*Eur J Med Chem* (1994) 29, 579–593




# Synthesis and *in vitro* pharmacology of a series of hybrid molecules possessing 1,4-dihydropyridine calcium-channel blocking activity and histamine $H_2$-agonistic properties*

JAM Christiaans, AD Windhorst, H van der Goot, H Timmerman

*Leiden/Amsterdam Center for Drug Research, Division of Medicinal Chemistry, Department of Pharmacochemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands*

(Received 18 February 1994; accepted 25 April 1994)

**Summary** — The synthesis and *in vitro* pharmacology of a series of new cardiovascular hybrid molecules, which could be useful for the treatment of certain types of hypertension and at the same time for the treatment of cardiac ischemic disease, are discussed. Two types of 1,4-dihydropyridine $Ca^{2+}$-channel blockers have been studied. In general, hybrid molecules possessing a diethyl 2-(ω-aminoalkylthio)methyl-2,6-dimethyl-4-[(substituted)phenyl]-1,4-dihydropyridine-3,5-dicarboxylic structural moiety and a histamine $H_2$-agonistic structural moiety are more potent L-type calcium-channel blockers and histamine $H_2$-agonists than hybrid molecules containing a diethyl 4-[2-(ω-aminoalkoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic structural moiety.

**hybrid molecule / calcium-channel blocker / 1,4-dihydropyridine / tiamdipine / histamine $H_2$-agonist / impromidine / cardiovascular disorders**

## Introduction

The 1,4-dihydropyridine-type $Ca^{2+}$-channel blockers (DHPs) have allowed a new direction in therapy of several cardiovascular disorders. Several DHP $Ca^{2+}$-channel blockers are now well established in antianginal and antihypertensive therapy, as well as in the treatment of certain cardiac arrhythmias and peripheral vascular disorders [1–5]. DHPs cause vasodilation by inhibiting the influx of extracellular $Ca^{2+}$ into the cell through slow calcium channels, but also decrease ventricular contractility by the same mechanism. Although nifedipine **1** and nicardipine **2** (fig 1) are widely used clinically, the rather short duration of action of this type of drugs is disadvantageous. Examples of the second generation DHPs, such as amlodipine **3** [6] and tiamdipine **4** [7], offer a different pharmacokinetic profile: longer plasma half-life and 100% oral availability. These pharmacokinetic properties are apparently responsible for longer duration of action and the maintenance of a sustained antihypertensive effect by once-a-day administration [8].

One of the mechanisms controlling myocardial contractility proceeds *via* the sympathetic nervous system. In congestive heart failure (CHF) the myocardial inotropic contractility is disturbed due to increased levels of circulating catecholamines, initiated by the sympathetic nervous system. Prolonged

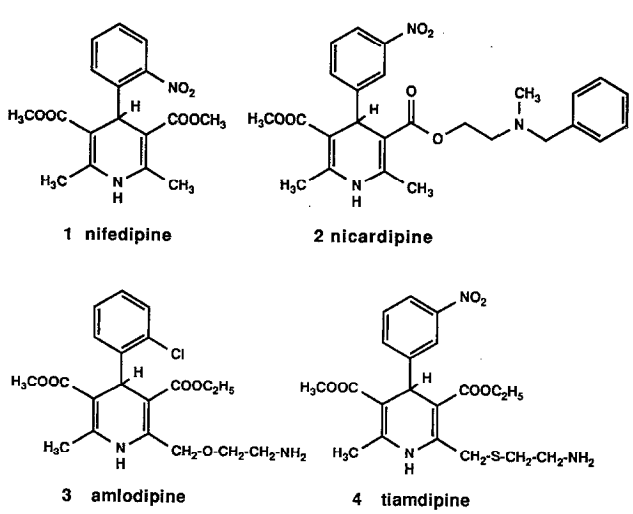



**Fig 1.** 1,4-Dihydropyridine-type calcium-channel blockers.

*Part of this work was presented at the *XIIth International Symposium on Medicinal Chemistry*, abstract book, poster presentation (P-277 A), page 189, Basel, Switzerland, September 1992.

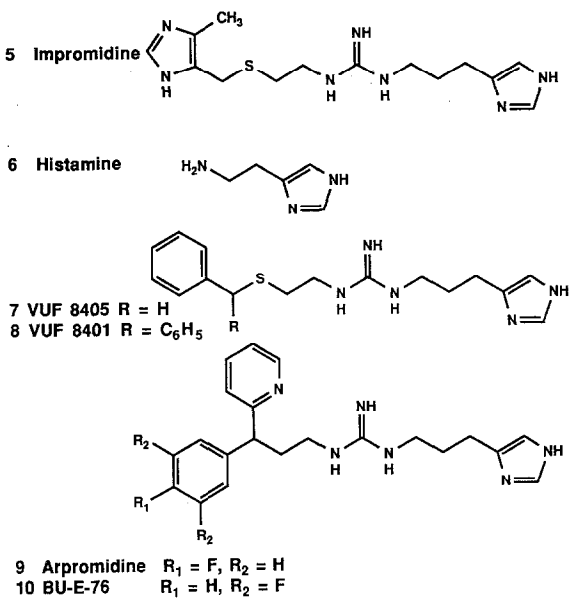



**Fig 2.** Histamine $H_2$-agonists.

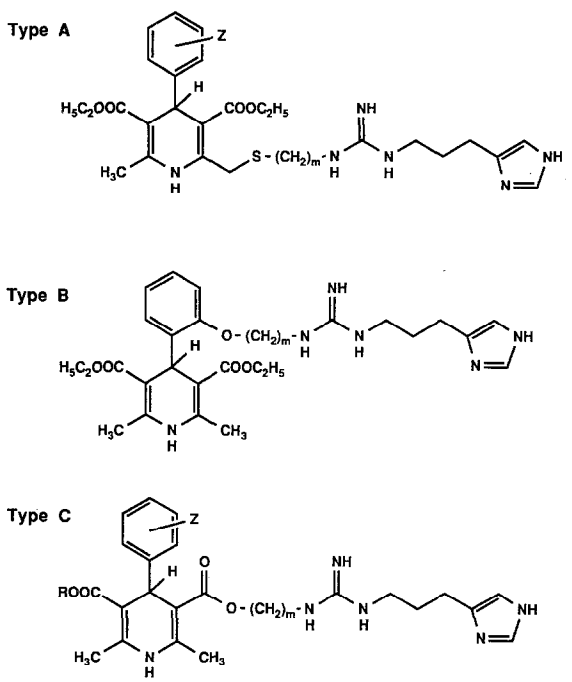



**Fig 3.** Hybrid molecules combining calcium-channel blocking activity and histamine $H_2$-agonistic activity.

exposure of the heart to excessive levels of endogenous catecholamines, in turn, results in a decreased number and sensitivity of myocardial $\beta_1$-adrenoceptors [9].

Although the heart becomes less sensitive to catecholamine stimulation, other mechanisms are able to increase cardiac contractility. One of the mechanisms proceeds *via* histamine $H_2$-receptors. Stimulation of the myocardial histamine $H_2$-receptor activates an adenylate cyclase system which, analogous to stimulation of the $\beta_1$-adrenoceptor adenylate cyclase system, results in stimulation of myocardial contractility [10]. The histamine $H_2$-agonist impromidine **5** is 48 times more potent than the natural agonist histamine **6** on the spontaneously beating guinea-pig right atrium [11] (fig 2). In human papillary muscle impromidine appears to be equally effective as the $\beta_1$-agonist isoproterenol in stimulating the myocardial adenylate cyclase system [12].

Baumann *et al* [13] have demonstrated that *in vivo* impromidine exhibits a positive inotropic effect in humans, resulting in an increased cardiac output. Simultaneously, a decrease in both systemic arterial blood pressure and vascular resistance is induced, while the heart rate remains constant. Unfortunately, impromidine is a potent stimulator of gastric acid secretion and like other cardiac stimulants may cause arrhythmias. More recently, new histamine $H_2$-agonists have been developed, which seem to have a more beneficial hemodynamic profile and are less arrhythmogenic than impromidine [14].

The imidazolylpropylguanidine structure of impromidine is believed to be responsible for the histamine $H_2$-agonistic activity, while the methylimidazole group contributes affinity. This concept is confirmed by Sterk *et al* (VUF 8405 **7** [15]; VUF 8401 **8** [16]) and Buschauer [17] (arpromidine **9**; BU-E-76 **10**) by replacing the methylimidazole group by lipophilic mono or diaryl structural moieties and/or heterocycles, affording compounds that are more potent than impromidine and have less stimulatory effects on gastric acid secretion. Arpromidine and BU-E-76 are guanidine type histamine $H_2$-agonists with additional histamine $H_1$-antagonistic properties due to the pharmacophoric moiety resembling pheniramine-like histamine $H_1$-antagonists. *In vivo*, in guinea pigs, BU-E-76 is more potent in reducing cardiac pre- and afterload and enhancing cardiac output and stroke volume than arpromidine or impromidine. Furthermore, BU-E-76 and arpromidine affect the chronotropy and cardiac rhythm less than impromidine. Therefore, BU-E-76 might be suitable for the treatment of patients suffering from severe heart failure [18].

Combination of positive inotropic and vasodilator effects into one drug might be beneficial in the treat-

ment of congestive heart failure, if the improved cardiac performance is achieved independently from the $\beta_1$-adrenergic system [19].

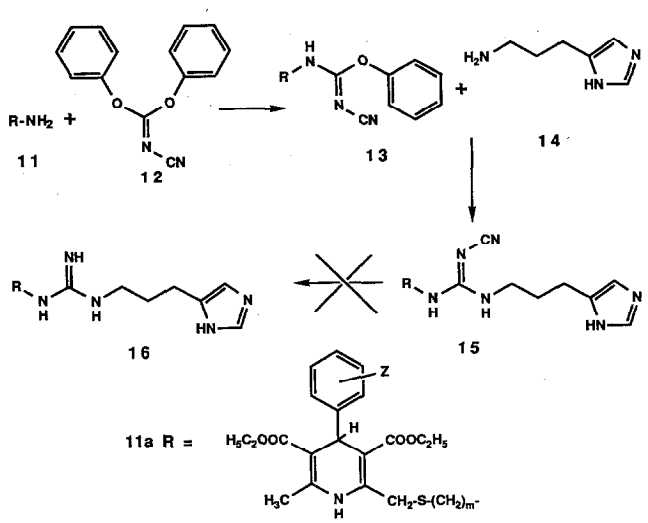
The goal of our project was to design DHPs which retain calcium-channel blocking activity and, in addition, possess cardiotonic activity to compensate for negative inotropic actions associated with DHP calcium-channel blockers. Such dual activities in a single molecule might offer some novel and attractive research tools. Our objective might be reached by combining in one molecule a 1,4-DHP, which possesses a vasodilator property and a negative inotropic action, with a histamine $H_2$-agonist having a positive inotropic activity. The structural modifications carried out on impromidine demonstrate that a certain tolerance exists about replacing the methylimidazole by lipophilic $H_2$-nonspecific structures (for reviews, see [14, 20–22]). Because of this tolerance, we decided to replace the $H_2$-nonspecific structural moiety by 1,4-dihydropyridines, introducing potential vasodilating properties.

On the basis of our previous results regarding the activity of thio-bioisosteres of amlodipine [23], we decided to synthesize several types of hybrid molecules (A and B; fig 3) by combining histamine $H_2$-agonists and 1,4-dihydropyridine-type calcium-channel blockers, following an idea proposed by Schickaneder *et al* [24] (type C; fig 3). In type A hybrid molecules the histamine $H_2$-agonistic part is coupled *via* an alkylthiomethyl chain to the 2-position of the DHP. In type B hybrid molecules the histamine $H_2$-agonistic part is coupled *via* an alkoxy chain to the 4-phenyl ring of the DHP. In type C hybrid molecules, the histamine $H_2$-agonistic moiety is coupled *via* an ester to the 1,4-DHP [24].

