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Synthesis and *in vitro* pharmacology of new 1,4-dihydropyridines. 1. 2-(ω-Aminoalkylthiomethyl)-1,4-dihydropyridines as potent calcium channel blockers

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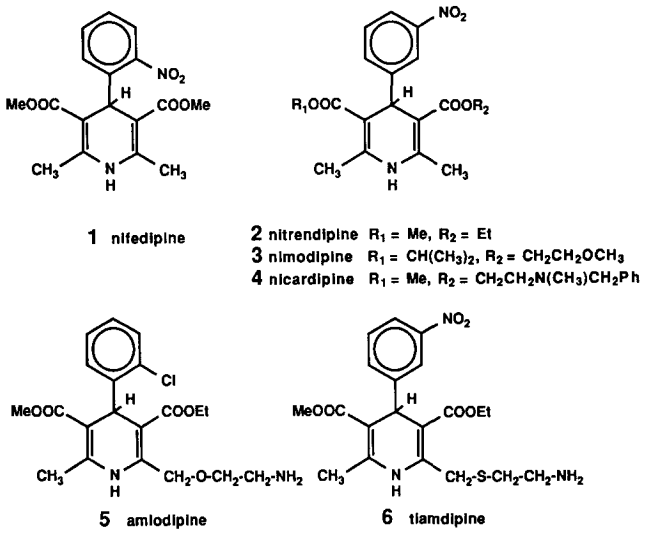
Summary — The synthesis and *in vitro* calcium channel blocking activities and binding of 2-(ω-aminoalkylthiomethyl)-4-(substituted)phenyl-1,4-dihydropyridines, by determination of the displacement of [^{3}H]nitrendipine from the calcium channel binding sites on rat cortex have been discussed. It has been shown that increasing the alkyl chain length on the 2-position of the 1,4-dihydropyridine ring from ethyl to pentyl does not affect the calcium channel blocking activity of 3-nitrophenyl substituted dihydropyridines, measured on K^+-depolarisation induced contractile responses in rat aorta strips. It did not seem to be important whether the 1,4-dihydropyridines bore 2 identical or different ester moieties on the 3- and 5-position of the 1,4-dihydropyridine ring.

1,4-dihydropyridines / tiamdipine / calcium channel blocker

Introduction

The 1,4-dihydropyridine (DHP) calcium channel blockers are important drugs in antianginal and antihypertensive therapy [1–5] because of their vasodilatory properties. Nifedipine **1** [6], nitrendipine **2** [7], nimodipine **3** [8] and nicardipine **4** [9] represent the classical 1,4-dihydropyridines and exert their pharmacologic activity by interaction with specific receptors present on L-type voltage-dependent calcium channels. 1,4-DHPs block calcium entry through slow calcium channels resulting in vasodilation and hence reduce vascular resistance. Coronary dilatation increases the oxygen and nutrient supply to the heart. Peripheral vasodilatation lowers the oxygen demand of the heart through a reduction in cardiac load and also accounts for the antihypertensive properties of these drugs.

Systematic modifications of the 2-position of the DHP ring have been carried out to increase the rather disadvantageous short duration of action and to improve the bioavailability of the classical DHPs, resulting in amlodipine **5** [10] and its derivatives and



their thio-bioisosters such as tiamdipine **6** [11, 12]. Amlodipine, presently under clinical investigation for treatment of angina pectoris and hypertension, was the first compound demonstrating that extended 2-substituents bearing a basic functionality were well tolerated at the DHP-receptor. Amlodipine has an affinity for the 1,4-DHP binding site comparable to that of

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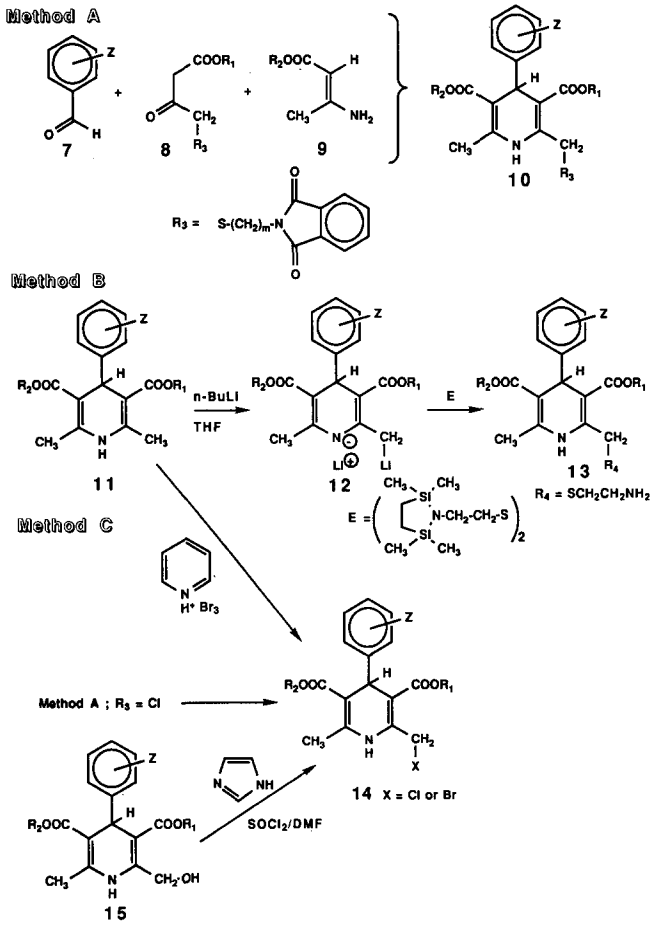
nifedipine, but has a longer duration of action and slower onset and offset kinetics. These kinetic effects of amlodipine/tiamdipine derivatives have to be ascribed to additional interaction with the binding site of the L-type calcium channel due to an ionic interaction between the protonated amino function and the negatively charged phosphate group of a phospholipid [13, 14].

Our objective was to determine whether DHPs with identical ester groups are as potent as the corresponding DHPs with non-identical ester groups. Furthermore, the influence of variation of the ω-aminoalkyl chain length in tiamdipine analogues on the calcium channel blocking activity was examined.

Chemistry

In general, the synthesis of 2-substituted dihydropyridines shown in table I can be accomplished by several modifications of the classical 3-component Hantzsch reaction shown in scheme 1.



Scheme 1.

