Cerebral Antihypoxic Activity of New Thienyldihydropyridines

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New thienyldihydropyridines were synthesized according to Hantzsch's method. The antihypoxic activity of these compounds was compared with that of three reference phenyldihydropyridines by means of the skin conductance reaction (SCR)-hypoxia test.

Keywords antihypoxic activity; thienyldihydropyridine; SCR-hypoxia test; skin conductance reaction

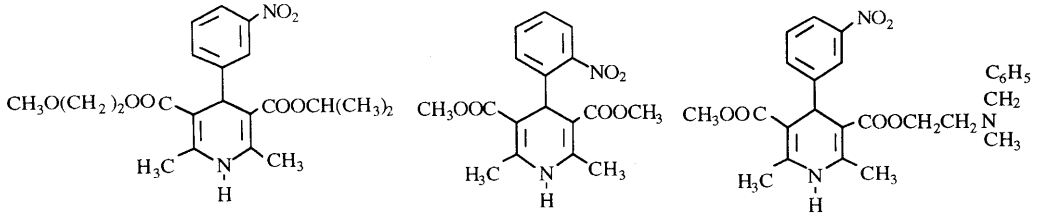
The calcium channel-blocking drugs include a various groups of substances, within which 1,4-dihydropyridines (DHPs) represent a therapeutic class widely used not only for hypertension but also for angina pectoris and peripherial or cerebral vascular diseases. In this class, nimodipine **1** can cross the blood-brain barrier and is used to treat vasospasm after subarachnoid haemorrhage.[1] The structural features leading to optimal activity of the 1,4-DHPs have been studied in detail.[2] Compounds with a cyclic substituent at the 4-position possess good activity. Many studies have been and are still being carried out on 4-(substituted phenyl)-DHPs. The effects of different substituents have been widely studied in this series; compounds substituted with phenyl groups bearing electron-withdrawing groups such as NO_2, CN, CF_3, and Cl at *o* and *m* positions exhibited higher activity. Extensive studies have also been done on DHPs with 4-heterocyclic or polycyclic substituents (pyridine,[3] benzoxadiazole[4] or tricyclic[5]). However the properties of 4-thienyl-1,4-DHP derivatives, especially 4-(substituted-thienyl) ones have never been studied in detail. We therefore prepared new 4-thienyl-DHPs in order to examine the influence of a thienyl group substituted (or not) by a nitro group. This study was carried out to evaluate the antihypoxic activity of new nifedipine (Chart 1) thiophenic analogs obtained by replacing the 4-phenyl moiety by a thiophene ring and to compare their activity with that of three phenyl-DHPs references, *i.e.* nimodipine **1**, nifedipine **2** and nicardipine **3**.

The antihypoxic activity was evaluated with the skin conductance reaction (SCR)-hypoxia test.[6] This test enables us to study drugs which are active against cerebral hypoxia and is an adaptation, under hypoxic normobaric conditions, of the SCR test.[7] The SCR corresponds to an increase of sudoral secretion under the influence of a sudden emotional change or a sufficiently strong sensory stimulation. This phenomenon is controlled by the reticular formation. Thus, SCR magnitude give us some information on reticular formation activity. Moreover, it has been demonstrated[8] that hypoxic normobaric conditions ($O_2 < 10\%$) induce a dramatic decrease in behavior of mice. So, under the same experimental conditions, the lowering of the SCR may be attributed to an oxygenation deficiency of the reticular formation, a phenomenon comparable to impaired consciousness (a well-known consequence of cerebral hypoxia). Recovery is related to better cerebral tissue oxygenation and thus shows the antihypoxic effect of drugs (such as naftidrofuryl, piracetam, nicergolin and meclofenoxate.[6] In our test, the SCR was measured with a palmar-skin conductance meter, in response to photostimulation in mice (see experimental section).

