Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 3423–3430
Bioorganic & Medicinal Chemistry

1,4-Dihydropyridine derivatives as calcium channel modulators: the role of 3-methoxy-flavone moiety

Roberta Budriesi, Alessandra Bisi, Pierfranco Ioan,* Angela Rampa, Silvia Gobbi, Federica Belluti, Lorna Piazzi, Piero Valenti and Alberto Chiarini

Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy

Received 7 January 2005; revised 24 February 2005; accepted 1 March 2005
Available online 30 March 2005

Abstract—It was earlier recognized that calcium antagonists, and in particular 1,4-dihydropyridines, exhibited distinct cardiovascular profiles. In addition two different splice variants of the L-type calcium channel were found in vascular and cardiac tissues. In this study, novel substituted 1,4-dihydropyridines with a 3-methoxy-flavone moiety were synthesized and structural modifications of the substituents in the dihydropyridine ring of nifedipine were carried out in order to find tissue specific compounds. The negative inotropic, chronotropic and vasorelaxant effects were investigated on guinea-pig left, right atria and aortic strips, respectively. The introduction of an heteroaromatic ring in 4-position of the 1,4-dihydropyridine nucleus led to compounds selective for cardiac tissues. Moreover, different residues in the 1,4-dihydropyridine ring could modulate the chronotropic versus inotropic activity.

1. Introduction

Calcium entry blockers (CEBs) are structurally divided in two large groups: the dihydropyridines (DHP), represented by Nifedipine (**1** Fig. 1), and the non-dihydropyridines, represented by Verapamil and Diltiazem.[1] Most of them are therapeutic agents used in the treatment of many cardiovascular diseases such as angina and hypertension. They elicit the therapeutic effects by reversibly blocking Ca^{2+} influx through L-type calcium channels (LCCs Ca_v1) found in cardiac and vascular smooth muscle.[2] In particular, splice variants $Ca_v1.2a$ and $Ca_v1.2b$ are associated with the heart and smooth muscle, respectively.[3]

Ca_v1 channels consist of a pore forming α_1-subunit and three auxiliary subunits, namely α_2-δ, β and γ.[4,5] The α_1-subunit is composed of four homologues domains (I–IV); each of which has six transmembrane segments (S1–S6).[6] Photoaffinity labelling provided the first evidence that the DHP binding site was located on the transmembrane strands IIIS6 and IVS6 and on the S5/S6 helices.[6]

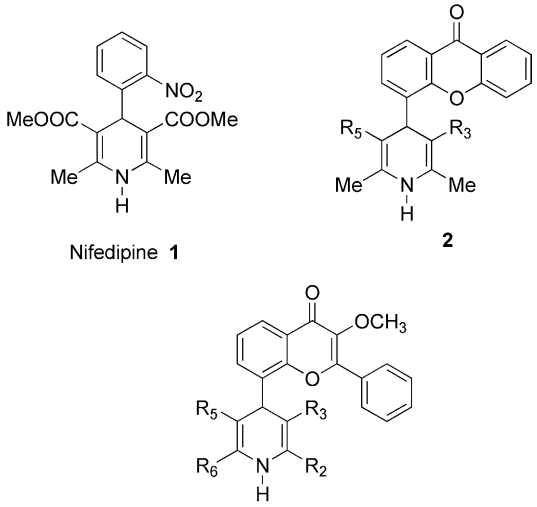


Figure 1.

For a long time we have been interested in the study of the 1,4-dihydropyridines bearing a different heteroaromatic ring in 4-position.[6–13] The elucidation of the structure–activity relationships of DHPs agreed that the substitution of *o*-nitrophenyl ring by aromatic or

Keywords: Calcium entry blockers; 1,4-Dihydropyridine; Cardiac selectivity; In vitro assays.
* Corresponding author. Tel.: +39 051 2099737; fax: +39 051 2099721; e-mail: pierfranco.ioan3@unibo.it

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.03.007

heteroaromatic ring is important to improve selectivity and activity.[7,8] In particular, the potent and selective negative chronotropic activity of compounds bearing a 4-xanthone moiety (**2** Fig. 1) might indeed open new perspectives in the search for more effective drugs for the control of cardiac arrhythmias.

Considering that the xantone[6,7,9] group can be successfully replaced with structurally related oxygen heterocycles such as fluorenone,[9–11] benzophenone[11,12] and coumarin[13] and taking into account a patent from 1985 reporting substituted chromone and flavone[14] derivatives as hypotensive and cardiotonic drugs, we prepared a series of 1,4-dihydropyridines bearing a 3-methoxyflavone ring with general formula **3** (Fig. 1).

The aim of this work is to evaluate the influence of the heteroaromatic ring in combination with different substituents in the 1,4 dihydropyridines nucleus. Though the enantioselective property of the binding site for 1,4-dihydropiridines in the α_1-subunits of $Ca_v1.2$ channels is well established, the compounds **3d**, **3f**, **3h**, **3i**–**k**, and **3m**, were synthesized and tested as racemates and, at this stage of the research no attempt was made to resolve the enantiomeric mixtures. Above all the goal of the present paper is to clarify the influence of the methoxyflavone moiety on the 1,4-dihydropyridine backbone.

The cardiovascular activities of all synthesized compounds were assayed on isolated tissues namely right and left guinea-pig atria and K^+-depolarized guinea-pig aortic strips.

2. Chemistry

The compounds listed in Table 1 were prepared according to Schemes 1 and 2.

In Scheme 1, the synthesis of the key intermediate 3-methoxy-4-oxo-2-phenyl-4*H*-chromene-8-carbaldehyde **6** is reported. *O*-Cresol was refluxed with acetic anhydride in the presence of $AlCl_3$ to give 1-(2-hydroxy-3-methylphenyl)-ethanone, which was treated with benzaldehyde and 50% NaOH to yield the corresponding 1-(2-hydroxy-3-methylphenyl)-3-phenyl-propenone. Cyclization with H_2O_2 in NaOH gave 3-hydroxy-8-methyl-2-phenylchromen-4-one,[15] which was methylated by means of dimethylsulfate to give compound **4**. Bromination with *N*-bromosuccinimide followed by reaction with hexamethylentetramine and HCl afforded 3-methoxy-4-oxo-2-phenyl-4*H*-chromene-8-carbaldehyde **6**.

