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Synthesis and *in vitro* pharmacology of a series of new 1,4-dihydropyridines. 2. Diethyl 4-[2-(ω-aminoalkoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates and their corresponding isothioureas as tools for determining structure–activity relationships

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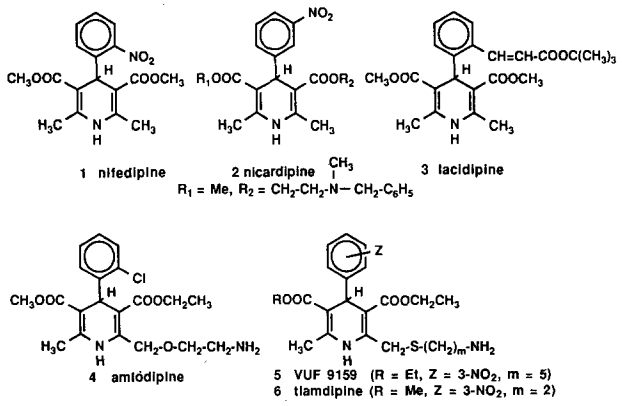
Summary — The synthesis, *in vitro* calcium channel blocking activities and the affinities for the Ca^{2+}-entry receptor protein, of a new series of diethyl 2,6-dimethyl-4-[2-(ω-substituted-alkoxy)phenyl]-1,4-dihydropyridine-3,5-dicarboxylates are discussed. Increasing the ω-substituted alkoxy chain length from pentoxy to decoxy is found not to affect the calcium channel blocking activity or affinity in both diethyl 4-[2-(ω-aminoalkoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates and diethyl 2,6-dimethyl-4-[2-(ω-thiourea ω-thiouronium substituted-alkoxy)phenyl]-1,4-dihydropyridine-3,5-dicarboxylates.

1,4-dihydropyridines / calcium channel blockers

Introduction

The introduction of 4-aryl-1,4-dihydropyridines (DHPs) with highly potent Ca^{2+}-channel blocking activity led to a new direction in cardiovascular therapy. Ca^{2+}-channel blockers are now well established in the treatment of angina pectoris, hypertension, certain cardiac arrhythmias and peripheral vascular disorders [1–5]. The 1,4-dihydropyridine Ca^{2+}-channel blockers inhibit the influx of extracellular Ca^{2+} *via* L-type potential-dependent calcium channels and reduce vascular resistance. Although nifedipine **1** [6] and nicardipine **2** [7] are widely used clinically, their rather short duration of action is disadvantageous. For this reason several DHPs have been synthesized in which variations in phenyl substitution were performed or modifications of ester substituents on the 3- and 5-position on the 1,4-dihydropyridine ring were carried out [8–11].



Qualitative and quantitative structure–activity investigations have been carried out by Loev *et al* (antihypertensive action in anaesthetized animals) [12] and Rodenkirchen *et al* (negative inotropic activity on isolated, isotonically contracted cat papillary muscle) [13]. In both studies the most potent DHPs carry an *ortho*-substituent in the 4-phenyl ring. Derivatives with *meta* or *para* substituents are less active. In the case of 4-aryl-substituted dihydropyri-

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dines the biological activity depends mainly on steric influences and appears generally to be independent of the electronic properties of the substituents.

For lacidipine **3** and analogues it has been found that increasing steric bulk and lipophilicity of the *ortho*-substituent on the 4-phenyl ring of the 1,4-DHP enhances *in vivo* calcium channel blocking activity [14]. The lacidipine analogue without a cinnamoyl ester is > 700 times less active than lacidipine. By increasing the lipophilicity of the cinnamoyl ester of lacidipine analogues, the *in vitro* calcium channel blocking activity decreases while the duration of action increases [15].

Systematic modifications at the 2-position of the DHP ring have been performed to increase the rather short duration of action and improve the bioavailability of the classical DHPs, resulting in amlodipine **4** [16] and its derivatives and their thio-bioisosters such as VUF 9159 **5** [17] and tiamdipine **6** [18]. These latter DHPs demonstrate that extended 2-substituents bearing a basic functionality are well tolerated at the receptor.

Calcium channel blocking activity of 1,4-DHPs is thought to be achieved by interaction with specific receptors present on the L-type voltage-dependent calcium channels. A major role for contribution to binding to these receptors is played by the 4-phenyl ring of the 1,4-DHPs. Thus, substituents on the 4-phenyl ring of nifedipine analogues significantly influence the affinity to the receptor. Amlodipine and tiamdipine analogues possess a basic side chain at the 2-position on the 1,4-DHP ring. It has been suggested that this protonated side chain is trapped in the membrane and the preferred conformation forces the phenyl ring to move from the position adopted by nifedipine analogues in such a way that the contribution of the 4-phenyl ring substituents to binding is reduced [19, 20]. This could then explain the rather small influence of the substituents on the 4-phenyl ring of amlodipine and tiamdipine analogues on their pharmacological potencies. In the present study we therefore established whether substituents of the O-$(CH_2)_m$-R type (with m = 5, 6 or 10 and R = NH_2 or thiourea or thiouronium bromide) at the *ortho*-position of the phenyl ring are tolerated. The choice of identical ester substitution at the 3- and 5-position on the 1,4-dihydropyridine ring has been made because of earlier results obtained showing that these DHPs were equally active as different ester substituted DHPs [17] and to avoid stereochemical complications.

Chemistry

The compounds listed in table I were prepared by a general method illustrated in scheme 1.