The antihypoxic activity (capacity to recover the SCR depressed by hypoxia) of new thienyl DHPs was compared with that of calcium antagonists selected as reference compounds. Two parameters were employed: minimal recovery dose (MRD), *i.e.*, the lowest dose (mg/kg) at which the SCR recovery percentage was statistically significant (minimum effective dose) and recovery Percentage (RP), *i.e.*, SCR recovery percentage in relation to the control (see experimental section for calculation). These two parameters gave information about the improvement in cerebral activity depressed by hypoxia.

Chemistry

The synthesis of 4-thienyl-1,4-dihydropyridines **4** to **10** was carried out according to Hantzsch's method starting from substituted or unsubstituted thiophen-2- and



nimodipine (1) nifedipine (2) nicardipine (3)

Chart 1

3-carboxaldehydes **11** to **17**. The nitrothiophenecarboxaldehydes **14**, **16** and **17** were prepared as follows (Chart 2). Nitration reactions were conducted with fuming HNO_3 and H_2SO_4 at $-10\,°C$, starting from the appropriate thiophenecarboxaldehydes. 4-Nitrothiophene-2-carboxaldehyde **14** must be separated from 5-nitrothiophen-2-carboxaldehyde by fractional crystallization.[9]

The synthesis of alkylpyridines according to the Hantzsch's method[10] proceeds through condensation of a β-ketoester with a carboxaldehyde in the presence of ammonia (Chart 3). This method was applied to the thiophenecarboxaldehydes **11** to **17** using methyl 3-aminocrotonate and methylacetoacetate, leading to the thienyldihydropyridines **4** to **10**. The reaction was run in refluxing ethanol for 6 h.

Pharmacological Results and Discussion

In the SCR-hypoxia test,[6] a box was used in which the O_2 level was maintained at 7.5%. Under these conditions, the SCR[7] was lowered to below normoxic conditions. The average rise of SCR in the control group was of $1.8+0.3\,\mu A$ in hypoxia compared to $3.5+0.4\,\mu A$ in normoxia.

Statistically significant recovery doses and corresponding recovery percentages, at these doses, of reference drugs and thienyldihydropyridines **4** to **10** are listed in Table I. In every case, the recovery percentages were not proportional to dosage. We were unable to calculated ED_{50}, but it was possible to determine the effective dose range. The MRD corresponds to the lowest dose (mg/kg) producing a statistically significant effect.

The SCR-hypoxia test revealed the antihypoxic activity of several calcium antagonists as reflected by their capacity to produce SCR recovery. This recovery has been demonstrated and quantified for nicardipine, nifedipine and

Table I. Doses and Recovery (%) Values Due to Thienyldihydropyridines and Reference Compounds (HS = $p<0.01$, S = $p<0.05$, NS = not significant)

Compound	Dose (mg/kg)	Recovery (%)	Compound	Dose (mg/kg)	Recovery (%)
4	0.1	NS	**9**	0.1	NS
	0.5	NS		0.5	NS
	1	NS		1	NS
	5	NS		5	30.0 S
	10	NS		10	NS
5	0.1	NS	**10**	0.01	NS
	0.5	NS		0.05	25.0 HS
	1	20.0 HS		0.1	45.0 HS
	5	22.5 HS		0.5	40.0 HS
	10	NS		1	35.0 HS
		NS		5	NS
6	0.5	NS		10	NS
	1	NS	Nimodipine	0.005	NS
	5	30.0 HS		0.01	35.0 S
	10	NS		0.05	67.5 HS
	20	NS		0.1	67.5 HS
7	0.1	NS		0.5	50.0 HS
	0.5	NS		1	30.0 S
	1	27.5 S	Nifedipine	0.05	NS
	5	20.0 S		0.1	48.0 HS
	10	55.0 HS		0.5	NS
	20	NS		1	NS
8	0.1	NS		5	NS
	0.5	NS	Nicardipine	0.01	NS
	1	32.5 HS		0.05	20.0 S
	5	22.5 S		0.1	28.0 S
	10	NS		0.5	NS
				1	NS
				5	NS