The compounds **3a**–**k** were prepared (Scheme 2) according to the classical Hantzsch reaction,[16] by heating the aldehyde with the appropriate reagents in isopropyl alcohol.

Table 1. Cardiovascular activity of compounds

Compound	Cardiovascular activity								
	Negative inotropy			Negative chronotropy			Vasorelaxant activity		
	Ia %[a] (±SEM)	EC_{50} (μM)[b]	(95% cl)	Ia %[c] (±SEM)	EC_{30} (μM)[b]	(95% cl)	Ia %[d] (±SEM)	IC_{50} (μM)[b]	(95% cl)
1	97 ± 2	0.26	(0.19–0.36)	85 ± 4.2[h]	0.025	(0.019–0.031)	82 ± 1.3[g]	0.009	(0.003–0.02)
3a	24 ± 0.7[e]			93 ± 3.7[i]	0.105	(0.09–0.126)	66 ± 4.1	1.11	(0.85–1.28)
3b	69 ± 3.5[e]	1.79	(1.52–2.05)	87 ± 1.3[h]	0.0097	(0.0074–0.013)	61 ± 2.8	0.46	(0.36–0.52)
3c	63 ± 3.2[f]	0.031	(0.028–0.033)	67 ± 3.4[f]	0.041	(0.038–0.043)	37 ± 1.5		
3d	38 ± 1.4			59 ± 2.1[f]	0.13	(0.09–0.21)	42 ± 3.4		
3e	47 ± 1.4			79 ± 2.3	0.35	(0.29–0.43)	29 ± 1.2[j]		
3f	61 ± 1.5	1.19	(0.82–1.61)	20 ± 1.5			19 ± 0.7[j]		
3g	20 ± 0.3[e]			25 ± 1.3[e]			47 ± 2.1		
3h	10 ± 0.3			90 ± 1.6	0.38	(0.27–0.53)	24 ± 1.4[j]		
3i	48 ± 2.7[g]			20 ± 1.4			24 ± 1.3[j]		
3j	63 ± 2	0.55	(0.39–0.62)	67 ± 1.7	2.25	(1.82–2.83)	24 ± 0.1		
3k	24 ± 1.3			74 ± 3.7	1.29	(1.07–1.55)	13 ± 0.9		
3l	10 ± 0.7			81 ± 1.3	0.46	(0.41–0.51)	38 ± 2.1[j]		
3m	8 ± 0.6			82 ± 4.7	0.81	(0.76–0.87)	58 ± 1.7[j]	0.80	(0.73–0.88)

[a] Decrease in developed tension in isolated guinea-pig left atrium at 10^{-5} M, expressed as percent changes from the control (n = 5–6). The left atria were driven at 1 Hz. 10^{-5} M gave the maximum effect for most compounds.

[b] Calculated from log concentration–response curves (Probit analysis by Litchfield and Wilcoxon with n = 6–7).[17] When the maximum effect was <50%, the EC_{50} ino., EC_{30} chrono., IC_{50} values were not calculated.

[c] Decrease in atrial rate on guinea-pig spontaneously beating isolated right atrium at 10^{-5} M, expressed as percent changes from the control (n = 7–8). Pretreatment heart rate ranged from 165 to 190 beats/min. 10^{-5} M gave the maximum effect for most compounds.

[d] Percent inhibition of calcium-induced contraction on K^+-depolarized guinea-pig aortic strip at 5×10^{-5} M (n = 5–6). 5×10^{-5} M gave the maximum effect for most compounds.

[e] At the 5×10^{-5} M.

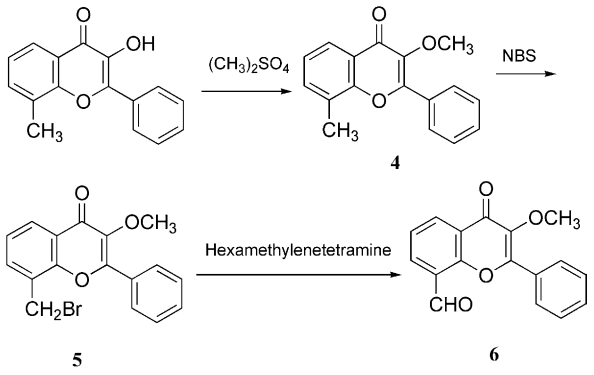
[f] At the 5×10^{-7} M.

[g] At the 10^{-6} M.

[h] At the 10^{-7} M.

[i] At the 5×10^{-6} M.

[j] At the 10^{-5} M.