2-(ω-Substituted-alkoxy)benzaldehydes **9** were available by reaction of 2-hydroxybenzaldehyde with *N*-(ω-bromoalkyl)phthalimides. The *N*-(ω-bromoalkyl)-phthalimides were synthesized according to Soine *et al* [21]. Hantzsch type condensation of a substituted benzaldehyde **9** with ethyl acetoacetate **7** and ethyl 3-aminocrotonate **8** afforded 4-[2-(ω-phthalimidoalkoxy)phenyl]-1,4-dihydropyridines **10**. Subsequent hydrazinolysis of the phthalimides with hydrazine monohydrate gave the 4-[2-(ω-aminoalkoxy)phenyl]-1,4-dihydropyridines **11**. The corresponding thiourea analogues **13** were obtained *via* reaction of the amino function of **11** with benzoyl isothiocyanate **12** [22] and subsequent basic hydrolysis. Reaction of the thiourea analogues **13** with ethyl bromide gave the thiouronium compounds **14**.

Pharmacology

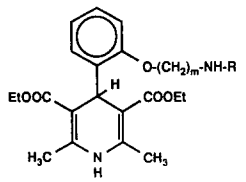
Calcium channel blocking activities were determined *in vitro* on rat aorta, and dihydropyridine receptor binding affinities were determined on isolated rat cortex as previously described by Christiaans *et al* [17]. The calcium channel blocking activities (expressed as pIC_{50}) were assessed as the concentration required to inhibit the K^+-depolarization-induced (50 mM) contractile responses in rat aorta strips by 50%. Concentration–response curves were utilized to determine pIC_{50} values.

The dihydropyridine receptor binding assay was performed on rat cortex microsomes which were incubated with [^{3}H]-nitrendipine and various concentrations of the compounds at 37°C for 60 min. The incubations were carried out according to Boer *et al* [23] in such a way that the final DMSO concentration never exceeded 1% (v/v), a concentration which did not affect the binding. The equilibrium dissociation constant (K_d) of the labelled compound and the maximal binding (B_{max}) were determined with the non-linear fitting program LIGAND 4.1 [24] being 0.75 nM and 270 fmol/mg protein respectively.

Results and discussion

All compounds described in this paper show *in vitro* calcium channel blocking activity within 5 min but do not reach complete equilibrium within 45 min. This is in accordance with diethyl 2-(5-aminopentylthiomethyl)-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (VUF 9159) and related compounds, described by Christiaans *et al* [17]. Complete inhibition of contractile responses was obtained for all compounds, as verified by addition of 1 mM papaverine.

Table I. Calcium blocking activities and radioligand binding affinities of diethyl 2,6-dimethyl-4-[2-(ω-substituted-alkoxy)-phenyl]-1,4-dihydropyridine-3,5-dicarboxylates.



Compound	*R*	*m*	pIC_{50}[a]	*Rat tissue*	pK_d[b]
VUF 4587	H	5	6.61 ± 0.08	Aorta	6.83 ± 0.08
VUF 4599	H	6	6.42 ± 0.04	Aorta	7.02 ± 0.05
VUF 4600	H	10	6.35 ± 0.20	Aorta	7.30 ± 0.06
VUF 4622	$C(S)NH_2$	5	6.44 ± 0.13	Aorta	7.00 ± 0.07
VUF 4671	$C(S)NH_2$	6	6.39 ± 0.09	Aorta	7.09 ± 0.09
VUF 4623	C(NH)SEt HBr	5	6.31 ± 0.10	Aorta	7.27 ± 0.15
VUF 4624	C(NH)SEt HBr	6	6.34 ± 0.11	Aorta	7.70 ± 0.11
1	Nifedipine		8.77 ± 0.08	Aorta	8.70 ± 0.14
3	Amlodipine		8.1[c]	Aorta	–
4	VUF 9159		7.96 ± 0.07	Aorta	8.55 ± 0.03

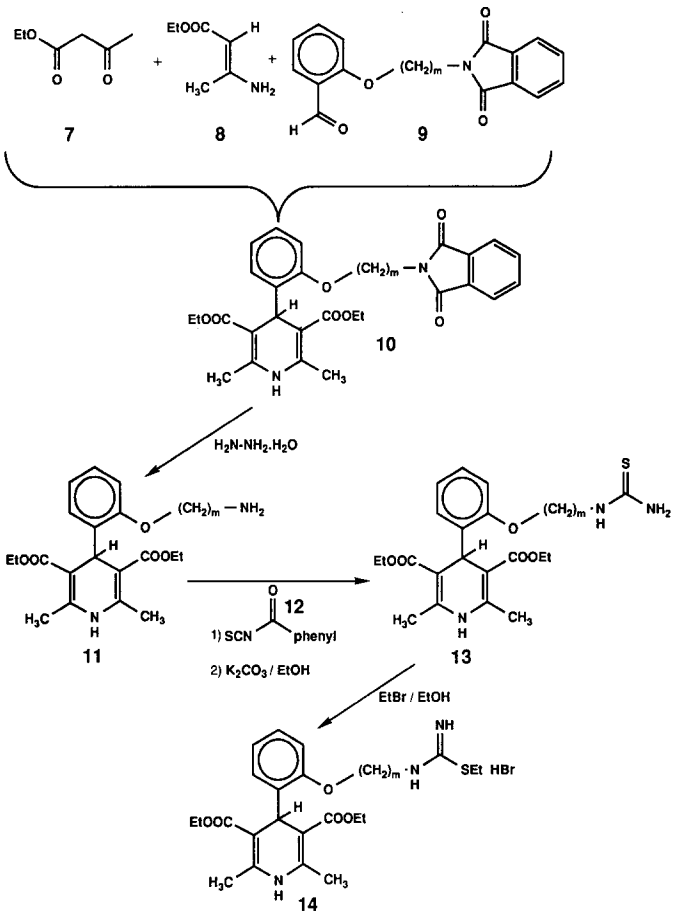
[a]pIC_{50} concentration required to produce 50% of its inhibitory effect; all values are means ± SD for 3 independent observations; [b]all values are means ± SD for 6-9 independent observations; all radioligand binding affinities (pK_d) were determined on isolated rat cortex membranes; [c]Alker *et al* [25].