Hybrid molecules of type A seem to be the most promising hybrid molecules as they combine a histamine $H_2$-agonistic activity with calcium-channel blockers of the second generation. Amlodipine **3** and tiamdipine **4** are examples of second-generation calcium-channel blockers with a longer duration of action than first-generation calcium-channel blockers, such as nifedipine **1**. Especially, because type A hybrid molecules incorporate the tiamdipine structuralmoiety, these hybrid molecules could offer a more beneficial pharmacokinetic profile than type C hybrid molecules.

## Chemistry

The general synthetic routes of type A and B hybrid molecules are given in schemes 1 and 2. The syntheses of the primary amines **11a** were carried out as previously described [23]. According to scheme 1, the appropriate primary amine **11a** was treated with




**Scheme 1.**

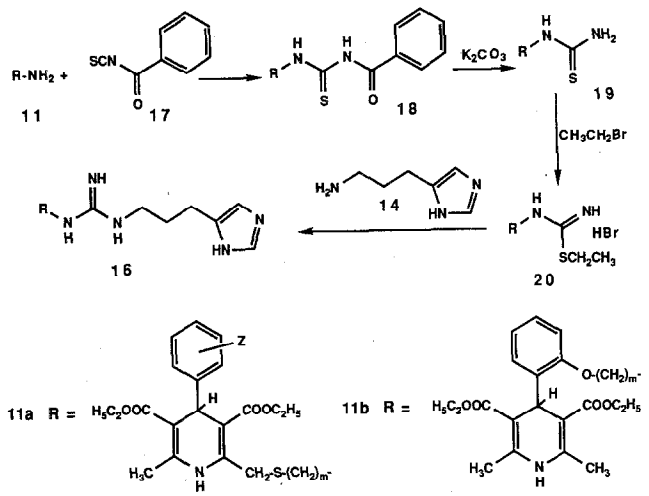
diphenyl *N*-cyanocarbonimidate **12** in diethyl ether or methylene chloride to afford the *N*-cyanoisoureas **13**. Subsequent reaction of the *N*-cyanoisoureas **13** with 3-(1*H*-imidazol-4-yl)propylamine **14** gave the *N*-cyanoguanidines **15** in only poor yields (10–22%). Acid hydrolysis with 1 M HCl to obtain the guanidines **16** failed because of hydrolysis of the thioether function and/or ester functions of the 1,4-dihydropyridines. Therefore, we have developed a new synthetic approach to achieve the proposed hybrid molecules.

The synthetic route in scheme 2 appears to be more convenient to obtain the guanidines **16**. The diethyl 2,6-dimethyl-4-[2-(ω-aminoalkoxy)phenyl]-1,4-dihydropyridine-3,5-dicarboxylates **11b** used as DHP building blocks in type B hybrid molecules were synthesized according to the previously described procedure [25]. Reaction of the appropriate primary amines **11a** or **11b** with benzoyl isothiocyanate **17** yielded the benzoylisothiourea derivatives **18**. Subsequent alkaline hydrolysis of the benzoylthiourea derivatives and direct alkylation with ethyl bromide, without isolation of **19**, gave the isothiourea hydrobromides **20**. Condensation of **20** with 3-(1*H*-imidazol-4-yl)propylamine **14** gave the guanidines **16**.

## Pharmacology

### *In vitro calcium-channel blocking activities and DHP receptor binding assay*

*In vitro* calcium-channel blocking activities and radioligand binding studies were carried out as previously described [23]. In this method calcium-channel blocking



**Scheme 2.**

activities were determined on rat aorta strips and were assessed as the concentration required to inhibit the $K^+$-depolarization-induced (50 mM) contractile responses by 50%.

The DHP receptor affinities were determined on isolated rat cortex by [$^3$H]nitrendipine displacement studies [26]. The equilibrium dissociation constant ($K_d$ = 0.75 nM) of the labelled compound and the maximal binding ($B_{max}$ = 270 fmol/mg protein) were determined with the non-linear fitting program Ligand 4.1 [27].

*In vitro histamine $H_2$-agonistic activities*

Male guinea pigs (300–350 g) were killed by a blow on the head. The spontaneously beating right atria were removed, attached to a tissue holder (load 0.5 g) connected to Grass 79D isometric equipment, and placed in a 20 ml organ bath with Krebs buffer and bubbled with oxygen containing 5% $CO_2$ at 37°C. The bath fluid contained 117.5 mM NaCl, 5.6 mM KCl, 1.18 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.28 mM $NaH_2PO_4$, 2.5 mM $NaHCO_3$ and 5.5 mM glucose, giving a pH of 7.4. The inotropy was measured on the isolated guinea-pig papillary muscle and was also performed on a Grass 79D isometric apparatus (load 0.5 g), using the same Krebs buffer as described for the determination of the chronotropic action. The papillary muscle was electrically stimulated with a squarewave stimulator with a frequency of 3 Hz and a duration of 0.5 ms.

*In vitro histamine $H_2$-receptor affinity measured by radioligand displacement*

Male guinea pigs (300–350 g) were killed by a blow on the head. The cerebral cortices were isolated and put into 10 ml sodium phosphate buffer (50 mM, pH 7.4). The cortices were cooled on ice and homogenized with a polytron (twice during 15 s at 8000 rpm). The homogenate was centrifuged for 1 min at 260 *g*. The supernatant was removed and centrifugated for 30 min at 20 000 *g*. The pellets were resuspended in 3 ml sodium phosphate buffer (50 mM pH 7.4) for each cortex. The protein concentration was determined by addition of 20 µl membrane suspension to 1 ml Merck–Biorad reagents (5 times diluted with nanopure water). Absorption was determined at 595 nm. The protein concentration was determined with bovine serum albumin as standard. The concentration of the membrane suspension was adjusted to 1 mg/ml and the suspension is kept at –80°C until use.

The incubations were performed in polypropylene tubes over 2 h at 25°C. Incubations were carried out with 100 µg protein and with a 0.4 nM concentration of [$^{125}$I]iodoaminopotentidine (synthesized according to the literature [28]) with a total volume of 400 µl. As incubation medium, a sodium phosphate buffer (50 mM, pH 7.4) with 1% bovine gelatine (final concentration) was added in order to prevent absorption of [$^{125}$I]iodoaminopotentidine. The incubation was stopped by addition of 4 ml ice-cold buffer (sodium phosphate buffer 20 mM, containing 0.1% bovine serum albumin to prevent filter binding). The membranes were filtered through Whatman GF/B filters, which were soaked for 3 h in a 0.3% polyethyleneimine solution in sodium phosphate buffer (50 mM, pH = 7.4), and washed twice with 4 ml ice-cold buffer using a Brandel filtration apparatus. The filters were isolated and the radioactivity was determined with an LKB Wallace 1282 compugamma S universal gamma counter at an efficiency of approximately 60%.

Non-specific binding was determined in the presence of 10 µM ranitidine. Specific binding was obtained by subtracting the non-specific binding from the total binding. The equilibrium dissociation constant ($K_d$ = 0.23 nM) of the labelled compound and the maximal binding ($B_{max}$ = 83 fmol/mg protein) were determined with the non-linear fitting program Ligand 4.1 [27].

## Results and discussion

*In vitro* calcium-channel blocking activities were assessed as the concentration required to inhibit the $K^+$-depolarization-induced (50 mM) contractile responses in rat aorta strips by 50%, and are expressed

as $pIC_{50}$ values. All compounds were tested as racemic mixtures and display a complete inhibition of contractile responses as was verified by addition of 1 mM papaverine. As reported by Christiaans *et al* [23], the onset of calcium-channel blocking activity of VUF 9159 (table I) and its analogues occurred slowly and did not reach equilibrium within 1 h. Additional increasing doses of the 1,4-DHP derivatives to generate dose–response curves were given every 45 min, so no complete equilibrium was reached, which could lead to underestimated values for the calcium-channel blocking activities.

*Calcium-channel blocking activities and affinities of type A hybrid molecules*

In table I, the calcium-channel blocking activities and affinities of the tiamdipine analogues are shown. The calcium-channel blocking activity of tiamdipine is not presented in table I because the pharmacological data reported by Kwon *et al* [8] are determined on a different tissue (rat tail artery).

As reported previously [23], 1,4-DHPs with a nitro substituent on the 4-phenyl ring are more potent than those compounds possessing a 2,3-dichloro substituent (VUF 9158; table I) or no substituent at all (VUF 9056; table I). Increasing the length of the 2-(ω-aminoalkylthio)methyl side chain on the 2-position of the 1,4-DHP ring from an ethyl chain to a hexyl chain does not affect calcium-channel blocking activity.

Affinities of the tiamdipine analogues ($pK_d$) determined by displacement of [$^3$H]nitrendipine from rat cortex membranes differ from the $pIC_{50}$ values. This is possibly because of underestimated calcium-channel blocking activities. Although tissue specificity cannot be ruled out, the established $pK_d$ value of nifedipine is in good correlation with its $pIC_{50}$ value.

In table II, the calcium-channel blocking activities and affinities of a series of type A hybrid molecules, containing a histamine $H_2$-agonistic structural moiety, are given. Within both series of type A hybrid molecules and 2-(ω-aminoalkylthio)methyl-1,4-DHPs, the 1,4-DHP derivatives without a substituent in the phenyl ring (VUF 4752; table II and VUF 9056; table I, respectively) are the least active calcium-channel blockers. The 2-(ω-aminoalkylthio)methyl-1,4-DHP derivative with a hexyl chain (VUF 4731; table I) is equally as potent as the DHP derivatives with an ethyl, propyl, and pentyl chain (table I), while in the series of type A hybrid molecules, VUF 4575 (table II), with a hexyl chain, is the most potent calcium-channel blocker (functional studies). The affinity of the original 1,4-DHP derivative VUF 4731 is similar to that of the type A hybrid molecule VUF 4575.

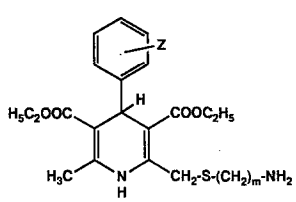
Comparing the activities of the hybrid molecules of table II with the original DHPs of table I, it is striking that all hybrid molecules are less potent than the 2-(ω-aminoalkylthio)methyl-1,4-DHPs. However, the difference in potency varies remarkably among the original 1,4-DHPs of table I and the type A hybrid molecules (table II). VUF 9055 is 45 times more potent as a calcium-channel blocker in functional studies than VUF 9065, while VUF 9158 is only approximately 4 times more potent than VUF 9160, as demonstrated in table III. This big difference in potency is not shared by the affinities established in radioligand binding studies; in these tests VUF 9056 was 8 times more potent than VUF 4572, while VUF 4731 was equally potent as VUF 4575.

A remarkable phenomenon was observed among the 4-(3-nitrophenyl)-1,4-DHP derivatives. Increasing the alkyl chain length from an ethyl chain to a hexyl chain resulted in a decrease of the difference between calcium-channel blocking activity of the 2-(ω-aminoalkylthio)methyl-1,4-DHPs and the hybrid molecules. The differences between the affinities nearly remained the same. Obviously, the underestimation of the activities differ among the several 1,4-DHPs.

*Histamine $H_2$-agonistic activity and $H_2$-receptor affinity of type A hybrid molecules*

In table II the histamine $H_2$-agonistic activities and affinities of type A hybrid molecules and some hista-

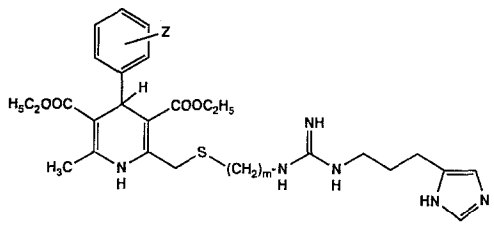
**Table I.** Calcium blocking activities and affinities of a series of tiamdipine analogues.




| *Compound* | *Z* | *m* | *$pIC_{50}$*[a] | *$pK_d$*[b] |
|---|---|---|---|---|
| VUF 9056 | H | 2 | 7.27 ± 0.08 | 8.57 ± 0.10 |
| VUF 9158 | 2,3 diCl | 2 | 7.47 ± 0.03 | 8.37 ± 0.10 |
| VUF 9055 | 3-$NO_2$ | 2 | 7.96 ± 0.07 | 8.61 ± 0.06 |
| VUF 9108 | 3-$NO_2$ | 3 | 7.82 ± 0.04 | 8.43 ± 0.05 |
| VUF 9159 | 3-$NO_2$ | 5 | 7.96 ± 0.12 | 8.55 ± 0.08 |
| VUF 4731 | 3-$NO_2$ | 6 | 7.85 ± 0.02 | 8.41 ± 0.15 |
| **1** | Nifedipine | | 8.77 ± 0.08 | 8.70 ± 0.14 |

[a]All $pIC_{50}$ values are means ± sd for 3 independent observations, determined on rat aorta strips; [b]all affinities ($pK_d$) were determined on isolated rat cortex membranes, and are means ± sd for 6–9 independent observations.