Thus, condensation of a benzaldehyde **7** with an alkyl 3-aminocrotonate **9** and a substituted β-keto ester **8** can afford the title compounds (*Method A*). The substituted keto esters are obtained by reaction of a suitable sodium thiolate with the enolate of alkyl 4-chloroacetoacetate. When alkyl 4-chloroacetoacetate, condensed with cysteamine or longer ω-mercaptoalkylamines are used in the Hantzsch condensation reaction, the amine function (*Method A*; R_3 contains an amine function) has to be prevented from participation in the condensation reaction with a benzaldehyde. For this reason the amine function must be protected by first transforming it into a phthalimide group.

According to Pointdexter *et al* [15] tiamdipine analogues can also be obtained *via* a metalation methodology (*Method B*). Metalation of DHPs **11** with 2 equivalents of *n*-butyllithium afforded dilithio species **12**. The cysteamine precursor *E* was used as an electrophile to yield the tiamdipine analogues **13** [16].

The third way to accomplish the synthesis of the tiamdipine analogues proceeds *via* the reaction of 2-halomethyl-1,4-dihydropyridines with sodium thiolate derivatives. Several methods have been described to afford the 2-halomethyl-1,4-dihydropyridines **14** (*Method C*). Young *et al* [17] reported that reaction of 1,4-dihydropyridines **11** with pyridinium bromide perbromide gives unstable brominated species. Alker and Swanson [18] used this method to react these 2-bromomethyl-1,4-dihydropyridines *in situ* with a range of nucleophiles. As expected, reaction of DHPs **11** with different 3- and 5-esters with pyridinium bromide perbromide followed by *in situ* reaction with nucleophiles give mixtures of the 2 possible isomeric 2- and 6-bromomethyl-1,4-dihydropyridines. For this reason Alker and Denton [19] developed an alternative synthetic route in which 2-hydroxymethyl-1,4-dihydropyridines **15** are chlorinated by reaction with thionyl chloride and imidazole in THF. Cho *et al* [20] performed the chlorination of 2-hydroxymethyl-1,4-dihydropyridines by refluxing in phosphorus oxychloride. A major disadvantage of this method is the long and complex reaction route to obtain the 2-hydroxymethyl-1,4-dihydropyridines.

Cupka *et al* [21] reported the synthesis of 2-chloromethyl-1,4-dihydropyridines by reaction of equimolar amounts of alkyl 4-chloroacetoacetate, alkyl 3-aminocrotonate and a benzaldehyde in refluxing methanol. On the other hand, several authors describe reactions in which 2-halomethyl-1,4-dihydropyridines **17** are refluxed in alcohol to afford 4-aryl-2-methyl-5-oxo-1,4,5,7-tetrahydrofuro[3,4b]pyridine-3-carboxylates **18** (scheme 2) [17, 22, 23].

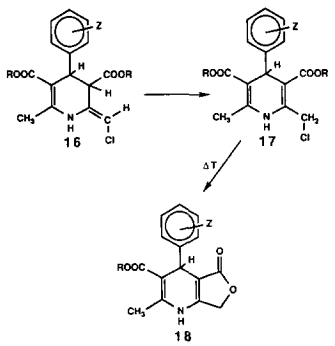
Our choice of synthesis was that of *Method A* based on the easy preparation of 2-chloromethyl-1,4-dihydropyridines using ethyl 4-chloroacetoacetate accord-

Table I. Chemical shifts of characteristic protons [^{1}H] and carbon atoms [^{13}C] determined with HH-cosy and CH-cosy NMR experiments in DMSO–d_6 of DHPs according to scheme 2.

		Z = 2,3-diCl; R = Et			*Z = 2-NO_2; R = Et*		
		16a	***17a***	***18a***	***16b***	***17b***	***18b***
^{1}H-NMR	Pyridine-CH_2-Cl	–	4.76 and 4.85 (AB, J_{ab} = 13.3 Hz, 2H)	–	–	4.67 and 4.75 (AB, J_{ab} = 13.1 Hz, 2H)	–
	CH_2-lactone	–	–	4.83 (s, 2H)	–	–	4.84 (s, 2H)
	Pyridine=CH-Cl	5.45 (s, 1H)	–		5.58 (s, 1H)	–	–
	Pyridine-H_3	3.45 (bs, 1H)	–	–	3.60 (bs, 1H)	–	–
	Pyridine-H_4	4.97 (bs, 1H)	5.54 (s, 1H)	5.3 (s, 1H)	4.79 (bs, 1H)	5.68 (s, 1H)	5.63 (s, 1H)
	CH_3-CH_2-O	1.01 and 1.15 (2x t, J = 7.6 Hz, 6H)	1.21 (m, 6H)	0.95 (t, J = 7.1 Hz, 3H)	0.93 and 1.15 (2x t, J = 7.1 Hz, 6H)	1.09 (m, 6H)	0.89 (t, J = 7.2 Hz, 3H)
	CH_3-CH_2-O	3.89 and 4.12 (2x q, J = 7.6 Hz, 4H)	4.12 (m, 4H)	3.85 (q, J = 7.1 Hz, 2H)	3.81 and 4.13 (2x q, J = 7.1 Hz, 4H)	3.96 (m, 4H)	3.86 (q, J = 7.2 Hz, 2H)
^{13}C-NMR							
	Pyridine-CH_2-Cl	–	39.46	–	–	39.5	–
	CH_2-lactone	–	–	65.0	–	–	65.0
	Pyridine=CH-Cl	97.4	–	–	98.2	–	–
	Pyridine-C_3/C_5	45.9 and 95.6	101.0 and 103.6	100.0 and 102.3	46.7 and 96.5	101.0 and 103.6	99.9 and 102.2
	Pyridine-C_4	36.4	38.40	35.2	35.5	33.6	31.6

ing to Archibald *et al* [24]. However, in the work-up procedures before acid treatment we discovered a product whose structure was identified as a 2-chloromethylene-1,2,3,4-tetrahydropyridine **16**. These DHP exoisomers were first described by Frigerio *et al* [25]. The ^{1}H-NMR and ^{13}C-NMR spectra of the exoisomers **16** clearly differed from the 1,4-DHPs **17**. In table I the ^{1}H-NMR and ^{13}C-NMR data for the 4-(2,3-dichlorophenyl) and 4-(2-nitrophenyl)-1,4-DHPs are shown.

