Diethyl 1,4-dihydro-2,6-dimethyl-4-[3-[4-(2,6-dimethylphenylcarbamoylmethyl)piperazin-1-ylmethyl]phenyl]pyridine-3,5-dicarboxylate (3c). Using the precedent procedure and starting from **7b** (1.1 g, 0.03 mol), 0.67 g (40%) of **3c**, mp 208–212 °C (toluene) were obtained; ^{1}H NMR: δ 1.2 (6, t), 2.2 (6, s), 2.3 (6, s), 2.5–2.7 (8, m), 3.2 (2, s), 3.5 (2, s), 4.1 (4, q), 5.0 (1, s), 5.6 (1, s), 7.1–7.3 (7, m), 8.7 (1, s). Anal. calcd ($C_{34}H_{44}N_4O_5$) C, H, N.

Dimethyl 1,4-dihydro-2-[4-(2,6-dimethylphenylcarbamoylmethyl)piperazin-1-ylmethyl]-6-methyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate (4a). Method B. To a cold solution of dimethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate[15] (1.2 g, 0.0035 mol) in methylene chloride (30 mL), pyridine (0.4 mL) and pyridinium bromide perbromide (1.2 g) were added. The reaction mixture was stirred for 40 min, diluted with methylene chloride (30 mL), and washed with 2 N HCl and cold water. The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was taken up with tetrahydrofuran and *N*-(2,6-dimethylphenyl)-2-piperazin-1-ylacetamide (1.73 g, 0.007 mol) and potassium carbonate (1 g) in dimethylformamide were added. The reaction mixture was stirred at 0 °C for 2 h, at room temperature for 12 h and then evaporated to dryness. The residue was taken up with methylene chloride and the organic layer washed with water, dried over sodium sulfate, and evaporated. The residue was purified by flash chromatography (eluent: toluene:acetone, 3:2) to yield 0.41 g (30%) of **4a**, mp 100–104 °C (ligroin); ^{1}H NMR: δ 2.2 (6, s), 2.4 (3, s), 2.7–2.9 (8, m), 3.3 (2, s), 3.65 (3, s), 3.7 (3, s), 3.8 (2, q), 5.1 (1, s), 7.1–8.05 (8, m), 8.6 (1, s). Anal. calcd ($C_{31}H_{37}N_5O_7$) C, H, N.

Dimethyl 1,4-dihydro-2-[4-(2,6-dimethylphenylcarbamoylmethyl)piperazin-1-ylmethyl]-6-methyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate (4b). Using the precedent procedure and starting from dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-3,5-dicarboxylate[15] (1.2 g, 0.0035 mol), 0.55 g (40%) of **4b** were obtained, mp 209–213 °C (ligroin); ^{1}H NMR: δ 2.25 (6, s), 2.4 (3, s), 2.6–2.9 (8, m), 3.3 (2, s), 3.6 (3, s), 3.65 (3, s), 3.8 (2, q), 5.8 (1, s), 7.1–7.7 (7, m), 7.9 (1, s), 8.6 (1, s). Anal. calcd ($C_{31}H_{37}N_5O_7$) C, H, N.

Diethyl 1,4-dihydro-2-[4-(2,6-dimethylphenylcarbamoylmethyl)piperazin-1-ylmethyl]-6-methyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate (4c). Using the precedent procedure and starting from diethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate[15] (1.12 g, 0.003 mol), 0.185 g (10%) of **4c** were obtained, mp 175–178 °C (ligroin); ^{1}H NMR: δ 1.2 (6, m), 2.2 (6, s), 2.4 (3, s), 2.6–2.9 (8, m), 3.3 (2, s), 3.8 (2, q), 4.1 (4, m), 5.1 (1, s), 7.1–8.1 (8, m), 8.6 (1, s). Anal. calcd ($C_{33}H_{41}N_5O_7$) C, H, N.

