Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXI, No. 12

SYNTHESIS OF [^{3}H]-VUF 4576; A NEW RADIOLABELLED Ca^{2+}-ANTAGONIST

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Summary

The new radiolabelled [^{3}H]-VUF 4576 has been prepared by an easy procedure using commercially available C^3H_3I. The new radiolabelled ligand [^{3}H]-VUF 4576 belongs to a subclass of prenylamine analogues which interfere with calcium regulated pathways. The compound has been synthesized in order to study the interaction of other compounds with these mechanisms and in particular the site of action of this subclass of calcium entry blockers and calmodulin antagonists.

Key Words. Prenylamine, diphenylalkylamine, calcium antagonists, calmodulin antagonists, tritium labelling.

Introduction

Prenylamine is the lead structure from which we have developed a new series of diphenylalkylamines [1]. Together with cinnarizine, flunarizine and other diphenylalkylamines, prenylamine (scheme 1) is a member of the so-called class-III type of calcium antagonists [2] with a broad spectrum of activity because of their capacity to interfere with intracellular calcium dependent pathways as well. The structural variations in our series of prenylamine analogues (scheme 1) were introduced in order to analyse the structural requirements responsible for the potency and specificity towards the different interaction sites.

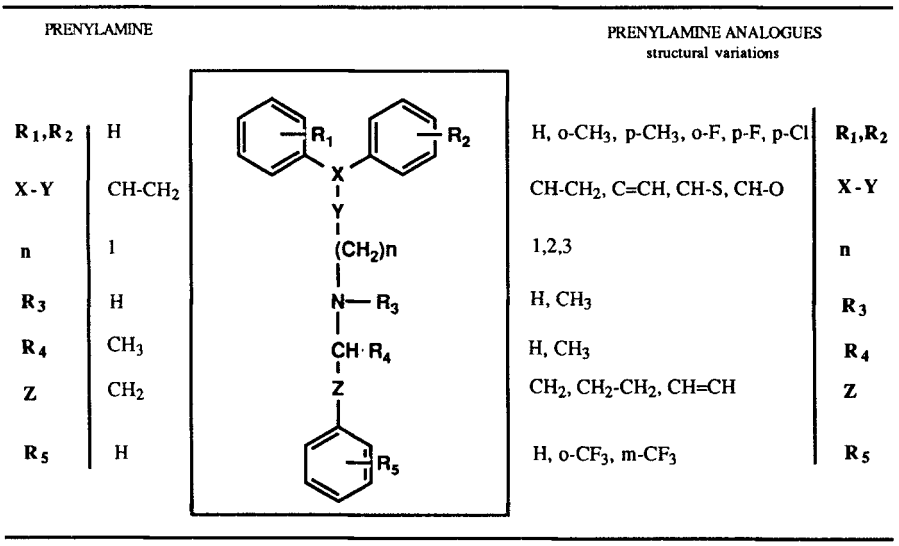
0362–4803/92/120987–07$08.50

Received 7 July, 1992
Revised 14 August, 1992

The calcium antagonistic profile of this class is currently under investigation [3]; they inhibit smooth muscle contractions. Consequently, we paid attention to their relatively high capacity to interfere with calmodulin [4], which modulates the calcium induced contractions responsible for the excitation-relaxation cycle in smooth muscle [5]. The key-role of the $4Ca^{2+}$-calmodulin complex in activating the contractile protein myosin in smooth muscles has been well documented. The interaction of this type of compound with calmodulin might be, at least partially, the basis for their pharmacological profile.

Because the interaction of calmodulin antagonists with calmodulin is largely dominated by hydrophobic forces [6], the lipophilic character of all these molecules has a positive influence on their level of activity. However, due to this lipophilicity, their mechanism of action can be masked by other non-specific effects, such as interactions with cell membranes. Consequently, the assessment of a clear pharmacological profile of such compounds is generally difficult.

SCHEME 1. General structure of diphenylalkylamine derivatives. Structural features of prenylamine and prenylamine analogues are presented on the right and left hand side of the scheme .



In the underlying study the synthesis (see scheme 2) of [^{3}H]-N-{2-[bis-(4-fluorophenyl)methylthio]ethyl}-N-methyl-2-[(3-trifluoromethyl)phenyl)] ethylamine, i.e. VUF 4576 as a representative compound of this class, is presented. Our new radiolabelled compound was synthesized from N-{2-[bis-(4-fluorophenyl)methylthio]ethyl}-2-(3-trifluoromethyl)phenylethylamine, VUF 9046. The isotopically labelled compound, [^{3}H]-VUF 4576, is not only a useful

tool for evaluating the mode of interaction with calmodulin, but it can also be used in pharmacokinetical studies, such as the determination of the distribution throughout the body and within the cells.