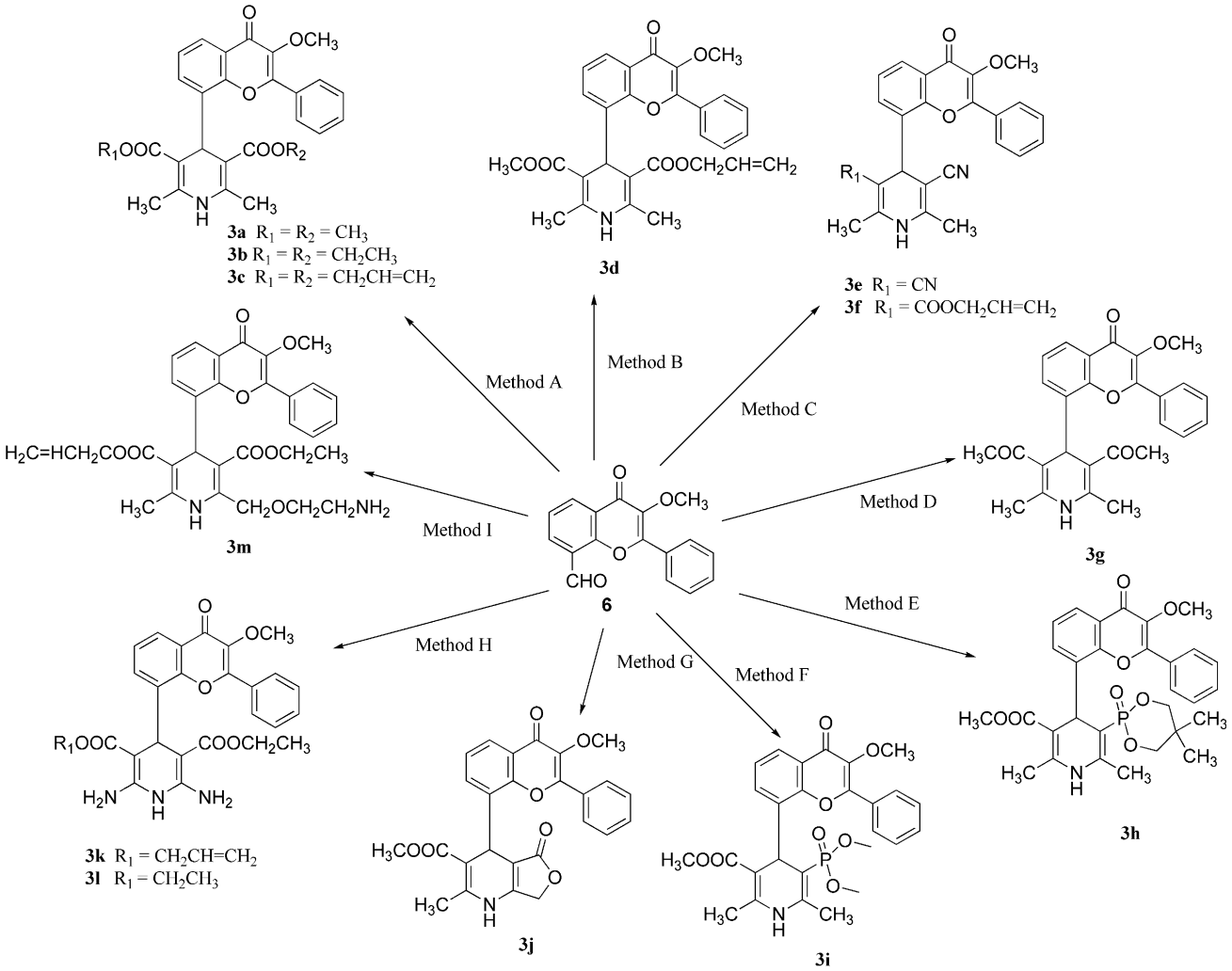
Scheme 1.

Compounds **3a–c** were prepared following method A, coupling the 3-methoxy-4-oxo-2-phenyl-4*H*-chromene-8-carbaldehyde **6** with the selected acetoacetic ester in the presence of ammonia. The asymmetric 1,4-dihydropyridine **3d** was synthesized by refluxing the aldehyde **6** with an equimolar amount of allyl acetoacetate and methyl 3-aminocrotonate (method B).

Compounds **3e–f** were obtained according to method C by the reaction of **6** with aminocrotononitrile and allyl acetoacetate, compound **3g** was prepared by reacting **6** with acetylacetone and ammonia (method D). Compounds **3h–i** were synthesized by refluxing the aldehyde and methyl 3-aminocrotonate with 2-acetonyl-5,5-dimethyl-2-oxo-1,3,2-dioxophosphorinane and β-ketopropionylphosphonate, respectively (methods E and F). Compound **3j** was obtained reacting **3a** with pyridinium bromide perbromide and heating the not isolable bromomethyl intermediate (method G). Compounds **3k–l** were prepared according to method H, by heating the aldehyde with an equimolar amount of allyl acetoacetate and ethoxy carbonyl acetamidine in dry ethanol with a catalytic amount of piperidine. Finally, compound **3m**, bearing the 2-aminoethoxymethyl group, was prepared by cleavage of the 2-phtalimido derivative (obtained starting from ethyl 4-(2-phtalimidoethoxy)acetoacetate and allyl 3-aminocrotonate) with aqueous methylamine (method I).

3. Pharmacology

The pharmacological profile of compounds was tested on guinea-pig isolated left and right atria to evaluate their inotropic and chronotropic effects, respectively,



Scheme 2.

and on K^+-depolarized guinea-pig aortic strips to assess calcium antagonist activity. At first, all compounds were checked at increasing doses to evaluate the percent decrease on developed tension on isolated left atrium driven at 1 Hz (negative inotropic activity), the percent decrease in atrial rate on spontaneously beating right atrium (negative chronotropic activity) and the percent inhibition of calcium-induced contraction on K^+-depolarized aortic strips (vasorelaxant activity). Data were analyzed by Student's *t*-test. The potency of drugs defined as EC_{50}, EC_{30} and IC_{50} was evaluated from log concentration–response curves (Probit analysis by Litchfield and Wilcoxon, $n = 6–8$) in the appropriate pharmacological preparations. All data are presented as mean ± SEM.[17–19]

4. Results and discussion

The activity of compounds, expressed as efficacy and potency, was determined on guinea-pig left and right atria to test negative inotropy and chronotropy, respectively, while vasorelaxant activity was assessed on guinea-pig aortic strips. All data are reported in Table 1 with those of parent compound **1**, taken as reference, to allow relevant reasoning on structure–activity relationships. It is well established that nifedipine is selective for the vascular activity over the heart, this is probably due to a different affinity for the different isoforms of $Ca_v1.2$ channels expressed on both tissues,[20] ($Ca_v1.2a$ expressed in the heart and $Ca_v1.2b$ expressed in the vascular tissue). The substitution of the *o*-nitrophenyl ring of nifedipine with a 3-methoxyflavone moiety (**3a**) reverses the selectivity of compounds **3a** being about a 123-fold less potent than nifedipine on vascular smooth muscles (EC_{50} = 1.11 μM and 0.0097 μM, respectively). Moreover, compound **3a** shows weak negative inotropic activity, while negative chronotropic potency is about four fold less if compared with that of the reference compound **1** (EC_{30} = 0.105 μM and 0.025 μM, respectively). The drastic change in the cardiovascular activity of compound **3a** with respect to that of nifedipine could be due to the position of the carbonyl group of the 3-methoxyflavone moiety. Indeed we have already shown in previous works that most of the compounds bearing a carbonyl group in the same position of 3-methoxyflavone moiety, as fluorenone[9] or benzophenone,[12] are endowed with the same cardiovascular profile as of **3a**. On the contrary, in coumarin derivatives,[13] where the carbonyl group is shifted, the activity slightly changes showing a reduced potency on heart and vascular preparations in respect to that of nifedipine. The metabolic breakdown of 1,4-dihydropyridine follows the principle of increasing hydrophilicity.[21] The first step is oxidation to the pyridine derivative, practically devoid of vasodilatory activity, which is followed by saponification of one of the two ester functions to give the pyridine-monocarboxylic acid. The final products of biotransformation are the hydroxymethyl-pyridine-carboxylic acids resulting from oxidative hydroxylation of the methyl group neighbouring the carboxylic acid function. These give the corresponding γ-lactones after acidification.