Diethyl 1,4-dihydro-2-[4-(2,6-dimethylphenylcarbamoylmethyl)piperazin-1-ylmethyl]-6-methyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate (4d). Using the precedent procedure and starting from diethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-3,5-dicarboxylate[15] (1.12 g, 0.003 mol), 0.37 g (20%) of **4d** were obtained, mp 155–159 °C (ligroin); ^{1}H NMR: δ 1.2 (6, s), 2.2 (6, s), 2.6–2.8 (8, m), 3.3 (2, s), 3.8 (2, q), 4.0–4.2 (4, m), 5.9 (1, s), 7.1–7.75 (7, m), 7.9 (1, s), 8.6 (1, s). Anal. calcd ($C_{33}H_{41}N_5O_7$) C, H, N.

Dimethyl 1,4-dihydro-2-[4-(2,6-dimethylphenylcarbamoylmethyl)piperazin-1-ylmethyl]-6-methyl-4-(2,3-dichlorophenyl)pyridine-3,5-dicarboxylate (4e). Using the precedent procedure and starting from dimethyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)pyridine-3,5-dicarboxylate[16] (1.11 g, 0.003 mol), 0.37 g (20%) of **4e** were obtained, mp 204–209 °C (ligroin); ^{1}H NMR: δ 2.2 (6, s), 2.35 (3, s), 2.6–2.85 (8, m), 3.25 (2, s), 3.55 (3, s), 3.6 (3, s), 3.8 (2, q), 5.5 (1, s), 7.1–7.25 (6, m), 7.9 (1, s), 8.6 (1, s). Anal. calcd ($C_{31}H_{36}Cl_2N_4O_5$) C, H, N.

Diethyl 1,4-dihydro-2-[4-(2,6-dimethylphenylcarbamoylmethyl)piperazin-1-ylmethyl]-6-methyl-4-(2,3-dichlorophenyl)pyridine-3,5-dicarboxylate (4f). Using the precedent procedure and starting from diethyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)pyridine-3,5-dicarboxylate[16] (1.12 g, 0.003 mol), 0.39 g (20%) of **4f** were obtained, mp 185–188 °C (ligroin); ^{1}H NMR: δ 1.2 (6, s), 2.25 (6, s), 2.4 (3, s), 2.6–2.9 (8, m), 3.3 (2, s), 3.8 (2, q), 4.1 (4, m), 5.5 (1, s), 7.1–7.3 (6, m), 7.9 (1, s), 8.6 (1, s). Anal. calcd ($C_{33}H_{40}Cl_2N_4O_5$) C, H, N.

Methyl 2-[4-(2,6-dimethylphenylcarbamoylmethyl)piperazin-1-yl]ethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (5a). Method C. A mixture of **10a** (0.71 g, 0.0021 mol) and 1,1′-carbonyldiimidazole (0.69 g, 0.0042 mol) in tetrahydrofuran (10 mL) was stirred at room temperature for 1 h. The solvent was evaporated, *N*-(2,6-dimethylphenyl)-2-[4-(2-hydroxyethylpiperazin-1-yl)]acetamide (0.63 g, 0.0021 mol) was added, and the mixture was heated at 120 °C for 40 min. After cooling the residue was purified by flash chromatography (eluent: toluene:acetone, 1:1) to give 0.38 g (30%) of **5a** mp 175–178 °C (ligroin); ^{1}H NMR: δ 2.2 (6, s), 2.35 (6, s), 2.5–2.7 (10, m), 3.2 (2, s), 3.65 (3, s), 4.2 (2, m), 5.1 (1, s), 6.0 (1, s), 7.1–8.1 (7, m), 8.7 (1, s). Anal. calcd ($C_{32}H_{39}N_5O_7$) C, H, N.

Methyl 2-[4-(2,6-dimethylphenylcarbamoylmethyl)piperazin-1-yl]ethyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)pyridine-3,5-dicarboxylate (5c). Using the precedent procedure and starting from **10c** (0.85 g, 0.0023 mol), 0.29 g (20%) of **5c** mp 220–222 °C (toluene) were obtained; ^{1}H NMR: δ 2.2 (6, s), 2.3 (6, s), 2.5–2.7 (10, m), 3.2 (2, s), 3.6 (3, s), 4.2 (2, m), 5.45 (1, s), 5.7 (1, s), 7.1–7.35 (6, m), 8.7 (1, s). Anal. calcd ($C_{32}H_{38}Cl_2N_4O_5$) C, H, N.

