Eur J Med Chem (1996) 31, 547–556

Synthesis and activities of a thienyl dihydropyridine series on intracellular calcium in a rat pituitary cell line (GH3/B6)

M Varache-Lembège[1], A Nuhrich[1], V Zemb[1], G Devaux[1]*, P Vacher[2], AM Vacher[2], B Dufy[2]

[1]*Laboratoire de pharmacie chimique et chimie thérapeutique, UFR des sciences pharmaceutiques, Université de Bordeaux-II, 3, place de la Victoire;*
[2]*CNRS URA 1200 , Laboratoire de neurophysiologie, Université de Bordeaux-II, 146, rue Léo-Saignat, 33076 Bordeaux cedex, France*

(Received 5 October 1995; accepted 29 January 1996)

Summary — The synthesis of a thienyl dihydropyridine series according to the Hantzsch method is described. The influence of these derivatives on intracellular calcium ($[Ca^{2+}]i$) in GH3 cells was evaluated in vitro using spectrofluorimetry with indo1 as Ca^{2+} fluorescent probe. We compared their effects on $[Ca^{2+}]i$ and hormone release with those of nifedipine. The most active tested compounds on $[Ca^{2+}]i$ were those methylated on the 3-position of the thienyl ring (activity was about 75% of nifedipine). Interestingly, the most efficient compounds on $[Ca^{2+}]i$ were also the most efficient on hormone release.

dihydropyridine / calcium channel blocker / spectrofluorimetry / hormone secretion / prolactin / growth hormone

Introduction

Calcium antagonists [1, 2] are a well-established therapeutic class of agents which are finding increasing use in the treatment of angina pectoris and hypertension. Among the major structural series with this mode of action are the 1,4-dihydropyridines (DHPs) [3] of which nifedipine is the prototype (fig 1). Most of the studied DHPs contain a substituted phenyl group at the 4-position [4–9]. Many authors report also DHPs where the substituted aryl group was replaced by an isosteric heteroaryl group: 2-, 3- or 4-pyridine [10, 11], 4-dihydropyridine [12], tetrahydropyridine [13], 2-furan [11, 14], benzoxadiazol [15], spirobenzothiophene [16] and xanthone [17]. But, to our knowledge, the replacement of the *o*-nitrophenyl substituent of nifedipine by a thiophen ring has rarely been described [11, 18, 36].

In this paper, we report the synthesis and the biological properties of new thienyldihydropyridines. We have investigated their effects on the calcium influx in a rat pituitary cell line (GH3/B6) that spontaneously secretes prolactin (PRL) and growth hormone (GH). Hormone release in anterior pituitary cells is dependent on intracellular calcium [19–21]. We have therefore also studied the effects of the dihydropyridines on PRL and GH release.

Chemistry

In general, the synthesis of 1,4-dihydro-4-arylpyridines can be accomplished by the classical three-component Hantzsch reaction [22]. Thus, condensation of a thiophene carboxaldehyde with a substituted β-ketoester and a concentrated ammonia solution afforded the title compounds (scheme 1). The 5-substituted-2-thiophene carboxaldehyde intermediates **III–V** required for the preparation of **18–20** (table I) were obtained as indicated in scheme 2 according to Carpenter et al [23, 24].

In this sequence, the 2-thiophene carboxaldehyde **I** was protected as its *N,N'*-dimethylimidazolidine deri-

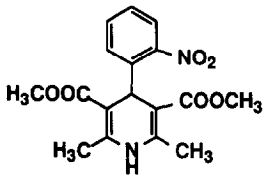
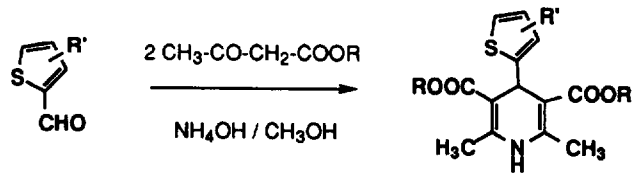


Fig 1. Nifedipine.

*Correspondence and reprints



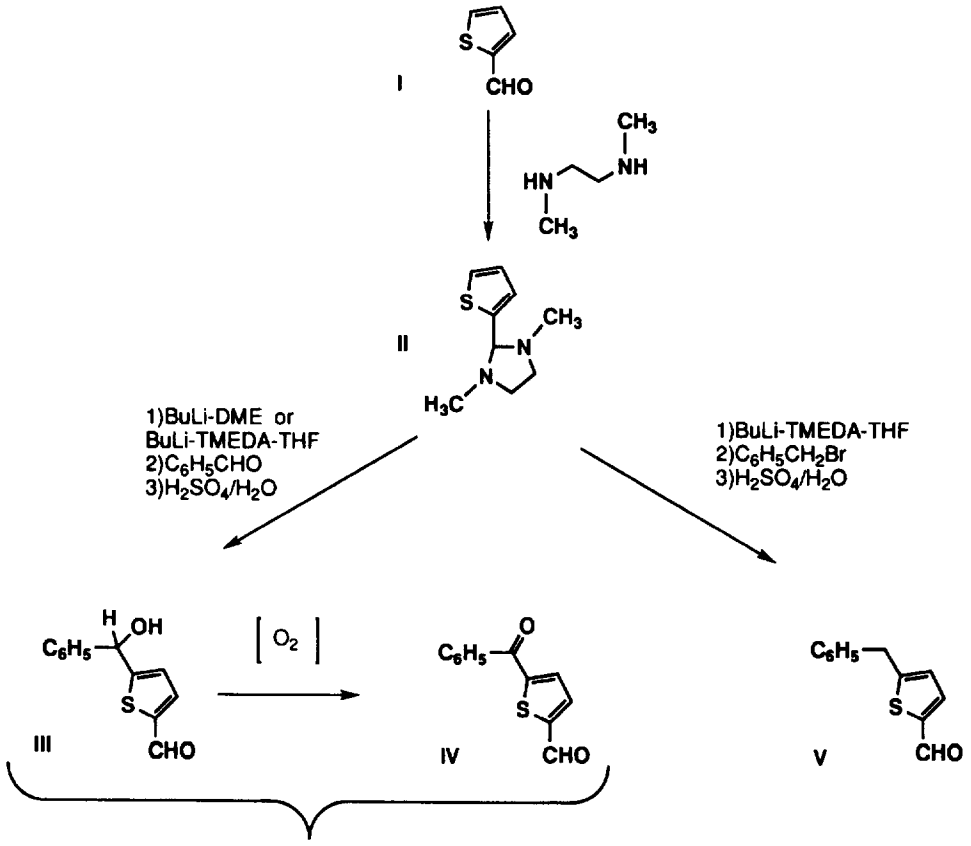
Scheme 1.

vative **II** by using *N,N'*-dimethylethylenediamine in refluxing benzene. The thiophene hydrogen at position 5 was selectively metallated with *n*-butyllithium. Lithiation of **II** with *n*-BuLi in 1,2-dimethoxyethane (DME) or TMEDA/THF (TMEDA = *N,N,N',N'*-tetramethylethylenediamine), followed by condensation with benzaldehyde, gave a mixture of products **III** and **IV**, readily separable by column chromatography. According to Carpenter and Chadwick [24], the formation of the ketoderivative **IV** could be explained by aerial oxidation during work-up. Under our experimental conditions, the alcohol **III** was obtained predominantly when lithiation was performed in TMEDA/THF mixture. With the same solvent system, the methylene analogue **V** was prepared using α-bromotoluene as electrophile.