With the aim to evaluate the effects of γ-lactones[22] of **3a** we decided to synthesize compound **3j**. As shown in Table 1, this compound was devoid of vasorelaxant activity and showed a clear decrease in the negative chronotropic potency (EC_{30} = 2.25 μM, c.l. 95% = 1.82–2.83). In contrast, it elicited a significant negative inotropic potency (EC_{50} = 0.546 μM, c.l. 95% = 0.392–0.617), only two times less than the reference compound **1**.

The substitution of the methyl ester in position C-3 and C-5 of compound **3a** with an ethyl ester, as in **3b**, improved the cardiovascular potency of the compound, which proved to be about 11- and 2-fold more potent than **3a** as negative chronotropic and vasorelaxant agent, respectively. This improved potency did not injure the negative chronotropic selectivity, indeed **3b** kept a good vasorelaxant/negative chronotropic and negative inotropic/chronotropic ratio (47 and 184, respectively). When the ethyl ester residues of **3b** were changed with allyl ester ones (**3c**), the compound became more selective for the cardiac tissue. If compared with **3b**, the vasorelaxant activity decreased significantly but the negative chronotropic over inotropic selective activity was lost.

The most active nifedipine analogues are characterized by the non-identical carboxylate group in C-3 and C-5. In most cases, dihydropyridines with non-identical ester group have a higher vasodilatory activity than their symmetrically substituted analogues.[23] In compound **3d** the methyl ester and allyl ester groups were combined. The cardiovascular profile of **3d** is similar to that of **3a**, although it is a weak vasorelaxant agent compared to the latter compound. The replacement of one allyl ester group of **3c** with a different electron withdrawing group as CN (**3f**) did not affect the cardiovascular profile, but when both C-3 and C-5 were substituted with a cyano group (**3e**), the compound became a weak but selective negative inotropic agent (EC_{50} = 1.19 μM, c.l. = 0.82–1.61). Decreasing the electron withdrawing effect of substituents in position C-3 and C-5 (**3g**) the cardiovascular activity was lost, in good agreement with our previous investigations.[8]

On the basis of the good results obtained with some phosphonate derivatives of 1,4-dihydropyridines,[24] we decided to introduce a cyclic or open phosphonate group in C-3 (compounds **3h** and **3i**, respectively). Surprisingly, while compound **3h** showed a good negative chronotropic potency and selectivity, the opening of the cyclic phosphonate group (**3i**), caused the loss of the cardiovascular activity.

It is well known that both the presence of small alkyl groups, such as methyl in C-2 and C-6, and the replacement of one alkyl group by amino are well tolerated without affecting activity.[25] The compounds **3k–m** were synthesized to investigate the influence of different substituents in position C-2 and C-6 of the 1,4-dihydropyridine ring in combination with the methoxyflavone moiety.

Compound **3l**, the 2,6-diamino substituted of **3b**, showed selective negative chronotropic activity but its

potency decreased to about 47-fold if compared with **3b** (EC_{30} = 0.46 μM and 0.0097 μM, respectively). On the other hand, the introduction of amino groups in C-2 and C-6 of the compound **3k** with nonidentical ester groups decreased the bradycardic potency without affecting the selectivity.

Restoring the methyl group in C-6 and introducing in 2-position a residue of amlodipine (**3m**), the negative chronotropic potency was improved with respect to **3k**, and vasorelaxant activity was restored.

5. Conclusions

In this work a series of 1,4-dihydropyridines bearing a 3-methoxyflavone ring in 4-position and different groups from C-2 to C-6 were synthesized. Once again we could demonstrate that the introduction of 4-heteroaromatic group in the 1,4-dihydropyridine ring of nifedipine could modulate the cardiovascular activity of compounds. In particular most of the new compounds showed a selective bradycardic activity in the guinea-pig isolated right atria.

Recently, Zamponi et al.[5] employed a 3D-QSAR model, to explain the SARs for 4-isoxazolyl-1,4-dihydropyridines. Our compounds could bind the same site in the domain region, namely the 3-methoxyflavone moiety could interact with tyrosine and methionine aminoacid residues.

The substitution in 2,3,5,6-position of 1,4-dihydropyridine ring could modulate the chronotropic/inotropic activity or make molecules inactive. In particular **3b** is 51 and 7-fold less potent than nifedipine as vasorelaxant and negative inotropic agent, respectively, while is 3-fold more potent as bradycardic. Of course, further studies are required, but this kind of compounds could be useful in the treatment of myocardial ischemia, where negative inotropic and hypotensive effects could be potentially deleterious.[26,27]

6. Experimental

6.1. Chemistry

6.1.1. General methods. Melting points were determined on a Buchi apparatus and are uncorrected. ^{1}H NMR spectra were obtained for $CDCl_3$ solutions on a Gemini 300 spectrometer. Chemical shifts are reported in parts per million (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-Institute and Springer.