Compounds **16a** (Z = 2,3-diCl; R = Et) and **16b** (Z = 2-NO_2; R = Et) reveal a singlet in ^{1}H-NMR (3.45 and 3.60 ppm respectively) with an integral of 1 proton. This signal is coupled to an sp^3 carbon atom signal in ^{13}C-NMR (at 45.9 and 46.7 ppm respectively) indicating a proton connected to carbon C_3. Both structures also have singlet signals in ^{1}H-NMR (5.45 and 5.58 ppm respectively) each having an integral of 1 proton. These signals are each coupled to an sp^2 carbon atom signal in ^{13}C-NMR (at 97.4 and 98.2 ppm respectively), indicating that these signals originate from the exocyclic carbon atom and the proton in the 2-chloromethylene group. The NMR spectra of the corresponding compounds **17a** and **17b** lack the signals from the proton and the sp^3 carbon atom at position 3. Both structures **17** give signals of an AB system to be ascribed to the protons on an sp^3 carbon atom of the 2-chloromethyl group. The NMR of the lactone **18** lacks signals from one ethyl ester group; instead methylene signals in the ^{1}H-NMR and ^{13}C-NMR from the lactone ring appear.



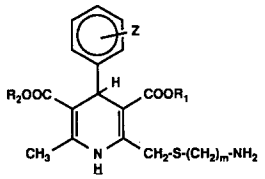
Scheme 2.

The exoisomers **16** are quite stable in pyridine or methanol solutions while they isomerize to the endo-isomers **17** in acidic alcohol.

All attempts to synthesize the 2-chloromethyl-4-(2-chlorophenyl)-1,4-DHP **17** (with Z = 2-Cl; R = Et) failed, and only the corresponding lactone **18** could be isolated (scheme 2).

The synthesis of *N*-(ω-mercaptoalkyl)phthalimides was performed according to a modification of the method of Gabriel *et al* [26]. *In situ* reaction of the thiolate anions of *N*-(ω-mercaptoalkyl)phthalimides with the 2-chloromethyl-1,4-dihydropyridines **19** afforded the corresponding thioethers **20**. Subsequent hydrazinolysis of the phthalimides **20** gave the tiamdipine analogues **21** shown in table II (scheme 3).

Table II. Calcium-blocking activities and radioligand binding affinities of tiamdipine analogues.



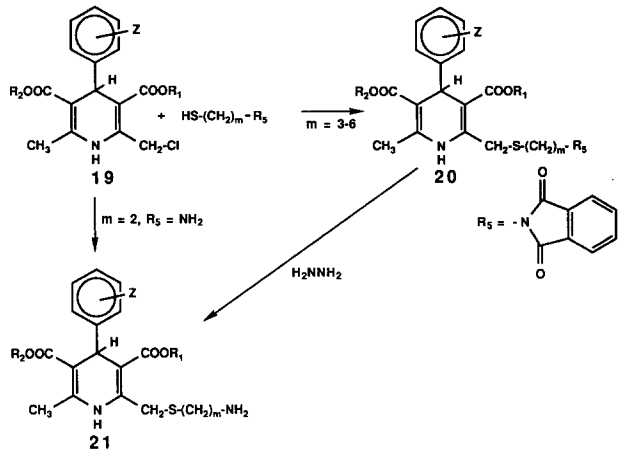
Compound	*Z*	*m*	R_1/R_2	pIC_{50}[1]	*Rat tissue*	pK_d[2]
VUF 9056	H	2	Et/Et	7.27 ± 0.08	Aorta	8.57 ± 0.10
VUF 4574	2-NO_2	2	Et/Et	8.02 ± 0.07	Aorta	8.58 ± 0.12
VUF 9158	2,3-diCl	2	Et/Et	7.47 ± 0.03	Aorta	8.37 ± 0.10
VUF 4621	2,3-diCl	3	Et/Et	6.77 ± 0.06	Aorta	8.00 ± 0.12
VUF 9055	3-NO_2	2	Et/Et	7.96 ± 0.07	Aorta	8.61 ± 0.06
VUF 9108	3-NO_2	3	Et/Et	7.82 ± 0.04	Aorta	8.43 ± 0.05
VUF 9159	3-NO_2	5	Et/Et	7.96 ± 0.12	Aorta	8.55 ± 0.08
VUF 9109[3]	3-NO_2	2	Et/Et	6.64 ± 0.11	Aorta	Not tested
–	2-Cl	2	Me/Me	7.8[a]	Aorta	–
–	2-Cl	2	Et/Me	7.6[a]	Aorta	–
1	Nifedipine			8.77 ± 0.08 8.4[a] / 8.9[b]	Aorta	8.70 ± 0.14 8.44[c]
5	Amlodipine			8.1[a]	Aorta	–
–	2-NO_2	2	Et/Me	7.08[d]	Tail artery	7.25[d1]/7.74[d2]/8.12[d3]
–	H	2	Et/Me	7.45[d]	Tail artery	7.44[d1]/7.68[d2]/8.01[d3]
6	3-NO_2	2	Et/Me	7.18[d]	Tail artery	7.70[d1]/8.03[d2]/8.44[d3]

[1]All values are means ± SD for 3 independent observations; [2]all values are means ± SD for 6 to 9 independent observations. All radioligand binding affinities (pK_d) were determined on isolated rat cortex membranes; [3]amine protected as a phthalimide; [a]Alker *et al* [12]; [b]Godfraind [33]; [c]Wibo *et al* [34]; [d]Kwon *et al* [29]; pIC_{50} data calculated from IC_{50} values; [d1]rat brain; [d2]rat heart; [d3]guinea-pig ileum; data expressed as pK_i.