In table I the influence of the variations in the ω-aminoalkoxy chain length at the *ortho* position of the 4-phenyl ring on calcium channel blocking activity show a small range of potencies. Compounds with ω-aminoalkoxy substituents at the *meta* position of the 4-phenyl ring are not active at all (data not shown).

Converting the amine function of the ω-aminoalkoxy substituent into thiourea affords species which are not protonated under physiological conditions. These compounds (VUF 4622 and VUF 4671) are as active as the corresponding amines (VUF 4599 and VUF 4600). Even introduction of an isothiouronium bromide, a polar group, does not alter the calcium channel blocking activities (VUF 4623 and VUF 4624). Although, as mentioned by Loev *et al* [12] and Rodenkirchen *et al* [13], the calcium channel blocking activities of 4-aryl-substituted-1,4-dihydropyridines depend mainly on steric influences, no such indications can be found for the compounds described in this paper.

Table I shows that there is a difference between pIC_{50} values and pK_d values. This difference can originate from the lipophilic character of the *ortho*-phenyl substituents.

Spampinato *et al* [26] investigated the role of lipophilicity of lacidipine on its calcium channel blocking activities. The potency of lacidipine as a calcium channel blocker proved not to be directly related to the amount of drug locked in the cell, indicating that lacidipine binds to the lipid bilayer of the cell membrane and then diffuses towards a specific binding site. This shows a similarity to the blockade of Na^+-channels by local anesthetics. The drug receptor for local anesthetics is situated in the sodium channel and ligands reach the receptor *via* the membrane phase (hydrophobic pathway) or *via* the channel (hydrophilic pathway). The DHP receptor is located within the calcium channel in the lipid bilayer near the external end of the channel [27, 28]. In the 3-compartment receptor model of Gaviraghi [14] (fig 1) the dihydropyridine receptor is seen as a protein compartment surrounded by a lipid compartment. Drugs in the aqueous compartment stay in contact with both lipid and protein compartments. Increase in the lipophilicity of a dihydropyridine can increase the affinity for the lipid compartment from which it is slowly released into the protein compartment. Increasing the affinity of dihydropyridines for the receptor enhances the potency but does not affect the duration of action [15].



Scheme 1.

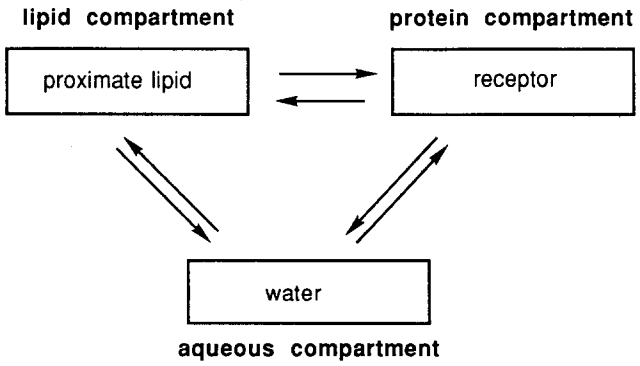


Fig 1. Three-compartment receptor model (from [14]).

By modifying the lipophilicity of dihydropyridines the competition for binding to the protein compartment or to the lipid compartment is influenced. Dihydropyridines with a slow onset of action and a long duration of effect are thought to compete strongly for the lipid compartment with respect to the receptor compartment when delivered by the aqueous compartment.

In accordance with the results obtained on calcium channel blocking activities a narrow potency range is also found in affinity for the binding site of the L-type calcium channel. All pK_d values are slightly higher than the corresponding pIC_{50} values. The phenomenon of differences in pK_d values and pIC_{50} values was also observed by Kwon *et al* [29]. A possible explanation for these differences may be that the calcium channel blocking activities and the affinities are determined on different organs.

Conclusions

The present diethyl 2,6-dimethyl-4-[2-(ω-substituted-alkoxy)phenyl]-1,4-dihydropyridine-3,5-dicarboxylates are moderate inhibitors of K^+-depolarization-induced contractile responses in rat aorta strips. Increasing the ω-aminoalkoxy chain length from pentoxy to decoxy does not enhance calcium channel blocking activity. Even replacement of the amino function by a thiouronium bromide does not alter the calcium channel blocking activity. It has been found that calcium channel blocking activity is independent of steric influences of the diethyl 2,6-dimethyl-4-[2-(ω-substituted-alkoxy)phenyl]-1,4-dihydropyridine-3,5-dicarboxylates.

There seems to be a tendency that with increasing alkyl chain length the differences between pIC_{50} values and pK_d values become more substantial. By increasing the alkyl chain length, the lipophilicity of the dihydropyridines is increased. According to the 3-compartment receptor model, the dihydropyridines with higher lipophilicity show a higher affinity for the lipid compartment and therefore a lower potency *in vitro*. This could then explain the more substantial differences between pIC_{50} values and pK_d values.

The narrow activity range does not permit quantitation of the structure–activity relationships of these novel 1,4-dihydropyridine derivatives.

Experimental protocols

Where indicated, crude reaction products were purified by flash chromatography on silica-gel (JT Baker 70242). Melting points were determined on a Mettler FP 52 with microscope. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AC 200. The chemical shifts are in ppm relative to tetramethylsilane. Mass spectra were determined on a Mat 90 (Finnigan

Mat) mass spectrometer with fast atom bombardment ionization (matrix: thioglycerol, Ion Tech saddlefield gun, 8 keV Xenon with xenon ioncurrent 0.2 mA). Furthermore, the purity of the compounds was checked by TLC (Merck silica gel 60, F254 0.25 mm). Nifedipine was obtained from Sigma Chemical Co. [^{3}H]-Nitrendipine (73 Ci/mmol) was purchased from Du Pont de Nemours (The Netherlands).