6.1.1.1. 3-Methoxy-8-methyl-2-phenyl-chromen-4-one (4). A suspension of 5 g (0.02 mol) of 3-hydroxy-8-methyl-2-phenyl-chromen-4-one[15], 2.5 mL of $(CH_3)_2SO_4$, 5 g of K_2CO_3 in 100 mL of acetone was heated under reflux 8 h, hot filtered and evaporated to dryness. The residue, on crystallizing from ethanol, gave 5 g (96%) of **4**, mp 147–150 °C. ^{1}H NMR δ 2.6 (s, 3H, *CH_3*), 3.9 (s, 3H, *OCH_3*), 7.25–8.2 (m, 8H, *Ar*). Anal. Calcd ($C_{17}H_{14}O_3$) C, H.

6.1.1.2. 8-Bromomethyl-3-methoxy-2-phenyl-chromen-4-one (5). A mixture of 3-methoxy-8-methyl-2-phenyl-chromen-4-one (5.5 g, 0.02 mol), *N*-bromosuccinimide (3.7 g, 0.02 mol) in the presence of a catalytic amount of benzoyl peroxide in 150 mL of carbon tetrachloride, was refluxed for 4 h and then hot filtered. The solution was evaporated to dryness and the residue, on crystallizing from ethyl acetate, gave 5 g (70%) of **5**, mp 156–159 °C. ^{1}H NMR δ 3.9 (s, 3H, *OCH_3*), 4.8 (s, 2H, *CH_2Br*), 7.3–8.25 (m, 8H, *Ar*). Anal. Calcd ($C_{17}H_{13}BrO_3$) C, H.

6.1.1.3. 3-Methoxy-4-oxo-2-phenyl-4*H*-chromene-8-carbaldehyde (6). A solution of 8-bromomethyl-3-methoxy-2-phenyl-chromen-4-one (3.44 g, 0.01 mol) and hexamethylenetetramine (2.8 g, 0.02 mol) in 32 mL of 50% acetic acid was refluxed for 5 h. Conc. HCl (15 mL) was added and the mixture was refluxed for 1 h, poured into ice and filtered. The solid was purified by flash-chromatography (eluent: petroleum ether/ethyl acetate 4.5:0.5) to give 2.23 g (80%) of **6** mp 153–154 °C. ^{1}H NMR δ 3.95 (s, 3H, *OCH_3*), 7.5–8.6 (m, 8H, *Ar*), 10.75 (s, 1H, *CHO*). Anal. Calcd ($C_{17}H_{11}O_4$) C, H.

6.1.1.4. 4-(3-Methoxy-4-oxo-2-phenyl-4*H*-chromen-8-yl)-2,6-dimethyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid dimethylester (3a). Method A: A solution of **6** (0.56 g, 0.002 mol), methyl acetoacetate (0.46 g, 0.004 mol) and ammonia (5 mL) in isopropyl alcohol (15 mL) was refluxed 30 h. The separated solid, on cooling, was filtered and crystallized from toluene to yield 0.28 g (30%) of **3a** mp 264–265 °C. ^{1}H NMR δ 2.3 (s, 6H, *CH_3*), 3.3 (s, 6H, COO*CH_3*), 3.9 (s, 3H, *OCH_3*), 5.7 (broad, 1H, *NH*), 5.8 (s, 1H, *H-4 dihydropyridine*), 7.2–8.3 (m, 8H, *Ar*). MS: 476 (M^{+1}). Anal. Calcd ($C_{27}H_{25}NO_7$) C, H, N.

6.1.1.5. 4-(3-Methoxy-4-oxo-2-phenyl-4*H*-chromen-8-yl)-2,6-dimethyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid diethylester (3b). Using the precedent procedure and starting from 0.56 g (0.002 mol) of **6**, 0.4 g (40%) of **3b** mp 229–230 °C (toluene) were obtained. ^{1}H NMR δ 0.8 (t, 6H, CH_2CH_3), 2.35 (s, 6H, *CH_3*), 3.8–3.9 (m, 4H, *CH_2CH_3*), 3.95 (s, 3H, *OCH_3*), 5.55 (broad, 1H, *NH*), 5.8 (s, 1H, *H-4 dihydropyridine*), 7.2–8.3 (m, 8H, *Ar*). MS: 504 (M^{+1}). Anal. Calcd ($C_{29}H_{29}NO_7$) C, H, N.

6.1.1.6. 4-(3-Methoxy-4-oxo-2-phenyl-4*H*-chromen-8-yl)-2,6-dimethyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid diallylester (3c). Using the precedent procedure and starting from 0.56 g (0.002 mol) of **6**, 0.47 g (45%) of **3c** mp 194–195 °C (toluene) were obtained. ^{1}H NMR δ 2.4 (s, 6H, *CH_3*), 3.95 (s, 3H, *OCH_3*), 4.2–4.4 (m, 4H,

CH=CH_2), 4.7–4.85 (m, 4H, COOCH_2), 5.3–5.5 (m, 2H, CH=CH_2), 5.85 (broad, 1H, *NH*), 5.95 (s, 1H, *H-4 dihydropyridine*), 7.25–8.3 (m, 8H, *Ar*). MS: 528 (M^{+1}). Anal. Calcd ($C_{31}H_{29}NO_7$) C, H, N.

6.1.1.7. 4-(3-Methoxy-4-oxo-2-phenyl-4*H*-chromen-8-yl)-2,6-dimethyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid-3-allylester-5-methylester (3d). A solution of **6** (0.56 g, 0.002 mol), allyl acetoacetate (0.28 g, 0.002 mol), methyl 3-aminocrotonate (0.23 g, 0.002 mol) in isopropyl alcohol (15 mL) was refluxed for 10 h. The solvent was evaporated to dryness and the residue was purified by flash-chromatography (eluent: toluene/acetone 4:1) to give 0.25 g (25%) of **3d** mp 232–233 °C (toluene). ^{1}H NMR δ 2.3 (d, 6H, CH_3), 3.3 (s, 3H, COOCH_3), 3.95 (s, 3H, OCH_3), 4.2–4.4 (m, 2H, CH=CH_2), 4.65–4.8 (m, 2H, COOCH_2), 5.3–5.5 (m, 1H, CH=CH_2), 5.65 (broad, 1H, *NH*), 5.8 (s, 1H, *H-4 dihydropyridine*), 7.2–8.3 (m, 8H, *Ar*). MS: 502 (M^{+1}). Anal. Calcd ($C_{29}H_{27}NO_7$) C, H, N.