Pharmacology

In vitro calcium channel blocking activities

Male Wistar rats (200–250 g, Harlan CPB, Zeist, The Netherlands) were killed by decapitation. The thorax was opened and thymus, lungs and oesophagus were removed. Next the heart was taken at the apex and removed together with the thoracial aorta, by gently cutting the latter from the spine up to the diaphragm and placed in Krebs Ringer solution (composition in mM: NaCl 118.5, KCl 4.74, $MgSO_4$ 1.18, KH_2PO_4 1.18, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 10) at room temperature. The aorta was excised free from heart and connective tissue. After removal of the aortic arch, the aorta was cut spirally by gently unwinding it from a horizontally placed metal bar. Thus 3 to 4 strips per animal were obtained. Strips of aortic tissue (length: 1–1.5 cm; width: 1–2 mm) were placed (using silk thread, Perma-Hand 0.7 metric, Ethicon, Nordestedt, Germany) in an organ bath (20 ml; Krebs Ringer medium bubbled with O_2/CO_2 (95:5%) at 37°C. A resting tension of 0.5 g was applied and the aortic strips were equilibrated for 100 min and placed in fresh buffer solution every 20 min. Next the strips were isotonically contracted by a modificated buffer solution (composition in mM: NaCl 73.2, KCl 50,



Scheme 3.

$MgSO_4$ 1.18, KH_2PO_4 1.18, $CaCl_2$ 1.25, $NaHCO_3$ 25, glucose 10). Concentration–response curves of tiamdipine analogues were provoked to determine pIC_{50} values. Measurements of inhibition were started 15 min after depolarisation. Additional increasing doses to generate dose–response curves were given every 45 min.

Dihydropyridine receptor binding assay

The cortices from the rats were isolated and homogenised in ice-cold Tris–HCl buffer (50 mM pH 7.4 at 0°C) in a ratio of 1:3 (v/v). The homogenate was centrifuged (48 000 *g* for 10 min) and this was repeated 3 times with resuspension of the pellet in the buffer and stored in liquid nitrogen until required.

Binding experiments were performed under sodium light because of the photolability of [^{3}H]nitrendipine.

All binding assays were carried out according to Boer *et al* [27] by adding in each incubation tube 200 µl Tris buffer (50 mM, pH 7.4, 37°C); 100 µl rat brain membrane suspension (170 µg protein/ml); incubated for 60 min at 37°C with 100 µl 1 nM [^{3}H]nitrendipine solution, and 100 µl of the drug concentration for a final volume of 0.5 ml. The final DMSO concentration never exceeded 1% (v/v) which did not affect binding. The incubation was stopped with 4 ml ice-cold Tris–HCl buffer filtered under reduced pressure onto Whatman GF/C filters and washed twice with 4 ml ice-cold buffer using a Brandel filtration apparatus. The radioactivity was counted after addition of 5 ml scintillation liquid (Optiphase Hi Safe-3) to the filter, by liquid scintillation spectrometry (Packard 1900 CA Tri-card liquid scintillation counter) at an efficiency of ≈ 55%.

Saturation analysis at equilibrium was performed by incubating increasing concentrations of [^{3}H]nitrendipine up to 2 nM with 50 µl purified protein and Tris–HCl buffer (pH 7.4, 37°C) for a total volume of 0.25 ml.

Non-specific binding was determined in the presence of 1 µM nifedipine. Specific binding was determined by subtracting the non-specific binding from the total binding. Equilibrium dissociation constant (K_d) 0.75 nM of the labelled compound and the maximal binding (B_{max}) of 270 fmol/mg protein were determined with the non-linear fitting program, LIGAND 4.1 [28].

Results and discussion

In vitro vascular calcium antagonistic activity (expressed as pIC_{50}) was assessed as the concentration of the compound required to inhibit the K^+-depolarisation induced (50 mmol/l) contractile responses in rat aorta strips by 50%. All compounds, tested as racemic mixtures, caused complete inhibition of contractile responses as verified by addition of 1 mM papaverine. As reported earlier, the development of antagonism of amlodipine and tiamdipine analogues occurred slowly and did not reach equilibrium after 2 h [29]. Because additional increasing doses to generate dose–response curves were given every 45 min, no complete equilibrium was reached and this could have led to underestimated calcium inhibitory activities.

The influence of substituents in the 4-phenyl ring of the compounds with a 2-aminoethylthiomethyl side-chain on the 2-position of the 1,4-dihydropyridine on the calcium channel blocking activity is rather small (table II). Compounds with a nitro substituent in the 4-phenyl ring and with m = 2 (VUF 4574 and VUF 9055) are almost as potent as amlodipine **5** and slightly more active than those compounds possessing 2,3-dichloro substituents (VUF 9158) or no substituent at all (VUF 9056).

The DHP with a 3-nitro substituent in the 4-phenyl ring was chosen to explore the influence of increasing ω-aminoalkylthiomethyl side-chain length. There is no difference in calcium channel blocking activity when the alkyl chain length is increased from an ethyl chain to a pentyl chain.

The phenomenon of unaltered potency with changing alkyl chain length is not shared for compounds VUF 9158 and VUF 4621. Increasing the alkyl chain length from ethyl to propyl results in a decrease in calcium channel blocking activity. VUF 9109, in which the amine function is replaced by a phthalimide group, is less active than the corresponding compound VUF 9055 with a primary amine group.

Alker and Denton [19] compared 2 4-(2-chlorophenyl)-1,4-dihydropyridines bearing identical (Me/Me) or different ester substitution (Me/Et). The calcium

Table III. *In vitro* inhibitory effect of racemates and their enantiomers on K+-induced contractions on different tissues expressed as pIC_{50}values.

Compound	*Tissue*	*Racemate*	*(–)-Enantiomer*	*(+)-Enantiomer*	*Ref*
Nimodipine	Rabbit aorta	8.24	8.52	7.80	[30]
Tiamdipine	Rat tail artery	7.19	7.51	5.35	[29]
Amlodipine	Rat aorta	8.10	8.70	5.80	[31]

All data were taken from the literature and calculated from IC_{50} values.

inhibitory potencies of both compounds against K+-depolarisation induced responses in rat aorta strips were almost equal. The DHPs with non-identical esters were tested as racemic mixtures.

Kwon *et al* [29] investigated the influence of substitution on the phenyl group in 2-(2-aminoethylthiomethyl)-3-carboethoxy-5-carbomethoxy-4-phenyl-1,4-dihydropyridines on their calcium inhibitory potencies against K+-depolarisation induced responses in rat tail artery. In their series, the DHP with Z = H (table II) seemed to be the most potent calcium channel inhibitor compared to the 2- or 3-nitrophenyl substituted DHP. In the case of 3-nitrophenyl substituted DHP, the individual enantiomers were examined.

In table III the inhibitory potencies of 3 different racemic DHPs and their enantiomers are given. Each racemate and its enantiomeric pair were tested on the same tissue. Although the pairs of DHPs were tested on different tissues, all (–)-enantiomers are 100 to 1000-fold more active than their (+)-enantiomers.