**Table II.** Histamine $H_2$-agonistic activities (chronotropic activity), calcium-channel blocking activities and affinities of type A hybrid molecules.




| *VUF* | *Z* | *m* | *Histamine $H_2$-agonism* | | | *$Ca^{2+}$-entry blocking activity* | |
|---|---|---|---|---|---|---|---|
| | | | *Functional* | | *Binding* | *Functional* | *Binding* |
| | | | *$pEC_{50}$*[a] | *ia* | *$pK_d$*[b] | *$pIC_{50}$*[c] | *$pK_d$*[d] |
| 4572 | H | 2 | nma | – | 6.19 ± 0.23 | 6.26 ± 0.03 | 7.67 ± 0.05 |
| 9160 | 2,3-diCl | 2 | nma | – | 5.51 ± 0.04 | 6.85 ± 0.07 | 7.97 ± 0.04 |
| 9065 | 3-$NO_2$ | 2 | nma | – | 5.82 ± 0.29 | 6.30 ± 0.07 | 8.46 ± 0.03 |
| 4570 | 3-$NO_2$ | 3 | nma | – | 5.77 ± 0.05 | 6.37 ± 0.02 | 8.15 ± 0.10 |
| 4573 | 3-$NO_2$ | 5 | 6.28 ± 0.13 | 0.6 | 5.93 ± 0.09 | 6.54 ± 0.06 | 8.07 ± 0.07 |
| 4575 | 3-$NO_2$ | 6 | 6.38 ± 0.14 | 0.9 | 6.04 ± 0.02 | 7.16 ± 0.05 | 8.39 ± 0.10 |
| Histamine | | | 6.06 ± 0.13 | 1.0 | 4.64 ± 0.35 | | |
| Impromidine | | | 7.63 ± 0.06 | 1.0 | 6.97 ± 0.07 | | |
| Ranitidine* | | | – | – | 7.12 ± 0.11 | | |
| Iodoaminopotentidine* | | | – | – | 9.52 ± 0.03 | | |

[a]All values are means ± sd for 3–50 independent observations, determined on isolated spontaneously beating guinea-pig right atrium; [b]all values are means ± sd for 3–6 independent observations. All affinities ($pK_d$) were determined on isolated guinea-pig cortex membranes, [$^{125}$I]iodoaminopotentidine was used as a hot ligand; [c]all values are means ± sd for 3–4 independent observations, determined on rat aorta strips; [d]all values are means ± sd for 5–9 independent observations. All affinities ($pK_d$) were determined on isolated rat cortex membranes, [$^3$H]nitrendipine was used as a hot ligand. nma: no measurable activity due to negative inotropic activity of the DHPs; ia: intrinsic activity relative to histamine; *histamine $H_2$-antagonist.

**Table III.** Relative calcium-channel blocking potencies (CCB) and affinities (CC-A) of the DHPs from table I compared with the corresponding hybrid molecules of table II.

| *DHP/hybrid* | *CCB rel act* | *CC-A rel aff* |
|---|---|---|
| VUF 9056/VUF 4572 | 10 | 8 |
| VUF 9158/VUF 9160 | 4 | 2.5 |
| VUF 9055/VUF 9065 | 45 | 1.4 |
| VUF 9108/VUF 4570 | 28 | 1.9 |
| VUF 9159/VUF 4573 | 26 | 3 |
| VUF 4731/VUF 4575 | 4.9 | 1 |

CCB rel act: relative calcium-channel blocking activity of a DHP from table I over the corresponding hybrid molecule; CC-A rel aff: relative affinity of a DHP from table I over the corresponding hybrid molecule.

mine $H_2$-agonists and $H_2$-antagonists are shown. It is remarkable in table II that some hybrid molecules do not display any activity, which can be explained as follows. Concentration–response curves (CR curves) of type A hybrid derivatives on isolated spontaneously beating guinea-pig right atria reveal an abnormal pattern. Normally, histamine $H_2$-agonists show a concentration-dependent increase in heart rate (positive chronotropy) of the isolated guinea-pig right atria. However, the positive chronotropic activity could not always be detected because of negative inotropic and chronotropic effects, which seem to produce a complete standstill of spontaneously beating right atria. The negative inotropic and chronotropic effects occurring in the CR curves of the hybrid molecules are due to the 1,4-DHP structural moiety. It is known that 1,4-DHPs exert a negative inotropic and chronotropic action, as is shown in table IV. The heart rate of some

**Table IV.** Negative inotropic and chronotropic activities of nifedipine and nisoldipine, determined on guinea-pig papillary muscle and guinea-pig right atrium, respectively (calculated from $EC_{50}$ values from Kazda *et al* [29]).

| | *Nifedipine 1* | *Nisoldipine*[a] |
|---|---|---|
| Negative inotropy; $pEC_{50}$ | 7.35 ± 0.35 | 7.30 ± 0.21 |
| Negative chronotropy; $pEC_{50}$ | 7.66 ± 0.24 | 7.19 ± 0.43 |

[a]Nisoldipine has a *sec*-butyl ester at the 3-position on the 1,4-DHP ring instead of a methyl ester, as in nifedipine.

of the right atria could be partially restored by noradrenaline or histamine stimulation, but this was only temporary.

By increasing the alkyl chain length of the type A hybrid molecules, carrying a 3-nitrophenyl substituent on the 4-position of the 1,4-DHP ring, we get molecules that show a chronotropic activity exhibited by the histamine $H_2$-agonistic structural moiety (table II). Obviously, the histamine $H_2$-agonistic effects can overcome the effects exerted by the 1,4-DHP calcium-channel blockers. Although VUF 4573 and VUF 4575 have a higher chronotropic activity than histamine, it remains unclear whether the histaminergic effects are increased or whether the negative inotropic and chronotropic effects of the 1,4-DHP structural moiety are decreased.

Comparison of the histamine $H_2$-agonistic activities of the type A hybrid molecules with impromidine shows that VUF 4573 and VUF 4575 are moderate histamine $H_2$-agonists. Although VUF 4573 and VUF 4575 have an intrinsic activity relative to histamine of 0.6 and 0.9, respectively, this does not necessarily indicate that these 2 compounds are partial agonists as verified by addition of $10^{-5}$ M histamine at the end of the CR curves of VUF 4573 and VUF 4575, which did not lead to the initial maximal contractile force produced in control experiments.

VUF 4573 was also pharmacologically tested on the guinea-pig left atrium (used in pharmacological studies to demonstrate histamine $H_1$-activity), but was devoid of any effect, demonstrating that VUF 4573 has no histamine $H_1$-activity.

In figure 4 the CR curves of nifedipine, histamine and of a 1:1 molar mixture of nifedipine/histamine are shown. Addition of $10^{-6}$ M isoprenaline is unable to restore any response at the end of the CR curve of nifedipine.

Figure 4 nicely demonstrates the idea behind hybrid molecules. While nifedipine affects inotropic activity at much lower concentrations than histamine, a 1:1 molar mixture of nifedipine/histamine has no overall effect on inotropic activity. Thus the combination of the 2 compounds in a 1:1 molar ratio, to mimic the fixed 1:1 molar ratio in hybrid molecules, does not simply result in summing the 2 distinct CR curves.

Inotropic activities of the histamine $H_2$-agonistic activities of type A hybrid molecules were also measured on the electrically stimulated guinea-pig papillary muscle. As already discussed in the section on chronotropic activity, the negative inotropic activity of the 1,4-DHP structural moiety hinders the determination of the positive inotropic action exhibited by the histamine $H_2$-agonistic structural moiety.

In table V, the positive inotropic activities of type A hybrid molecules are shown. Determination of the positive inotropic activity was only possible for VUF 4573 (VUF 4575 was not tested). The explanation of the poor intrinsic activity of VUF 4573 relative to histamine is the same as that for the poor intrinsic activities in the section of chronotropic studies. When the CR curve of VUF 4573 is determined in presence of 1 µM cimetidine (a histamine $H_2$-antagonist; $pA_2$ = 6.1), the CR curve is shifted to the right (higher concentration), confirming that the exhibited effect is a histamine $H_2$-effect.

By adjusting the experimental procedure, it is possible to avoid the problems exhibited by the DHP structural moiety. At the beginning of each CR curve, 1 µM Bay k 8644 (a DHP-type calcium-channel activator) is added. Bay k 8644 has a positive inotropic and chronotropic activity, thus the initial contractile force and beat frequency is increased. At the same time, the effects originating from the DHP structural moiety of the hybrid molecules are suppressed by Bay k 8644. So, by neutralizing the calcium-blocking effect, it is possible to determine the inotropic activities of a number of type A hybrid molecules.

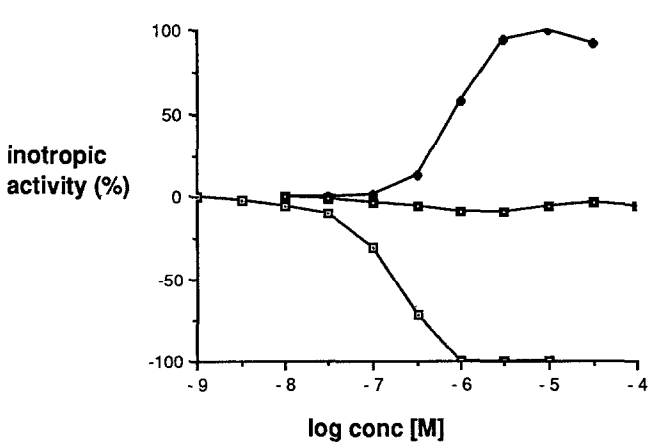
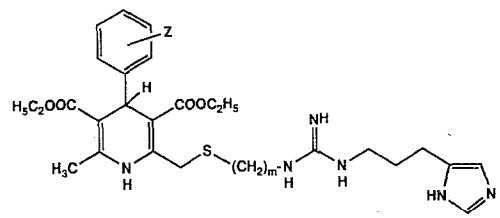



**Fig 4.** Inotropic activity of histamine (HA), nifedipine (nif), and a 1:1 molar mixture of histamine/nifedipine on the electrically stimulated guinea-pig papillary muscle. ◆: Histamine; ▣: nifedipine; ■: HA/nif 1:1.

**Table V.** Histamine $H_2$-agonistic activities on the electrically stimulated guinea-pig papillary muscle (inotropic activity).



| *Compound* | *Z* | *m* | *$pEC_{50}$* | *ia* | *In the presence of 1 μM Bay k 8644* *$pEC_{50}$** | *ia** |
|---|---|---|---|---|---|---|
| VUF 4572 | H | 2 | nt | | 5.73 ± 0.30 | 1.0 |
| VUF 9160 | 2,3-diCl | 2 | nt | – | < 5 | – |
| VUF 9065 | 3-$NO_2$ | 2 | nma | – | nma | – |
| VUF 4570 | 3-$NO_2$ | 3 | nma | – | 5.25 | 1.0 |
| VUF 4573 | 3-$NO_2$ | 5 | 5.95 | 0.3 | 6.15 ± 0.18 | 1.0 |
| VUF 4575 | 3-$NO_2$ | 6 | nt | – | 6.16 ± 0.13 | 1.0 |
| Histamine | | | 6.17 ± 0.03 | 1.0 | 6.65 ± 0.20 | 1.0 |
| Impromidine | | | 7.24 | 0.95 | 7.32 | 1.0 |

$pEC_{50}$: concentration required to produce 50% of its maximal effect, all values are means ± sd for 3–20 independent observations; *data obtained in presence of 1 μM Bay k 8644 (a calcium-channel activator) in order to neutralize the calcium-channel blocking activities of the DHP structural moieties; nt: not tested; nma: no measurable activity at $10^{-5}$ M; ia: intrinsic activity relative to histamine.