We report that the final compounds (**1–22**) are light sensitive like nifedipine [25] and must be stored in darkness and under nitrogen. Poor yields of allylic compounds, particularly **4**, **8**, **12** and **16**, were found due to the difficulty of the work-up as a result of their high instability.

Pharmacology

Intracellular calcium was assayed using dual-emission microfluorimetry. Emission light was monitored at two wavelengths, 405 (*F*405) and 480 (*F*480) nm, by photon counting (fig 2). The autofluorescence of the cells at 405 and 480 nm was substracted from *F*405 and *F*480 respectively. From the ratio $R = F405/F480$,



Scheme 2.

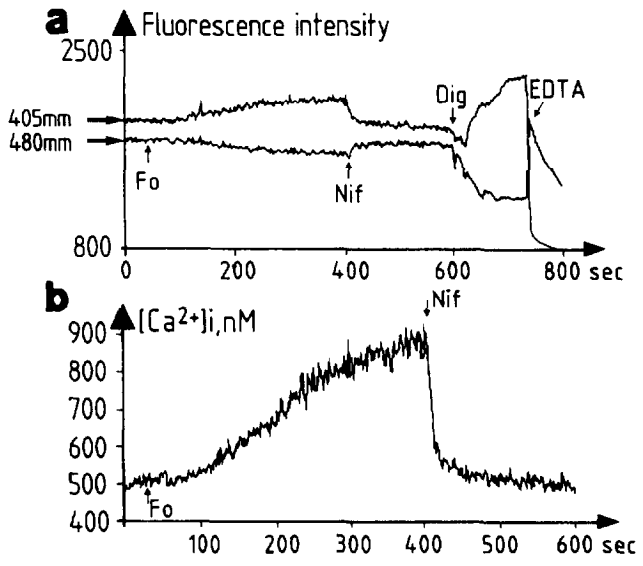


Fig 2. Determination of the intracellular calcium concentration by spectrofluorimetry using Indo 1 as fluorescent Ca^{2+} probe. Effect of 1 μM nifedipine on 10 μM forskoline-induced Ca2+ increase. **a**. Measurement of fluorescence intensities emitted by the Indo-1-loaded cells at two wavelengths, 405 and 480 nm. 100 μM Digitonine and 10 mM EDTA were added to calibrate the traces. Antiparallelism of the traces at 405 and 480 nm points out variations in $[Ca^{2+}]i$. **b**. $[Ca^{2+}]i$ was determined by using the Grynkiewicz's equation after subtraction of the cell autofluorescence.

$[Ca^{2+}]i$ was calculated using the formula: $[Ca^{2+}]i = Kd\beta\ (R - R\text{min})/(R\text{max} - R)$. $Kd\beta$ for Indo 1 was 406 nM as previously determined. The values for *R*max and *R*min were calculated from measurements using respectively 25 μM digitonine and 5mM 1,2-di(2-aminoethoxy)ethane-*N*,*N*,*N*',*N*'-tetraacetic acid (EGTA) (pH 8.5). Four compounds (**5**, **9**, **17** and **20**) were autofluorescent and thus were not used for this study.

To amplify the dihydropyridine effects, Ca^{2+} influx was stimulated by an application of forskoline, a potent activator of adenyl cyclase [26]. cAMP is known to increase $[Ca^{2+}]i$ via a stimulation of protein kinase A that phosphorylates L-type Ca^{2+} channels and thus favours their opening in GH3 cells [27, 28]. These effects on $[Ca^{2+}]i$ were related to cAMP-induced PRL and GH release [29].

Results and discussion

Calcium influx inhibition

Intracellular free Ca^{2+} levels were monitored in populations of GH3 cells by spectrofluorimetry using the fluorescent Ca^{2+} probe Indo 1. Ca^{2+} levels were monitored 2–4 days after cell replating. Basal $[Ca^{2+}]i$ is about 120 nM when determined in single cells [28]. However these cells are spontaneously active and exhibit spontaneous $[Ca^{2+}]i$ oscillations due to Ca^{2+} influx during action potential [20]. The amplitude of these Ca^{2+} spikes varies from 300–800 nM [28]; therefore the basal $[Ca^{2+}]i$ measured in cell population experiments (~500 nM) corresponds to the mean amplitude of the asynchronous spontaneous Ca^{2+} oscillations of all the cells. In addition, we have recently described [30] steady-state Ca^{2+} currents sensitive to dihydropyridines that control Ca^{2+} influx at resting potentials in GH3 cells. Dihydropyridines (nifedipine and thienyldihydropyridines) reduce spontaneous (unstimulated) Ca^{2+} influx (fig 4). However, in order to amplify the dihydropyridines effects, we stimulated Ca^{2+} influx with several products: phorbol myristate acetate, forskoline and KCl. Dihydropyridines reduced Ca^{2+} influx induced by all three, but the most reproducible data were obtained with forskoline. In this paper we report only the data obtained with forskoline as Ca^{2+} influx activator.

The 22 compounds listed in table I were evaluated as calcium-influx inhibitors and compared with nifedipine as reference. Some examples (nifedipine, and compounds **3**, **7** and **15** with high, moderate and weak activity respectively) are shown in figure 3.

Dihydropyridines were applied when the effect of forskoline was maximal. We used a concentration of 1 μM, known to inhibit calcium influx through L-type calcium channels [31, 32]. We measured the mean amplitude of the dihydropyridine-induced decreases in $[Ca^{2+}]i$ obtained in between four and eight independent experiments. All data are summarized in table I, where they are expressed as percentage of inhibition of basal $[Ca^{2+}]i$ and forskoline-stimulated Ca^{2+} increase.

It appears that nifedipine was the most active on intracellular calcium and that inhibition of calcium influx differed according to different parameters: position and nature of the thienyl substituent (R') and the nature of the ester group R [8]. The position of the sulfur atom in the 4-(1,4-dihydropyridinyl) ring system did not have a large effect on the biological activity; in fact the most interesting compounds belonged to the 2-thienyl series (**2**, **3** and **4**) and to the 3-thienyl series (**21** and **22**). The position of the substituent on the thienyl nucleus was important. In fact, the introduction of a substituent in the 3-position was the most favourable; compound **2** with a methyl group in the 3-position was more active than the unsubstituted compound **1**.

When a bromo substituent was introduced in position 4, a small decrease in activity resulted; compound **10** was a weaker calcium inhibitor than compound **1**. When the thienyl nucleus was substituted in the 5-

