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Coumarin 1,4-Dihydropyridine Derivatives

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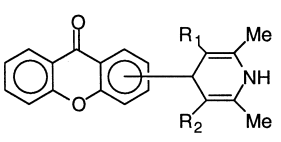
Abstract—A series of 1,4-dihydropyridines bearing a coumarin moiety in 4-position was synthesized. The compounds were evaluated for inotropic, chronotropic and calcium antagonist activities. The replacement of the *o*-nitrophenyl moiety of nifedipine with a coumarin or phenylcoumarin system is accompained by a decrease of the activity on myocardial and vascular parameters, but the synthesized compounds showed selective inhibiting effects on cardiac contractility and frequency.

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] 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 1,4-dihydropyridine-type calcium antagonists, in spite of the extensive SAR studies, further deserve the interest of the medicinal chemists. For a long time we have been interested[9–16] in the study of the 1,4-dihydropyridines, of general formula **1**, bearing a xanthone moiety in 4-position. The presence in the synthesized derivatives of a potent and selective chronotropic negative activity might indeed open new perspectives in the search of more effective drugs for the control of cardiac arrhythmias. With this aim we have prepared a series of coumarin 1,4-dihydropyridines of general formula **2**:



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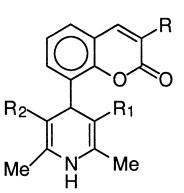

2a–p

The rationale for these modifications is based on the theory that the xanthone moiety can be successfully replaced with structurally related oxygen heterocycles such as chromone, flavone and coumarin moieties. In fact, in a number of cases this replacement has allowed us to obtain important results with regard to analeptics,[17] adrenergic β-blocking agents,[18] clofibrate-like hypolipemic drugs,[19] and more recently, antitumor drugs.[20–22] The asymmetric compounds were tested as racemates.

Chemistry

The studied compounds, collected in Table 1, were prepared as shown in Schemes 1 and 2.

In Scheme 1 is reported the synthesis of the key intermediates **4a**,**b**. *o*-Cresol was treated with formaldehyde in presence of $SnCl_4$ to give the corresponding salicylaldehyde,[23] which was condensed with sodium acetate or sodium phenylacetate, and acetic anhydride to afford 8-methylcoumarin and 8-methyl-3-phenylcoumarin, respectively. Bromination with *N*-bromosuccinimide (compounds **3a**,**b**), followed by reaction with hexamethylenetetramine afford the aldehydes **4a**,**b**.

In Scheme 2 is reported the synthesis of the studied derivatives.

The compounds, listed in Table 1, were prepared according to the classical Hantzsch reaction,[24] heating the selected aldehyde in isopropyl alcohol with the appropriate reagents. Compounds **2a**–**f** were prepared

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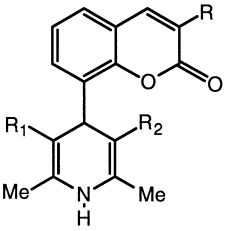
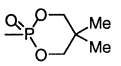
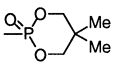
Table 1. Inotropic, chronotropic and calcium antagonist activities