Table IV show the organ dependency of nifedipine for its calcium channel blocking activity. On all 3 different smooth muscle preparations the calcium inhibitory potency of nifedipine varies only within a small range (pIC_{50} = 8.1 – 8.8). Therefore, the calcium inhibitory potencies of the tiamdipine analogues tested on rat aorta strips and rat tail artery might be regarded as equipotent. In table II the tiamdipine analogues with identical esters appeared to be as potent as the tiamdipine analogues with different esters, although it should be borne in mind that they were tested on different smooth muscle preparations.

Table IV. *In vitro* calcium inhibitory activities of nifedipine against K+-depolarisation induced contractions on different tissues.

Tissue	*pIC_{50}*	*Ref*
Rabbit aorta	8.09 ± 0.12 (95% CL)	[32]
Rat aorta	8.77 ± 0.08	
	8.40 – 8.88	[12; 33]
Rat tail artery	8.17 ± 0.17 (95% CL)	[29]

95% confidence limit; number of observations, 4–6; all data were taken from literature and calculated from IC_{50}values.

Radioligand binding affinities of the tiamdipine analogues (pK_d), determined by displacement of [3H]nitrendipine from rat cortex membranes, exhibit similar binding affinities and are almost equal to that of nifedipine **1** (table II). The differences between the pIC_{50} values (determined on rat aorta) and pK_d values (determined on rat cortex membranes) might be caused by the possible underestimated calcium channel blocking activities or might be explained by organ specificity. Thus, Kwon *et al* [29] determined the affinities on rat brain, rat heart and guinea-pig ileum membranes and found an increase in their pK_i values.

Additional interaction of the protonated aminoalkyl side chain of amlodipine and tiamdipine analogues with the 1,4-DHP binding site might lead to a conformation in which the 4-phenyl ring is displaced from the binding site, seen for nifedipine and related 1,4-DHPs. This conformation diminishes the contribution of 4-phenyl ring substituents in the mode of interaction with the 1,4-dihydropyridine binding site. This explains why VUF 9109, without a primary aminoalkyl group, does bind to a lesser extent than the DHPs with a free amine group which under physiological conditions can be protonated.

Conclusions

All tiamdipine analogues discussed are potent inhibitors of K+-depolarisation induced contractions in isolated rat aorta. This activity is relatively independent of the nature of the substituent in the 4-phenyl ring. It can be concluded that identical or non-identical ester substitution does not play a major role for potency. Furthermore, concerning the 4-(3-nitrophenyl)-1,4-dihydropyridines, calcium channel blocking activities are sustained with increasing alkyl chain length (*m* = 2–5; table II).

The rather small number of compounds and especially the narrow activity range do not permit quantification of the structure–activity relationships of the presented 1,4-dihydropyridines.

Experimental protocols

Melting points were determined on a Mettler FP 52 with a 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 ionisation (matrix: glycerol + ammonium acetate, thioglycerol or 3-nitrobenzylalcohol, Ion Tech saddlefield gun, 8 keV Xenon with xenon ion current 0.2 mA). All compounds gave the expected $(M+H)^+$ and to a lesser extent $(M-H)^-$ peaks (negative ions). Furthermore, the purity of the compounds was assessed by thin-layer chromatography (Merck silica gel 60, F254 0.25 mm).

General procedure

2-Chloromethyl-3,5-dicarboethoxy-6-methyl-4-(substituted phenyl)-1,4-dihydropyridine

0.1 mol 3-nitrobenzaldehyde, 0.1 mol ethyl 4-chloroacetoacetate, 6.6 mmol benzylamine and 6.6 mmol acetic acid were stirred for 24 h in 2-propanol at room temperature. Then 0.1 mol ethyl 3-aminocrotonate was added and the reaction mixture was stirred for another 24 h at room temperature. Then 5 ml concentrated hydrochloric acid was added and stirring was continued for 2 h. The precipitate was filtered off and the filtrate evaporated. The residue was dissolved in ethyl acetate and diethyl ether was added until no further precipitation occurred. The solid material was filtered off, the filtrate was evaporated and the residue crystallised from methanol.

2-Chloromethyl-3,5-dicarboethoxy-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine

Yield: 58%, mp: 120.2–121.1°C. ^{1}H-NMR (DMSO–d_6): 1.10 ppm (t, J = 6.6 Hz, 6H, 2x CH_3-CH_2-O), 2.40 ppm (s, 3H, pyridine–CH_3), 4.12 ppm (m, 4H, 2x CH_3-CH_2-O), 4.90 ppm (AB, J_{AB} = 13.2 Hz, 2H, pyridine–CH_2-Cl), 5.14 ppm (s, 1H, pyridine–H_4), 6.68 ppm (s, 1H, pyridine–NH), 7.63 ppm (t, J = 7.3 Hz, 1H, phenyl–H_5), 7.74 ppm (d, J = 7.3 Hz, 1H, phenyl–H_6), 8.05 ppm (d, J = 7.3 Hz, 1H, phenyl–H_4), 8.14 ppm (s, J = 7.3 Hz, 1H, phenyl–H_2).

^{13}C-NMR (DMSO–d_6): 13.58 and 13.76 ppm (2x CH_3-CH_2-O), 17.99 ppm (pyridine–CH_3), 38.96 (pyridine–C_4), 39.41 ppm (pyridine–CH_2-Cl), 59.15 and 59.70 ppm (2x CH_3-CH_2-O), 100.55 and 102.80 ppm (pyridine–C_3 and C_5), 121.12 and 121.75 and 129.42 and 133.97 ppm (phenyl–C_2, C_4, C_5 and C_6), 144.35 and 146.22 and 147.26 and 149.12 ppm (phenyl–C_1 and C_3 and pyridine–C_2 and C_6), 165.12 and 165.99 ppm (2x carbonyl-C).

2-Chloromethyl-3,5-dicarboethoxy-6-methyl-4-(phenyl)-1,4-dihydropyridine

Yield: 48%, mp: 111.7–112.3°C. ^{1}H-NMR (DMSO–d_6): 1.14 ppm (m, 6H, 2x CH_3-CH_2-O), 2.31 ppm (s, 3H, pyridine-CH_3), 3.95–4.11 ppm (m, 4H, 2x CH_3-CH_2-O), 4.72 and 4.82 ppm (AB, J_{AB} = 10.7 Hz, 2H, pyridine–CH_2-Cl), 4.95 ppm (s, 1H, pyridine–H_4), 7.11–7.22 ppm (m, 5H, 5x phenyl-H), 9.25 ppm (s, 1H, pyridine–NH).