Results and Discussion

The choice of VUF 4576 as a representative compound of the prenylamine analogues series, was based on its potency to interfere with calmodulin. This was demonstrated by the inhibition of the calmodulin-dependent activation of phosphodiesterase [4]. Within this series, VUF 4576 combines the structural requirements for an optimal calmodulin antagonistic activity. In fact, from qualitative structure-activity relationship studies, we have discovered the importance of the fluorine substitution in *para* position of the benzhydryl moiety and the trifluoromethyl group in *meta* position of the single aromatic ring. The chiral centre as originally present in prenylamine was found to be not essential. Moreover, in those compounds in which the lower part (scheme 1) is linear, no difference in potency was detected between secondary and tertiary amines. This fact allowed the introduction of the label on the nitrogen atom in the secondary amine VUF 9046, using the commercially available C^3H_3I.

We were interested in preparing the compound with specific activity >30 Ci/mmol, in order to apply it in binding and pharmacokinetical studies. Therefore, the introduction of the N-[C^3H_3] group using C^3H_3I seemed to be the best and most straightforward choice.

To avoid a substantial quaternarization reaction, a high excess of the secondary amine was used (1000-fold).

The formation of the desired compound was checked by TLC using the previously synthesized unlabelled N-{2-[bis(4-fluorophenyl)methylthio]-ethyl}-N-methyl-[2-(m-trifluoromethyl)phenyl]ethylamine, VUF 4576, as reference; the isotopic effect due to the tritium present in the final compound has been taken into consideration [7]. In comparison with the unlabelled VUF 4576, the basic strength of the labelled compound is increased by tritiation, resulting in a slight decrease in retention time on the silica.

Experimental section

Materials. C^3H_3I (10mCi/mL, of which specific activity has been claimed to be 85 Ci/mmol), was purchased from Amersham.

Analytical Thin Layer Chromatography was performed on Merk silica gel type

60 F_{254} aluminium plates. Column chromatography was performed using Baker 30-60 μM silica or aluminium oxide ICN Alumina B deactivated with 5% water. ^1H NMR spectra were recorded on Bruker AC 200 or on Bruker AM 360 spectrometers, chemical shifts are given in part per million downfield from tetramethylsilane. Mass spectra were obtained on Finnigan MAT 90, Finnigan MAT TSQ 70 mass/spectrometers or a HP 5971A GC/MS. Melting points were obtained on a Mettler FP5 apparatus.

The synthetic steps shown in scheme 2 have been carried out as follows. The primary amine (**1**) was prepared by the alkylation of the sulphur of the cysteamine hydrochloride with the corresponding benzhydrol [8]. The other fragment (**2**) for VUF 9046 was prepared by nucleophilic substitution of the hydroxy-group with bromide. VUF 9046 was obtained by coupling (**1**) and (**2**), using a conventional alkylation method under basic conditions [9]. The radiolabelled compound [^3H]-VUF 4576 was synthesized by methylation of VUF 9046 with C^3H_3I.
The related and cold VUF 4576 was prepared by reductive alkylation of VUF 9046 according to the Leuckard reaction [10]. This procedure was not applied in preparing the labelled compound because of the low specific activity of the corresponding labelled reagents.