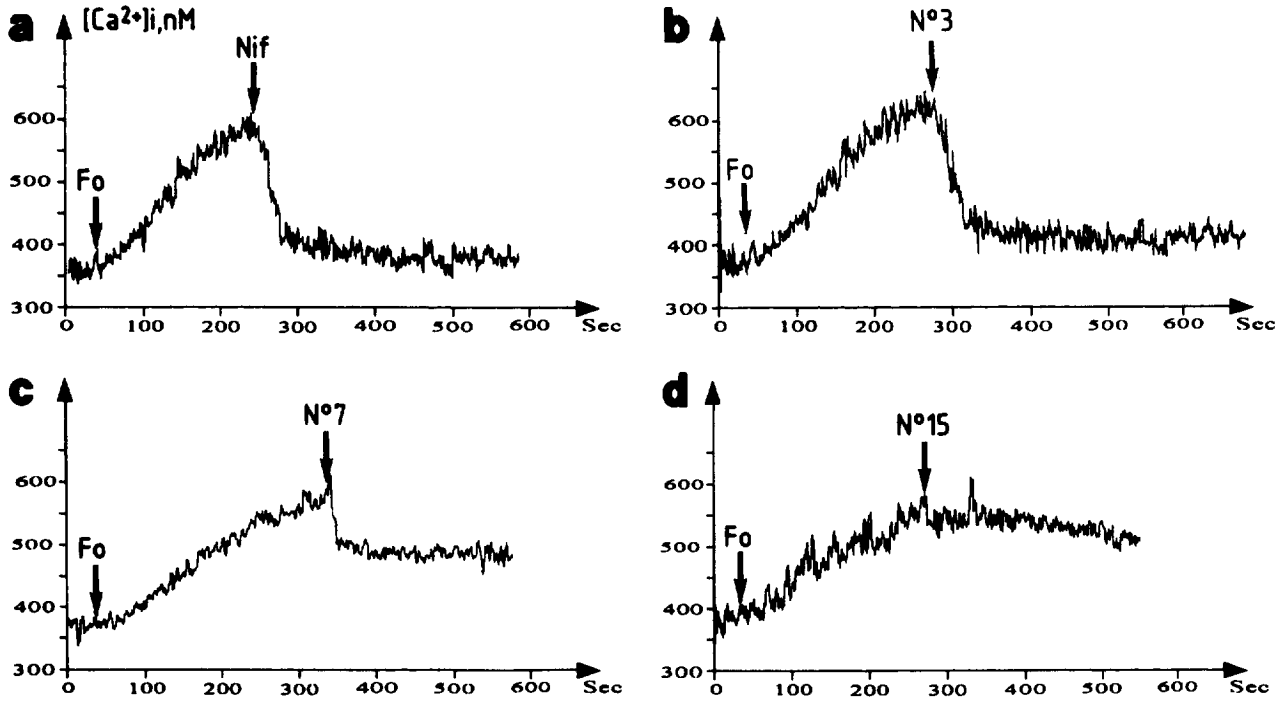


Fig 3. Comparison of the effects of nifedipine (a) and compounds **3** (b), **7** (c) and **15** (d) on forskoline-induced Ca^{2+} increase. The four dihydropyridines were used at 1 μM.

position, the activity depended on two factors: steric bulk and electronic properties. The introduction of a very bulky group in the 5-position, such as an α-hydroxybenzyl (**19**), cancelled the biological effect. However it appears that steric bulk was not the only implicated factor. The phenyl and thienyl rings of **19** are linked through an sp^3-hybridized carbon, while for **18** the junction between these same rings is secured via an sp^2-hybridized carbon. This could explain why the biological effect of **18** was partially restored. The frequency of the vibration $\nu_{C=O}$ observed for compound **18** (1630 cm^{-1}) is indicative of electronic delocalization in the benzoylthienyl group.

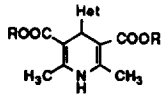
In compound **1**, the introduction of an electron-withdrawing group, such as a bromo atom (**14**) decreased the activity. In contrast, an electron-donating group, such as a methyl group (**6**), allowed part of the biological activity of the unsubstituted derivative **1** to remain. However, the inhibition of calcium influx was dependent on the nature of the ester group [12]; in each series, the ethyl compounds **3**, **7**, **11**, **15** and **22** were more active than the corresponding methyl derivatives **2**, **6**, **10** and **21**, or at least as active as them (**14**). The *tert*-butyl compounds were not classified because their measurements have not been made on account of their autofluorescence. Excepting the compound **16**, the other allyl esters had a lower activity than the analogous ethyl derivatives **4**, **8** and **12**.

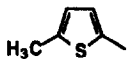
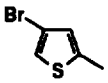
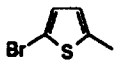
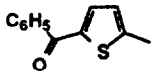
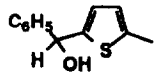
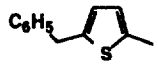
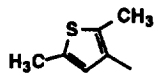
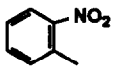
It can be concluded from this biological study that the presence of a methyl group adjacent to the junction of the two heterocycles plays a primary role. Its effect is essentially steric and allows a loss of coplanarity of the two heterocyclic systems. Currently this hypothesis [12, 13] is accepted to explain the activity of the nifedipine analogues.

Secretory responses

Hormone release by pituitary cells is dependent on $[Ca^{2+}]i$ [19]. We have therefore studied the effects of the more efficient dihydropyridines (nifedipine and compounds **2**–**5**) on basal and forskoline-stimulated PRL and GH release. Because the effects we observed when dihydropyridines were applied to forskoline-stimulated cells correspond to an inhibition of both basal and stimulated release, we have subtracted the amplitude of this inhibition from the amplitude of basal release inhibition, in order to compare the effects of each dihydropyridine in stimulated and unstimulated conditions. Results are expressed as

Table I. Percentage of inhibition of calcium influx for compounds **1**– **22**.



Compounds	Het.	R	% $[Ca^{2+}]_i$
1		CH_3	50 ±3.0
2		CH3	73 ± 4.8
3		C2H5	88 ± 3.5
4		CH_2-CH=CH_2	76 ± 5.4
5		$C(CH_3)_3$	(b)
6		CH3	39 ± 3.6
7		C2H5	53 ± 4.1
8		CH_2-CH=CH_2	52 ± 3.5
9		$C(CH_3)_3$	(b)
10		CH3	34 ± 2.7
11		C2H5	85 ± 3.8
12		CH_2-CH=CH_2	43 ± 2.5
13		$C(CH_3)_3$	(b)
14		CH3	27 ± 2.4
15		C2H5	29 ± 3.0
16		CH_2-CH=CH_2	38 ± 3.2
17		$C(CH_3)_3$	(b)
18		CH_3	12 ± 2.4
19		CH_3	0
20		CH_3	(b)
21		CH_3	66 ± 3.2
22		C2H5	80 ± 3.5
Nifedipine		CH_3	97 ± 3.0

(a) The activity of the different derivatives was expressed in percentage of inhibition of basal $[Ca^{2+}]i$ and forskoline-stimulated Ca^{2+}increase. Data represent mean ± SD of 8 experiments.

(b) Non measurable because compounds were autofluorescent.

percentage of control, where control was basal release for unstimulated cells, and forskoline-stimulated release for stimulated cells (fig 4).

The same calculations were performed for $[Ca^{2+}]i$ (fig 4). The *tert*-butyl compound **5** was the most efficient dihydropyridine on PRL and GH release. Unfortunately, it cannot be used for spectrofluorimetry experiments because of its autofluorescence. Its activity on calcium channels could be determined using electrophysiological techniques. For the other dihydropyridines it can be noted that all the compounds that reduced hormone release also reduced $[Ca^{2+}]i$. This result questions once more the calcium-dependence of the hormone release by pituitary cells [19, 20].