Compound	R	R_1	R_2	ED_{50} ino	95% Conf. lim.	ED_{50} chrono	95% Conf. lim	ED_{50} CCB	95% Conf. lim.
2a	H	COOMe	COOMe	7.90	6.60–9.37	2.70	2.55–2.91	1.31	1.04–1.59
2b	H	COOEt	COOEt	0.54	0.46–0.64	0.055	0.042–0.071	0.056	0.048–0.071
2c	H	$COOCH_2CH=CH_2$	$COOCH_2CH=CH_2$	1.76	1.47–2.15	0.60	0.51–0.71	0.25	0.19–0.32
2d	Ph	$COOCH_2CH=CH_2$	$COOCH_2CH=CH_2$	0.41	0.33–0.50	2.47	2.30–2.63	34 ± 1.5% at 50 μM	
2e	Ph	COOMe	COOMe	0.93	0.75–1.15	14 ± 0.3% at 10 μM		15 ± 0.4% at 50 μM	
2f	Ph	COOEt	COOEt	0.89	0.81–1.04	0.27	0.19–0.38	35 ± 1.5% at 50 μM	
2g	H	COOMe	$COOCH_2CH=CH_2$	38 ± 1.4% at 50 μM		2.36	2.18–2.53	12 ± 0.4% at 50 μM	
2h	Ph	COOMe	$COOCH_2CH=CH_2$	2.69	2.11–3.40	45 ± 1.9% at 10 μM		0.57	0.49–0.65
2i	H	COOMe		34 ± 1.4% at 50 μM		3.05	2.87–3.25	39 ± 2.7% at 50 μM	
2j	Ph	COOMe		1.25	0.96–1.60	2.95	2.77–3.15	39 ± 1.4% at 50 μM	
2k	H	COOMe		5 ± 0.3% at 50 μM		7 ± 0.3% at 50 μM		45 ± 2.3% at 50 μM	
2l	H	COOMe	NO_2	5 ± 0.1% at 50 μM		8 ± 0.5% at 10 μM		10 ± 0.3% at 50 μM	
2m	Ph	COOMe	NO_2	41 ± 2.3% at 1 μM		18 ± 0.6% at 50 μM		2.63	2.11–3.34
2n	H	COOMe	COMe	2.06	1.57–2.75	24 ± 1.3% at 10 μM		45 ± 2.7% at 50 μM	
2o	H	COMe	COMe	10 ± 0.3% at 50 μM		19 ± 0.7% at 50 μM		37 ± 1.4% at 50 μM	
2p	*	*	*	19 ± 0.1% at 50 μM		33 ± 1.4% at 50 μM		14 ± 0.8% at 50 μM	
Nifedip.				0.26	0.2–0.3	0.039	0.031–0.048	0.009	0.003–0.02

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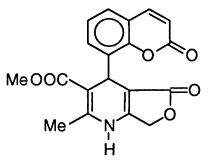
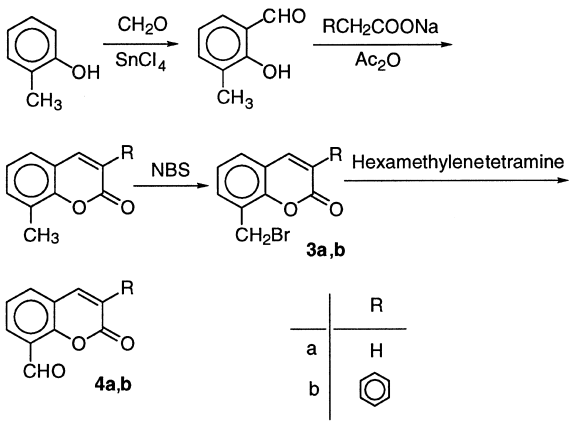


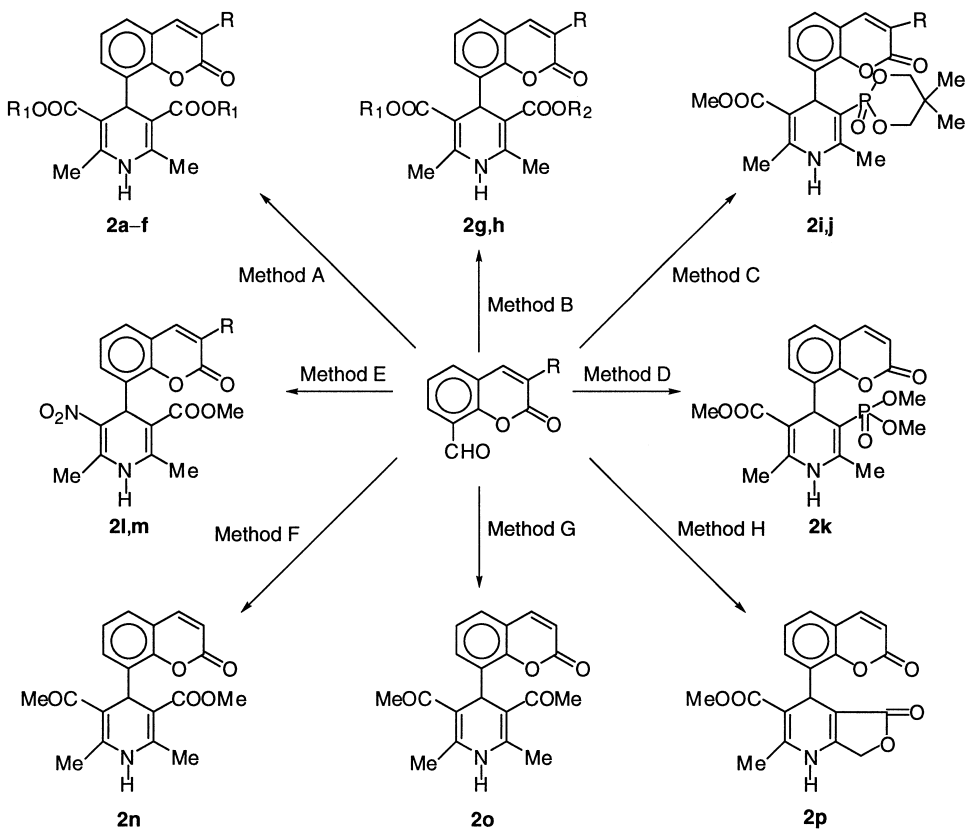
ED_{50} values with 95% conf. lim. are calculated from log-concn in response curves (probit analysis by Litchfield and Wilcoxon).[30] Each compound was tested at least five times. 'ED_{50} ino' shows the ED_{50} values for the negative inotropic potency of tested compounds on stimulated guinea pig left 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.