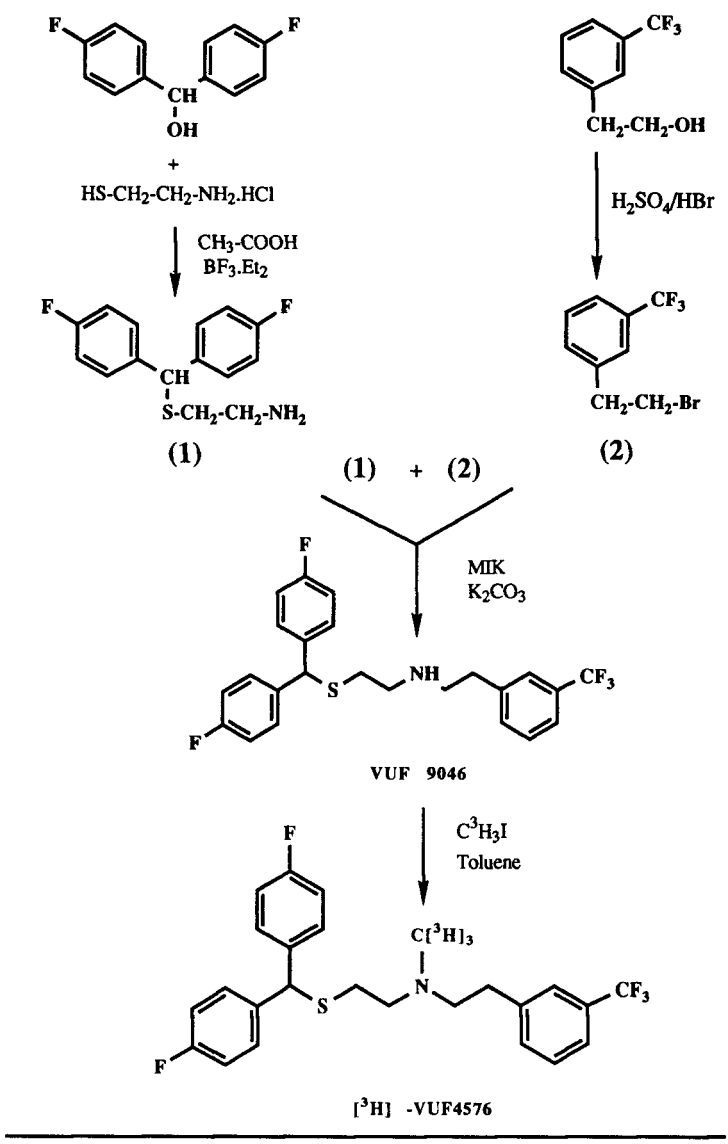
- Synthesis of N-2-[bis(p-fluorophenyl)methylthio]ethylamine (**1**)

2-mercaptoethylamine hydrochloride (0.10 mol) and bis-(4-fluorophenyl)-methanol (0.105 mol) were dissolved in glacial acetic acid (100 mL) followed by the addition of $BF_3.Et_2O$ (15 mL, 0.12 mol of BF_3). The stirred solution was heated at 80-90 °C for 15 min. The crude product was precipitated by the addition of ether (1.2 L), filtered and dried *in vacuo* over NaOH pellets.
This salt was dissolved in ethanol (50 °C), filtered and concentrated to yield 80% of the hydrochloride. The product was recrystallized from EtOAc, m.p. 165-167 °C. The free base was obtained by dissolving the hydrochloride salt in hot water, adding of Na_2CO_3 to pH 10, followed by extraction with ether.
^1H NMR ($CDCl_3$) ∂:1.30 (s; 2H, NH_2); 2.47(t; 2H J=7.3, $-CH_2-N$); 2.82 (t; 2H J =7.3, $S-CH_2$); 5,15 (s; 1H, -CH-S); 7.0 (t; 4H J=8.0, arom); 7.31-7.48 (m; 4H, arom).
HRMS ($C_{15}H_{15}NSF_2$); found: 279.088; calc: 279.0893.

- Synthesis of 2-[3-(trifluoromethyl)phenyl]ethyl bromide (**2**)

5g (0.026 mol) of 2-[3-(trifluoromethyl)phenyl]ethylalcohol was refluxed overnight in 50 mL of a 3:7 mixture of H_2SO_4/HBr 48%.

SCHEME 2. Synthesis of VUF 9046 and [^{3}H]-VUF 4576



The reaction mixture was cooled and neutralized with aqueous K_2CO_3 (1N). The bromide was extracted with ether (3 x 50 mL), the organic phase was dried over Na_2SO_4, filtered and concentrated to yield a brown oil. Distillation *in vacuo* (54°C, 0.1 mm Hg) to afford the pure bromide as colourless oil (85%).

^{1}H NMR ($CDCl_3$) ∂: 3.0 (t; 2H J=7.3, Ph-CH_2); 3.44 (t; 2H J=7.3, CH_2-Br); 7.20-7.78 (m; 4H, arom).

GS/MS; m/z: 252 ($M^{+\cdot}$, 0.013%); 172 ($M^{+\cdot}$-HBr, 100%).