Although the combined presence of both activities does not allow us to test whether the compounds are full or partial histamine $H_2$-agonists, the data in table V demonstrate that under these particular conditions VUF 4573 and VUF 4575 are full agonists as they have about the same intrinsic activity as histamine and impromidine. The inotropic activities (as $pEC_{50}$) of the hybrid molecules are lower than that of histamine or impromidine. VUF 4573 exhibits a positive inotropic activity with or without Bay k 8644. It seems that the alkyl chain length reaches a point where the overall inotropic activity is positive. VUF 4573 and VUF 4575 are equally active in the pharmacological assay in which Bay k 8644 is administered.

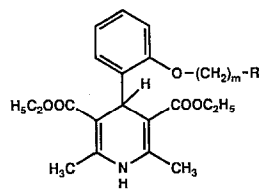
Although it is tenable to assume that the increased inotropic activity of histamine in presence of Bay k 8644 might be explained by an improved availability of intracellular calcium for the contractile process, this property is not observed for impromidine (table V).

*Calcium-channel blocking activities and affinities of type B hybrid molecules*

In table VI the calcium-channel blocking activities and affinities of a number of diethyl 2,6-dimethyl-4-[2-(ω-substituted-alkoxy)phenyl]-1,4-dihydropyridine-3,5-dicarboxylate derivatives are shown. In this series of compounds a remarkable tolerance exists upon substitution without affecting calcium-channel blocking activity.

Only the hybrid molecules VUF 4612 and VUF 4730, with a histamine $H_2$-agonistic structural moiety, are less active than the non-hybrid molecules (table VI). It is not illogical to assume that the lower calcium-channel blocking activities are caused by the histamine $H_2$-agonistic activity. However, this assumption is in contrast with the findings of Tenner *et al* [30], who investigated the influence of histamine $H_2$-agonists on rabbit aorta strips. In rabbit aorta strips contracted by 60 mM KCl, no significant relaxation is produced by histamine or the histamine $H_2$-agonists impromidine and dimaprit. This was confirmed in our experiments in which impromidine did not affect the KCl-induced contractions in rat aorta strips (data not shown). The affinities of the hybrid molecules VUF 4612 and VUF 4730 are equal to the affinities of the non-hybrid molecules (table VI), indicating that the histamine $H_2$-agonistic structural moiety does not affect the conformation adopted by the DHP structure to bind to its receptor. We do not have an explanation

**Table VI.** Calcium-channel blocking activities ($pIC_{50}$) and affinities ($pK_d$) of a series of diethyl 2,6-dimethyl-4-[2-(ω-substituted-alkoxy)phenyl]-1,4-dihydropyridine-3,5-dicarboxylates.




| *R* | | *m* = 5 | | | *m* = 6 | |
|---|---|---|---|---|---|---|
| | *Compound* | $pIC_{50}$[a] | $pK_d$[b] | *Compound* | $pIC_{50}$[a] | $pK_d$[b] |
| $NH_2$ | VUF 4587 | 6.61 ± 0.08 | 6.83 ± 0.08 | VUF 4599 | 6.42 ± 0.04 | 7.02 ± 0.05 |
| $NHC(S)NH_2$ | VUF 4622 | 6.44 ± 0.13 | 7.00 ± 0.07 | VUF 4671 | 6.39 ± 0.09 | 7.09 ± 0.09 |
| NHC(NH)SEt | VUF 4623 | 6.31 ± 0.10 | 7.27 ± 0.15 | VUF 4624 | 6.34 ± 0.11 | 7.70 ± 0.11 |
| ipg | VUF 4612 | 5.58 ± 0.16 | 7.52 ± 0.11 | VUF 4730 | 5.39 ± 0.11 | 7.42 ± 0.17 |
| | Nifedipine | 8.77 ± 0.08 | 8.70 ± 0.14 | | | |

$pIC_{50}$: concentration required to produce 50% of its inhibitory effect; [a]all values are means ± sd for 3 independent observations; [b]all values are means ± sd for 5–9 independent observations; all affinities ($pK_d$) were determined on isolated rat cortex membranes; ipg: imidazolylpropylguanidine structural moiety as in **16** (scheme 2).

for the differences in the observed *in vitro* calcium-channel blocking activities (functional studies) and the affinities (radioligand binding studies) of VUF 4612 and VUF 4730.

The affinities of the compounds shown in table VI are only marginally affected by the nature of the substituents. Compounds with an isothiourea or a guanidino group have a little higher affinity than the derivatives with a thiourea or a primary amine function. The higher affinity of the derivatives with an isothiourea or a guanidino function might be ascribed to additional interaction with the binding site of the L-type calcium-channel due to an ionic interaction between the protonated isothiourea or guanidino group and the negatively charged phosphate group of a phospholipid. However, this would not explain the lower calcium-channel blocking activities ($pIC_{50}$ values). Although compounds with a primary amine function should obey the same additional interaction described for isothiourea and guanidino functions, the affinities of the 1,4-DHPs with a primary amine group are as active as the derivatives with a thiourea function (not protonated at physiological pH).

In table VII the calcium-channel blocking activity and affinity of the hybrid molecule with a propyl chain (VUF 4588) demonstrates that increasing the alkyl chain length of the 2-(ω-substituted-alkoxy)phenyl hybrid molecules from propyl to hexyl results in a decrease of calcium-channel blocking activity, while the affinity slightly increases. We have no explanation for this phenomenon.

*Histamine $H_2$-agonistic activity and $H_2$-receptor affinity of type B hybrid molecules*

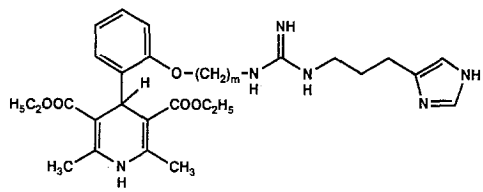
The only type B hybrid molecule having a positive chronotropic activity is VUF 4730, possessing a hexyl chain. The hybrid molecules with a propyl or a pentyl chain show no activity (table VII). However, all 3 type B hybrid molecules have an affinity for the histamine $H_2$-receptor. The inactivity of VUF 4588 and VUF 4612 is most likely due to the calcium-channel blocking activity of the hybrid molecule. Increasing the alkyl chain length, from a propyl to a hexyl chain, decreases the calcium-channel blocking activity, while increasing histamine $H_2$-agonistic activity.

VUF 4730 is also the only type B hybrid molecule with a positive inotropic action. The inotropic activities of VUF 4588 and VUF 4612 were not determined in absence of 1 μM Bay k 8644 because they did not demonstrate any activity in the pharmacological system that contained Bay k 8644 (table VIII).

There is no statistical difference in inotropic activities of VUF 4730 in the absence or presence of the calcium-channel activator Bay k 8644.

In the series of tiamdipine analogues, it has been shown that increasing the alkyl chain length on the 2-position of the 1,4-DHP ring from ethyl to hexyl

**Table VII.** Histamine $H_2$-agonistic activity (chronotropic activity), calcium-channel blocking activities and affinities of a series of type B hybrid structures.




| *VUF* | *m* | *Histamine $H_2$-agonism* | | | *$Ca^{2+}$-entry blocking activity* | |
|---|---|---|---|---|---|---|
| | | *Functional* $pEC_{50}$[a] | *ia* | *Binding* $pK_d$[b] | *Functional* $pIC_{50}$[c] | *Binding* $pK_d$[b] |
| 4588 | 3 | na | – | 5.76 ± 0.15 | 5.88 ± 0.14 | 7.03 ± 0.14 |
| 4612 | 5 | na | – | 5.96 ± 0.22 | 5.58 ± 0.16 | 7.52 ± 0.11 |
| 4730 | 6 | 6.20 ± 0.06 | 0.75 | 6.69 ± 0.15 | 5.39 ± 0.11 | 7.42 ± 0.17 |

$pEC_{50}$: concentration required to produce 50% of its maximal contractile effect; $pIC_{50}$: concentration required to produce 50% of its inhibitory effect; na: not active; [a]all values are means ± sd for 3–4 independent observations; [b]all values are means ± sd for 5–9 independent observations; [c]all values are means ± sd for 3–4 independent observations.

**Table VIII.** Histamine $H_2$-agonistic activity (inotropic activity) of type B hybrid molecules on the electrically stimulated guinea-pig papillary muscle.

| *VUF* | *$pD_2$* | *ia* | *pEC50*[a] | *ia* |
|---|---|---|---|---|
| 4588 | Not tested | – | Not active | – |
| 4612 | Not tested | – | Not active | – |
| 4730 | 5.63 ± 0.23 | 1.0 | 5.86 ± 0.05 | 1.0 |

[a]In presence of 1 μM Bay k 8644 in order to neutralize the negative inotropic activities of the DHP structural moieties; all number of experiments = 2.

only marginally affects the potent calcium-channel blocking activities and affinities.

In the series of type A hybrid molecules the affinities for the DHP binding site are almost equal to the affinities of the tiamdipine analogues. However, the *in vitro* calcium-channel blocking activities of type A hybrid molecules are lower than those of the tiamdipine analogues. The decrease in calcium-channel blocking activity must be ascribed to the unfavourable bulky structural moiety and not to any histamine $H_2$-effect. By increasing the alkyl chain length from ethyl to hexyl in type A hybrid molecules, the calcium-channel blocking activities increases. The increased flexibility of the hybrid molecule with an extended alkyl chain makes it possible for the DHP structure to adopt a conformation that allows the DHP structural moiety to interact with the DHP binding site. At the same time, increasing the alkyl chain length of type A hybrid molecules leads to an overall moderate positive chronotropic and inotropic activity.

The histamine $H_2$ chronotropic and inotropic activities of type A hybrid molecules with an ethyl chain length cannot be determined because of the negative inotropic and chronotropic effects exhibited by the DHP structural moiety. This complicated attribution on overall inotropic activity makes it impossible to verify the contribution of phenyl ring substituents, as described by Rodenkirchen *et al* [31]. They demonstrated that in a series of nifedipine analogues, the negative inotropic activity on cat papillary muscle mainly depends on steric and lipophilic substituent properties for aryl derivatives and/or on the steric nature of the ester substituents. Bulky ester substituents decrease the negative inotropic activities in cat papillary muscle. In general the negative inotropic activity of phenyl substituted DHP derivatives decrease in the order of *ortho* > *meta* > *para* substitution. These structure–activity relationship studies were confirmed by Boyd *et al* [32], who used rabbit papillary muscle. The *in vitro* and *in vivo* pharmacology of a series of felodipine (ethyl methyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate) analogues with different ester groups in 3- and 5-positions on the DHP ring have been evaluated on rat papillary muscle and portal vein, leading to the conclusion that the DHP effector site in vascular smooth muscle must be structurally different from the DHP effector site in cardiac tissue [33].

Type B hybrid molecules have much lower calcium-channel blocking activities than type A hybrid molecules. The explanation of the lower calcium-channel blocking activities can be reduced to the discussion on the structure–activity relationship of the tiamdipine analogues from table I and the 4-(ω-substituted-alkoxy)phenyl DHPs from table VI. In general, calcium-channel blocking activity decreases for 4-(substituted-phenyl)-1,4-DHPs in the order of *ortho* > *meta* > *para* substitution, and this effect becomes more pronounced when the substituent becomes bulkier. This might explain the reduction of calcium-channel blocking activity of type B hybrid molecules with increasing alkyl substituents on the 4-phenyl ring, although this is not confirmed by the radioligand binding studies.