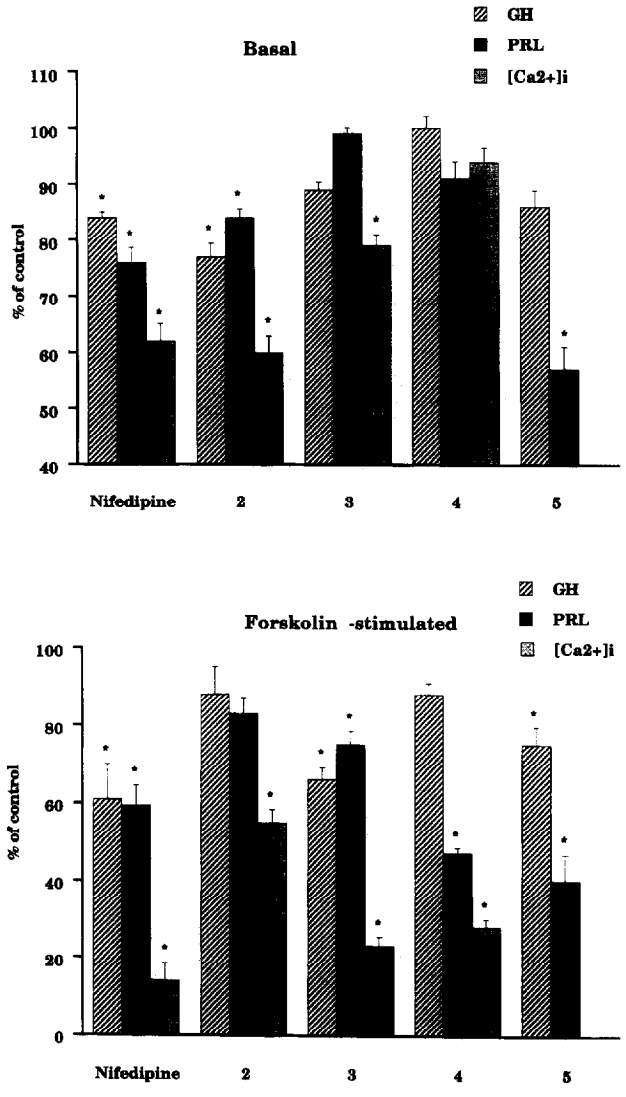


Fig 4. Effects of nifedipine and compounds **2**, **3**, **4**, **5** on basal (upper panel) and forskoline-stimulated (lower panel) GH and PRL release and $[Ca^{2+}]i$. Values are means ± SD of four experiments in quadruplicate. $*P < 0.05$ vs control by analysis of variance and Neuman-Keul multiple comparison test.

On the other hand, compounds that decreased $[Ca^{2+}]i$ did not inevitably inhibit hormone release. Inhibition of basal hormone release should require more than 35% inhibition of basal $[Ca^{2+}]i$, whereas inhibition of forskoline-stimulated PRL release should require more than 70% inhibition of forskoline-stimulated $[Ca^{2+}]i$. GH release, and particularly forskoline-stimulated GH release should be less Ca^{2+}-dependent. A likely explanation for this discrepancy between PRL and GH secretion is that dihydropyridines reduced PRL synthesis but not GH synthesis [33]. Thus, by decreasing intracellular calcium the dihydropyridines reduced the release of stored and newly synthesized PRL, but only the release of stored GH [34].

Obviously, the increase in hormone synthesis by forskoline and the subsequent stimulation of protein kinase A activity amplified the effects of dihydropyridines on hormone release. We show that the different derivatives do not all act in the same way. For example, compounds **3** and **4** did not significantly affect basal intracellular calcium and hormone release, whereas they strongly reduced forskoline-stimulated intracellular calcium and hormone release. On the other hand, nifedipine and compound **5** reduced intracellular calcium and hormone release in both cases. These results suggest that the derivatives have distinct action mechanisms.

It was found that the inhibitory action of nifedipine on the L-type Ca^{2+} current was greatly enhanced when cells were held at relatively depolarized membrane potentials in neurons [35]. It can be postulated that dihydropyridine derivatives act differently according to the membrane potential, compounds **3** and **4** would not be active at resting membrane potential. Electrophysiological experiments could confirm this hypothesis.

Experimental protocols

Chemistry

Melting points were determined in open capillary tubes on an Electrothermal digital melting point apparatus and are uncorrected. The IR spectra were recorded on a Shimadzu IR 470 spectrometer using KBr pellets. The ^{1}H-NMR spectra were obtained at 60 MHz on a Varian EM 360 L spectrometer instrument, with chloroform-*d* as the solvent. Chemical shifts are reported in parts per million relative to tetramethylsilane as internal standard. Elemental analyses were carried out by the Service central de microanalyse du CNRS (Vernaison, France) and were within ± 0.4% of the calculated values.

1,4-Dihydro-2,6-dimethyl-4-(2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-methyl ester) ***1*** *[36]*
A mixture of 2-thiophenecarboxaldehyde (1.12 g, 10 mmol) and methyl acetoacetate (2.16 mL, 20 mmol) in methanol (25 mL) was added to a 20% ammonia solution (9 mL) and refluxed for 12 h in darkness. After cooling to 0 °C, the resulting precipitate was filtered and recrystallized from ethanol to give 1.53 g (50% yield) of **1**. Mp 194 °C. IR (KBr) cm^{-1}: 3320 ν_{NH}; 1675 and 1660 $\nu_{C=O}$; 1620 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.30 (s, 6H, 2 x pyridine-CH_3); 3.75 (s, 6H, 2 x $COOCH_3$); 5.35 (s, 1H, pyridine-H_4); 6.40 (s, 1H, NH); 6.70–7.10 (m, 3H, thiophene-H_3, -H_4 and -H_5). Anal: $C_{15}H_{17}NO_4S$ (C, H, N, S).

1,4-Dihydro-2,6-dimethyl-4-(3-methyl-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-methyl ester) ***2*** *[36]*
A mixture of 3-methyl-2-thiophenecarboxaldehyde (1.26 g, 10 mmol) and methyl acetoacetate (2.16 mL, 20 mmol) in methanol (25 mL) was added to a 20% ammonia solution (9 mL) and refluxed for 12 h in darkness. After cooling to 0 °C, the formed solid was filtered then recrystallized from hexane/toluene (50:50) to give 1.09 g (34% yield) of **2**. Mp 220 °C. IR (KBr) cm^{-1}: 3320 ν_{NH}; 1680 and 1645 $\nu_{C=O}$; 1615 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.30 (s, 9H, 2 x pyridine-CH_3 and thiophene-CH_3); 3.75 (s, 6H, 2 x $COOCH_3$); 5.35 (s, 1H, pyridine-H_4); 6.15 (s, 1H, NH); 6.55–7.00 (AB system, 2H, J = 4 Hz, thiophene-H_4 and -H_5). Anal: $C_{16}H_{19}NO_4S$ (C, H, N, S).

1,4-Dihydro-2,6-dimethyl-4-(3-methyl-2-thienyl)-3,5-pyridinedicarboxylic acid bis-1,1-ethyl ester) ***3***
To a stirred solution of 3-methyl-2-thiophenecarboxaldehyde (1.26 g, 10 mmol) and ethyl acetoacetate (2.54 mL, 20 mmol) in methanol (25 mL) was added a 20% ammonia solution (9 mL). The mixture was refluxed for 12 h in darkness. After evaporation of methanol, the residue was extracted with dichloromethane (3 x 30 mL). The combined organic layers were dried over sodium sulfate and concentrated in vacuo. A recrystallization from cyclohexane in the presence of charcoal afforded 1.50 g of **3** (43%). Mp 141 °C. IR (KBr) cm^{-1}: 3350 ν_{NH}; 1690 and 1650 $\nu_{C=O}$; 1635 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.10 (t, 6H, 2 x CH_2CH_3); 2.30 (s, 9H, 2 x pyridine-CH_3 and thiophene-CH_3); 4.15 (q, 4H, 2 x CH_2CH_3); 5.35 (s, 1H pyridine-H_4); 6.40 (s, 1H, NH); 6.50–7.10 (AB system, 2H, J_{AB} = 4 Hz, thiophene-H_4 and -H_5). Anal: $C_{18}H_{23}NO_4S$ (C, H, N, S).