6.1.1.8. 4-(3-Methoxy-4-oxo-2-phenyl-4*H*-chromen-8-yl)-2,6-dimethyl-1,4-dihydro-pyridine-3,5-dicarbonitrile (3e). 5-Cyano-4-(3-methoxy-4-oxo-2-phenyl-4*H*-chromen-8-yl)-2,6-dimethyl-1,4-dihydro-pyridine-3-carboxylic acid allyl ester (3f). Method C: A solution of **6** (0.56 g, 0.002 mol), allyl acetoacetate (0.28 g, 0.002 mol), aminocrotonitrile (0.23 g, 0.002 mol) in isopropyl alcohol (15 mL) was refluxed for 20 h. The solvent was evaporated to dryness and the residue was purified by flash-chromatography (eluent: toluene/acetone 4:1) to give first 0.19 g (20%) of **3f** mp 128–129 °C (toluene), then 0.4 g (50%) of **3e** mp 214–215 °C (toluene).

3f: ^{1}H NMR δ 2.05 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 3.9 (s, 3H, OCH_3), 4.25 (m, 2H, CH=CH_2), 4.7 (m, 2H, COOCH_2), 5.4 (m, 1H, CH=CH_2), 5.55 (s, 1H, *H-4 dihydropyridine*), 6.7 (broad, 1H, *NH*), 7.15–8.15 (m, 8H, *Ar*). MS: 470 (M^{+1}). Anal. Calcd ($C_{28}H_{24}N_2O_5$) C, H, N.

3e: ^{1}H NMR δ 2.1 (s, 6H, CH_3), 3.9 (s, 3H, OCH_3), 5.2 (s, 1H, *H-4 dihydropyridine*), 6.6 (broad, 1H, *NH*), 7.2–8.2 (m, 8H, *Ar*). MS: 409 (M^{+1}). Anal. Calcd ($C_{25}H_{19}N_3O_3$) C, H, N.

6.1.1.9. 8-(3,5-Diacetyl-2,6-dimethyl-1,4-dihydro-pyridin-4-yl)-3-methoxy-2-phenyl-chromen-4-one (3g). Method D: Acetylacetone (0.4 g, 0.004 mol) was added to a solution of **6** (0.56 g, 0.002 mol) in isopropyl alcohol (15 mL) and ammonia (5 mL) with stirring. The reaction mixture was refluxed for 12 h and then evaporated to dryness. The residue, on crystallizing from toluene, gave 0.4 g (40%) of **3g** mp 216–219 °C. ^{1}H NMR δ 2.2 (s, 6H, CH_3), 2.31 (s, 6H, CH_3), 3.9 (s, 3H, OCH_3), 5.8 (broad, 1H, *NH*), 5.9 (s, 1H, *H-4 dihydropyridine*), 7.2–8.3 (m, 8H, *Ar*). MS: 445 (M^{+1}). Anal. Calcd ($C_{27}H_{25}NO_5$) C, H, N.

6.1.1.10. 5-(5,5-Dimethyl-2-oxo-1,3,2-dioxophosphorinan-2-yl)-4-(3-methoxy-4-oxo-2-phenyl-4*H*-chromen-8-yl)-2,6-dimethyl-1,4-dihydro-pyridine-3-carboxylic acid methyl ester (3h). Method E: A solution of **6** (0.56 g, 0.002 mol), 2-acetonyl-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinane (0.44 g, 0.002 mol) and methyl 3-aminocrotonate (0.23 g, 0.002 mol) in isopropyl alcohol (15 mL) was refluxed for 10 h and evaporated to dryness. The residue was purified by flash-chromatography (eluent: toluene/ethyl acetate 7:3) to yield 0.23 g (20%) of **3h** mp 260–262 °C (toluene). ^{1}H NMR δ 0.5 (s, 3H, PCH_3), 0.75 (s, 3H, PCH_3), 2.3 (s, 6H, CH_3), 3.35 (m, 5H, COOCH_3 and OCH_2), 3.95 (m, 5H, OCH_3 and OCH_2), 5.6 (d, 1H, *H-4 dihydropyridine*), 6.15 (broad, 1H, *NH*), 7.8–8.25 (m, 8H, *Ar*). MS: 565 (M^{+1}). Anal. Calcd ($C_{30}H_{32}NO_8P$) C, H, N.

6.1.1.11. 5-(Dimethoxy-phosphoryl)-4-(3-methoxy-4-oxo-2-phenyl-4*H*-chromen-8-yl)-2,6-dimethyl-1,4-dihydro-pyridine-3-carboxylic acid methyl ester (3i). Method F: A solution of **6** (0.56 g, 0.002 mol), dimethyl-β-ketopropylphosphonate (0.33 g, 0.002 mol) and methyl 3-aminocrotonate (0.23 g, 0.002 mol) in isopropyl alcohol (15 mL) was refluxed for 10 h and evaporated to dryness. The residue was purified by flash-chromatography (eluent: toluene/ethyl acetate 7:3) to yield 0.20 g (20%) of **3i** as oily compound. ^{1}H NMR δ 2.4 (s, 6H, CH_3), 2.9 (d, 3H, POCH_3), 3.4 (d, 3H, POCH_3), 3.45 (s, 3H, COOCH_3), 3.95 (s, 3H, OCH_3), 5.6 (d, 1H, *H-4 dihydropyridine*), 6.1 (broad, 1H, *NH*), 7.3–8.4 (m, 8H, *Ar*). MS: 526 (M^{+1}). Anal. Calcd ($C_{27}H_{28}NO_8P$) C, H, N.