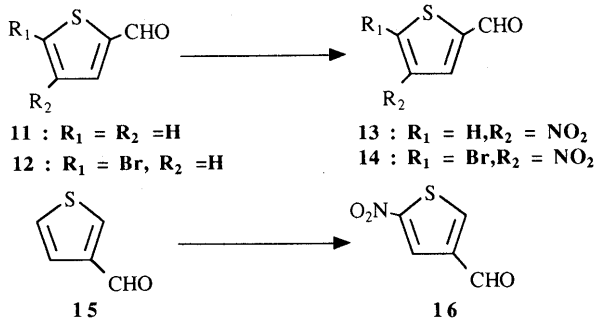


Chart 2

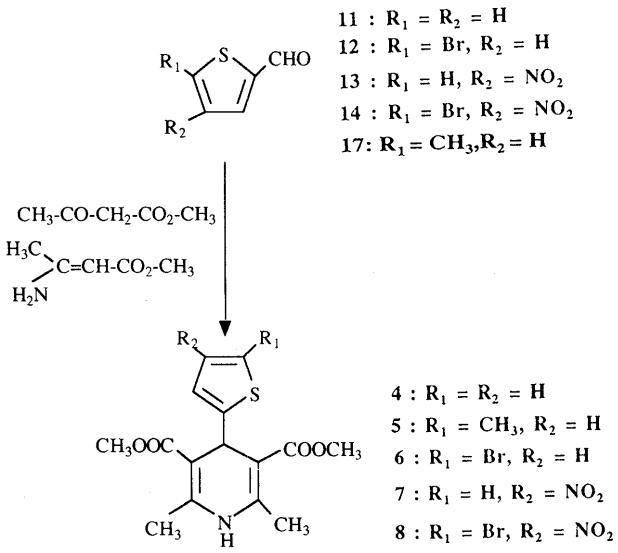
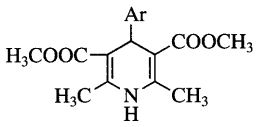
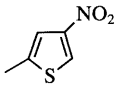
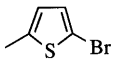
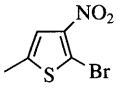
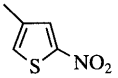


Chart 3

nimodipine. A comparative study showed that nimodipine exhibited antihypoxic activity from 10 μg/kg, while doses of 50 or 100 μg/kg were effective for nicardipine and nifedipine. The maximum recovery percentage was obtained with nimodipine (67.5% at 50 μg/kg). These results are consistent with the fact that nimodipine acts preferentially in cerebral arteries and in this way confers a greater resistance to hypoxia. Every tested derivative, except **4**, also revealed antihypoxic activity after peripheral administration. The replacement of the phenyl ring of the latter compounds by a thiophene ring was favourable: thienyldihydropyridines **5**, **7**, **8**, **10** gave at a recovery closely similar to that reference phenyldihydropyridine (20 to 50%) at doses as high as 1 mg/kg.

TABLE II. Influence of Substitution of Thiophene Ring by a Nitro Group: Comparison of Minimal Recovery Doses (MRD) or Minimal Effective Dose of Derivatives **4**, **6**, **9** and Their Nitro-Homologues **7**, **8**, **10**



Compound	Ar	MRD (mg/kg)	Nitro compound	Ar	MRD (mg/kg)
4		—	**7**		1
6		5	**8**		0.5
9		5	**10**		0.05

In particular, **10** showed antihypoxic activity comparable to that of nifedipine at a dose 2 times lower (50 to 100 μg/kg).

As in phenyl DHP, substitution of the aromatic ring of the thiophenic homologous derivatives by a nitro group seemed necessary for antihypoxic activity since **9** had an active dose 100 times higher than that of its nitro homologue **10** (Table II).

In conclusion, the present experiments confirmed the antihypoxic activity of nimodipine **1** and enabled us to select a thienyl-DHP **10** which seems as active as nicardipine **3** and more active than nifedipine **2**. Substitution of the ester functions of the derivative **10** by lengthening the ester chain, as with nimodipine **1**, might increase the activity.