1,4-Dihydro-2,6-dimethyl-4-(3-methyl-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-propen-2-yl ester) ***4***. This was obtained in the same manner as **3** from 3-methyl-2-thiophene carboxaldehyde and propen-2-yl acetoacetate and purified on a silica-gel column eluted with dichloromethane. A recrystallization from hexane afforded pure **4** in 14% yield. Mp 97 °C. IR (KBr) cm^{-1}: 3340 ν_{NH}; 1700 and 1660 $\nu_{C=O}$; 1650 $\nu_{CH=CH_2}$; 1640 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.30 (s, 9H, 2 x pyridine-CH_3 and thiophene-CH_3); 4.60 (d, 4H, 2 x $CH_2CH=CH_2$); 4.95–5.50 (m, 4H, 2 x $CH_2CH=CH_2$); 5.55–6.25 (m, 2H, 2 x $CH_2CH=CH_2$-); 5.30 (s, 1H, pyridine-H_4); 6.45 (s, 1H, NH); 6.55–7.10 (AB system, 2H, J_{AB} = 4 Hz, thiophene-H_4 and H_5). Anal: $C_{20}H_{23}NO_4S$ (C, H, N, S).

1,4-Dihydro-2,6-dimethyl-4-(3-methyl-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-tert-butyl ester) ***5***. This was obtained in the same manner as **3** from 3-methyl-2-thiophene carboxaldehyde and *tert*-butyl acetoacetate. After chromatography through a silica-gel column eluted with dichloromethane/diethyl ether (98:2), recrystallization from cyclohexane gave 1.34 g of **5** (33%). Mp 187 °C. IR (KBr) cm^{-1}: 3330 ν_{NH}; 1690 and 1640 $\nu_{C=O}$; 1620 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.45 (s, 18H, 2 x $C(CH_3)_3$); 2.25 (s, 9H, 2 x pyridine-CH_3 and thiophene-CH_3); 5.30 (s, 1H, pyridine-H_4); 6.10 (s, 1H, NH); 6.45–6.95 (AB system, 2H, J = 6 Hz, thiophene-H_4 and -H_5). Anal: $C_{22}H_{31}NO_4S$ (C, H, N, S).

1,4-Dihydro-2,6-dimethyl-4-(5-methyl-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-methyl ester) ***6***. This compound, previously described [36], was obtained in the same manner as **3** from 3-methyl-2-thiophenecarboxaldehyde and methyl acetoacetate and recrystallized from ethanol in 42% yield. Mp 188 °C. IR (KBr) cm^{-1}; 3340 ν_{NH}; 1690 and 1640 $\nu_{C=O}$; 1620 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.30 (s, 6H, 2 x pyridine-CH_3); 2.35 (s, 3H, thiophene-CH_3); 3.70 (s, 6H, 2 x $COOCH_3$); 5.25 (s, 1H, pyridine-H_4); 6.10 (s, 1H, NH); 6.45–6.60 (m, 2H, thiophene-H_3 and -H_4). Anal: $C_{16}H_{19}NO_4S$ (C, H, N, S).

1,4-Dihydro-2,6-dimethyl-4-(5-methyl-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-ethyl ester) ***7***. This was obtained from 5-methyl-2-thiophene carboxaldehyde and ethyl acetoacetate in the same manner as **3** in 43% yield after recrystallization from cyclohexane. Mp 136 °C. IR (KBr) cm^{-1}: 3340 ν_{NH}; 1690 and 1645 $\nu_{C=O}$; 1620 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.25 (t, 6H, 2 x CH_2CH_3); 2.30 (s, 6H, 2 x pyridine-CH_3); 2.35 (s, 3H, thiophene-CH_3); 4.15 (q, 4H, 2 x CH_2CH_3); 5.25 (s, 1H, pyridine-H_4); 6.25 (s, 1H, NH); 6.40–6.45 (m, 2H, thiophene-H_3 and H_4). Anal: $C_{18}H_{23}NO_4S$ (C, H, N, S).

1,4-Dihydro-2,6-dimethyl-4-(5-methyl-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-propen-2-yl-ester) ***8***. This was obtained in the same manner as **3** from 5-methyl-2-thiophenecarboxaldehyde and propen-2-yl acetoacetate. After chromatography through a silica-gel column eluted with dichloromethane/diethyl ether (80:20), recrystallization from hexane afforded **8** in 16% yield. Mp 118 °C. IR (KBr) cm^{-1}: 3350 ν_{NH}; 1680 and 1655 $\nu_{C=O}$; 1635 $\nu_{CH=CH2}$; 1615 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.30 (s, 9H, 2 x pyridine-CH_3 and thiophene-CH_3); 4.64 (d, 4H, 2 x $CH_2CH=CH_2$); 5.00–5.45 (m, 4H, 2 x $CH_2CH=CH_2$); 5.60–6.25 (m, 2H, 2 x $CH_2CH=CH_2$); 5.30 (s, 1H, pyridine-H_4; 6.40 (s, 1H, NH); 6.45–6.65 (m, 2H, thiophene-H_3 and -H_4). Anal: $C_{20}H_{23}NO_4S$ (C, H, N, S).

1,4-Dihydro-2,6-dimethyl-4-(5-methyl-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-tert-butyl ester) ***9***. This was obtained in the same manner as **3** from 5-methyl-2-thiophene carboxaldehyde and *tert*-butyl acetoacetate and chromatographed over a silica gel column eluted with dichloromethane/diethyl ether (98:2). Recrystallization from hexane gave **9** in 20% yield. Mp 165–166 °C. IR (KBr) cm^{-1}: 3360 ν_{NH}; 1690 and 1645 $\nu_{C=O}$; 1625 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.45 (s, 18H, 2 x $C(CH_3)_3$); 2.25 (s, 6H, 2 x pyridine-CH_3); 2.35 (s, 3H, thiophene-CH_3); 5.20 (s, 1H, pyridine-H_4); 6.20 (s, 1H, NH); 6.45–6.60 (m, 2H, thiophene-H_3 and -H_4). Anal: $C_{22}H_{31}NO_4S$ (C, H, N, S).

1,4-Dihydro-2,6-dimethyl-4-(4-bromo-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-methyl ester) ***10***. This was obtained in the same manner as **3** from 4-bromo-2-thiophenecarboxaldehyde and methyl acetoacetate and recrystallized from toluene in 52% yield. Mp 173 °C. IR (KBr) cm^{-1}: 3335 ν_{NH}; 1690 and 1645 $\nu_{C=O}$; 1620 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.35 (s, 6H, 2 x pyridine-CH_3); 3.70 (s, 6H, 2 x $COOCH_3$); 5.25 (s, 1H, pyridine, H_4); 6.50 (s, 1H, NH); 6.65 (d, J = 2 Hz, 1H, thiophene-H_3); 6.95 (d, J = 2 Hz, 1H, thiophene-H_5). Anal: $C_{15}H_{16}BrNO_4S$ (C, H, Br, N, S).

1,4-Dihydro-2,6-dimethyl-4-(4-bromo-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-ethyl ester) ***11***. This was obtained from 4-bromo-2-thiophene carboxaldehyde and ethyl acetoacetate in the same manner as **3** and recrystallized from cyclohexane in 51% yield. Mp 153 °C. IR (KBr) cm^{-1}: 3350 ν_{NH}; 1690 and 1650 $\nu_{C=O}$; 1620 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.25 (t, 6H, 2 x CH_2CH_3); 2.35 (s, 6H, 2 x pyridine-CH_3); 4.20 (q, 4H, 2 x CH_2CH_3); 5.30 (s, 1H, pyridine-H_4); 6.45 (s, 1H, NH); 6.75 (d, J = 2 Hz, 1H, thiophene-H_3); 6.95 (d, J = 2 Hz, 1H, thiophene-H_5). Anal: $C_{17}H_{20}BrNO_4S$ (C, H, Br, N, S).