In a patent concerning a series of type C hybrid molecules, Schickaneder *et al* [24] claimed that the compound with a hexyl chain, a methyl ester, and a 3-nitro substituent on the 4-phenyl, is a potent histamine $H_2$-agonist ($pD_2$ = 7.4) and calcium-channel blocker ($pA_2$ = 7.0). Unfortunately, no pharmacological methods are described, making it impossible to compare the activities of type C hybrid molecules with those of type A and B hybrid molecules. Furthermore, it is not clear whether the authors of type C hybrid molecules have encountered the same problems in determining the individual actions of the 2 pharmacophoric groups.

## Conclusion

Of the proposed type A and B hybrid molecules combining histamine $H_2$-agonistic properties and calcium-channel blocking activities, the type A hybrid molecule VUF 4575 is the most promising compound, which could provide interesting leads in the treatment of myocardial heart failure or some hypertensive disorders, because it combines good *in vitro* $Ca^{2+}$-blocking action and an overall positive inotropic and chronotropic activity in one molecule. VUF 4573 has a nearly equivalent histamine $H_2$-agonistic potency as VUF 4575, but has a lower calcium-channel blocking activity. Introduction of an imidazolylpropylguanidine structure on the 4-phenyl ring on the DHP structural moiety (type B hybrid molecules) affords VUF 4730, which exhibits both overall positive chronotropic and inotropic activity, but is less interesting than VUF 4575 or VUF 4573 because it has a lower calcium-channel blocking activity.

## Experimental protocols

If 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. $^{13}$C-NMR spectra were verified by CH-cosy NMR experiments. 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). The purity of the compounds was checked by thin-layer chromatography (Merck silica gel 60, F254 0.25 mm).

*General synthetic procedure*

*Diethyl 2-[(6-aminohexyl)thiomethyl]-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate fumarate* ***VUF 4731***

VUF 4731 was synthesized according to VUF 9159 as described by Christiaans *et al* [23]. Yield = 24% (starting from diethyl 2-chloromethyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate); melting point = 98.4–100.1°C. Mass spectrum, glycerol + ammonium acetate as matrix (FAB$^+$) 506 $[M+H]^+$, (FAB$^-$) 504 $[M-H]^-$.

$^1$H-NMR (DMSO-$d_6$): 1.11–1.65 ppm (m, 14H, 2 x $CH_3$-$CH_2$-O and S-C-$(CH_2)_4$-C-N), 2.32 ppm (s, 3H, pyridine-$CH_3$), 2.44–2.56 ppm (m, 2H, $CH_2$), 2.73–2.78 ppm (m, 2H, $CH_2$), 3.70 and 4.09 ppm (AB, $J_{AB}$ = 13.1 Hz, 2H, pyridine-$CH_2$-S), 3.98–4.05 ppm (m, 4H, 2 x $CH_3$-$CH_2$-O), 5.01 ppm (s, 1H, pyridine-$H_4$), 6.48 ppm (s, 2H, fumaric acid-C*H*), 7.52–8.02 ppm (m, 6.5H, 4 x phenyl-*H* and $NH_2$), 9.36 ppm (bs, 1H, pyridine-N*H*).

$^{13}$C-NMR (DMSO-$d_6$): 13.73 and 13.83 ppm (q, 2 x $CH_3$-$CH_2$-O), 18.00 ppm (q, pyridine-$CH_3$), 25.20 and 26.74 and 27.57 and 28.87 and 29.14 and 30.85 ppm (t, S-$(CH_2)_6$-N), 38.28 ppm (t, pyridine-$CH_2$-S), 39.06 ppm (d, pyridine-$C_4$), 59.05 and 59.33 ppm (t, 2 x $CH_3$-$CH_2$-O), 100.26 and 101.36 ppm (s, pyridine-$C_3$ and $C_5$), 120.99 and 121.66 and 129.30 and 133.93 ppm (d, 4 x phenyl-*C*H [$C_2$, $C_4$, $C_5$, $C_6$]), 134.98 ppm (d, fumaric acid *C*H), 146.53 and 147.25 and 147.69 and 149.74 ppm (s, phenyl-$C_1$ and $C_3$ and pyridine-$C_2$ and $C_6$), 165.92 and 166.15 ppm (s, 2 x carbonyl-*C*), 167.91 ppm (s, 2 x carbonyl-*C* fumaric acid).

*N-Benzoyl-N'-{ω-[3,5-diethoxycarbonyl-1,4-dihydro-6-methyl-4-(substituted-phenyl)pyridin-2-yl]methylthio}alkylthiourea*

Under a nitrogen atmosphere at 0°C 20 mmol benzoyl isothiocyanate in 150 ml dichloromethane was added dropwise to a solution of 20 mmol diethyl 2-(ω-aminoalkyl)thiomethyl-6-methyl-4-(substituted-phenyl)-1,4-dihydropyridine-3,5-dicarboxylate in 150 ml dichloromethane. Stirring was continued for 2 h while the temperature was raised to room temperature. The reaction mixture was evaporated and the residue was washed 4 times with 50 ml diethyl ether.

*N-Benzoyl-N'-{2-[(3,5-diethoxycarbonyl-1,4-dihydro-6-methyl-4-phenylpyridin-2-yl)methylthio]ethyl}thiourea*

Yield = 82%; melting point = 52.8–54.1°C. The product was purified by column chromatography, using petroleum ether 60–80°C/ethyl acetate 3:2 as eluent ($R_f$ = 0.5).

$^1$H-NMR ($CDCl_3$): 1.15–1.28 ppm (m, 6H, 2 x $CH_3$-$CH_2$-O), 2.39 ppm (s, 3H, pyridine-$CH_3$), 2.79–2.88 ppm (m, 2H, S-$CH_2$-C-N), 3.89–3.96 ppm (m, 2H, S-C-$CH_2$-N), 4.02–4.18 ppm (m, 6H, pyridine-$CH_2$-S and 2 x $CH_3$-$CH_2$-O), 5.02 ppm (s, 1H, pyridine-$H_4$), 6.74 ppm (s, 1H, pyridine-N*H*), 7.10–7.90 ppm (m, 10H, 5 x benzoyl-*H* and 5 x phenyl-*H*), 9.03 ppm (bs, 1H, -C(S)-N*H*-C(O)-), 11.01 ppm (bs, 1H, S-C-C-N*H*-).

*N-Benzoyl-N'-{2-{[4-(2,3-dichlorophenyl)-3,5-diethoxycarbonyl-1,4-dihydro-6-methylpyridin-2-yl]methylthio}ethyl}thiourea*
Yield = 65%; melting point = 54.5–55.6°C. The product was purified by column chromatography, using petroleum ether 60–80°C/ethyl acetate 2:1 as eluent ($R_f$ = 0.5).

$^1$H-NMR ($CDCl_3$): 1.14–1.29 ppm (m, 6H, 2 x $CH_3$-$CH_2$-O), 2.37 ppm (s, 3H, pyridine-$CH_3$), 2.88 ppm (t, $J$ = 6.7 Hz, 2H, S-$CH_2$-C-N), 3.91–4.15 ppm (m, 8H, S-C-$CH_2$-N and pyridine-$CH_2$-S and 2 x $CH_3$-$CH_2$-O), 5.51 ppm (s, 1H, pyridine-$H_4$), 6.81 ppm (s, 1H, pyridine-N*H*), 7.06–7.08 ppm (m, 1H, phenyl-*H*), 7.22–8.08 ppm (m, 7H, 5 x benzoyl-*H* and 2 x phenyl-*H*), 9.06 ppm (bs, 1H, -C(S)-N*H*-C(O)-), 11.03 ppm (bs, 1H, S-C-C-N*H*-).

*N-Benzoyl-N'-{2-{[3,5-diethoxycarbonyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)pyridin-2-yl]methylthio}ethyl}thiourea*
Yield = 91%; melting point = 56.0–57.8°C. The product was purified by column chromatography, using petroleum ether 60–80°C/ethyl acetate 2:1 as eluent ($R_f$ = 0.3; diethyl ether as eluent).

$^1$H-NMR ($CDCl_3$): 1.17–1.30 ppm (m, 6H, 2 x $CH_3$-$CH_2$-O), 2.40 ppm (s, 3H, pyridine-$CH_3$), 2.87 ppm (t, $J$ = 7.3 Hz, 2H, S-$CH_2$-C-N), 3.85–4.22 ppm (m, 8H, S-C-$CH_2$-N and pyridine-$CH_2$-S and 2 x $CH_3$-$CH_2$-O), 5.10 ppm (s, 1H, pyridine-*H*), 7.03 ppm (s, 1H, pyridine-N*H*), 7.28–7.81 ppm (m, 7H, 5 x benzoyl-*H* and 2 x phenyl-*H*), 8.00–8.03 ppm (m, 1H, phenyl-*H*), 8.10 ppm (bs, 1H, phenyl-$H_2$), 9.04 ppm (bs, 1H, -C(S)-N*H*-C(O)-), 11.03 ppm (bs, 1H, S-C-C-N*H*-).

*N-Benzoyl-N'-{3-{[3,5-diethoxycarbonyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)pyridin-2-yl]methylthio}propyl}thiourea*
Yield = 65% (obtained as an oil). The product was purified by column chromatography, using ethyl acetate/dichloromethane 5:1 as eluent ($R_f$ = 0.8).

$^1$H-NMR ($CDCl_3$): 1.11–1.25 ppm (m, 6H, 2 x $CH_3$-$CH_2$-O), 1.83–1.87 ppm (m, 2H, C-$CH_2$-C), 2.25 ppm (s, 3H, pyridine-$CH_3$), 2.47–2.54 ppm (m, 2H, S-$CH_2$-C-C-N), 3.58–3.67 ppm (m, 2H, S-C-C-$CH_2$-N), 3.82–4.15 ppm (m, 6H, pyridine-$CH_2$-S and 2 x $CH_3$-$CH_2$-O), 5.03 ppm (s, 1H, pyridine-$H_4$), 7.04 ppm (s, 1H, pyridine-N*H*), 7.23–7.58 ppm (m, 5H, 3 x benzoyl-*H* and 2 x phenyl-*H*), 7.62–7.74 ppm (m, 2H, 2 x benzoyl-*H*), 7.83–7.86 ppm (m, 1H, phenyl-*H*), 8.00 ppm (s, 1H, phenyl-$H_2$), 9.13 ppm (bs, 0.9H, -C(S)-N*H*-C(O)-), 10.77 ppm (bs, 0.9H, S-C-C-C-N*H*-).

*N-Benzoyl-N'-{5-{[3,5-diethoxycarbonyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)pyridin-2-yl]methylthio}pentyl} thiourea*
Yield = 83% (obtained as an oil). The product was purified by column chromatography, using ethyl acetate/dichloromethane 1:1 as eluent ($R_f$ = 0.9).

$^1$H-NMR ($CDCl_3$): 1.22 ppm (t, $J$ = 7.1 Hz, 3H, $CH_3$-$CH_2$-O), 1.23 ppm (t, $J$ = 7.1 Hz, 3H, $CH_3$-$CH_2$-O), 1.40–1.52 ppm (m, 2H, C-C-$CH_2$-C-C), 1.60–1.75 ppm (m, 4H, S-C-$CH_2$-C-$CH_2$-C-N), 2.41 ppm (s, 3H, pyridine-$CH_3$), 2.51 ppm (t, $J$ = 7.1 Hz, 2H, S-$CH_2$-$(C)_4$-N), 3.65 ppm (t, $J$ = 6.8 Hz, 2H, S-$(C)_4$-$CH_2$-N), 3.98 and 4.17 ppm (AB, $J_{AB}$ = 14.8Hz, 2H, pyridine-$CH_2$-S), 3.92–4.08 ppm (m, 4H, 2 x $CH_3$-$CH_2$-O), 5.14 ppm (s, 1H, pyridine-$H_4$), 7.10 ppm (s, 1H, pyridine-N*H*), 7.35–7.39 ppm (m, 1H, phenyl-*H*), 7.43–7.86 ppm (m, 6H, 5 x benzoyl-*H* and phenyl-*H*), 8.00–8.03 ppm (m, 1H, phenyl-*H*), 8.11–8.16 ppm (m, 1H, phenyl-$H_2$), 9.12 ppm (bs, 1H, -C(S)-N*H*-C(O)-), 10.78 ppm (bs, 1H, S-$(C)_5$-N*H*-).