Experimental

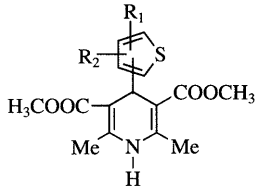
Chemistry Melting points were taken on a Köfler block without correction. Infrared spectra were recorded on a Philips PU 9716 apparatus. NMR spectra were recorded on a JEOL FX 200 in dimethyl sulfoxide-d_6 (DMSO-d_6) solution using tetramethylsilane as an internal standard (Table III).

General Procedure for Synthesis of Methyl 2,6-Dimethyl-4-(2-thienyl)- and -(3-thienyl)-1,4-dihydropyridine-3,5-dicarboxylates (4 to 10) These compounds were synthesized by Hantzsch's method.[10] A mixture of an appropriate thiophene-2 or 3-carbaldehyde (0.25 mol), methyl 3-aminocrotonate (28.75 g, 0.25 mol) and methyl acetoacetate (29 g, 0.25 mol) in ethanol (200 ml) was refluxed for 6 h. The reaction mixture was then left at room temperature for 24 h and the precipitate was collected by filtration, dried and crystallized from ether or methanol.

Dimethyl 2,6-Dimethyl-4-(2-thienyl)-1,4-dihydropyridine-3,5-dicarboxylate (4) From 2-thiophene carbaldehyde **11** (28 g, 0.25 mol). Crystallized from methanol as a white solid (23 g, 30%), mp 200 °C (dec.). *Anal.* Calcd for $C_{15}H_{17}NO_4S$: C, 58.62; H, 5.58; N, 4.56. Found: C, 58.85; H, 5.65; N, 4.52. IR (KBr) cm^{-1}: 3320 (NH), 1670, 1640 (CO), 1490, 1330, 1220, 1020, 730. ^{1}H-NMR (DMSO-d_6) δ: 2.27 (6H, s), 3.60 (6H, s), 5.15 (1H, s), 6.62 (1H, dd), 6.80 (1H, dd), 7.13 (1H, dd), 9.00 (1H, s).

Dimethyl 2,6-Dimethyl-4-(5-methyl-2-thienyl)-1,4-dihydropyridine-3,5-dicarboxylate (5) From 5-methyl-2-thiophene carbaldehyde **17** (31.6 g,

TABLE III. Spectral Data of (Substituted Thienyl)-1,4,-dihydropyridines **4** to **10**



Compound	R_1	R_2	Binding position of 4-thienyl to 1,4-DHP[a]	IR ν cm^{-1} (KBr)	^{1}H-NMR (DMSO-d_6) δ (ppm)
4	H	H	2	3320, 1670, 1640, 1490, 1330, 1220, 1020, 730	2.27 (6H, s), 3.60 (6H, s), 5.15 (1H, s), 6.22 (1H, dd), 6.80 (1H, dd), 7.13 (1H, dd), 9 (1H, s)
5	5-Me	H	2	3340, 1690, 1640, 1430, 1305, 1552, 1020, 805	2.26 (3H, s), 2.30 (6H, s), 3.60 (6H, s), 5.06 (1H, s), 6.40 (2H, m), 8.97 (1H, s)
6	5-Br	H	2	3340, 1690, 1675, 1640, 1480, 1265, 1215, 1100, 800	2.27 (6H, s), 3.63 (6H, s), 5.07 (1H, s), 6.40 (1H, d), 6.83 (1H, d), 9.05 (1H, s)
7	4-NO_2	H	2	3360, 1700, 1430, 1375, 1310, 1270, 1150, 1090, 785	2.27 (6H, s), 3.60 (6H, s), 5.10 (1H, s), 7.03 (1H, s), 9.23 (1H, s)
8	5-Br	4-NO_2	2	3340, 1700, 1480, 1430, 1380, 1220, 1110, 780	2.27 (6H, s), 3.60 (6H, s), 5.05 (1H, s), 7.03 (1H, s), 9.23 (1H, s)
9	H	H	3	3345, 2950, 1695, 1480, 1425, 1340, 1210, 1110, 760	2.21 (6H, s), 3.60 (6H, s), 4.90 (1H, s), 6.82 (2H, m), 7.23 (1H, dd), 8.83 (1H, s)
10	5-NO_2	H	3	3320, 1700, 1500, 1335, 1220, 1130, 1100, 790	2.27 (6H, s), 3.60 (6H, s), 4.93 (1H, s), 7.47 (1H, d), 7.63 (1H, d), 9.03 (1H, s)

a) Binding position of 4-(substituted thienyl)group to 1,4-dihydropyridine.