1,4-Dihydro-2,6-dimethyl-4-(4-bromo-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-propen-2-yl ester) ***12***. This was obtained in the same manner as **3** from 4-bromo-2-thienylcarboxaldehyde and propen-2-yl acetoacetate. After chromatography on a silica-gel column eluted with dichloromethane/diethyl ether (98:2), recrystallization from hexane gave **12** in 31% yield. Mp 130 °C. IR (KBr) cm^{-1}: 3350 ν_{NH}; 1690 and 1645 $\nu_{C=O}$; 1630 $\nu_{pyridine\ C=C}$; 1610 $\nu_{CH=CH_2}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.35 (s, 6H, 2 x pyridine-CH_3); 4.65 (d, 4H, 2 x $CH_2CH=CH_2$); 5.00–5.50 (m, 4H, 2 x $CH_2CH=CH_2$); 5.65–6.35 (m, 2H, 2 x $CH_2CH=CH_2$); 5.40 (s, 1H, pyridine-H_4); 6.05 (s, 1H, NH); 6.75 (d, J = 2 Hz, 1 H, thiophene-H_3); 6.95 (d, J = 2 Hz, 1 H, thiophene-H_5). Anal: $C_{19}H_{20}BrNO_4S$ (C, H, Br, N, S).

1,4-Dihydro-2,6-dimethyl-4-(4-bromo-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-tert-butyl ester) ***13***. This was obtained from 4-bromo-2-thiophenecarboxaldehyde and *tert*-butyl acetoacetate in the same manner as **3** in 30% yield after recrystallization from ethanol. Mp 186 °C. IR (KBr) cm^{-1}: 3340 ν_{NH}; 1690 and 1640 $\nu_{C=O}$; 1620 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.45 (s, 18H, 2 x $C(CH_3)_3$); 2.35 (s, 6H, 2 x pyridine-CH_3); 5.25 (s, 1H, pyridine-H_4); 6.10 (s, 1H, NH); 6.75 (d, J = 2 Hz, 1H, thiophene-H_3); 7.05 (d, J = 2 Hz, 1H, thiophene-H_5). Anal: $C_{21}H_{28}BrNO_4S$ (C, H, Br, N, S).

1,4-Dihydro-2,6-dimethyl-4-(5-bromo-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-methyl ester) ***14***. This compound, previously described [36], was obtained in the same manner as **3** from 5-bromo-2-thiophenecarboxaldehyde and methyl acetoacetate and recrystallized from ethanol in 30% yield. Mp 200 °C. IR (KBr) cm^{-1}; 3340 ν_{NH}; 1680 and 1640 $\nu_{C=O}$; 1620 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.35 (s, 6H, 2 x pyridine-CH_3); 3,75 (s, 6H, 2 x $COOCH_3$); 5.25 (s, 1H, pyridine-H_4); 6.10 (s, 1H, NH); 6.45–6.85 (AB system , 2H, J_{AB} = 4 Hz, thiophene-H_3 and -H_4). Anal: $C_{15}H_{16}BrNO_4S$ (C, H, Br, N, S).

1,4-Dihydro-2,6-dimethyl-4-(5-bromo-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-ethyl ester) ***15***. This was obtained in the same manner as **3** from 5-bromo-2-thiophenecarboxaldehyde and ethyl acetoacetate. After chromatography on a silica-gel column eluted with dichloromethane/diethyl ether (90:10), recrystallization from cyclohexane gave **15** in 17% yield. Mp 137 °C. IR (KBr) cm^{-1}: 3340 ν_{NH}; 1690 and 1650 $\nu_{C=O}$; 1620 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.25 (t, 6H, 2 x CH_2CH_3); 2.30 (s, 6H, 2 x pyridine-CH_3); 4.15 (q, 4H, 2 x CH_2CH_3); 5.25 (s, 1H, pyridine-H_4); 6.65 (s, 1H, NH); 6.45–6.85 (AB system, 2H, J_{AB} = 4 Hz, thiophene-H_3 and -H_4). Anal: $C_{17}H_{20}BrNO_4S$ (C, H, Br, N, S).

1,4-Dihydro-2,6-dimethyl-4-(5-bromo-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-propen-2-yl ester) ***16***. This was obtained in the same manner as **3** from 5-bromo-2-thiophenecarboxaldehyde and propen-2-yl acetoacetate, and chromatographed on a silica-gel column eluted with dichloromethane. Recrystallization from hexane afforded **16** in 12% yield. Mp 110 °C. IR (KBr) cm^{-1}: 3350 ν_{NH}; 1690 and 1650 $\nu_{C=O}$; 1640 $\nu_{CH=CH_2}$; 1620 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.35 (s, 6H, 2 x pyridine-CH_3); 4.65 (d, 4H, 2 x $CH_2CH=CH_2$); 5.00–5.35 (m, 4H, 2 x $CH_2CH=CH_2$); 5.60–6.25 (m, 2H, 2 x $CH_2CH=CH_2$); 5.40 (s, 1H, pyridine-H_4); 6.40 (s, 1H, NH); 6.45–6.85 (AB system, 2H, J_{AB} = 4 Hz, thiophene-H_3 and -H_4). Anal: $C_{19}H_{20}BrNO_4S$ (C, H, Br, N, S).

1,4-Dihydro-2,6-dimethyl-4-(5-bromo-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-tert-butyl ester) ***17***. This was obtained from 5-bromo-2-thiophenecarboxaldehyde and *tert*-butyl acetoacetate in the same manner as **3**, in 17% yield after chromatography on a silica-gel column eluted with dichloromethane, and recrystallization from hexane. Mp 162 °C. IR (KBr) cm^{-1}: 3350 ν_{NH}; 1695 and 1640 $\nu_{C=O}$; 1620 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.40 (s, 18H, 2 x $C(CH_3)_3$); 2.20 (s, 6H, 2 x pyridine-CH_3); 5.15 (s, 1H, pyridine-H_4); 5.85 (s, 1H, NH); 6.40–6.75 (AB system, 2H, J = 4 Hz, thiophene-H_3 and -H_4). Anal: $C_{21}H_{28}BrNO_4S$ (C, H, Br, N, S).

1,4-Dihydro-2,6-dimethyl-4-(5-benzoyl-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-methyl ester) ***18***

(5-Formyl-2-thienyl)phenylmethanone was synthesized according to a modified method [24]. In a round-bottomed flask equipped with a Dean and Stark apparatus a mixture of 2-thiophenecarboxaldehyde (3.36 g, 30 mmol) and *N,N'*-dimethylethylenediamine (2.7 g, 30 mmol) in benzene (50 mL) was refluxed for 12 h. The benzene was removed by evaporation and the residue distilled (81 °C, 0.5 mmHg) to give 4.30 g of 1,3-dimethyl-2-(2-thienyl) imidazolidine (80%) kept under nitrogen. To a solution of this crude product (2.68 g, 15 mmol) in dry dimethoxyethane (150 mL) at –78 °C was added slowly a solution of 1.6 M *n*-butyllithium (10.3 mL, 16.5 mmol) in hexane. The reaction mixture was stirred for 2 h. Benzaldehyde (1.9 mL, 18.5 mmol) was then added; the mixture was allowed to warm to 20 °C and then left for 12 h at this temperature under stirring. The mixture was hydrolyzed by a sulfuric acid solution 10% (200 mL) for 12 h at room temperature. After extraction with dichloromethane (3 x 30 mL), the organic layers were washed with water until neutrality and dried over Na_2SO_4. The solvent was then evaporated under reduced pressure, and the obtained product was purified by chromatography on a silica-gel column eluted with dichloromethane. The first fraction afforded 0.65 g (5-formyl-2 thienyl)phenylmethanone in 20% yield.