*N-Benzoyl-N'-{6-{[3,5-diethoxycarbonyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)pyridin-2-yl]methylthio}hexyl}thiourea*
Yield = 61% (obtained as an oil). The product was purified by column chromatography, using ethyl acetate/dichloromethane 1:1 as eluent ($R_f$ = 0.8).

$^1$H-NMR ($CDCl_3$): 1.13–1.58 ppm (m, 14H, 2 x $CH_3$-$CH_2$-O and S-C-$(CH_2)_4$-C-N), 2.31 ppm (s, 3H, pyridine-$CH_3$), 2.44–2.55 ppm (m, 2H, S-$CH_2$-$(C)_5$-N), 3.63–3.71 ppm (m, 2H, S-$(C)_5$-$CH_2$-N), 3.93–4.04 ppm (m, 6H, pyridine-$CH_2$-S and 2 x $CH_3$-$CH_2$-O), 5.02 ppm (s, 1H, pyridine-$H_4$), 7.07 ppm (s, 1H, pyridine-N*H*), 7.25–7.83 ppm (m, 7H, 5 x benzoyl-*H* and 2 x phenyl-*H*), 8.00–8.04 ppm (m, 1H, phenyl-*H*), 8.09 ppm (bs, 1H, phenyl-$H_2$), 9.07 ppm (bs, 1H, -C(S)-N*H*-C(O)-), 10.75 ppm (bs, 0.9H, S-$(C)_5$-N*H*-).

*N-{ω-{[3,5-Diethoxycarbonyl-1,4-dihydro-6-methyl-4-(substituted-phenyl)pyridin-2-yl]methylthio}alkyl}-S-ethylisothiourea HBr*
A sample of 10 mmol $K_2CO_3$ in 40 ml water was added to a solution of 10 mmol *N*-benzoyl-*N'*-{ω-{[3,5-diethoxycarbonyl-1,4-dihydro-6-methyl-4-(substituted-phenyl)pyridin-2-yl]methylthio}alkyl}thiourea in 135 ml ethanol. The reaction mixture was refluxed for 45 min. The reaction mixture was then diluted with 270 ml water and extracted 6 times with 50 ml dichloromethane. The organic layer was dried and evaporated. The residue was dissolved in 110 ml ethanol and 0.1 mol ethyl bromide was added. The reaction mixture was stirred overnight followed by 3 h refluxing. The solvent was evaporated and the residue was washed 5 times with 100 ml hot diethyl ether.

*N-{2-[(3,5-Diethoxycarbonyl-1,4-dihydro-6-methyl-4-phenylpyridin-2-yl)methylthio]ethyl}-S-ethylisothiourea HBr*
Yield = 57% (obtained as an oil). The product was purified by column chromatography, using ethyl acetate/methanol 4:1 as eluent ($R_f$ = 0.6).

$^1$H-NMR (DMSO-$d_6$): 1.13 ppm (t, $J$ = 7.0 Hz, 6H, 2 x $CH_3$-$CH_2$-O), 1.26 ppm (t, $J$ = 7.3 Hz, 3H, S-$CH_2$-$CH_3$), 2.28 ppm (s, 3H, pyridine-$CH_3$), 2.69–2.80 ppm (m, 2H, S-$CH_2$-C-N), 3.19 ppm (q, $J$ = 7.3 Hz, 2H, S-$CH_2$-$CH_3$), 3.45–3.54 ppm (m, 2H, S-C-$CH_2$-N), 3.83–4.06 ppm (m, 6H, pyridine-$CH_2$-S and 2 x $CH_3$-$CH_2$-O), 4.89 ppm (s, 1H, pyridine-$H_4$), 7.10–7.26 ppm (m, 5H, 5 x phenyl-*H*), 8.97 ppm (s, 1H, pyridine-N*H*), 9.26 ppm (bs, 2H, 2 x N*H*).

*N-{2-[3,5-Diethoxycarbonyl-1,4-dihydro-6-methyl-4-(2,3-dichlorophenyl)pyridin-2-yl]methylthio}ethyl}-S-ethylisothiourea HBr*
Yield = 56%; melting point = 84.1–85.3°C. The product was purified by column chromatography, using ethyl acetate/methanol 1:1 as eluent ($R_f$ = 0.7).

$^1$H-NMR($CDCl_3$): 1.15–1.22 ppm (m, 6H, 2 x $CH_3$-$CH_2$-O), 1.41 ppm (t, $J$ = 7.4 Hz, 3H, S-$CH_2$-$CH_3$), 2.42 ppm (s, 3H, pyridine-$CH_3$), 2.80–2.87 ppm (m, 2H, S-$CH_2$-C-N), 3.38–3.57 ppm (m, 4H, S-C-$CH_2$-N and S-$CH_2$-$CH_3$), 3.97–4.12 ppm (m, 6H, pyridine-$CH_2$-S and 2 x $CH_3$-$CH_2$-O), 5.48 ppm (s, 1H, pyridine-$H_4$), 7.03–7.36 ppm (m, 3H, 3 x phenyl-*H*), 7.71–8.15 ppm (bs, 1.4H, N*H*), 8.91 ppm (bs, 0.7H, N*H*), 9.50 ppm (bs, 1H, S-C-C-N*H*-).

*N-{2-{[3,5-Diethoxycarbonyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)pyridin-2-yl]methylthio}ethyl}-S-ethylisothiourea HBr*
***VUF 9113***
Yield = 90%; melting point = 73.2–74.1°C.

$^1$H-NMR ($CDCl_3$): 1.12–1.28 ppm (m, 6H, 2 x $CH_3$-$CH_2$-O), 1.39 ppm (t, $J$ = 7.3 Hz, 3H, S-$CH_2$-$CH_3$), 2.45 ppm (s, 3H,

pyridine-$CH_3$), 2.78–2.83 ppm (m, 2H, S-$CH_2$-C-N), 3.36 ppm (q, $J$ = 7.3 Hz, 3H, S-$CH_2$-$CH_3$), 3.88–4.33 ppm (m, 8H, S-C-$CH_2$-N and pyridine-$CH_2$-S and 2 x $CH_3$-$CH_2$-O), 5.10 ppm (s, 1H, pyridine-$H_4$), 7.39–7.42 ppm (m, 1H, phenyl-$H_5$), 7.63–7.67 ppm (m, 1H, phenyl-$H_6$ or $H_4$), 7.95 ppm (bs, 1H, pyridine-N*H*), 8.00–8.03 ppm (m, 1H, phenyl-$H_4$ or $H_6$), 8.30 ppm (bs, 1H, N*H*), 8.49–8.84 ppm (bs, 1.4H, N*H*), 8.93 ppm (s, 0.9H, N*H*), 9.51 ppm (bs, 0.6H, S-C-C-N*H*-).

*N-{3-{[3,5-Diethoxycarbonyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)pyridin-2-yl]methylthio}propyl}-S-ethylisothiourea HBr* ***VUF 9110***

Yield = 66%; melting point = 65.6–67.9°C. The product was purified by column chromatography, using ethyl acetate/methanol 7:3 as eluent ($R_f$ = 0.6).

$^1$H-NMR ($CDCl_3$): 1.00–1.49 ppm (m, 9H, 2 x $CH_3$-$CH_2$-O and S-$CH_2$-$CH_3$), 1.77–1.85 ppm (m, 2H, C-$CH_2$-C), 2.33 ppm (s, 3H, pyridine-$CH_3$), 2.58–2.66 ppm (m, 2H, S-$CH_2$-C-C-N), 3.12–3.57 ppm (m, 4H, S-C-C-$CH_2$-N and S-$CH_2$-$CH_3$), 3.78–4.17 ppm (m, 6H, pyridine-$CH_2$-S and 2 x $CH_3$-$CH_2$-O), 5.00 ppm (s, 1H, pyridine-$H_4$), 7.38 ppm (bs, 1H, phenyl-*H*), 7.60 ppm (bs, 1H, phenyl-*H*), 7.90–7.93 ppm (m, 1H, phenyl-*H*), 8.01–8.03 ppm (m, 1H, phenyl-*H*), 8.27–8.65 ppm (m, 1.9H, pyridine-N*H* and N*H*), 9.26 ppm (bs, 1H, S-$(C)_5$-N*H*-).

*N-{5-{[3,5-Diethoxycarbonyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)pyridin-2-yl]methylthio}pentyl}-S-ethylisothiourea HBr*

Yield = 75%; melting point = < 38°C (hygroscopic). The product was purified by column chromatography, using petroleum ether 60–80°C/ethyl acetate 7:3 as eluent ($R_f$ = 0.7).

$^1$H-NMR ($CDCl_3$): 1.23 ppm (t, $J$ = 7.1 Hz, 3H, $CH_3$-$CH_2$-O), 1.24 ppm (t, $J$ = 7.0 Hz, 3H, $CH_3$-$CH_2$-O), 1.43–1.62 ppm (m, 9H, C-$CH_2$-$CH_2$-$CH_2$-C and S-$CH_2$-$CH_3$), 2.44 ppm (s, 3H, pyridine-$CH_3$), 2.55 ppm (t, $J$ = 6.7 Hz, 2H, S-$CH_2$-$(C)_4$-N), 3.30–3.41 ppm (m, 4H, S-$(C)_4$-$CH_2$-N and S-$CH_2$-$CH_3$), 3.95 and 4.18 ppm (AB, $J_{AB}$ = 15.1 Hz, 2H, pyridine-$CH_2$-S), 4.01–4.12 ppm (m, 4H, 2 x $CH_3$-$CH_2$-O), 5.12 ppm (s, 1H, pyridine-$H_4$), 7.40 ppm (bs, 1H, phenyl-*H*), 7.66–7.68 ppm (m, 1H, phenyl-*H*), 8.00–8.02 ppm (m, 1H, phenyl-*H*), 8.11–8.14 ppm (m, 1H, phenyl-$H_2$), 8.35–8.73 ppm (bs, 2H, pyridine-N*H* and N*H*), 9.20 ppm (bs, 1H, S-$(C)_5$-N*H*-).

*N-{6-{[3,5-Diethoxycarbonyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)pyridin-2-yl]methylthio}hexyl}-S-ethylisothiourea HBr*

Yield = 68% obtained as an oil (very hygroscopic). The product was purified by column chromatography, using petroleum ether 60:80°C/ethyl acetate 7:3 as eluent.

$^1$H-NMR ($CDCl_3$): 1.13–1.24 ppm (m, 6H, 2 x $CH_3$-$CH_2$-O), 1.39–1.76 ppm (m, 11H, S-C-$(CH_2)_4$-C-N and S-$CH_2$-$CH_3$), 2.36 ppm (s, 3H, pyridine-$CH_3$), 2.49–2.55 ppm (m, 2H, S-$CH_2$-$(C)_5$-N), 3.27–3.44 ppm (m, 4H, S-$(C)_5$-$CH_2$-N and S-$CH_2$-$CH_3$), 3.88–4.22 ppm (m, 6H, pyridine-$CH_2$-S and 2 x $CH_3$-$CH_2$-O), 5.07 ppm (s, 1H, pyridine-$H_4$), 7.40 ppm (bs, 1H, phenyl-*H*), 7.63–7.65 ppm (m, 1H, phenyl-*H*), 7.98 ppm (bs, 1H, phenyl-*H*), 8.05–8.08 ppm (m, 1H, phenyl-*H*), 8.41–8.81 ppm (m, 2.1H, pyridine-N*H* and N*H*), 9.37 ppm (m, 1H, S-$(C)_6$-N*H*-).

*N-{ω-{[3,5-Diethoxycarbonyl-1,4-dihydro-6-methyl-4-(substituted-phenyl)pyridin-yl]methylthio}alkyl}-N'-[3-(imidazol-4(5)-yl)propyl]guanidine 2HBr*

A mixture of 5 mmol 4(5)-(3-aminopropyl)imidazole and 5 mmol *N*-{ω-{[3,5-diethoxycarbonyl-1,4-dihydro-6-methyl-4-(substituted-phenyl)pyridin-2-yl)methylthio]}alkyl}-*S*-ethylisothiourea was refluxed in 150 ml pyridine for 72 h. The pyridine was then evaporated and the residue was 3 times coevaporated with ethanol. The residue was then crystallized from ethyl acetate and the precipitate was filtered off. The filtrate was purified by column chromatography, using ethyl acetate/ethanol 4:1 as eluent. The obtained fractions were crystallized from ethyl acetate/methanol and the precipitate was filtered off. The product was obtained as a foam by evaporating the solvent.