General procedure

2-[ω-(Phthalimido)alkoxy]benzaldehyde ***9***
50 mmol of the appropriate *N*-(ω-bromoalkyl)phthalimide, 50 mmol salicylaldehyde and 50 mmol potassium carbonate were stirred in 50 ml DMF for 5 h at 110°C under nitrogen. After cooling to room temperature, the reaction mixture was filtered. The filtrate was evaporated and the residue was crystallized from methanol.

2-[5-(Phthalimido)pentoxy]benzaldehyde
Yield: 100%, mp: 92.1–93.8°C. ^{1}H-NMR ($CDCl_3$): 1.57 ppm (m, 2H, C-C-CH_2-C-C), 1.77 and 1.90 ppm (m, 4H, O-C-CH_2-C-CH_2-CH_2-C-N), 3.73 ppm (t, *J* = 7.0 Hz, 2H, O-C-C-C-C-CH_2-N), 4.07 ppm (t, *J* = 6.3 Hz, 2H, O-CH_2-C-C-C-C-N), 6.92–7.04 ppm (m, 2H, 2 x phenyl-*H*), 7.46–7.58 ppm (m, 1H, phenyl-*H*), 7.69–7.88 ppm (m, 5H, 4 x phthalimide-*H* and 1 x phenyl-*H*), 10.47 ppm (s, 1H, -*H*C=O).

2-[6-(Phthalimido)hexyloxy]benzaldehyde
Yield: 85%, mp: 85.7–86.6°C. ^{1}H-NMR ($CDCl_3$): 1.25–2.04 ppm (m, 8H, O-C-(CH_2)$_4$-C-N), 3.74 ppm (t, *J* = 7.1 Hz, 2H, O-(C)$_5$-CH_2-N), 4.07 ppm (t, *J* = 6.3 Hz, O-CH_2-(C)$_5$-N), 6.92–7.05 ppm (m, 2H, 2 x phenyl-*H*), 7.47–7.57 ppm (m, 1H, phenyl-*H*), 7.71–7.84 ppm (m, 5H, 4 x phthalimide-*H* and phenyl-*H*), 10.50 ppm (s, 1H, -*H*C=O).

2-[10-(Phthalimido)decyloxy]benzaldehyde
Yield: 75%, mp: 65.0–67.1°C. ^{1}H-NMR ($CDCl_3$): 1.10–1.58 ppm (m, 12H, O-C-C-(CH_2)$_6$-C-C-N), 1.58–1.77 ppm (m, 2H, O-(C)$_8$-CH_2-C-N), 1.77–1.94 ppm (m, 2H, O-C-CH_2-(C)$_8$-N), 3.68 ppm (t, *J* = 7.0 Hz, 2H, O-(C)$_9$-CH_2-N), 4.05 ppm (t, *J* = 6.2 Hz, O-CH_2-(C)$_9$-N), 6.93–7.06 ppm (m, 2H, 2 x phenyl-*H*), 7.44–7.61 ppm (m, 1H, phenyl-*H*), 7.61–7.77 ppm (m, 2H, 2 x phthalimide-*H*), 7.77–7.80 ppm (m, 3H, phenyl-*H* and 2 x phthalimide-*H*), 10.50 ppm (s, 1H, -*H*C=O).

Diethyl 2,6-dimethyl-4-{2-[ω-(phthalimido)alkoxy]phenyl}-1,4-dihydropyridine-3,5-dicarboxylate ***10***
One equivalent of the appropriate 2-[ω-(phthalimido)alkoxy]-benzaldehyde, 1 equivalent ethyl acetoacetate and 0.06 equivalents of glacial acetic acid and benzylamine were refluxed in absolute ethanol (2 l/mol) under nitrogen. After 3 h, 1 equivalent of ethyl aminocrotonate was added and refluxing was continued overnight. After cooling to room temperature, the solvent was evaporated and the residue was crystallized from 2-propanol.

Diethyl 2,6-dimethyl-4-{2-[5-(phthalimido)pentoxy]phenyl}-1,4-dihydropyridine-3,5-dicarboxylate
Yield: 43%, mp: 140.4–141.4°C. ^{1}H-NMR ($CDCl_3$): 1.16 ppm (t, *J* = 7.2 Hz, 6H, 2 x CH_3-CH$_2$-O), 1.46–1.58 ppm (m, 2H, C-C-CH_2-C-C), 1.70–1.88 ppm (m, 4H, O-C-CH_2-C-CH_2-C-N), 2.28 ppm (s, 6H, 2 x pyridine-CH_3), 3.66–3.78 ppm (m, 2H, O-C-C-C-C-CH_2-N), 3.88 ppm (t, *J* = 6.65 Hz, 2H, O-CH_2-C-C-C-C-N), 4.00 ppm (q, *J* = 7.2 Hz, 4H, 2 x CH$_3$-CH_2-O), 5.15 ppm (s, 1H, pyridine-H_4), 6.13 ppm (s, 1H, pyridine-N*H*), 6.69–6.82 ppm (m, 2H, 2 x phenyl-*H*), 7.07 ppm (d, *J* = 7.5 Hz, 1H, phenyl-*H*), 7.21 ppm (d, *J* = 7.5 Hz, 1H, phenyl-*H*), 7.70–7.73 ppm (m, 2H, 2 x phthalimide-*H*), 7.81–7.87 ppm (m, 2H, 2 x phthalimide-*H*).