18 was obtained from (5-formyl-2-thienyl)phenylmethanone and methyl acetoacetate in the same manner as **3**, in 12% yield after recrystallization from hexane. Mp 186 °C. IR (KBr) cm^{-1}: 3340 ν_{NH}; 1700, 1680 and 1650 $\nu_{C=O\ ester}$; 1630 $\nu_{C=O\ ketone}$; 1625 $\nu_{pyridine\ C=C}$; 1600 $\nu_{phenyl\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.30 (s, 6H, 2 x pyridine CH_3); 3.65 (s, 6H, 2 x $COOCH_3$); 5.35 (s, 1H, pyridine-H_4); 6.85–7.75 (AB system, 2H, J = 4 Hz, thiophene-H_3 and -H_4); 7.00–7.90 (m, 5H, Ar); 7.15 (s, 1H, NH). Anal: $C_{22}H_{21}NO_5S$ (C, H, N, S).

1,4-Dihydro-2,6-dimethyl-4-(5-phenyl-methanol-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-methyl ester) ***19***

(5-Formyl-2-thienyl)phenylmethanol was prepared according to a modified method [24]. To a stirred mixture of 1,3-dimethyl-2-(2-thienyl) imidazolidine (2.68 g, 15 mmol) in dry tetrahydrofurane (150 mL) and *N,N,N',N'*-tetramethylethylenediamine (2.35 mL, 15 mmol), maintained at –78 °C under nitrogen, was added dropwise, via a syringe, a solution of

n-butyllithium 1.6 M (10.3 mL, 16.5 mmol) in hexane. The resulting mixture was stirred to −78 °C over a 2 h period. Benzaldehyde (1.8 mL, 18 mmol) was then added; the mixture was allowed to warm to 20 °C and then left for 12 h at this temperature under stirring. After evaporation of the solvents, the mixture was hydrolyzed by a sulfuric acid solution 10% (100 mL) for 12 h at room temperature, and then extracted with dichloromethane (3 x 30 mL). The organic layer was washed with water until neutrality and dried over sodium sulfate. After evaporation in vacuo of the solvent, the crude product was chromatographed on a silica-gel column eluted with hexane/ethylacetate (75:25). The second fraction gave 1.9 g of (5-formyl-2-thienyl) phenylmethanol in 17% yield.

19 was obtained from (5-formyl-2-thienyl)phenylmethanol and methyl acetoacetate in the same manner as **3**, in 27% yield after chromatography on a silica-gel column eluted with hexane/toluene (50:50). Mp 180 °C. IR (KBr) cm^{-1}: 3500 ν_{OH}; 3340 ν_{NH}; 1685 and 1650 $\nu_{C=O}$; 1620 $\nu_{pyridine\ C=C}$; 1600 $\nu_{phenyl\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.25 (s, 6H, 2 x pyridine-CH_3); 3.65 (s, 6H, 2 x $COOCH_3$); 5.25 (s, 1H, pyridine-H_4); 5.85 (br s, 1H, C*H*OH); 6.15 (s, 1H, NH); 6.45–6.65 (m, 2H, thiophene-H_3 and -H_4); 7.15–7.60 (m, 5H, Ar); 7.35 (br s, 1H, OH). Anal: $C_{22}H_{23}NO_5S$ (C, H, N, S).

1,4-Dihydro-2,6-dimethyl-4-(5-phenylmethyl-2-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-ethyl ester) ***20***
(5-Formyl-2-thienyl)phenylmethane was synthesized as the same manner as the previous (5-formyl-2-thienyl) phenylmethanol, from α-bromotoluene (2.7 mL, 22 mmol) instead of benzaldehyde. The residue was purified by chromatography on a silica-gel column eluted with hexane/diethylether (65:35) to afford 1.5 g of (5-formyl-2-thienyl)phenylmethane in 40% yield.

20 was obtained from (5-formyl-2-thienyl)phenylmethane and methylacetoacetate in the same manner as **3**, in 33% yield after chromatography on a silica-gel column eluted with hexane/toluene (50:50). Mp 194 °C. IR (KBr) cm^{-1}: 3330 ν_{NH}; 1700 and 1650 $\nu_{C=O}$; 1630 $\nu_{pyridine\ C=C}$; 1600 $\nu_{phenyl\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.25 (s, 6H, 2 x pyridine-CH_3); 3.65 (s, 6H, 2 x $COOCH_3$); 4.00 (s, 2H, CH_2Ar); 5.25 (s, 1H, pyridine-H_4); 6.10 (s, 1H, NH); 6.40–6.65 (m, 2H, thiophene-H_3 and -H_4); 7.15–7.40 (m, 5H, Ar). Anal: $C_{22}H_{23}NO_4S$ (C, H, N, S).

1,4-Dihydro-2,6-dimethyl-4-(2,5-dimethyl-3-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-methyl ester) ***21***. This was prepared by the procedure described for **3**, from 2,5-dimethyl-3-thienyl carboxaldehyde [37] and methyl acetoacetate. This was purified by recrystallization from hexane to give 1.17 g of pure product in 35% yield. Mp 212 °C. IR (KBr) cm^{-1}: 3320 ν_{NH}; 1690 and 1650 $\nu_{C=O}$; 1630 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.30 (s, 9H, 2 x pyridine-CH_3 and thiophene-CH_3); 2.45 (s, 3H, thiophene-CH_3); 3.65 (s, 6H, 2 x $COOCH_3$); 4.95 (s, 1H, pyridine-H_4); 6.25 (s, 1H, NH); 6.45 (s, 1H, thiophene-H_4). Anal: $C_{17}H_{21}NO_4S$ (C, H, N, S).

1,4-Dihydro-2,6-dimethyl-4-(2,5-dimethyl-3-thienyl)-3,5-pyridinedicarboxylic acid bis-(1,1-ethyl ester) ***22***. This was obtained in the same manner as **21** from ethyl acetoacetate and purified by recrystallization from hexane to give a pure product in 42% yield. Mp 168 °C. IR (KBr) cm^{-1}: 3340 ν_{NH}; 1695 and 1650 $\nu_{C=O}$; 1630 $\nu_{pyridine\ C=C}$. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.25 (t, 6H, 2 x CH_2CH_3); 2.30 (s, 9H, 2 x pyridine-CH_3 and thiophene-CH_3); 2.45 (s, 3H, thiophene-CH_3); 4.15 (q, 4H, 2 x CH_2CH_3); 4.95 (s, 1H, pyridine-H_4); 6.40 (s, 1H, NH); 6.45 (s, 1H, thiophene-H_4). Anal: $C_{19}H_{25}NO_4S$ (C, H, N, S).

Pharmacology

Cell culture conditions
The material for this study consisted of GH_3/B6 cells. A subclone of the GH_3 cell line was originally obtained from A Tixier-Vidal (Collège de France, Paris, France) and stored frozen until used or passaged. Cells were cultured in HAM's F-10 nutrient medium supplemented with 15% heat-inactivated horse serum (IBF, Villeneuve-La-Garenne, France) and 2.5% fetal bovine serum (FBS, GIBCO, Grand Island, NY), and maintained at 37 °C in a humidified atmosphere gassed with 95% air/5% CO_2. Experiments were conducted three to seven days after replating. The medium was changed every two or three days.