according to method A, coupling the coumarinic aldehydes with the selected acetoacetic ester in presence of ammonia.

The asymmetric 1,4-dihydropyridines **2g**–**h** were synthesized according to method B, by refluxing the aldehydes with an equimolar amount of selected acetoacetic ester and 3-aminocrotonate. According to method C compounds **2i**,**j** were prepared by reaction of the aldehydes with 2-acetonyl-5,5-dimethyl-2-oxo-1,3,2-dioxophosphorinane[25] and methyl 3-aminocrotonate. Compound **2k** was synthesized using dimethyl β-chetopropionylphosphonate[26] and methyl 3-aminocrotonate (Method D). Compounds **2l**,**m** were obtained by reaction with



Scheme 1.



Scheme 2.

nitroacetone[27] and methyl 3-aminocrotonate (Method E). Compounds **2n**,**o** were prepared by reaction of coumarinic aldehyde with acetylacetone, methyl 3-aminocrotonate and, respectively, ammonia (Methods F and G). Finally, compound **2p** was obtained reacting **2a** with pyridinium bromide perbromide and heating the not isolable bromomethyl intermediate (Method H).

Pharmacology

The biological activity of all new compounds was tested by functional studies on isolated guinea pig cardiac preparations to evaluate the inotropic and chronotropic effects in left driven atria and in spontaneously beating right atria, respectively. The vasorelaxing activity,

expression of calcium antagonism, was assessed in potassium depolarized guinea pig aortic strips.

The potency of the compounds was expressed as ED_{50} and IC_{50} values accordingly to the different functional experiments or as percent inhibition at a particular concentration if no ED_{50} or IC_{50} values have been measured.

Results and discussion

Table 1 lists negative inotropic, chronotropic and vasorelaxing activities of studied compounds in comparison with nifedipine used as reference standard.

A first analysis of the results reveals that the replacement of the *o*-nitrophenyl moiety of nifedipine with coumarin or phenylcoumarin system is accompanied by a decrease of the activity on myocardial and vascular parameters. The derivatives possessing the coumarin system (**2a**, **2b**, **2c**, and **2g**) are more potent than the correspondent phenylcoumarin analogues (**2d**, **2e**, **2f**, and **2h**), but less selective. In fact **2d**, **2e**, and **2f** show appreciable negative inotropic potency whereas **2b** displays fairly good negative chronotropic efficacy and potency.

Generally speaking, the most active compounds of the series appear to be compounds **2b**, **2d**, and **2f**. The ratio between ED_{50} (negative inotropic activity) and ED_{50} (negative chronotropic activity) and the corresponding values of nifedipine are 3.3 and 6.9 for **2f**; 2 and 1.4 for **2b**; 1.5 and 63 for **2d**; 6.5 and 15 for **2c**; 29 and 69 for **2a**; 4.6 and 76 for **2j**.

Taking a closer look at these potency ratio it emerges that in these derivatives there is an increase of negative inotropic activity with respect to the other effects except for **2b** which shows a negative chronotropic potency comparable to nifedipine. Furthermore, the efficacy, expressed by % decreases both in left atrium [$92 \pm 4.2\%$ at 50 μM] and in right atrium [$69 \pm 1.2\%$ at 0.1 μM], is similar in the corresponding figures of nifedipine [$97 \pm 2\%$ at 10 μM; $85 \pm 4.2\%$ at 0.1 μM], but this latter compound triggers these effects at lower concentration.