^{13}C-NMR (DMSO–d_6): 13.68 and 13.86 ppm (2x CH_3-CH_2-O), 17.91 ppm (pyridine–CH_3), 38.67 (pyridine–C_4), 39.61 ppm (pyridine–CH_2-Cl), 58.91 and 59.47 ppm (2x CH_3-CH_2-O), 101.30 and 103.84 ppm (pyridine–C_3 and C_5), 125.95 ppm (phenyl–C_4), 127.15 and 127.72 ppm (phenyl–C_2, C_3, C_5 and C_6), 143.25 and 145.22 and 147.07 ppm (phenyl–C_1 and pyridine–C_2 and C_6), 165.61 and 166.43 ppm (2x carbonyl-C).

2-Chloromethyl-3,5-dicarboethoxy-6-methyl-4-(2-nitrophenyl)-1,4-dihydropyridine

Yield: 60%, mp: 99.8–100.4°C. ^{1}H-NMR (DMSO–d_6): 1.07–1.11 ppm (m, 6H, 2x CH_3-CH_2-O), 2.27 ppm (s, 3H, pyridine–CH_3), 3.80–4.15 ppm (m, 4H, 2x CH_3-CH_2-O), 4.67 and 4.75 ppm (AB, J_{AB} = 13.1 Hz, 2H, pyridine–CH_2-Cl), 5.67 ppm (s, 1H, pyridine–H_4), 7.32–7.83 ppm (m, 4H, 4x phenyl-H), 9.38 ppm (s, 1H, pyridine–NH).

^{13}C-NMR (DMSO–d_6): 13.51 and 13.67 ppm (2x CH_3-CH_2-O), 18.02 ppm (pyridine–CH_3), 33.62 ppm (pyridine–C_4), 39.50 ppm (pyridine–CH_2-Cl), 59.05 and 59.65 ppm (2x CH_3-CH_2-O), 101.02 and 103.58 ppm (pyridine–C_3 and C_5), 123.54 and 127.38 and 130.49 and 133.13 ppm (phenyl–C_3, C_4, C_5 and C_6), 141.29 and 143.94 ppm (phenyl–C_1 and C_2), 146.80 and 146.82 ppm (pyridine–C_2 and C_6), 156.32 and 166.04 ppm (2x carbonyl-C).

2-Chloromethyl-3,5-dicarboethoxy-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine

The crude reaction product was purified by flash chromatography (eluents: petroleum ether 60–80°C/ethyl acetate, 7:3) on silica gel (JT Baker 70242). Yield: 52%, mp: 135.4–136.6°C. Mass spectrum (Electron Impact (70eV); $C_{19}H_{20}{}^{35}Cl_2{}^{37}ClNO_4$, mass calculated: 433.043, mass found: 433.044 ± 0.003).

^{1}H-NMR (DMSO–d_6): 1.21 ppm (m, 6H, 2x CH_3-CH_2-O), 2.33 ppm (s, 3H, pyridine–CH_3), 4.06–4.18 ppm (m, 4H, 2x CH_3-CH_2-O), 4.76 and 4.85 ppm (AB, J_{AB} = 13.3 Hz, 2H, pyridine–CH_2-Cl), 5.54 ppm (s, 1 H, pyridine–H_4), 6.95 ppm (br s, 1H, pyridine–NH), 7.05–7.38 ppm (m, 3H, 3x phenyl-H).

^{13}C-NMR (DMSO–d_6): 13.74 and 13.84 ppm (2x CH_3-CH_2-O), 18.58 ppm (pyridine–CH_3), 38.40 ppm (pyridine–C_4), 39.46 ppm (pyridine–CH_2-Cl), 58.43 and 60.66 ppm (2x CH_3-CH_2-O), 100.98 and 103.56 ppm (pyridine–C_3 and C_5), 126.83 and 127.70 and 128.66 ppm (phenyl–C_4, C_5 and C_6), 131.29 and 131.60 ppm (phenyl–C_2 and C_3), 145.63 and 147.88 and 150.35 ppm (phenyl–C_1 and pyridine–C_2 and C_6), 150.35 (phenyl–C_1), 169.17 ppm (2x carbonyl ester-C).

Method A. 2-(2-Aminoethylthio)methyl-3,5-dicarboethoxy-6-methyl-4-(substitutedphenyl)-1,4-dihydropyridine fumarate

4 ml 5 M NaOH solution in water was added to 10 mmol cysteamine•HCl in ethanol. To this solution, 10 mmol 2-chloromethyl-3,5-dicarboethoxy-6-methyl-4-(substituted phenyl)-1,4-dihydropyridine in 10 ml ethanol/dimethoxyethane (5:1) was added dropwise at –15°C. After stirring for 15 min at –15°C the reaction mixture was slowly heated to room temperature. The solution was brought to pH = 4.5 with acetic acid. After evaporation of the solvent, the reaction mixture was dissolved in water and washed with diethyl ether (3x 30 ml). The water layer was made basic with a sodium bicarbonate solution and washed 5 times with 50 ml ethyl acetate/diethyl ether (1:1). The combined organic layers were dried with $MgSO_4$ and the solvent evaporated. The free base was dissolved in ethyl acetate at 40°C. Then fumaric acid in methanol 60°C was added. The product was obtained after cooling.

2-(2-Aminoethylthio)methyl-3,5-dicarboethoxy-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine fumarate ***VUF 9055***

Yield: 24%, mp: 151.4–152.8°C. Mass spectrum (matrix = glycerol + ammonium acetate) (FAB^+) 450 $[M+H]^+$, (FAB^-) 448 $[M-H]^-$.