Methyl 2-[4-(2,6-dimethylphenylcarbamoylmethyl)piperazin-1-yl]ethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate (5b). Method D. To a cold solution of **10b** (0.6 g, 0.0018 mol) 4-dimethylaminopyridine (DMAP; 0.23 g, 0.0018 mol), *N*-(2,6-dimethylphenyl)-2-[4-(2-hydroxyethylpiperazin-1-yl)]acetamide (0.52 g, 0.0018 mol), and 1,3-dicyclohexylcarbodiimide (DCC; 0.38 g, 0.0018 mol) were added. The reaction mixture was stirred at 0 °C for 5 min and then at room temperature for 6 h. After filtration the solvent was evaporated and the residue was taken up in methylene chloride. The organic layer was washed with $NaHCO_3$ solution, H_2O, dried, and evaporated. The residue was purified by flash chromatography (eluent:

toluene:acetone, 1:1) to afford 0.21 g (20%) of **5b**, mp 79–82 °C (toluene); ^{1}H NMR: δ 2.22 (6, s), 2.28 (3, s), 2.3 (3, s), 2.45–2.7 (10, m), 3.15 (2, s), 3.6 (3, s), 4.05–4.25 (2, m), 5.78 (1, s), 6.1 (1, broad), 7.1–7.75 (7, m), 8.7 (1, broad). Anal. calcd ($C_{32}H_{39}N_5O_7$) C, H, N.

Pharmacology

Isolated guinea-pig left and right atrial preparations. Guinea-pig (350–400 g male and female) were sacrified by cervical dislocation. After thoracotomy, the hearts were immediately removed and washed by perfusion through the aorta with oxygenated Tyrode solution of the following composition (mmol/L): 136.9 NaCl; 5.4 KCl; 2.5 $CaCl_2$; 1.0 $MgCl_2$; 0.4 $NaH_2PO_4 \cdot H_2O$; 11.9 $NaHCO_3$; 5.5 glucose. The physiological salt solution (PSS) 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 atrium were dissected from ventricles, cleaned of excess tissue, hung vertically in a 15 mL organ bath containing the PSS continously 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 (FT. 0.3, Grass Instrument, Quincy, Massachusetts, U.S.A.) connected to a pen recorder (KV 380), Battaglia-Rangoni, Bologna, Italy). The left atria were stimulated by rectangular pulses of 0.6–0.8 ms duration and ca. 50% threshold-voltage through two platinum contact electrodes in the lower holding clamp (Grass S88 stimulator). After the tissue was beating for several minutes, a length-tension curve was determined and the muscle length was maintained at that which elicited 90% of maximum contractile force observed at the optimal length. A stabilization period of 45–60 min was allowed before the atria were challenged by various agents. During this equilibration period, the beating 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 (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, and 100 μmol/L) first dissolved in dimethylformamide (DMF). According to this procedure the concentration of DMF in the bath solution never exceeded 0.3%, a concentration which did not produce appreciable inotropic and/or chronotopic effects. During the generation of a cumulative dose–response curve the next higher 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 Tyrode solution of the following composition (mmol/L): 118 NaCl; 4.75 KCl; 2.54 $CaCl_2$; 1.20 $MgSO_4$; 1.19 KH_2PO_4; 25 $NaHCO_3$; 11 glucose equilibrate with 95% O_2 and 5% CO_2 gas at pH 7.4. Vessel was cleaned of extraneous connective tissue. Two helicoidal strips (15 mm × 3 mm) were cut from each aorta beginning 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 at 35 °C. Strips were secured at once end to plexiglass hooks and connected via the surgical thread to a force displacement (FT. 0.3, Grass) transducer for monitoring changes in isometric contraction. Aortic strips were subjected to a resting force of 1 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 mmol/L KCl (equimolar substitution of K^+ for Na^+).

Subsequent to the contraction reaching a plateau (approximately 30 min) the compounds (0.1, 0.5, 1, 5, 10, and 50 μmol/L) were added cumulatively to the bath allowing for any relaxation to obtain an equilibrate 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 analysed by Student's t-test. The criterion for significance was a p value less than 0.05. The ED_{50} values were calculated from log concentration–response curves (Probit analysis by Litchfield and Wilcoxon, $n = 6–8$). All data are presented as mean ± SEM.[22]

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