*N-{[2-(3,5-Diethoxycarbonyl-1,4-dihydro-6-methyl-4-phenylpyridin-2-yl)methylthio]ethyl}-N'-[3-(imidazol-4(5)-yl)propyl]guanidine 2HBr* ***VUF 4572***

Yield = 17%; melting point = < 30°C; Mass spectrum, thioglycerol as matrix ($FAB^+$) 555 $[M + H]^+$, ($FAB^-$) 553 $[M - H]^-$.

$^1$H-NMR (DMSO-$d_6$): 1.05–1.20 ppm (m, 6H, $CH_3$-$CH_2$-O), 1.66–1.78 ppm (m, 5H, N-C-$CH_2$-C-imidazole and $CH_3$-COO), 2.30 ppm (s, 3H, pyridine-$CH_3$), 2.47–2.53 ppm (m, 2H, N-C-C-$CH_2$-imidazole), 2.69 ppm (t, $J$ = 6.9 Hz, 2H, S-$CH_2$-C-N), 3.12 and 3.44 ppm (m, 4H, S-C-$CH_2$-N and N-$CH_2$-C-C-imidazole), 3.81 and 4.07 ppm (AB, $J_{AB}$ = 13.8 Hz, 2H, pyridine-$CH_2$-S), 3.93–4.04 ppm (m, 4H, 2 x $CH_3$-$CH_2$-O), 4.88 ppm (s, 1H, pyridine-$H_4$), 6.75 ppm (bs, 1H, imidazole-*H*), 7.02–7.28 ppm (m, 5H, 5 x phenyl-*H*), 7.52 ppm (bs, 1H, imidazole-*H*).

*N-{2-{[3,5-Diethoxycarbonyl-1,4-dihydro-6-methyl-4-(2,3-dichlorophenyl)pyridin-2-yl]methylthio}ethyl}-N'-[3-(imidazol-4(5)-yl)propyl]guanidine 2HBr* ***VUF 9160***

Yield = 24%; melting point = 97.9–101.1°C; Mass spectrum, glycerol/ammonium acetate as matrix ($FAB^+$) 625 $[M + H]^+$, ($FAB^-$) 623 $[M - H]^-$.

$^1$H-NMR (DMSO-$d_6$): 1.08 ppm (t, $J$ = 7.1 Hz, 6H, 2 x $CH_3$-$CH_2$-O), 1.67–1.77 ppm (m, 2H, N-C-$CH_2$-C-imidazole), 1.82 ppm (s, 3H, $CH_3$-COO), 2.30 ppm (s, 3H, pyridine-$CH_3$), 2.47–2.53 ppm (m, 2H, N-C-C-$CH_2$-imidazole), 2.61–2.69 ppm (m, 2H, S-$CH_2$-C-N), 3.10 and 3.30 ppm (m, 4H, S-$(C)_4$-$CH_2$-N and N-$CH_2$-C-C-imidazole), 3.88 and 4.04 ppm (AB, $J_{AB}$ = 13.3 Hz, 2H, pyridine-$CH_2$-S), 3.91–4.03 ppm (m, 4H, 2 x $CH_3$-$CH_2$-O), 5.35 ppm (s, 1H, pyridine-$H_4$), 6.77 ppm (bs, 1H, imidazole-*H*), 7.21–7.42 ppm (m, 3H, 3 x phenyl-*H*), 7.54 ppm (bs, 1H, imidazole-*H*), 7.90 ppm (bs, 0.6H, N*H*), 9.36 ppm (bs, 0.6H, N*H*).

*N-{2-{[3,5-Diethoxycarbonyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)pyridin-2-yl]methylthio}ethyl}-N'-[3-(imidazol-4(5)-yl)propyl]guanidine 2HBr* ***VUF 9065***

Yield = 21%; melting point = 76.5–77.1°C; Mass spectrum, glycerol/ammonium acetate as matrix ($FAB^+$) 600 $[M + H]^+$, ($FAB^-$) 598 $[M - H]^-$, 678/680 $[M + Br]^-$.

$^1$H-NMR (DMSO-$d_6$): 1.00–1.13 ppm (m, 6H, $CH_3$-$CH_2$-O), 1.46–1.87 ppm (m, 2H, N-C-$CH_2$-C-imidazole), 2.32 ppm (s, 3H, pyridine-$CH_3$), 2.45–2.52 ppm (m, 4H, S-$CH_2$-C-N and N-C-C-$CH_2$-imidazole), 2.70 and 3.20 ppm (m, 4H, S-C-$CH_2$-N and N-$CH_2$-C-C-imidazole), 3.92 and 4.18 ppm (AB, $J_{AB}$ = 13.2 Hz, 2H, pyridine-$CH_2$-S), 3.97–4.12 ppm (m, 4H, 2 x $CH_3$-$CH_2$-O), 4.98 ppm (s, 1H, pyridine-$H_4$), 6.82 ppm (bs, 1H, imidazole-*H*), 7.40–7.73 ppm (m, 6H, 3 x phenyl-*H* and 3 x N*H*), 7.87 ppm (bs, 0.7H, pyridine-N*H*), 8.01–8.06 ppm (m, 2H, imidazole-*H* and phenyl-*H*), 9.30 ppm (bs, 0.7H, imidazole-N*H*).

*N-{3-{[3,5-Diethoxycarbonyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)pyridin-2-yl]methylthio}propyl}-N'-3-(imidazol-4(5)-yl)propyl]guanidine 2HBr* ***VUF 4570***

Yield = 20%; melting point = 87.3–88.2°C; Mass spectrum, thioglycerol as matrix ($FAB^+$) 614 $[M + H]^+$, ($FAB^-$) 612 $[M - H]^-$, 692/694 $[M + Br]^-$.

$^1$H-NMR (DMSO-$d_6$): 1.12 ppm (t, $J$ = 7.0 Hz, 3H, C$H_3$-$CH_2$-O), 1.14 ppm (t, $J$ = 7.0 Hz, 3H, C$H_3$-$CH_2$-O), 1.68–1.83 ppm (m, 4H, S-C-C$H_2$-C-N and N-C-C$H_2$-C-imidazole), 2.32 ppm (s, 3H, pyridine-C$H_3$), 2.50–2.63 ppm (m, 4H, S-C$H_2$-C-C-N and N-C-C-C$H_2$-imidazole), 3.11–3.24 ppm (m, 4H, S-C-C-C$H_2$-N and N-C$H_2$-C-C-imidazole), 3.89 and 4.08 ppm (AB, $J_{AB}$ = 13.0 Hz, 2H, pyridine-C$H_2$-S), 3.93–4.06 ppm (m, 4H, 2 x $CH_3$-C$H_2$-O), 5.00 ppm (s, 1H, pyridine-$H_4$), 6.85 ppm (s, 1H, imidazole-*H*), 7.52–7.65 ppm (m, 6.75H, 3 x phenyl-*H* and 3.75 x N*H*), 8.02–8.11 ppm (m, 2H, imidazole-*H* and phenyl-*H*), 9.25 ppm (bs, 1H, N*H*).

$^{13}$C-NMR (DMSO-$d_6$): 13.73 and 13.84 ppm (q, 2 x C$H_3$-$CH_2$-O), 18.03 ppm (q, pyridine-C$H_3$), 22.94 and 28.19 ppm (t, S-C$H_2$-C-C-N and N-C-C-C$H_2$-imidazole), 28.54 and 29.43 ppm (t, S-C-C$H_2$-C-N and N-C-C$H_2$-C-imidazole), 39.05 ppm (d, pyridine-$C_4$), 39.77 and 40.16 (t, S-C-C-C$H_2$-N and N-C$H_2$-C-C-imidazole), 55.77 ppm (t, pyridine-C$H_2$-S), 59.08 and 59.40 ppm (t, 2 x $CH_3$-C$H_2$-O), 100.49 and 101.34 ppm (s, pyridine-$C_3$ and $C_5$), 115.55 and 121.68 (d, 2 x imidazole-C*H*), 121.04 and 129.41 and 133.96 and 134.02 ppm (d, 4 x phenyl-C*H* [$C_2$, $C_4$, $C_5$, $C_6$]), 136.04 ppm (s, imidazole-*C*), 146.40 and 147.21 and 147.67 and 149.71 ppm (s, phenyl-$C_1$ and $C_3$ and pyridine-$C_2$ and $C_6$), 155.56 ppm (s, guanidine-*C*), 165.87 and 166.13 ppm (s, 2 x carbonyl-*C*).

*N-{5-{[3,5-Diethoxycarbonyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)pyridin-2-yl]methylthio}pentyl}-N'-[3-(imidazol-4(5)-yl)propyl]guanidine 2HBr* ***VUF 4573***

Yield = 33%; melting point = < 40°C (hygroscopic); Mass spectrum, thioglycerol as matrix (FAB$^+$) 642 [M + H]$^+$, (FAB$^-$) 640 [M – H]$^-$, 720/722 [M + Br]$^-$.

$^1$H-NMR (DMSO-$d_6$): 1.11 ppm (t, $J$ = 7.1 Hz, 3H, C$H_3$-$CH_2$-O), 1.13 ppm (t, $J$ = 7.1 Hz, 3H, C$H_3$-$CH_2$-O), 1.20–1.29 ppm (m, 2H, C-C-C$H_2$-C-C), 1.38–1.49 ppm (m, 4H, C-C$H_2$-C-C$H_2$-C), 1.72–1.78 ppm (m, 2H, N-C-C$H_2$-C-imidazole), 2.30 ppm (s, 3H, pyridine-C$H_3$), 2.46–2.61 ppm (m, 4H, S-C$H_2$-$(C)_4$-N and N-C-C-C$H_2$-imidazole), 3.08–3.19 ppm (m, 4H, S-$(C)_4$-C$H_2$-N and N-C$H_2$-C-C-imidazole), 3.79 and 4.03 ppm (AB, $J_{AB}$ = 13.2 Hz, 2H, pyridine-C$H_2$-S), 3.88–4.00 ppm (m, 4H, 2 x $CH_3$-C$H_2$-O), 5.00 ppm (s, 1H, pyridine-$H_4$), 6.94 ppm (bs, 1H, imidazole-*H*), 7.41–7.65 ppm (m, 6H, 2 x phenyl-*H* and 4 x N*H*), 7.85 ppm (d, $J$ = 0.9 Hz, 1H, imidazole-*H*), 7.98–8.06 ppm (m, 2H, 2 x phenyl-*H*), 9.16 ppm (bs, 1H, S-$(C)_5$-N*H*-)

$^{13}$C-NMR (DMSO-$d_6$): 13.73 and 13.82 ppm (q, 2 x C$H_3$-$CH_2$-O), 18.00 ppm (q, pyridine-C$H_3$), 22.59 ppm (t, C-C-C$H_2$-imidazole), 25.18 ppm (t, C-C-C$H_2$-C-C), 27.99 ppm (t, C-C$H_2$-C-imidazole), 28.68 and 29.48 ppm (t, C-C$H_2$-C-C$H_2$-C), 30.91 ppm (t, S-C$H_2$-$(C)_4$-N), 39.04 ppm (d, pyridine-$C_4$), 40.07 and 40.55 ppm (t, 2 x -C$H_2$-N), 56.08 ppm (t, pyridine-C$H_2$-S), 59.07 and 59.36 ppm (t, 2 x $CH_3$-C$H_2$-O), 100.03 and 101.39 ppm (s, pyridine-$C_3$ and $C_5$), 115.49 ppm (d, imidazole-C*H*), 121.00 ppm (d, imidazole-C*H*), 121.65 and 129.35 and 133.91 and 134.17 ppm (d, 4 x phenyl-C*H* [$C_2$, $C_4$, $C_5$, $C_6$]), 136.20 ppm (s, imidazole-*C*), 146.44 and 147.25 and 147.64 and 149.70 ppm (s, phenyl-$C_1$ and $C_3$ and pyridine-$C_2$ and $C_6$), 155.49 ppm (s, guanidine-*C*), 165.91 and 166.13 ppm (s, 2 x carbonyl-*C*).