^{1}H-NMR (DMSO–d_6):1.02-1.23 ppm (m, 6H, 2x CH_3-CH_2-O), 2.23 ppm (s, 3H, pyridine–CH_3), 2.82 ppm (br s, 2H, CH_2), 3.00 ppm (br s, 2H, CH_2), 3.73–4.12 ppm (m, 6H,

pyridine–CH_2-S and 2x CH_3-CH_2-O), 4.98 ppm (s, 1H, pyridine–H_4), 6.47 ppm (s, 2H, fumaric acid), 7.47–7.71 ppm (m, 2H, 2x phenyl-*H*), 7.93–8.07 ppm (m, 2H, 2x phenyl–*H*), 7.47–8.67 (br s, 2H, NH_2), 9.48 ppm (br s, 0.8 H, pyridine–N*H*).

^{13}C-NMR (DMSO–d_6): 13.65 and 13.80 ppm (2x CH_3-CH_2-O), 17.94 ppm (pyridine–CH_3), 28.46 and 29.59 ppm (S-CH_2-CH_2-N and S-CH_2-CH_2-N), 38.49 ppm (pyridine–CH_2-S), 38.96 ppm (pyridine–C_4), 59.08 and 59.39 ppm (2x CH_3-CH_2-O), 100.77 and 101.11 ppm (pyridine–C_3 and C_5), 120.98 and 121.72 and 129.37 and 133.98 ppm (phenyl–C_2, C_4, C_5 and C_6), 135.03 ppm (2x *C*H fumaric acid), 146.29 and 147.21 and 147.86 and 149.69 ppm (phenyl–C_1 and C_3 and pyridine–C_2 and C_6), 165.80 and 166.15 ppm (2x carbonyl ester–*C*), 168.13 (2x carbonyl–*C* fumaric acid).

2-(2-Aminoethylthio)methyl-3,5-dicarboethoxy-6-methyl-4-phenyl-1,4-dihydropyridine fumarate ***VUF 9056***

Yield: 50%, mp: 120.5–123.0°C. Mass spectrum (matrix = glycerol + ammonium acetate) (FAB$^+$) 405 [M+H]$^+$, (FAB$^+$) 403 [M-H]$^+$.

^{1}H-NMR (DMSO–d_6): 1.15 ppm (t, J = 6.6 Hz, 6H, 2x CH_3–CH_2-O), 2.29 (s, 3H, pyridine–CH_3), 2.80 ppm (t, J = 7.3 Hz, 2H, S-CH_2-CH_2-N), 3.02 ppm (t, J = 7.3 Hz, 2H, S-CH_2-CH_2-N), 3.88 ppm (s, 2H, pyridine–CH_2-S), 3.99 ppm (q, J = 6.6 Hz, 4H, 2x CH_3-CH_2-O), 4.90 ppm (s, 1H, pyridine–H_4), 6.52 ppm (s, 2H, fumaric acid), 7.05–7.30 ppm (m, 5H, 5x phenyl-*H*), 9.33 ppm (br s, 0.8 H, pyridine-N*H*).

^{13}C-NMR (DMSO–d_6): 13.75 and 13.89 ppm (2x CH_3-CH_2-O), 17.89 ppm (pyridine–CH_3), 28.48 and 29.68 ppm (S-CH_2-CH_2-N and S-CH_2-CH_2-N), 38.46 ppm (pyridine–CH_2-S), 38.60 ppm (pyridine–C_4), 58.84 and 59.16 ppm (2x CH_3-CH_2-O), 101.54 and 102.15 ppm (pyridine–C_3 and C_5), 125.78 ppm (phenyl–C_4), 127.10 and 127.65 ppm (phenyl–C_2, C_3, C_5 and C_6), 135.00 ppm (2x *C*H fumaric acid), 145.27 and 146.58 and 147.56 ppm (phenyl–C_1 and pyridine–C_2 and C_6), 166.24 and 166.56 ppm (2x carbonyl ester–*C*), 168.03 (2x carbonyl–*C* fumaric acid).

2-(2-Aminoethylthio)methyl-3,5-dicarboethoxy-6-methyl-4-(2-nitrophenyl)-1,4-dihydropyridine fumarate ***VUF 4574***

Yield: 67%, mp: 101.7–102.0°C. Mass spectrum (matrix = glycerol + ammonium–acetate) (FAB$^+$) 450 [M+H]$^+$, (FAB$^-$) 448 [M-H]$^-$.

^{1}H-NMR (DMSO–d_6): 1.02–1.33 ppm (m, 6H, 2x CH_3-CH_2-0), 2.29 (s, 3H, pyridine–CH_3), 2.77 and 2.98 ppm (m, 4H, S-CH_2-CH_2-N and S-CH_2-CH_2-N), 3.78-4.08 ppm (m, 6H, pyridine–CH_2-S and 2x CH_3-CH_2-O), 5.65 ppm (s, 1H, pyridine–H_4), 6.48 ppm (s, 2H, fumaric acid), 7.33-7.79 ppm (m, 4H, 4x phenyl–*H*), 9.50 ppm (br s, 0.7 H, pyridine–N*H*).

2-(2-Aminoethylthio)methyl-3,5-dicarboethoxy-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine fumarate ***VUF 9158***

Yield: 57%, mp: 147.6–150.3°C. Mass spectrum (Electron Impact (70eV); $C_{21}H_{26}{}^{35}Cl_2N_2O_4S$: mass calculated: 472.099; mass found: 472.100 ± 0.003).

^{1}H-NMR (DMSO–d_6): 1.01–1.24 ppm (m, 6H, 2x CH_3-CH_2-O), 2.26 ppm (s, 3H, pyridine–CH_3), 2.80 ppm (t, J = 6.6 Hz, 2H, CH_2), 3.00 ppm (t, J = 6.6 Hz, 2H, CH_2), 3.80 and 3.91 ppm (AB, J_{AB} = 13.3 Hz, 2H, pyridine–CH_2-S), 3.90–4.15 ppm (m, 4H, 2x CH_3-CH_2-O), 5.35 ppm (s, 1H, pyridine–H_4), 6.62 ppm (s, 2H, 2x *C*H fumaric acid), 7.08–7.48 ppm (m, 3H, 3x phenyl–*H*), 9.30 ppm (br s, 1H, pyridine–N*H*).