6.1.1.12. 4-(3-Methoxy-4-oxo-2-phenyl-4*H*-chromen-8-yl)-2-methyl-5-oxo-1,4,5,7-tetrahydro-furo[3,4-*b*] pyridine-3-carboxylic acid methyl ester (3j). Method G: To a cold solution of **3a** (0.24 g, 0.0005 mol) in chloroform (10 mL), pyridine (0.06 g, 0.0008 mol) and pyridinium bromide perbromide (0.19 g, 0.0006 mol) were added. The solution was stirred at 0 °C for 20 min and then it was refluxed for 90 min. After cooling, chloroform was added and the solution was washed with 2 N HCl and brine, dried and evaporated to dryness. The residue was crystallized form ethanol to give 0.11 (50%) of **3j** mp 298–302 °C. ^{1}H NMR (DMSO) δ 2.4 (s, 3H, CH_3), 3.4 (s, 3H, COOCH_3), 3.95 (s, 3H, OCH_3), 4.9 (s, 2H, CH_2), 5.55 (s, 1H, *H-4 dihydropyridine*), 7.2–8.2 (m, 8H, *Ar*), 9.9 (s, 1H, *NH*).

MS: 460 (M^{+1}). Anal. Calcd ($C_{26}H_{21}NO_7$) C, H, N.

6.1.1.13. 2-Amino-4-(3-methoxy-4-oxo-2-phenyl-4*H*-chromen-8-yl)-6-methyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid 5-allylester 3-ethylester (3k). 2,6-Diamino-4-(3-methoxy-4-oxo-2-phenyl-4*H*-chromen-8-yl)-1,4-dihydro-pyridine-3,5-dicarboxylic acid diethylester (3l). Method H: A solution of **6** (0.6 g, 0.0021 mol) in 20 mL of dry ethanol was prepared and ethoxy carbonyl acetamidine hydrochloride (0.273 g, 0.0021 mol), allyl acetoacetate (0.298 g, 0.0021 mol) and piperidine (six drops) were added with stirring. The reaction mixture was heated under reflux for 6 h and evaporated to dryness. The residue was purified by flash chromatography (eluent: toluene/acetone 4:1) to give 0.32 g (30%) of **3k** mp 235–238 °C. ^{1}H NMR (DMSO) δ 0.5 (t, 3H, CH_3), 2.4 (s, 3H, CH_3), 3.6 (m, 2H, COOCH_3), 3.9 (s, 3H, OCH_3), 4.0–4.3 (m, 2H, COOCH_2), 4.55–4.7 (m, 2H, CH=CH_2), 5.3 (m, 1H, CH=CH_2), 5.6 (s, 1H, *H-4 dihydropyridine*),

6.75 (broad, 2H, NH_2), 7.1–8.3 (m, 8H, *Ar*), 8.8 (s, 1H, *NH*). MS: 517 (M^{+1}). Anal. Calcd ($C_{29}H_{28}N_2O_7$) C, H, N.

Compound **3l** (0.11 g, 10%) was obtained from the same column washing with methanol, mp 175–179 °C. ^{1}H NMR δ 0.6 (t, 6H, CH_3), 3.7 (m, 4H, COOCH_2), 3.8 (s, 3H, OCH_3), 5.6 (d, 1H, *H-4 dihydropyridine*), 6.4 (broad, 5H, NH_2 and *NH*), 7.2–8.3 (m, 8H, *Ar*).

MS: 506 (M^{+1}). Anal. Calcd ($C_{27}H_{27}N_3O_7$) C, H, N.

6.1.1.14. 2-[2-Aminoethoxymethyl]-4-(3-methoxy-4-oxo-2-phenyl-4*H*-chromen-8-yl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylic acid allyl ethyl ester (3m). Method I: 1.8 g of **6** (0.0064 mol) were dissolved in 20 mL of isopropyl alcohol. Ethyl 4-(2-phtalimido)ethoxyacetoacetate (2.05 g, 0.0064 mol) and allyl-3-aminocrotonate (0.913 g, 0.0064 mol) were added with stirring and the reaction mixture was refluxed for 20 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (eluent: toluene/ethyl acetate 7:3), to yield 1.8 g (40%) g of oily compound, which was used in the subsequent step without further purification. ^{1}H NMR δ 0.7 (t, 3H, CH_3), 2.3 (s, 3H, CH_3), 3.8 (m, 4H, COOCH_2 + OCH_2), 3.9 (m, 7H, OCH_3 + CH_2 + CH_2N), 4.2–4.4 (m, 2H, CH=CH_2), 4.65–4.8 (m, 2H, COOCH_2), 5.1 (s, 1H, *H-4 dihydropyridine*), 5.3–5.5 (m, 1H, CH=CH_2), 5.8 (broad, 1H, *NH*), 7.5–8.4 (m, 12H, *Ar*).

1.8 g of previous compound (0.026 mol) was suspended in 50 mL of aqueous methylamine (35% w/v) and stirred at room temperature for 72 h. After filtration, the solid was purified by flash chromatography (eluent: methanol), to give 0.3 g of **3m**, mp 235–238 °C. ^{1}H NMR δ 0.7 (t, 3H, CH_3), 2.4 (s, 3H, CH_3), 3.0 (m, 2H, COOCH_2), 3.6 (m, 2H, CH_2), 3.8 (m, 2H, OCH_2), 3.9 (s, 3H, OCH_3), 4.1–4.4 (m, 2H, CH=CH_2), 4.75–4.85 (m, 4H, COO$CH2$ + CH_2N), 5.4 (m, 1H, *H-4 dihydropyridine*), 5.3–5.5 (m, 1H, CH=CH_2), 5.8 (broad, 1H, *NH*), 7.2–8.4 (m, 10H, *Ar* + NH_2). MS: 575 (M^{+1}). Anal. Calcd ($C_{32}H_{34}N_2O_8$) C, H, N.