It is to note that these results are in keeping with our previous studies,[11,12] which underlined, as far as cardiac activity is concerned, the relevant importance of allyl and ethyl functions to the ester moiety at 3- and 5-positions of the dihydropyridine ring.

Another additional interesting finding is the clear selectivity of **2i** and **2b** (ED_{50} values 3.05 μM and 0.055 μM, respectively) for myocardial parameter chronotropism appearing as nearly pure negative chronotropic agents. Considering vasorelaxing activity, which is a measure of calcium antagonist action on smooth muscle again compounds **2b**, **2c** and **2h** possessing allyl and ethyl radicals to the ester moiety elicit significant calcium antagonist activity being from about 6–63 fold less potent than nifedipine. For all the other derivatives, except **2a** and **2m** bearing methyl ester groups, calcium antagonism is poor or undetectable.

Finally, some of these new compounds showed a very intriguing behaviour. In fact, in our experiments on left and right atria a rapid and transient positive inotropic effect appeared and was not changed both by pretreatment with a non-selective β-antagonist (1 μM propranolol) and by employing reserpine-treated guinea pigs (data not shown). The mechanism involved in this phenomenon is elusive and further research on this topic is needed. In conclusion the replacement of the *o*-nitrophenyl moiety of nifedipine with a coumarin or phenylcoumarin system is accompanied by a decrease of the activity on myocardial and vascular parameters, but the synthesized compounds show selective cardiodepressant effects expecially negative chronotropic action. Furthermore, the results confirm our hypotesis that the xanthone moiety can be successfully replaced with a coumarin system.

Experimental

Chemistry

Melting points were determined on a Büchi apparatus and are uncorrected. ^1H 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 $\pm 0.4\%$ 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.

8-Methyl-2*H*-chromen-2-one. A mixture of 2-hydroxy-3-methylbenzaldehyde[23] (6.8 g, 0.05 mol), anhydrous sodium acetate (4.1 g, 0.05 mol) and acetic anhydride (10.2 g, 0.1 mol) was heated at 180–190 °C for 5 h. After cooling the mixture was poured in 10% solution of K_2CO_3. The separated solid was collected by filtration, dried and crystallized from ligroin to give 5.2 g (65%) of

product mp 112–115 °C [lit.[28] mp 109.6–109.9 °C]. ^{1}H NMR δ 2.45 (s, 3H, C*H*$_3$), 6.4 (d, 1H, H-3), 7.15–7.4 (m, 3H, Ar), 7.7 (d, 1H, H-4).

8-Methyl-3-phenyl-2*H*-chromen-2-one. Using the precedent procedure and starting from 2-hydroxy-3-methylbenzaldehyde (6.8 g, 0.05 mol) and anhydrous sodium phenylacetate (7.9 g, 0.05 mol), 8.26 g (70%) of product mp 114–115 °C (ligroin) [lit.[29] mp 107.5–109 °C] were obtained. ^{1}H NMR δ 2.5 (s, 3H, C*H*$_3$), 7.2–7.8 (m, 9H, Ar and H-4).

8-Bromomethyl-2*H*-chromen-2-one (3a). A mixture of 8-methyl-2*H*-chromen-2-one (8 g, 0.05 mol), *N*-bromosuccinimide (8.9 g, 0.05 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 ligroin, gave 8 g (70%) of **3a** mp 90–92 °C. ^{1}H NMR δ 4.75 (s, 2H, C*H*$_2$), 6.5 (d, 1H, H-3), 7.2–7.75 (m, 4H, Ar and H-4). Anal. calcd ($C_{10}H_7BrO_2$) C, H.

8-Bromomethyl-3-phenyl-2*H*-chromen-2-one (3b). Using the precedent procedure and starting from 8-methyl-3-phenyl-2*H*-chromen-2-one (11.8 g, 0.05 mol), 11 g (70%) of **3b** mp 157–160 °C were obtained. ^{1}H NMR δ 4.78 (s, 2H, C*H*$_2$), 7.2–7.8 (m, 9H, Ar and H-4). Anal. calcd ($C_{16}H_{11}BrO_2$) C, H.