0.25 mol). Crystallized from ethanol, as white crystals (15.1 g, 19%), mp 190 °C (dec.). *Anal.* Calcd for $C_{16}H_{19}NO_4S$: C, 59.85; H, 5.92; N, 4.36. Found: C, 59.73; H, 5.93; N, 4.26. IR (KBr) cm^{-1}; 3340 (NH), 1690 (CO), 1640, 1430, 1305, 1220, 1020, 805. ^{1}H-NMR (DMSO-d_6) δ: 2.26 (3H, s), 2.30 (6H, s), 3.60 (6H, s), 5.06 (1H, s), 6.40 (2H, m), 8.97 (1H, s).

Dimethyl 4-(5-Bromo-2-thienyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (6) From 5-bromo-2-thiophene carbaldehyde **12** (48 g, 0.25 mol). Crystallized from ethanol as white crystals (38.5 g, 39%), mp 208 °C (dec.). *Anal.* Calcd for $C_{15}H_{16}BrNO_4S$: C, 44.64; H, 4.15; N, 3.59. Found: C, 44.60; H, 4.17; N, 3.59. IR (KBr) cm^{-1}: 3340 (NH) 1690, 1675 (CO) 1480, 1265, 1215, 1100, 800. ^{1}H-NMR (DMSO-d_6) δ: 2.27 (6H, s), 3.63 (6H, s), 5.07 (1H, s), 6.40 (1H, d), 6.83 (1H, d), 9.05 (1H, s).

Dimethyl 2,6-Dimethyl-4-(4-nitro-2-thienyl)-1,4-dihydropyridine-3,5-dicarboxylate (7) From 4-nitro-2-thiophene carbaldehyde **13** (28 g, 0.25 mol). Crystallized from methanol as yellow crystals (20 g, 23%), mp 170 °C (dec.). *Anal.* Calcd for $C_{15}H_{16}N_2O_6S$: C, 51.13; H, 4.51; N, 7.95; S, 8.00. Found: C, 50.97; H, 4.58; N, 8.04; S, 7.92. IR (KBr) cm^{-1}: 3360 (NH), 1700 (CO), 1430, 375, 1310, 1270, 1150, 1090, 795. ^{1}H-NMR (DMSO-d_6) δ: 2.27 (6H, s), 3.60 (6H, s), 5.10 (1H, s), 7.08 (1H, d), 8.46 (1H, d), 9.20 (1H, s).

Dimethyl 4-(5-Bromo-4-nitro-2-thienyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (8) From 5-bromo-4-nitro-2-thiophene carbaldehyde **14** (60 g, 0.25 mol). Crystallized from ethanol as yellow crystals (26 g, 60%), mp 210 °C (dec.) *Anal.* Calcd for $C_{15}H_{15}BrN_2O_6S$: C, 41.77; H, 3.48; Br, 18.52; S, 7.43. Found: C, 41.73; H, 3.41; Br, 18.29; S, 7.62. IR (KBr) cm^{-1}: 3340 (NH), 1700 (CO), 1480, 1430, 1380, 1220, 1110, 780. ^{1}H-NMR (DMSO-d_6) δ: 2.27 (6H, s), 3.60 (6H, s), 5.05 (1H, s), 7.03 (1H, s), 9.23 (1H, s).