Diethyl 2,6-dimethyl-4-{2-[6-(phthalimido)hexoxy]phenyl}-1,4-dihydropyridine-3,5-dicarboxylate
Yield: 50%, mp: 131.2–133.3°C. ^{1}H-NMR ($CDCl_3$): 1.16 ppm (t, *J* = 7.1 Hz, 6H, 2 x CH_3-CH$_2$-O), 1.44–1.84 ppm (m, 8H, O-C-(CH_2)$_4$-C-N), 2.27 ppm (s, 6H, 2 x pyridine-CH_3), 3.72 ppm (t, *J* = 7.1 Hz, 2H, O-(C)$_5$-CH_2-N), 3.87 ppm (t, *J* = 6.8 Hz, 2H, O-CH_2-(C)$_5$-N), 4.01 ppm (q, *J* = 7.1 Hz, 4H, 2 x CH$_3$-CH_2-O), 5.16 ppm (s, 1H, pyridine-H_4), 6.12 ppm (bs, 1H, pyridine-N*H*), 6.70–6.83 ppm (m, 2H, 2 x phenyl-*H*), 7.01–7.10 ppm (m, 1H, phenyl-*H*), 7.19–7.27 ppm (m, 1H, phenyl-*H*), 7.69–7.74 ppm (m, 2H, 2 x phthalimide-*H*), 7.83–7.87 ppm (m, 2H, 2 x phthalimide-*H*).

Diethyl 2,6-dimethyl-4-{2-[10-(phthalimido)decoxy]phenyl}-1,4-dihydropyridine-3,5-dicarboxylate
Yield: 60%, mp: 125.5–128.0°C. ^{1}H-NMR ($CDCl_3$): 1.17 ppm (t, *J* = 7.2 Hz, 6H, 2 x CH_3-CH$_2$-O), 1.22–1.88 ppm (m, 16H, O-C-(CH_2)$_8$-C-N), 2.29 ppm (s, 6H, 2 x pyridine-CH_3), 3.60–3.73 ppm (m, 2H, O-(C)$_9$-CH_2-N), 3.90 ppm (t, *J* = 6.7 Hz, 2H, O-CH_2-(C)$_9$-N), 4.04 ppm (q, *J* = 7.2 Hz, 4H, 2 x CH$_3$-CH_2-O), 5.20 ppm (s, 1H, pyridine-H_4), 5.65 ppm (s, 1H, pyridine-N*H*), 6.70–6.83 ppm (m, 2H, 2 x phenyl-*H*), 7.01–7.10 ppm (m, 1H, phenyl-*H*), 7.21–7.29 ppm (m, 1H, phenyl-*H*), 7.65–7.78 ppm (m, 2H, 2 x phthalimide-*H*), 7.78–7.90 ppm (m, 2H, 2 x phthalimide-*H*).

Diethyl 4-[2-(ω-aminoalkoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate ***11***
45 mmol phthalimide and 3 equivalents hydrazine monohydrate were refluxed in 300 ml ethanol for 6 h. After cooling to room temperature the reaction mixture was filtered and the solvent evaporated. The residue was dissolved in dichloromethane and extracted with 1 M NaOH. The organic layer was evaporated and the residue dissolved in ethanol/water (5:1) and acidified with acetic acid. After evaporation of the solvent the residue was dissolved in water and washed with diethyl ether (3 x 50 ml). The water layer was made basic with sodium bicarbonate solution and extracted with ethyl acetate. The combined organic layers were dried with $MgSO_4$ and the solvent evaporated. The free base was obtained as a solid.

Diethyl 4-[2-(5-aminopentoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate ***(VUF 4587)***
Yield: 77%, mp: 146.8–147.1°C, MS (FAB$^+$) 431 [M + H]$^+$, (FAB$^-$) 429 [M – H]$^-$. ^{1}H-NMR ($CDCl_3$): 1.16 ppm (t, *J* = 7.12 Hz, 6H, 2 x CH_3-CH$_2$-O), 1.58–1.76 ppm (m, 6H, O-C-CH_2-CH_2-CH_2-C-N), 2.25 ppm (s, 6H, 2 x pyridine-CH_3), 2.81 ppm (t, *J* = 5.7 Hz, 2H, O-(C)$_4$-CH_2-N), 3.85–3.92 ppm (m, 2H, O-CH_2-(C)$_4$-N), 4.01 ppm (q, *J* = 7.12 Hz, 4H, 2 x CH$_3$-CH_2-O), 5.14 ppm (s, 1H, pyridine-H_4), 6.68–6.79 ppm (m, 2H, 2 x phenyl-*H*), 7.01–7.09 ppm (m, 1H, phenyl-*H*), 7.19–7.26 ppm (m, 1H, phenyl-*H*), 7.58 ppm (s, 1H, pyridine-N*H*).

Diethyl 4-[2-(6-aminohexoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate ***(VUF 4599)***
Yield: 73%, mp: 113.9–116.8°C, MS (FAB$^+$) 445 [M + H]$^+$, (FAB$^-$) 443 [M – H]$^-$. ^{1}H-NMR ($CDCl_3$): 1.15 ppm (t, *J* = 7.1 Hz, 6H, 2 x CH_3-CH$_2$-O), 1.44–1.81 ppm (m, 8H, O-C-(CH_2)$_4$-C-N), 2.27 ppm (s, 6H, 2 x pyridine-CH_3), 2.68–2.76 ppm (m, 2H, O-(C)$_5$-CH_2-N), 3.89 ppm (t, *J* = 6.6 Hz, 2H, O-CH_2-(C)$_5$-N), 4.02 ppm (q, *J* = 7.1 Hz, 4H, 2 x CH$_3$-

CH_2-O), 5.19 ppm (s, 1H, pyridine-H_4), 5.85 ppm (bs, 1H, pyridine-N*H*), 6.72–6.83 ppm (m, 2H, 2 x phenyl-*H*), 7.01–7.07 ppm (m, 1H, 1 x phenyl-*H*), 7.19–7.25 ppm (m, 1H, 1 x phenyl-*H*).