8-Formyl-2*H*-chromen-2-one (4a). A solution of **3a** (4.78 g, 0.02 mol) and hexamethylenetetramine (5.6 g, 0.04 mol) in 40 mL of 50% acetic acid was refluxed for 4 h. Twenty milliliters of conc. HCl were added and the mixture was refluxed for 15 min. After cooling the mixture was filtered and crystallized from ligroin to give 2.78 g (80%) of **4a** mp 114–116 °C. ^{1}H NMR δ 6.55 (d, 1H, H-3), 7.4–8.15 (m, 4H, Ar and H-4), 10.75 (s, 1H, *CHO*). Anal. calcd ($C_{10}H_6O_3$) C, H.

8-Formyl-3-phenyl-2*H*-chromen-2-one (4b). Using the precedent procedure and starting from 3.15 g (0.01 mol), 1.86 g (75%) of **4b** mp 165–168 °C were obtained. ^{1}H NMR δ 7.4–8.15 (m, 9H, Ar and H-4), 10.8 (s, 1H, CHO). Anal. calcd ($C_{16}H_{10}O_3$) C, H.

Dimethyl 1,4-dihydro-4-(2-oxo-2*H*-chromen-8-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (2a). Method A. A solution of **4a** (1.74 g, 0.01 mol), methyl acetoacetate (2.32 g, 0.02 mol) and ammonia (10 mL) in isopropyl alcohol (30 mL) was refluxed 30 h. After cooling the reaction mixture was filtered and the solid obtained was crystallized from toluene to yield 0.74 g (20%) of **2a** mp 242–245 °C. ^{1}H NMR δ 2.32 (s, 6H, *CH*$_3$), 3.6 (s, 6H, COOCH$_3$), 5.4 (s, 1H, H-4 dihydropyridine), 6.15 (broad, 1H, NH), 6.35 (d, 1H, H-3 coumarin), 7.15–7.7 (m, 4H, Ar and H-4 coumarin). MS: *m*/*z* (relative abundance): 369 (M$^+$, 7.19), 225 (13.96), 224 (100). Anal. calcd ($C_{20}H_{19}NO_6$) C, H, N.

Diethyl 1,4-dihydro-4-(2-oxo-2*H*-chromen-8-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (2b). Using the precedent procedure and starting from 1.74 g (0.01 mol) of **4a**, 0.99 g (25%) of **2b** mp 197–200 °C (EtOH) were obtained. ^{1}H NMR δ 1.15 (t, 6H, CH$_2$*CH*$_3$) 2.32 (s, 6H, *CH*$_3$), 4.05 (m, 4H, *CH*$_2$CH$_3$), 5.4 (s, 1H, H-4 dihydropyridine), 6.1 (s, 1H, NH), 6.38 (d, 1H, H-3 coumarin), 7.1–7.7 (m, 4H, Ar and H-4 coumarin). MS: *m*/*z* (relative abundance): 397 (M$^+$, 39.65), 252 (100), 106 (11.95), 89 (15.74). Anal. calcd ($C_{22}H_{23}NO_6$) C, H, N.

Diallyl 1,4-dihydro-4-(2-oxo-2*H*-chromen-8-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (2c). Using the precedent procedure and starting from 1.74 g (0.01 mol) of **4a**, 0.63 g (15%) of **2c** mp 157–160 °C (EtOH) were obtained. ^{1}H NMR δ 2.32 (s, 6H, *CH*$_3$), 4.45 (m, 4H, *CH*=*CH*$_2$), 5.1 (m, 4H, COO*CH*$_2$), 5.42 (s, 1H, H-4 dihydropyridine), 5.75 (m, 2H, *CH*=CH$_2$), 6.0 (broad, 1H, NH), 6.35 (d, 1H, H-3 coumarin), 7.1–7.7 (m, 4H, Ar and H-4 coumarin). MS: *m*/*z* (relative abundance): 421 (M$^+$, 8.73), 277 (20.16), 276 (100). Anal. calcd ($C_{24}H_{23}NO_6$) C, H, N.