6.2. Functional studies

6.2.1. Guinea-pig atrial preparations. Guinea-pigs (300–400 g female) were sacrificed by cervical dislocation. After thoracotomy the heart was immediately removed and washed by perfusion through the aorta with oxygenated Tyrode solution of the following composition (mM): 136.9 NaCl, 5.4 KCl, 2.5 $CaCl_2$, 1.0 $MgCl_2$, 0.4 $NaH_2PO_4xH_2O$, 11.9 $NaHCO_3$ and 5.5 glucose. The physiological salt solution (PSS) was buffered at pH 7.4 by saturation with 95% O_2–5% CO_2 gas, and the temperature was maintained at 37 °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, and hung vertically in a 15 mL organ bath containing the PSS continuously bubbled with 95% O_2–5% CO_2 gas at 37 °C, pH 7.4. The contractile activity was recorded isometrically by means of a force transducer (FT 0.3, Grass Instruments, Quincy, MA) using Power Lab software (Basile, Italy). 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 S88 stimulator). The right atrium was in spontaneous activity. After the tissue was beating for several minutes, a length–tension curve was determined, and the muscle length was maintained at 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 used to test compounds. During the 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 activity of the compounds (0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 and 100 μM), first dissolved in DMSO and then diluted with PSS. According to this procedure, the concentration of DMSO in the bath solution never exceeded 0.3%, a concentration that did not produce appreciable inotropic and chronotropic effects. During the construction of cumulative dose–response curves, the next higher concentration of the compounds was added only after the preparation reached a steady state.

6.2.2. Guinea-pig aortic strips. The thoracic aorta was removed and placed in 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 $NaHCO_3$ and 11 glucose equilibrated with 95% O_2–5% CO_2 gas at pH 7.4. The vessel was cleaned of extraneous connective tissue. Two helicoidal strips (10 mm × 1 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 pharmacological salt solution (PSS) at 37 °C. Strips were secured at one end 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 have been contracted by washing in PSS containing 80 mM KCl (equimolar substitution of K^+ for Na^+). After the contraction reached a plateau (about 45 min) the compounds (0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 and 100 μM) 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 K^+-induced contraction (DMSO for all compounds).

Acknowledgements

This research was supported by grants from M.I.U.R. (P.R.I.N. 2003) and the University of Bologna.

References and notes

1. Campiani, G.; Garofalo, A.; Fiorini, I.; Botta, M.; Nacci, V.; Tafi, A.; Chiarini, A.; Budriesi, R.; Bruni, G.; Romeo, M. R. *J. Med. Chem.* **1995**, *38*, 4393.

2. Schleifer, K. J. *J. Med. Chem.* **1999**, *42*, 2204.
3. Triggle, D. J. *Cell. Mol. Neurobiol.* **2003**, *23*, 293.
4. Stephen, J. W. *J. Bioenerg. Biomembr.* **1998**, *30*, 299.
5. Zamponi, G. W.; Stotz, S. C.; Staples, R. J.; Andro, T. M.; Nelson, J. K.; Hulubei, V.; Blumenfeld, A.; Natale, N. R. *J. Med. Chem.* **2003**, *46*, 87.
6. Huber, I.; Wappl, E.; Herzog, A.; Mitterdorfer, J.; Glossmann, H.; Langer, T.; Striessnig, J. E. *Biochem. J.* **2000**, *347*, 829.
7. Valenti, P.; Chiarini, A.; Gasperi, F.; Budriesi, R. *Arzneim.-Forsch./Drug Res.* **1990**, *40*, 122.
8. Valenti, P.; Chiarini, A.; Rampa, A.; Bisi, A.; Budriesi, R. *Arzneim.-Forsch./Drug Res.* **1991**, *41*, 705.
9. Chiarini, A.; Valenti, P.; Rampa, A.; Bisi, A.; Budriesi, R. *Arzneim.-Forsch./Drug Res.* **1992**, *42*, 797.
10. Bisi, A.; Budriesi, R.; Chiarini, A.; Rampa, A.; Valenti, P. *Il Farmaco* **1993**, *48*, 1491.
11. Budriesi, R.; Aicardi, G.; Campana, G.; Spampinato, S.; Zaza, A.; Bisi, A.; Rampa, A.; Valenti, P.; Chiarini, A. *Eur. J. Pharmacol.* **1998**, *359*, 161.
12. Rampa, A.; Budriesi, R.; Bisi, A.; Chiarini, A.; Valenti, P. *Arzneim.-Forsch./Drug Res.* **1992**, *42*, 1284.
13. Valenti, P.; Rampa, A.; Budriesi, R.; Bisi, A.; Chiarini, A. *Bioorg. Med. Chem.* **1998**, *6*, 803.
14. Goldmann, S.; Franckowisk, G.; Schranun, M.; Thomas, G.; Groos, R. *Chem. Abstr.* **1985**, *102*, 62091.
15. Auwers, K. v. *Chem. Ber* **1916**, *49*, 815.
16. Hantzsch, A. *Justus Liebigs Ann. Chem.* **1882**, *1*, 215.
17. Tallarida, R. J.; Murray, R. B. *Manual of Pharmacologic Calculations with Computer Programs*, 2nd ed.; Springer: New York, 1987.
18. Motulsky, H.; Christopoulos, A. *Fitting Models to Biological Data Using Linear and Non Linear Regression* **2003**, www.GraphPad.com.
19. Motulsky, H. *Statistic guide: Statistical Analysis for Laboratory and Clinical Research: A Pratical Guide to Curve Fitting* **2003**, www.GraphPad.com.
20. Hu, H.; Marban, E. *Mol. Pharmacol.* **1998**, *53*, 902.
21. Shibanuma, T.; Iwanami, M.; Fujimoto, M.; Takenaka, T.; Muratami, M. *Chem. Pharm. Bull. (Tokyo)* **1980**, *28*, 2809.
22. Rampa, A.; Budriesi, R.; Bisi, A.; Fabbri, G.; Barili, P. G.; Chiarini, A.; Valenti, P. *Arzneim.-Forsch./Drug Res.* **1995**, *45*, 957.
23. Opie, L. H. In *Calcium Antagonists and Cardiovascular Disease*; Katz, A. M., Ed.; Raven: New York, 1984.
24. Budriesi, R.; Rampa, A.; Bisi, A.; Fabbri, G.; Chiarini, A.; Valenti, P. *Arzneim.-Forsch./Drug Res.* **1996**, *46*, 374.
25. Meyer, H.; Wehinger, E.; Bosset, F.; Stoepel, K.; Vater, W. *Arzneim.-Forsch./Drug Res.* **1981**, *31*, 1173.
26. Opie, L. H. *Cardiovasc. Drugs Ther.* **1989**, *3*, 482.
27. Kern, M. J.; Deligonul, U.; Labovitz, A. *Am. Heart J.* **1989**, *118*, 361.