Diethyl 4-[2-(10-aminodecoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate ***(VUF 4600)***
Yield: 72%, mp: 117.5–119.2°C, MS (FAB+) 501 [M + H]+, (FAB–) 499 [M – H]–. ^{1}H-NMR ($CDCl_3$): 1.18 ppm (t, J = 7.2 Hz, 6H, 2 x CH_3-CH_2-O), 1.22–1.88 ppm (m, 16H, O-C-$(CH_2)_8$-C-N), 2.26 ppm (s, 6H, 2 x pyridine-CH_3), 3.61–3.72 ppm (m, 2H, O-$(C)_9$-CH_2-N), 3.89 ppm (t, J = 6.7 Hz, 2H, O-CH_2-$(C)_9$-N), 4.04 ppm (q, J = 7.2 Hz, 4H, 2 x CH_3-CH_2-O), 5.19 ppm (s, 1H, pyridine-H_4), 5.82 ppm (s, 1H, pyridine-N*H*), 6.69–6.83 ppm (m, 2H, 2 x phenyl-*H*), 7.01–7.08 ppm (m, 1H, phenyl-*H*), 7.25–7.33 ppm (m, 1H, phenyl-*H*).

N-Benzoyl-N'-{ω-[2-(3,5-dicarboethoxy-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]alkyl}thiourea
A solution of 30 mmol of a suitable 3,5-dicarboethoxy-2,6-dimethyl-4-[2-(ω-aminoalkoxy)phenyl]-1,4-dihydropyridine in 150 ml dichloromethane was added dropwise to a solution of an equimolar amount of benzoyl isothiocyanate [22] in 150 ml dichloromethane followed by stirring for 4 h. Then the solvent was evaporated and the residue was purified by column chromatography, using dichloromethane/ethyl acetate 9:1 as eluent.

N-Benzoyl-N'-{5-[2-(3,5-dicarboethoxy-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]pentyl}thiourea
Yield: 51%, mp: 143.2–145.7°C. ^{1}H-NMR ($CDCl_3$): 1.16 ppm (t, J = 7.15 Hz, 6H, 2 x CH_3-CH_2-O), 1.45–1.59 ppm (m, 2H, O-C-C-C-CH_2-C-C-N), 1.77–1.91 ppm (m, 4H, O-C-CH_2-C-CH_2-C-N), 2.27 ppm (s, 6H, 2 x pyridine-CH_3), 3.67–3.76 ppm (m, 2H, O-$(C)_4$-CH_2-N), 3.91 ppm (t, J = 6.53 Hz, 2H, O-CH_2-$(C)_4$-N), 4.00 ppm (q, J = 7.15 Hz, 4H, 2 x CH_3-CH_2-O), 5.18 ppm (s, 1H, pyridine-H_4), 5.99 ppm (s, 1H, pyridine-N*H*), 6.74–6.81 ppm (m, 2H, 2 x phenyl-*H*), 7.01–7.08 ppm (m, 1H, phenyl-*H*), 7.21–7.29 ppm (m, 1H, phenyl-*H*), 7.58–7.73 ppm (m, 3H, 3 x benzoyl-*H*), 7.81–7.92 ppm (m, 2H, 2 x benzoyl-*H*), 9.04 ppm (bs, 1H, -C(S)-N*H*-C(O)-), 10.83 ppm (t, J = 4.9 Hz, 1H, C-C-C-N*H*-C(S)-).

N-Benzoyl-N'-{6-[2-(3,5-dicarboethoxy-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]hexyl}thiourea
Yield: 85%, mp: 154.2–157.2°C. ^{1}H-NMR ($CDCl_3$): 1.16 ppm (t, J = 7.1 Hz, 6H, 2 x CH_3-CH_2-O), 1.44–1.59 ppm (m, 4H, O-C-C-C-CH_2-CH_2-C-C-N), 1.71–1.90 ppm (m, 4H, O-C-CH_2-C-C-CH_2-C-N), 2.28 ppm (s, 6H, 2 x pyridine-CH_3), 3.68–3.77 ppm (m, 2H, O-$(C)_5$-CH_2-N), 3.91 ppm (t, J = 6.6 Hz, 2H, O-CH_2-$(C)_5$-N), 4.02 ppm (q, J = 7.1 Hz, 4H, 2 x CH_3-CH_2-O), 5.18 ppm (s, 1H, pyridine-H_4), 5.83 ppm (bs, 1H, pyridine-N*H*), 6.70–6.83 ppm (m, 2H, 2 x phenyl-*H*), 7.02–7.08 ppm (m, 1H, phenyl-*H*), 7.19–7.25 ppm (m, 1H, phenyl-*H*), 7.49–7.86 ppm (m, 5H, 5 x benzoyl-*H*), 9.00 ppm (bs, 1H, -C(S)-N*H*-C(O)-), 10.78 ppm (t, 1H, C-C-C-N*H*-C(S)-NH-C(O)-).

N-{ω-[2-(3,5-Dicarboethoxy-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]alkyl}thiourea ***13***
5 mmol K_2CO_3 in 20 ml water was added to a solution of 5 mmol of a suitable *N*-benzoyl-*N'*-{ω-[2-(3,5-dicarboethoxy-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]alkyl}thiourea in 60 ml ethanol. The reaction mixture was refluxed for 4 h. Then 50 ml water was added and the ethanol was evaporated. The water layer was extracted 3 times with 50 ml ethyl acetate and the organic layer was dried with $MgSO_4$ and subsequently evaporated.