Diallyl 1,4-dihydro-4-(3-phenyl-2-oxo-2*H*-chromen-8-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (2d). Using the precedent procedure and starting from 1.25 g (0.005 mol) of **4b**, 1.12 g (45%) of **2d** mp 213–215 °C (toluene) were obtained. ^{1}H NMR δ 2.35 (s, 6H, *CH*$_3$), 4.5 (m, 4H, *CH*=*CH*$_2$), 5.1 (m, 4H, COO*CH*$_2$), 5.45 (s, 1H, H-4 dihydropyridine), 5.8 (m, 2H, *CH*=CH$_2$), 6.2 (s, 1H, NH), 7.1–7.8 (m, 9H, Ar and H-4 coumarin). MS: *m*/*z* (relative abundance): 497 (M$^+$, 3.21), 276 (32.58), 91 (100). Anal. calcd ($C_{30}H_{28}NO_6$) C, H, N.

Dimethyl 1,4-dihydro-4-(3-phenyl-2-oxo-2*H*-chromen-8-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (2e). Using the precedent procedure and starting from 1.25 g (0.005 mol) of **4b**, 0.44 g (20%) of **2e** mp 265–267 °C (toluene) were obtained. ^{1}H NMR δ 2.35 (s, 6H, *CH*$_3$), 3.6 (s, 6H, COO*CH*$_3$), 5.4 (s, 1H, H-4 dihydropyridine), 6.1 (s, 1H, NH), 7.15–8.8 (m, 9H, Ar and H-4 coumarin). MS: *m*/*z* (relative abundance): 445 (M$^+$, 6.46), 224 (100), 223 (21.73). Anal. calcd ($C_{26}H_{24}NO_6$) C, H, N.

Diethyl 1,4-dihydro-4-(3-phenyl-2-oxo-2*H*-chromen-8-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (2f). Using the precedent procedure and starting from 1.25 g (0.005 mol) of **4b**, 1.2 g (50%) of **2f** mp 180–183 °C (toluene) were obtained. ^{1}H NMR δ 1.15 (t, 6H, CH$_2$*CH*$_3$), 2.3 (s, 6H, *CH*$_3$), 4.03 (m, 4H, *CH*$_2$CH$_3$), 5.4 (s, 1H, H-4 dihydropyridine), 6.1 (s, 1H, NH), 7.1–7.8

(m, 9H, Ar and H-4 coumarin). MS: *m/z* (relative abundance): 473 (M^+, 9.52), 252 (100), 45 (60.71). Anal. calcd ($C_{28}H_{28}NO_6$) C, H, N.

Methyl, allyl 1,4-dihydro-4-(2-oxo-2*H*-chromen-8-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (2g). Method B. A solution of **4a** (1.74 g, 0.01 mol), allyl acetoacetate (1.42 g, 0.01 mol), methyl 3-aminocrotonate (1.15 g, 0.01 mol) in isopropyl alcohol (30 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 1.58 g (40%) of **2g** mp 190–193 °C (toluene). ^{1}H NMR δ 2.35 (s, 6H, CH_3), 3.6 (s, 3H, COOCH_3), 4.45 (m, 2H, CH = CH_2), 5.1 (m, 2H, COOCH_2), 5.4 (s, 1H, H-4 dihydropyridine), 5.8 (m, 1H, CH = CH_2), 6.1 (broad, 1H, NH), 6.35 (d, 1H, H-3 coumarin), 7.1–7.7 (m, 4H, Ar and H-4 coumarin). MS: *m/z* (relative abundance): 395 (M^+, 7.14), 250 (100), 249×(9.33). Anal. calcd ($C_{22}H_{21}NO_6$) C, H, N.

Methyl, allyl 1,4-dihydro-4-(3-phenyl-2-oxo-2*H*-chromen-8-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (2h). Using the precedent procedure and starting from 1.25 g (0.005 mol) of **4b**, 0.34 g (20%) of **2h** mp 200–203 °C (toluene) were obtained. ^{1}H NMR δ 2.33 (s, 6H, CH_3), 3.58 (s, 3H, COOCH_3), 4.48 (m, 2H, CH = CH_2), 5.1 (m, 2H, COOCH_2), 5.4 (s, 1H, H-4 dihydropyridine), 5.8 (m, 1H, CH = CH_2), 6.1 (broad, 1H, NH), 7.1–7.8 (m, 9H, Ar and H-4 coumarin). MS: *m/z* (relative abundance): 471 (M^+, 8.44), 251 (16.38), 250 (100). Anal. calcd ($C_{28}H_{26}NO_6$) C, H, N.