^{13}C-NMR (DMSO–d_6): 13.80 and 13.89 ppm (2x CH_3-CH_2-O), 17.86 ppm (pyridine–CH_3), 28.90 and 29.56 ppm (S-CH_2-CH_2-N and S-CH_2-CH_2-N), 37.65 ppm (pyridine–C_4), 38.50 (pyridine–CH_2-S), 58.83 and 59.20 ppm (2x CH_3-CH_2-O), 101.20 and 101.86 ppm (pyridine–C_3 and C_5), 127.80 and 128.0 and 129.15 ppm (phenyl–C_4, C_5 and C_6), 131.17 ppm (phenyl–C_2 and C_3), 135.04 ppm (2x *C*H fumaric acid), 145.72 and 146.53 ppm (pyridine–C_2 and C_6), 148.44 (phenyl–C_1), 166.09 and 166.31 ppm (2x carbonyl ester–*C*), 167.98 ppm (2x carbonyl–*C* from fumaric acid).

Method B. In situ preparation of 2-(2-aminoalkylthio)methyl-3,5-dicarboethoxy-6-methyl-4-(substituted phenyl)-1,4-dihydropyridine fumarate

2 ml 10 M NaOH solution in water was added dropwise to a solution of 20 mmol NaSH and 20 mmol N-(ω-bromoalkyl) phthalimide in 75 ml DMSO. After stirring for 4 h, 20 mmol 2-chloromethyl-3,5-dicarboethoxy-6-methyl-4-(substituted phenyl)-1,4-dihydropyridine in 50 ml DMSO was added dropwise. The reaction mixture was stirred for 1 d at room temperature. The reaction mixture was poured into water and extracted with ethyl acetate. The combined organic layers were dried with $MgSO_4$ and the solvent evaporated. The crude reaction mixture was dissolved in 150 ml ethanol and 3 equivalents of hydrazine monohydrate was added. After refluxing for 3 h, the reaction mixture was cooled, filtered and 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 40 ml). The water layer was made basic with a 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 dissolved in ethyl acetate at 40°C. Then fumaric acid in methanol 60°C was added. The product was obtained after cooling. According to this method the following compounds were prepared.

2-(3-Aminopropylthio)methyl-3,5-dicarboethoxy-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine fumarate ***VUF 9108***

Yield: 54%, mp: 124.3–127.2°C. Mass spectrum (matrix = glycerol + ammonium acetate) (FAB$^+$) 464 [M + H]$^+$, (FAB$^-$) 462 [M-H]$^-$.

^{1}H-NMR (DMSO–d_6): 1.07–1.27 ppm (m, 6H, CH_3-CH_2-O), 1.77–1.96 ppm (m, 2H, C-CH_2-C), 2.32 ppm (s, 3H, pyridine–CH_3), 2.47–2.72 (m, 5.9 H, CH_2 + DMSO), 2.85 ppm (t, J = 6.6 Hz, 2H, CH_2), 3.69–4.12 ppm (m, 6H, pyridine–CH_2-S and 2x CH_3-CH_2-O), 5.01 (s, 1H, pyridine–H_4), 6.48 ppm (s, 2H, fumaric acid), 7.50–7.70 ppm (m, 2H, 2x phenyl-*H*), 7.93–8.10 ppm (m, 2H, 2x phenyl–*H*), 8.51–9.67 ppm (br s, 2H, NH_2), 9.47 ppm (s, 1H, pyridine–N*H*).

2-(5-Aminopentylthio)methyl-3,5-dicarboethoxy-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine fumarate ***VUF 9159***

Yield: 69%, mp: 100.6–102.5°C. Mass spectrum (matrix = glycerol + ammonium acetate) (FAB$^+$) 492 [M+H]$^+$, (FAB$^-$) 490 [M-H]$^-$.

^{1}H-NMR (DMSO–d_6): 1.10–1.18 ppm (m, 6H, 2x CH_3-CH_2-O), 1.21–1.30 ppm (m, 2H, C-C-CH_2-C-C), 1.48 ppm (m, 4H, C-CH_2-C-C-C and C-C-C-CH_2-C), 2.31 ppm (s, 3H, pyridine–CH_3), 2.42–2.52 ppm (m, 2H, CH_2), 2.72 ppm (t, J = 7.35 Hz, 2H, CH_2), 3.73 and 4.07 ppm (AB, J_{AB} = 13.1 Hz, 2H, pyridine–CH_2-S), 3.99 ppm (m, 4H, 2x CH_3-CH_2-O), 5.00 ppm (s, 1H, pyridine–H_4), 6.44 ppm (s, 2H, fumaric acid), 7.51–8.01 ppm (m, 4H, 4x phenyl–*H*), 9.42 ppm (br s, 1H, pyridine–N*H*).

2-(3-Aminopropylthio)methyl-3,5-dicarboethoxy-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine fumarate ***VUF 4621***
Yield: 35%, mp: 127.9–129.3°C. Mass spectrum (matrix = thioglycerol) (FAB^+) 487 $[M+H]^+$, (FAB^-) 485 [M-H]1.

^{1}H-NMR (DMSO–d_6): 1.10 ppm (t, J = 6.6 Hz, 6H, CH_3-CH_2-O), 1.83 ppm (m, 2H, C-CH_2-C), 2.27 ppm (s, 3H, pyridine–CH_3), 2.60 (m, 2 H, S-CH_2-C-C-N), 3.20 ppm (m, 2H, S-C-C-CH_2-N), 3.74 and 3.92 ppm (AB, J_{AB}= 12.9 Hz, 2H, pyridine–CH_2-S), 3.98 ppm (m, 4H, 2x CH_3-CH_2-O), 5.36 (s, 1H, pyridine–H_4), 6.46 ppm (s, 2H, fumaric acid), 7.22–7.41 ppm (m, 3H, 3x phenyl–*H*), 9.39 ppm (s, 1H, pyridine–N*H*).

^{13}C-NMR (DMSO–d_6): 13.91 ppm (2x CH_3-CH_2-O), 17.93 ppm (pyridine–CH_3), 27.31, 28.07 and 29.61 ppm (S-CH_2-CH_2-CH_2-N), 58.80 and 59.15 ppm (2x CH_3-CH_2-O), 100.97 and 102.11 ppm (pyridine–C_3 and C_5), 127.41, 128.04 and 129.22 ppm (phenyl–C_4, C_5, and C_6), 131.15 ppm (phenyl–C_2 and C_3), 135.13 ppm (2x *C*H fumaric acid), 145.78 and 146.65 ppm (pyridine–C_2 and C_6), 148.55 (phenyl–C_1), 166.34 ppm (2x carbonyl ester–*C*), 168.19 ppm (2x carbonyl–*C* from fumaric acid).

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