N-{5-[2-(3,5-Dicarboethoxy-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]pentyl}thiourea ***(VUF 4622)***
Yield: 92%, mp: 65.6–66.0°C, MS (FAB+) 490 [M + H]+, (FAB–) 488 [M – H]–. ^{1}H-NMR ($CDCl_3$): 1.18 ppm (t, J = 7.1 Hz, 6H, 2 x CH_3-CH_2-O), 1.50–1.75 ppm (m, 6H, O-C-$(CH_2)_3$-C-N), 2.24 ppm (s, 6H, 2 x pyridine-CH_3), 3.17 and 3.55 ppm (m, 2H, O-$(C)_4$-CH_2-N), 3.97–4.21 ppm (m, 6H, 2 x CH_3-CH_2-O and O-CH_2-$(C)_4$-N), 5.18 ppm (s, 1H, pyridine-H_4), 6.15 ppm (s, 1H, pyridine-N*H*), 6.52 ppm (bs, 1H, N*H*), 6.71–6.86 ppm (m, 2H, 2 x phenyl-*H*), 7.05–7.30 ppm (m, 3H, 2 x phenyl-*H* and N*H*).

N-{6-[2-(3,5-Dicarboethoxy-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]hexyl}thiourea ***(VUF 4671)***
Yield: 60%, mp: 175.6–175.8°C, MS (FAB+) 504 [M + H]+, (FAB–) 502 [M – H]–. ^{1}H-NMR (DMSO–d_6) 1.07 ppm (t, J = 7.0 Hz, 6H, 2 x CH_3-CH_2-O), 1.39–1.71 ppm (m, 8H, O-C-$(CH_2)_4$-C-N), 2.18 ppm (s, 6H, 2 x pyridine-CH_3), 3.03 and 3.33 ppm (m, 2H, O-$(C)_5$-CH_2-N), 3.86–3.97 ppm (m, 6H, 2 x CH_3-CH_2-O and O-CH_2-$(C)_5$-N), 5.09 ppm (s, 1H, pyridine-H_4), 6.71–7.09 ppm (m, 5H, 4 x phenyl-*H* and N*H*), 7.55 ppm (bs, 1H, N*H*), 8.61 ppm (s, 1H, pyridine-N*H*).

N-{ω-[2-(3,5-Dicarboethoxy-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]alkyl}-S-ethylisothiourea hydrobromide ***14***
A suitable *N*-{ω-[2-(3,5-dicarboethoxy-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]alkyl}thiourea was dissolved in 75 ml absolute ethanol; 3 equivalents ethyl bromide were added and the reaction mixture was stirred for 8 h at 60°C under nitrogen. Then the solvent was evaporated and the residue was washed with 30 ml diethyl ether.

N-{5-[2-(3,5-Dicarboethoxy-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]heptyl}-S-ethylisothiourea hydrobromide ***(VUF 4623)***
The product was crystallized from ethanol. Yield: 66%, mp: 155.2–156.4°C, MS (FAB+) 518 [M + H]+, (FAB–) 596/598 [M + Br]–. ^{1}H-NMR (DMSO–d_6): 1.07 ppm (t, J = 7.0 Hz, 6H, 2 x CH_3-CH_2-O), 1.26 ppm (t, J =7.3 Hz, 3H, CH_3-CH_2-S), 1.37–1.48 ppm (m, 2H, O-C-C-C-CH_2-C-C-N), 1.67–1.80 ppm (m, 4H, O-C-CH_2-C-CH_2-C-N), 2.20 ppm (s, 6H, 2 x pyridine-CH_3), 3.22 ppm (q, J = 7.3 Hz, 2H, CH_3-CH_2-S), 3.32–3.41 ppm (m, 2H, O-$(C)_4$-CH_2-N), 3.84–3.97 ppm (m, 6H, 2 x CH_3-CH_2-O and O-CH_2-$(C)_4$-N), 5.09 ppm (s, 1H, pyridine-H_4), 6.72–6.86 ppm (m, 2H, 2 x phenyl-*H*), 7.02–7.09 ppm (m, 2H, 2 x phenyl-*H*), 8.70 ppm (bs, 1H, pyridine-N*H*), 9.18 ppm (bs, 2H, -C-N-C(SEt)-NH_2+•Br–), 9.57 ppm (m, 1H, -C-N*H*-C(NH)-SEt).

N-{6-[2-(3,5-Dicarboethoxy-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]hexyl}-S-ethylisothiourea hydrobromide ***(VUF 4624)***
The product was crystallized from ethanol. Yield: 69%, mp: 154.0–154.3°C, MS (FAB+) 532 [M + H]+, (FAB–) 610/612 [M + Br]–. ^{1}H-NMR (DMSO–d_6): 1.07 ppm (t, J = 7.1 Hz, 6H, 2 x CH_3-CH_2-O), 1.26 ppm (t, J = 7.3 Hz, 3H, CH_3-CH_2-S), 1.39–1.48 ppm (m, 4H, O-C-C-CH_2-CH_2-C-C-N), 1.55–1.64 ppm (m, 2H, O-$(C)_4$-CH_2-C-N), 1.70–1.82 ppm (m, 2H, O-C-CH_2-$(C)_4$-N), 2.19 ppm (s, 6H, 2 x pyridine-CH_3), 3.22 ppm (q, J = 7.3 Hz, 2H, CH_3-CH_2-S), 3.29–3.37 ppm (m, 2H, O-$(C)_5$-CH_2-N), 3.79–3.86 ppm (m, 2H, O-CH_2-$(C)_5$-N), 3.91 ppm (q, J = 7.1 Hz, 4H, 2 x CH_3-CH_2-O), 5.10 ppm (s, 1H, pyridine-H_4), 6.71–6.82 ppm (m, 2H, 2 x phenyl-*H*), 7.01–7.08 ppm (m, 2H, 2 x phenyl-*H*), 8.68 ppm (bs, 1H, pyridine-N*H*), 9.15 ppm (bs, 2H, -C-N-C(SEt)-NH_2+•Br–), 9.57 ppm (bs, 1H, -C-N*H*-C(NH)-SEt).