Microfluorimetric assay of cytosolic calcium
These experiments were performed using the fluorescent probe Indo 1 as already described [38]. GH_3 cells were loaded with Indo 1AM as follows. Cells, grown as described above, were washed twice in Ca^{2+}/Mg^{2+}-free Hank's solution followed by a brief incubation in PBS/EDTA. Cells were centrifuged at 300 *g*. The cell pellet was washed twice in Hank's solution containing 142.6 mM NaCl, 5.6 mM KCl, 2 mM $CaCl_2$, 0.8 mM $MgCl_2$, 5 mM glucose, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), and buffered to pH 7.3 with NaOH (HBSS). The cells were resuspended in the same buffer with 5 μM Indo pentaacetoxymethyl ester (Indo 1/AM, Sigma) and 0.02% Pluronic F-127 (Molecular Probes) for 30 min at 22 ± 1 °C, then washed twice and stored on ice in the same saline solution prior to the fluorescence measurements. $[Ca^{2+}]i$ was measured on cell populations using a Hitachi F2000 spectrofluorimeter. Drugs and reagents were added directly to the cuvette under continuous stirring. The Indo 1 fluorescence response to the intracellular calcium concentration was calibrated as described in pharmacology section.

Measurement of secretory responses
For hormone-release experiments, GH3/B6 cells were cultured in 24-well plates, 2 x 10^5 cells per well. After culture for four or five days, the culture media were discarded. The cells were rinsed three times with prewarmed HAM's F10 nutrient medium, then exposed in quadruplicate to the different concentrations of pharmacological agents for 30 min at 37 °C. PRL and GH content were determined by RIA using the reagents provided by the National Pituitary Agency. All samples from the same experiment were assayed in duplicate in the same assay. Hormone concentrations were expressed in ng per mL/30 min as the mean ± SD.

Data analysis
All biological data (secretory responses and cytosolic calcium measurements) were expressed as mean ± SD when appropriate. Each experiment was repeated several times and the data shown in figures 2 and 3 are representative. Statistical analysis was performed by analysis of variance (Anova) followed by Neumar–Keuls multiple comparison tests as post test for comparison between two means. Differences with $P < 0.05$ were considered significant.

Acknowledgments

Financial support from the Conseil régional d'Aquitaine to use a Hitachi F 2000 spectrofluorimeter is gratefully acknowledged. The authors thank A Lagardère, S Larrouture and N Vilay-

leck for their able technical assistance, and A Carrère for secretarial help.

References

1 Godfraind T, Polster P (1968) *Thérapie* 23, 1209–1220
2 Fleckenstein A, Grun G (1969) *Pflügers Arch* 307, R 26
3 Janis RA, Triggle DJ (1983) *J Med Chem* 26, 775–785
4 Tamazawa K, Arima H, Kojima T et al (1986) *J Med Chem* 29, 2504–2511
5 Baldwin JJ, Claremon DA, Lumma PK et al (1987) *J Med Chem* 30, 690–695
6 Alker D, Campbell SF, Cross PE, Burges RA, Carter AJ, Gardiner DG (1990) *J Med Chem* 33, 585–591
7 Sunkel CE, Fau de Casa-Juana M, Santos L et al (1992) *J Med Chem* 35, 2407–2414
8 Christiaans JAM, Windhorst AD, Groenenberg PM, Van der Goot H, Timmerman H (1993) *Eur J Med Chem* 28, 859–867
9 Christiaans JAM, Van der Goot H, Timmerman H (1993) *Eur J Med Chem* 28, 935–941
10 Dagnino L, Li-Kwong-Ken MC, Wolowyk MW, Wynn H, Triggle CR, Knauss EE (1986) *J Med Chem* 29, 2524–2529
11 Sadanandam YS, Shetty MM, Ram Mohan Reddy K, Leelavathi P (1994) *Eur J Med Chem* 29, 975–979
12 Dagnino L, Li-Kwong-Ken MC, Wynn H, Wolowyk MW, Triggle CR, Knaus EE (1987) *J Med Chem* 30, 640–646
13 Dagnino L, Li-Kwong-Ken MC, Wolowyk MW, Triggle CR, Knaus EE (1987) *Eur J Med Chem* 22, 499–503
14 Kastron V, Duburs G, Vitolins R, Kimenis A (1979) *Khim-Farm Zh* 13, 57–62; *Chem Abstr* (1979) 91, 91468b
15 Prous J, Blancafort P, Castaner J, Serradell MN, Mealy N (1981) *Drugs Fut* VI (7), 427–440
16 Goldmann S, Born L, Kazda S, Pittel B, Schramm M (1990) *J Med Chem* 33, 1413–1418
17 Bisi A, Budriesi R, Chiarini A, Rampa A, Valenti P (1993) *Farmaco* 48, 1491–1502
18 Cupka P, Bella J, Martron A (1987) *Coll Czech Chem Commun* 52, 742–751
19 Ronning SA, Martin TFJ (1986) *J Biol Chem* 261, 7834–7839
20 Schlegel W, Winiger BP, Mollard P et al (1987) *Nature (Lond)* 329 (6141), 719–721
21 Chiavaroli C, Vacher P, Vecsey A et al (1991) *J Neuroendocrinol* 3, 253–258
22 Phillips AP (1949) *J Am Chem Soc* 71, 4003–4006
23 Carpenter AJ, Chadwick DJ (1985) *J Chem Soc, Perkin Trans I*, 173–181
24 Carpenter AJ, Chadwick DJ (1985) *Tetrahedron* 41, 3803–3812
25 Jolly SR, Gross GJ (1979) *Eur J Pharmacol* 54, 289–293
26 Seamon KB, Daly JW (1986) In: *Advances in Cyclic Nucleotide and Protein Phosphorylation Research*, Vol 20 (Greengard P, Robinson GA, eds) Raven, New York 1–150
27 Armstrong D, Eckert R (1987) *Proc Natl Acad Sci USA* 84, 2518–2522
28 Chiavaroli C, Vacher P, Schlegel W (1992) *Eur J Pharmacol* 227, 173–180
29 Holl RW, Thorner MO, Leong DA (1988) *Endocrinology* 122, 2927–2932
30 Mollard P, Theler JM, Guerineau N, Vacher P, Chiavaroli C, Schegel W (1994) *J Bio Chem* 269, 25158–25164
31 Fox AP, Nowycky MC, Tsien RW (1987) *J Physiol (Lond)* 394, 149–172
32 Regan LJ, Sah DWY, Bean BP (1991) *Neuron* 6, 269–280
33 Enyeart JJ, Sheu SS, Hinkle PM (1987) *J Biol Chem* 262, 3154–3159
34 Hinkle PM, Jackson AE, Thompson TM, Zavacki AM, Coppola DA, Barcroft C (1988) *Mol Endocrinology* 2, 1132–1138
35 Rane SG, Holz GG, Dunlap K (1987) *Pflügers Arch* 409, 361–366
36 Lemesle F (1989) *PhD thesis, Université de Caen*
37 Glaze AP, Harris SA, Heller HG et al (1985) *J Chem Soc, Perkin Trans I*, 957–961
38 Mollard P, Guerineau N, Audin J, Dufy B (1990) *Biochem Biophys Res Commun* 164, 1045–1062