*N-{6-{[3,5-Diethoxycarbonyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)pyridin-2-yl]methylthio}hexyl}-N'-[3-(imidazol-4(5)-yl)propyl]guanidine 2HBr* ***VUF 4575***

Yield = 27%; melting point = < 40°C (hygroscopic); Mass spectrum, 3-nitrobenzyl alcohol as matrix (FAB$^+$) 656 [M + H]$^+$, (FAB$^-$) 654 [M – H]$^-$, 734/736 [M + Br]$^-$.

$^1$H-NMR (DMSO-$d_6$): 1.13 ppm (t, $J$ = 7.1 Hz, 3H, C$H_3$-$CH_2$-O), 1.14 ppm (t, $J$ = 7.1 Hz, 3H, C$H_3$-$CH_2$-O), 1.22–1.44 ppm (m, 8H, S-C-$(CH_2)_4$-C-N), 1.46–1.57 ppm (m, 2H, N-C-C$H_2$-C-imidazole), 2.32 ppm (s, 3H, pyridine-C$H_3$), 2.48–2.58 ppm (m, 4H, S-C$H_2$-$(C)_5$-N and N-C-C-C$H_2$-imidazole), 3.08–3.27 ppm (m, 4H, S-$(C)_5$-C$H_2$-N and N-C$H_2$-C-C-imidazole), 3.64 and 4.02 ppm (AB, $J_{AB}$ = 13.2 Hz, 2H, pyridine-C$H_2$-S), 3.87–4.00 ppm (m, 4H, 2 x $CH_3$-C$H_2$-O), 5.01 ppm (s, 1H, pyridine-$H_4$), 6.85 ppm (s, 1H, imidazole-*H*), 7.46–7.63 ppm (m, 7H, 3 x phenyl-*H* and 4 x N*H*), 8.02–8.11 ppm (m, 2H, imidazole-*H* and phenyl-*H*), 9.20 ppm (bs, 1H, N*H*).

*N-{ω-{[2-(3,5-Diethoxycarbonyl-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]}alkyl}-N'-[3-(imidazol-4(5)-yl)propyl]guanidine 2HBr*

The *N*-{ω-{[2-(3,5-diethoxycarbonyl-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]}alkyl}-*N'*-[3-(imidazol-4(5)-yl)propyl]guanidine hydrobromide derivatives were synthesized according to the procedure described for the *N*-{ω-{[3,5-diethoxycarbonyl-1,4-dihydro-6-methyl-4-(substitutedphenyl)pyridin-2-yl]methylthio}alkyl}-*N'*-[3-(imidazol-4(5)-yl)propyl]guanidine hydrobromide derivatives, starting from the *N*-{ω-{[2-(3,5-diethoxycarbonyl-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]}alkyl}-*S*-ethylisothiourea hydrobromide derivatives described by Christiaans *et al* [25].

*N-{3-{[2-(3,5-Diethoxycarbonyl-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]}propyl}-N'-[3-(imidazol-4(5)-yl)propyl]guanidine 2HBr* ***VUF 4588***

Yield = 17%; melting point = 103.0–104.3°C; Mass spectrum, thioglycerol as matrix (FAB$^+$) 553 [M + H]$^+$, (FAB$^-$) 551 [M – H]$^-$, 631/633 [M + Br]$^-$.

$^1$H-NMR (DMSO-$d_6$): 1.01–1.13 ppm (m, 6H, 2 x C$H_3$-$CH_2$-O), 1.79–1.97 ppm (m, 4H, O-C-C$H_2$-C-N and N-C-C$H_2$-C-imidazole), 2.22 ppm (s, 6H, 2 x pyridine-C$H_3$), 2.59 ppm (t, 2H, N-C-C-C$H_2$-imidazole), 3.22–3.36 ppm (m, 4H, O-C-C-C$H_2$-N and N-C$H_2$-C-C-imidazole), 3.92–4.05 ppm (m, 6H, 2 x $CH_3$-C$H_2$-O and O-C$H_2$-C-C-N), 5.14 ppm (s, 1H, pyridine-$H_4$), 6.74–7.11 ppm (m, 5H, 4 x phenyl-*H* and N*H*), 7.54–7.70 ppm (m, 5H, imidazole-*H* [$J$ = 0.95 Hz] and 4 x N*H*), 8.76 ppm (s, 1H, imidazole-*H*).

*N-{5-{[2-(3,5-Diethoxycarbonyl-2 ,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]}pentyl}-N'-[3-(imidazol-4(5)-yl)propyl]guanidine 2HBr* ***VUF 4612***

Yield = 28%; melting point = 91.8–93.3°C; Mass spectrum, 3-nitrobenzyl alcohol as matrix (FAB$^+$) 581 [M + H]$^+$, (FAB$^-$) 579 [M – H]$^-$, 659/661 [M + Br]$^-$.

$^1$H-NMR ($CD_3OD$): 1.10–1.21 ppm (m, 6H, 2 x C$H_3$-$CH_2$-O), 1.56–2.00 ppm (m, 8H, O-C-$(CH_2)_3$-C-N and N-C-C$H_2$-C-imidazole), 2.24 ppm (s, 6H, 2 x pyridine-C$H_3$), 2.71 ppm (t, $J$ = 7.5 Hz, 2H, N-C-C-C$H_2$-imidazole), 3.26 ppm (2 x t, each $J$ = 7.0 Hz, 4H, O-$(C)_4$-C$H_2$-N and N-C$H_2$-C-C-imidazole), 3.90–4.04 ppm (m, 6H, 2 x $CH_3$-C$H_2$-O and O-C$H_2$-$(C)_4$-N), 5.16 ppm (s, 1H, pyridine-$H_4$), 6.71–6.84 ppm (m, 2H, 2 x phenyl-*H*), 7.02–7.16 ppm (m, 3H, 2 x phenyl-*H* and 1 x imidazole-*H*), 7.99 ppm (s, 1H, imidazole-*H*).

*N-{6-{[2-(3,5-Diethoxycarbonyl-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy]hexyl}-N'-[3-(imidazol-4(5)-yl)propyl]guanidine 2HBr* ***VUF 4730***

Yield = 21%; melting point = 83.1–85.3°C; Mass spectrum, 3-nitrobenzyl alcohol as matrix (FAB$^+$) 595 [M + H]$^+$, (FAB$^-$) 593 [M – H]$^-$, 673/675 [M + Br]$^-$.

$^1$H-NMR ($CD_3OD$): 1.10–1.17 ppm (m, 6H, 2 x C$H_3$-$CH_2$-O), 1.45–1.97 ppm (m, 10H, O-C-$(CH_2)_4$-C-N and N-C-C$H_2$-C-

imidazole), 2.23 ppm (s, 6H, 2 x pyridine-$CH_3$), 2.65 ppm (t, $J$ = 7.3 Hz, 2H, N-C-C-$CH_2$-imidazole), 3.23 ppm (2 x t, each $J$ = 7.0 Hz, 4H, O-$(C)_5$-$CH_2$-N and N-$CH_2$-C-C-imidazole), 3.89–4.04 ppm (m, 6H, 2 x $CH_3$-$CH_2$-O and O-$CH_2$-$(C)_5$-N), 5.16 ppm (s, 1H, pyridine-$H_4$), 6.71–6.84 ppm (m, 3H, phenyl-$H$ and 1 x imidazole-$H$), 7.01–7.17 ppm (m, 2H, 2 x phenyl-$H$), 7.59 ppm (d, $J$ = 1.0 Hz, 1H, imidazole-$H$).

## Acknowledgments

The results described originate from a research project which was financially supported by Cedona Pharmaceuticals BV, Haarlem, The Netherlands. MEJ Veerman (MSc), JF van der Werf, GJ Sterk and MP Caldirola and HP Voss (MSc) are kindly acknowledged for their help in carrying out certain pharmacological studies. We are grateful to BLM van Baar for measuring the mass spectrometric data.

## 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) *In: Clinical Use of Calcium-Channel Antagonist Drugs* (2nd ed) Kluwer Acad Publ, London
4 Naylor WG (1992) *Biochem Pharmacol* 43, 39–46
5 Van Zwieten PA, Pfaffendorf M (1993) *J Hypertension* 11, S3–S11
6 Arrowsmith JE, Campbell SF, Cross PE *et al* (1986) *J Med Chem* 29, 1696–1702
7 Gandolfi AC, Figerio M, Spinelli S, Tofanetti O, Tognella S (1987) *PCT/EP86/00445*
8 Kwon YW, Zhong Q, Wei XY, Zheng W, Triggle DJ (1990) *Arch Pharmacol* 341, 128–136
9 Bristow HR, Ginsburg R, Minobe WBS *et al* (1982) *New Engl J Med* 307, 205–211
10 Baumann G, Buschauer A, Permanetter B, Schunack W (1988) *Inn Mediz* 15, 30–38
11 Durant GJ, Duncan WAM, Ganellin CR, Parsons ME, Blakemore RC, Rasmussen AC (1978) *Nature (Lond)* 276, 403–405
12 Permanetter B, Baumann G, Dörner J, Schunack W, Blömer H (1985) *Agents Actions* 16, 215–218
13 Baumann G, Permanetter B, Wirtzfeld A (1984) *Pharmacol Ther* 24, 165–177
14 Van der Goot H, Bast A, Timmerman H (1991) *In: Handbook of Experimental Pharmacology; Histamine and Histamine Antagonists* (Uvnäs B, Ed) Springer-Verlag, Berlin Heidelberg, Vol 97, 573–748
15 Sterk GJ, Van der Goot H, Timmerman H (1986) *Eur J Med Chem* 21, 305–309
16 Sterk GJ, Koper J, Van der Goot H, Timmerman H (1987) *Eur J Med Chem* 22, 491–498
17 Buschauer A (1989) *J Med Chem* 32, 1963–1970
18 Baumann G, Buschauer A, Felix S (1992) *In: Agents & Actions, Special Conference Issue*, Birkhäuser Verlag, Basel, 329–332
19 Felix SB, Buschauer A, Baumann G (1991) *Agents Actions Suppl* 33, 257–269
20 Buschauer A, Baumann G (1991) *Agents Actions Suppl* 33, 231–256
21 Ganellin CR (1992) *In: The Histamine receptor; Pharmacochemistry of $H_1$ and $H_2$ Receptors* (Schwartz JC, Haas HL, eds) Wiley-Liss, New York, 1–56
22 Hill SJ (1990) *Pharmacol Rev* 42, 45–83
23 Christiaans JAM, Windhorst AD, Groenenberg PM, Van der Goot H, Timmerman H (1993) *Eur J Med Chem* 28, 859–867
24 Schickaneder H, Mörsdorf P, Buschauer A *et al* (1986) *EP 0 250 725 A1*
25 Christiaans JAM, Van der Goot H, Timmerman H (1993) *Eur J Med Chem* 28, 935–941
26 Bast A, Leurs R, Timmerman H (1987) *Drugs* 33 (Suppl 2), 67–74
27 Munson PJ, Rodbard D (1980) *Anal Biochem* 107, 220–239
28 Ruat M, Traiffort E, Bouthenet ML *et al* (1990) *Proc Natl Acad Sci USA* 87, 1658–1662
29 Kazda S, Garthoff B, Meyer H *et al* (1980) *Arzneim Forsch/Drug Res* 30, 2144–2164
30 Tenner TE Jr, McCully (1981) *Eur J Pharmacol* 73, 293-300
31 Rodenkirchen R, Bayer R, Steiner R, Bossert F, Meyer H, Möller E (1979) *Arch Pharmacol* 310, 69–78
32 Boyd RA, Giacomini JC, Wong FM, Nelson WL, Giacomini KM (1987) *J Pharmacol Exp Ther* 243, 118–125
33 Edgar B (1992) *Quant Struct-Act Relat* 11, 228–231