Dimethyl 2,6-Dimethyl-4-(3-thienyl)-1,4-dihydropyridine-3,5-dicarboxylate (9) From 3-thiophene carbaldehyde **15** (28.3 g, 0.25 mol). Crystallized from methanol as white crystals (37.3 g, 50%), mp 180 °C (dec.). *Anal.* Calcd for $C_{15}H_{17}NO_4S$: C, 58.62; H, 5.54; N, 4.56; S, 10.43. Found: C, 58.51; H, 5.58; N, 4.48; S, 10.48. IR (KBr) cm^{-1}: 3345 (NH), 1695 (CO), 2950, 1480, 1425, 1340, 1210, 1110, 760. ^{1}H-NMR (DMSO-d_6) δ: 2.21 (6H, s), 3.53 (6H, s), 4.90 (1H, s), 6.82 (2H, 2m), 7.23 (1H, dd), 8.83 (1H, s).

Dimethyl 2,6-Dimethyl-4-(5-nitro-3-thienyl)-1,4-dihydropyridine-3,5-dicarboxylate (10) From 5-nitro-3-thiophene carbaldehyde **16** (35 g, 0.25 mol). Crystallized from ethanol as pale yellow specks (17.5 g, 20%), mp 176 °C (dec.). *Anal.* Calcd for $C_{15}H_{16}N_2O_6S$: C, 51.13; H, 4.55; N, 7.95; S, 9.09. Found: C, 51.16; H, 4.48; N, 7.92; S, 9.05. IR (KBr) cm^{-1}: 3320 (NH), 1700 (CO), 1500, 1335, 1220, 1130, 1100, 790. ^{1}H-NMR (DMSO-d_6) δ: 2.27 (6H, s), 3.60 (6H, s), 4.93 (1H, s), 7.47 (1H, d), 7.63 (1H, d), 9.03 (1H, s).

Pharmacology Animals: Male and female OF1 mice, weighing 18 to 24 g, were used as the experimental aminals. They were randomized into batches of 20.

Drugs: Drugs were administered intraperitoneally. They were suspended in 0.5% aqueous carboxymethylcellulose except for nifedipine which was dissolved in a 25% glycerine+PEG 400 solution. The concentration level was adjusted in order to administer 0.4 ml per 20 g of body weight. Every animal in a given batch received an equal amount of the drug.

Apparatus: The box, made of polyvinyl chloride, was flexible and transparent, with four cuffs and gloves with which to work inside the box. On one side, was a lock enabling us to transfer subjects for experimentation. The desired atmosphere (O_2=7.5%) was obtained by allowing adequate nitrogen to enter the box. The oxygen level was measured with an oxygen analyzer.

The Palmar Skin Conductance Meter: This apparatus, especially designed and built for use with mice,[7] enabled us to evaluate the SCRs. The essential feature of this apparatus is two electrodes 2 mm in diameter. The mouse, taken by its nuchal skin and placed in front of the electrodes, immediately grasps them by reflex. The intensity of the current (μA), proportionate to the skin conductance level of the animals (on the palmar sides), was measured.

The Photostimulator: A 100 W (220 V) glow lamp located 10 cm above the head of the mouse was switched on for 7 to 10 s. The lamp was switched off when the maximal response was obtained. The photostimulus (PS) had to be given in a dark room.

Experimental Procedure: The whole test was conducted in the dark. Mice were treated, out of the box 20 min before the reading. Then, they were brought into the hypoxic atmosphere of the box, 10 min after the treatment, *i.e.*, 10 min before the reading. Each recorder SCR included two consecutive readings (r) of the skin conductance level: the first in the dark (r_d) immediately followed by the second during the PS (r_p) the skin conductance level: the first in the dark (r_d) immediately followed by the second during the PS (r_p) (the skin conductance level reached its highest value during the PS). The difference, ($r_p - r_d$), represented the SCR.

Recovery Percentage: The SCR recovery percentage was calculated for each treated animal in relation to control animal:

$$\text{recovery } (\%) = \frac{(r_p - r_d)\,\text{treated} - (r_p - r_d)\,\text{control}}{(r_p - r_d)\,\text{control}} \times 100$$

Then, for each batch of animals, *i.e.*, each dose, the mean percentage of recovery (%) was calculated.

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