^{1}H-NMR (CD_3OD–d_4): 1.14 ppm (t, J = 7.1 Hz, 6H, 2 x CH_3-CH_2-O), 1.36 ppm (t, J = 7.3 Hz, 3H, CH_3-CH_2-S), 1.48–1.55 ppm (m, 4H, O-C-C-C-CH_2-CH_2-C-C-N), 1.70–1.79 ppm (m, 2H, O-$(C)_4$-CH_2-C-N), 1.82–1.91 ppm (m, 2H, O-C-CH_2-$(C)_4$-N), 2.24 ppm (s, 6H, 2 x pyridine-CH_3), 3.19 ppm (q, J = 7.3 Hz, 2H, CH_3-CH_2-S), 3.39 ppm (t, J = 7.1 Hz, 2H, O-$(C)_5$-CH_2-N), 3.90–4.04 ppm (m, 6H, 2 x CH_3-CH_2-O and O-CH_2-$(C)_5$-N), 5.16 ppm (s, 1H, pyridine-H_4), 6.75–6.84 ppm (m, 2H, 2 x phenyl-*H*), 7.02–7.17 ppm (m, 2H, 2 x phenyl-*H*), 8.25 ppm (bs, 1H, pyridine-N*H*).

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References

1 Godfraind T, Miller R, Wibo M (1986) *Pharmacol Rev* 38, 321–416
2 van Zwieten PA (1989) *Am J Cardiol* 64, 117I–121I
3 Opie LH (1990) *Clinical Use of Calcium Channel Antagonist Drugs*. Kluwer Academic Publ, London, 2nd edn
4 Naylor WG (1992) *Biochem Pharmacol* 43, 39–46
5 van Zwieten PA, Pfaffendorf M (1993) *J Hypertens* 11, S3–S11
6 Vater W, Kroneberg G, Hoffmeister F, Kaller H, Meng K, Oberdorf A, Puls W, Schloßmann K, Stoepel K (1972) *Arzneim-Forsch/Drug Res* 22, 1–14
7 Takenaka T, Usuda S, Nomura T, Maeno H, Sado T (1976) *Arzneim-Forsch/Drug Res* 26, 2172–2178
8 Wehinger E (1987) *Structure and Physiology of the Slow Inward Calcium Channel* (Venter JC, Triggle DJ, eds) Alan R Liss Inc, NY, 1–28
9 Triggle DJ, Janis RA (1987) *Structure and Physiology of the Slow Inward Calcium Channel* (Venter JC, Triggle DJ, eds) Alan R Liss Inc, NY, 29–50
10 Bossert F, Vater W (1989) *Med Res Rev* 9, 291–324
11 Goldmann S, Stoltefuß J (1991) *Angew Chem* 103, 1587–1605
12 Loev B, Goodman MM, Snader KM, Tedeschi R, Macko E (1978) *J Med Chem* 17, 956–965
13 Rodenkirchen R, Bayer R, Steiner R, Bossert F, Meyer H, Möller E (1979) *Arch Pharmacol* 310, 69–78
14 Gaviraghi G (1989) *Trends in Medicinal Chemistry 1988* (Van der Goot H, Domány G, Pallos L, Timmerman H, eds) Elsevier Sci Publ BV, Amsterdam, 675–690
15 Feriani A, Gaviraghi G (1991) *QSAR: Rational Approaches to the Design of Bioactive Compounds* (Silipo C, Vittoria A, eds) Elsevier Sci Publ BV, Amsterdam, 427–430
16 Arrowsmith JE, Campbell SF, Cross PE, Stubbs JK, Burges RA, Gardiner DG, Blackburn KJ (1986) *J Med Chem* 29, 1696–1702
17 Christiaans JAM, Windhorst AD, Groenenberg PM, Van der Goot H, Timmerman H (1993) *Eur J Med Chem* 28, 859–867
18 Gandolfi AC, Frigerio M, Spinelli S, Tofanetti O, Tognella S (1987) PCT WO 87/00836
19 Chester DW, Herbette LG, Mason RP, Joslyn AF, Triggle DJ, Koppel DE (1985) *Biophys J* 52, 1021–1030
20 Mason RP, Chester DW, Gonye GE, Herbette LG (1988) *Biophys J* 53, 348a
21 Soine TO, Buchdahl MR (1963) *Org Synth* 4, 120–124
22 Bögemann M, Petersen S, Schultz OE, Söll H (1955) *Houben Weyl Methods Org Chem* 9, 879
23 Boer R, Grassegger A, Schudt C, Glossmann H (1989) *Eur J Pharmacol* 172, 131–145
24 Munson PJ, Rodbard D (1980) *Anal Biochem* 107, 220–239
25 Alker D, Burges A, Campbell SF, Carter AJ, Cross PE, Gardiner DG, Humphrey MJ, Stopher DA (1992) *J Chem Soc Perkin Trans* 2, 1137–1140
26 Spampinato S, Bachetti T, Carboni L, Ratti E, Van Amsterdam FTM, Ferri S (1993) *Eur J Pharmacol Mol Pharmacol* 244, 139–144
27 Kass RS, Arena JP (1989) *J Gen Physiol* 93, 1109–1127
28 Valdivia H, Coronado R (1988) *Biophys J* 53, 555a
29 Kwon YW, Zhong Q, Wei XY, Zheng W, Triggle DJ (1990) *Arch Pharmacol* 341, 128–136