Methyl 1,4-dihydro-5-(5,5-dimethyl-2-oxo-1,3,2-dioxophosphorinan-2-yl)-4-(2-oxo-2*H*-chromen-8-yl)-2,6-dimethylpyridine-3-carboxylate (2i). Method C. A solution of **4a** (1.74 g, 0.01 mol), 2-acetonyl-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinane (2.2 g, 0.01 mol) and methyl 3-aminocrotonate (1.15 g, 0.01 mol) in isopropyl alcohol (30 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 0.92 g (20%) of **2i** mp 252–255 °C (toluene). ^{1}H NMR δ 0.85 (s, 3H, PCH_3), 1.05 (s, 3H, PCH_3), 2.25 (s, 6H, CH_3), 3.6 (s, 3H, $COOCH_3$), 3.7 (m, 2H, OCH_2), 4.2 (m, 2H, OCH_2), 5.28 (d, 1H, H-4 dihydropyridine), 6.15 (broad, 1H, NH), 6.35 (d, 1H, H-3 coumarin), 7.1–7.7 (m, 4H, Ar and H-4 coumarin). MS: *m/z* (relative abundance): 459 (M^+, 12.82), 314 (100), 228 (19.69). Anal. calcd ($C_{23}H_{26}NO_7P$) C, H, N.

Methyl 1,4-dihydro-5-(5,5-dimethyl-2-oxo-1,3,2-dioxophosphorinan-2-yl)-4-(3-phenyl-2-oxo-2*H*-chromen-8-yl)-2,6-dimethylpyridine-3-carboxylate (2j). Using the precedent procedure and starting from 1.25 g (0.005 mol) of **4b**, 0.27 g (10%) of **2j** mp 290–292 °C (EtOH) were obtained. ^{1}H NMR δ 0.9 (s, 3H, PCH_3), 1.03 (s, 3H, PCH_3), 2.3 (s, 6H, CH_3), 3.6 (s, 3H, COOCH_3), 3.7 (m, 2H, OCH_2), 4.2 (m, 2H, OCH_2), 5.28 (d, 1H, H-4 dihydropyridine), 6.15 (broad, 1H, NH), 7.1–7.8 (m, 9H, Ar and H-4 coumarin). MS: *m/z* (relative abundance): 535 (M^+, 13.06), 314 (100), 228 (25.62). Anal. calcd ($C_{29}H_{31}NO_7P$) C, H, N.

Methyl 5-dimethoxyphosphinyl-2,6-dimethyl-4-(2-oxo-2*H*-chromen-8-yl)-1,4-dihydropyridine-3-carboxylate (2k). Method D. A solution of **4a** (1.74 g, 0.01 mol), dimethyl β-chetopropylphosphonate (1.64 g, 0.01 mol) and methyl 3-aminocrotonate (1.15 g, 0.01 mol) in isopropyl alcohol (30 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 0.42 g (10%) of **2k** mp 227–230 °C (toluene). ^{1}H NMR δ 2.3 (s, 6H, CH_3), 3.2 (d, 3H, POCH_3), 3.55 (d, 3H, POCH_3), 3.6 (s, 3H, $COOCH_3$), 5.15 (d, 1H, H-4 dihydropyridine), 6.15 (broad, 1H, NH), 6.35 (d, 1H, H-3 coumarin), 7.1–7.7 (m, 4H, Ar and H-4 coumarin). MS: *m/z* (relative abundance): 419 (M^+, 6.60), 274 (100), 273 (10.86). Anal. calcd ($C_{20}H_{22}NO_7P$) C, H, N.

Methyl 1,4-dihydro-2,6-dimethyl-3-nitro-(2-oxo-2*H*-chromen-8-yl)-pyridine-5-carboxylate 2l. Method E. Nitroacetone (1 g, 0.01 mol) was added to a solution of **4a** (1.74 g, 0.01 mol) in isopropyl alcohol (30 mL) and then methyl 3-aminocrotonate (1.15 g, 0.01 mol) was added with stirring. The reaction mixture was refluxed for 10 h and evaporated to dryness. The residue was crystallized from toluene to yield 1.42 g (40%) of **2l** mp 266–269 °C. ^{1}H NMR δ 2.35 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 3.6 (s, 3H, COOCH_3), 5.65 (s, 1H, H-4 dihydropyridine), 6.35 (d, 1H, H-3 coumarin), 6.95 (broad, 1H, NH), 7.1–7.75 (m, 4H, Ar and H-4 coumarin). MS: *m/z* (relative abundance): 356 (M^+, 14.50), 211 (100), 91 (91.36). Anal. calcd ($C_{18}H_{16}N_2O_6$) C, H, N.

Methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(3-phenyl-2-oxo-2*H*-chromen-8-yl)-pyridine-5-carboxylate (2m). Using the precedent procedure and starting from 1.25 g (0.005 mol) of **4b**, 0.65 g (30%) of **2m** mp 272–275 °C (toluene) were obtained. ^{1}H NMR δ 2.3 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 3.6 (s, 3H, COOCH_3), 5.62 (s, 1H, H-4 dihydropyridine), 6.95 (broad, 1H, NH), 7.1–7.8 (m, 9H, Ar and H-4 coumarin). MS: *m/z* (relative abundance): 432 (M^+, 17.71), 211 (100), 165 (26.65). Anal. calcd ($C_{24}H_{20}NO_6$) C, H, N.

Methyl 1,4-dihydro-2,6-dimethyl-3-acetyl-4-(2-oxo-2*H*-chromen-8-yl)-pyridine-5-carboxylate (2n). Method F. Acetylacetone (1 g, 0.01 mol) was added to a solution of **4a** (1.74 g, 0.01 mol) in isopropyl alcohol (30 mL) and then methyl 3-aminocrotonate (1.15 g, 0.01 mol) was added with stirring. The reaction mixture was refluxed for 10 h and evaporated to dryness. The residue was

purified by flash-chromatography (eluent: methylene chloride:ethyl acetate, 1:1) to yield 0.88 g (25%) of **2n** mp 242–246 °C (toluene). ^{1}H NMR δ 2.28 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 3.15 (s, 3H, CH_3), 5.5 (s, 1H, H-4 dihydropyridine), 6.1 (broad, 1H, NH), 6.4 (d, 1H, H-3 coumarin), 7.15–7.7 (m, 4H, Ar and H-4 coumarin). MS: *m*/*z* (relative abundance): 353 (M^+, 15.61), 208 (100), 207 (12.54). Anal. calcd ($C_{20}H_{19}NO_5$) C, H, N.

1,4-Dihydro-2,6-dimethyl-4-(2-oxo-2*H*-chromen-8-yl)-3,5-diacetylpyridine (2o). Method G. Acetylacetone (2 g, 0.02 mol) was added to a solution of **4a** (1.74 g, 0.01 mol) in isopropyl alcohol (30 mL) and ammonia (10 mL) with stirring. The reaction mixture was refluxed for 12 h and then evaporated to dryness. The residue, on crystallizing from toluene, gave 0.34 g (10%) of **2o** mp 135–138 °C. ^{1}H NMR δ 2.28 (s, 6H, CH_3), 2.31 (s, 6H, CH_3), 5.52 (s, 1H, H-4 dihydropyridine), 6.02 (broad, 1H, NH), 6.38 (d, 1H, H-3 coumarin), 7.15–7.7 (m, 4H, Ar and H-4 coumarin). MS: *m*/*z* (relative abundance): 337 (M^+, 25.21), 294 (12.92), 192 (100). Anal. calcd ($C_{20}H_{19}NO_4$) C, H, N.

Methyl 2-methyl-5-oxo-4-(2-oxo-2*H*-chromen-8-yl)-1,4,-5,7-tetrahydrofuro-[3,4-*b*]-pyridine-3-carboxylate (2p). Method H. To a cold solution of **2a** (0.74 g, 0.002 mol) in chloroform (20 mL), pyridine (0.26 g, 0.0033 mol) and pyridinium bromide perbromide (0.74 g, 0.0023 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 0.07 g (10%) of **2p** mp 158–162 °C. ^{1}H NMR δ 2.4 (s, 3H, CH_3), 3.55 (s, 3H, COOCH_3), 4.75 (s, 2H, CH_2), 5.25 (s, 1H, H-4 dihydropyridine), 6.4 (d, 1H, H-3 coumarin), 7.2–7.8 (m, 4H, Ar and H-4 coumarin). MS: *m*/*z* (relative abundance): 353 (M^+, 33.69), 208 (100), 179 (16.57). Anal. calcd ($C_{19}H_{13}NO_6$) 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 $NaH_2PO_4 \times H_2O$; 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, MA, USA) 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 50% above 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, and 50 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 chronotropic effects. During generation of 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. The 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 one 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 (0.01, 0.05, 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 analyzed 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 $\pm$ SEM.[30]

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