- Synthesis of VUF 9046

A mixture of 2-[3-(trifluoromethyl)phenyl]ethyl bromide (**2**) (0.1 mol), N-2-[bis-(4-fluorophenyl)methylthio]ethylamine (**1**) (0.08 mol) and 10 g of anhydrous K_2CO_3 in methyl isobutyl ketone (MIK) (50 mL) was refluxed under nitrogen for 40 hours. The reaction mixture was cooled, poured onto ice, followed by the addition of EtOAc. The organic phase was washed with water, brine, dried over K_2CO_3 and concentrated to an oil. The product was purified by flash column chromatography using petroleum ether(40-60)/EtOAc (7:3), as eluent, previously saturated with ammonia. TLC (SiO_2, PE-EtOAc-NH_3 80-20-sat) R_f 0.31. The free base was converted into the maleate and crystallized from methanol/ether (yield 70%); m.p. 138-140 °C.

^{1}H-NMR ($CDCl_3$) ∂: 2.75 (m; 2H, CH_2-Ph); 3.0-3.35 (m; 6H, CH_2-N-CH_2, CH_2-S); 5.2 (s; 1H, CH-S); 6.25 (s; 2H, mal); 6.9 (t; 4H J=8.0, arom); 7.25-7.65 (m; 8H, arom).

HRMS ($C_{24}H_{22}F_5NS$) found 451.139; calc.451.1393.

- Synthesis of VUF 4576

The secondary amine, VUF 9046 (6.3 mmol), was added with cooling (0 °C), to formic acid (15.7 mmol, 90%), followed by the addition of 7.0 mmol of formaldehyde (37% solution). The mixture was heated at 100 °C overnight. HCl (1N) was added, the formic acid and any excess formaldehyde were evaporated under reduced pressure. The colourless residue was quenched with water and rendered alkaline with an aqueous NaOH (0.6 mol.dm^{-3}) solution followed by extraction with ether. The ether phase was dried over Na_2SO_4 and concentrated. The tertiary amine was isolated by column chromatography using light petroleum ether (40-60)/EtOAc (8:2) as eluent (yield 80%). TLC (SiO_2 PE-EtOAc-NH_3 80-20-sat) R_f 0.45.

^{1}H NMR ($CDCl_3$) ∂: 2.25 (s; 3H, CH_3-N); 2.4-2.5 (m; 2H, CH_2-Ph); 2.5-2.6 (m; 4H, CH_2-S; CH_2-N); 2.7-2.85 (m, 2H, CH_2-N); 5.15 (s; 1H, CH-S); 7.0 (t; 4H J=8.0, arom); 7.3-7.5 (m; 8H, arom).

HRMS ($C_{25}H_{24}F_5NS$) found 465.154; calc.465.1550.

- Synthesis of [^{3}H]-VUF 4576

50 mg (0.11mmol) secondary amine, VUF 9046 , was dissolved in toluene (2 mL) and added to a pre-cooled (liquid nitrogen) solution of 10 mCi C^3H_3I in toluene (1 mL). The reaction mixture was set aside in the refrigerator for 10 days. The course of the reaction was followed by measuring the radioactivity of small samples of the reaction mixture after evaporation of the unreacted methyl iodide. The reaction mixture was purified when no further increase in

radioactivity was detected. The unreacted methyl iodide was removed under vacuum and led through a cooled trap with pyridine. The crude product was purified by column chromatography on basic aluminium oxide using exane/EtOAc (9:1) as eluent. The purity of [^{3}H]-VUF 4576 (2.6 mCi/mL) was checked on TLC-radioscan by co-elution with a sample of VUF 4576 (SiO_2, CH_2Cl_2/-Aceton (90:10); R_f 0.5 and R_f 0.42 for VUF 4576 and [^{3}H]-VUF 4576 respectively and SiO_2, CH_2Cl_2/Aceton/$N(CH_3)_3$ 90:10:1; R_f 0.76 and R_f 0.67 respectively) and proved to be >97%.

The presence of a substantial amount of unlabelled material was revealed by mass spectometric analysis ($^3H_0$: $^3H_1$: $^3H_3$ are 56%: 0%: 42% respectively), resulting in a specific activity of 37 Ci/mmol (1.4 TBq/mmol). As this cannot be explained so far, we may assume that the radioactivity of the methyl iodide has been indicated at too high a level (see Materials) .

^{3}H-NMR ($CDCl_3$) δ: 2.25 (s; N-C$^3H_3$).

Acknowledgements

The authors wish to thank F.M.G. Sperling (Organon International) for his assistance during the labelling procedure, J.D.M. Herscheid for his enthusiasm during the discussion of our labelling problems, J. Vader (Organon International) for reading the manuscript scrupulously.

References

1. Caldirola P., and Timmerman H. -*Eur. J. Med. Chem.* in press
2. Spedding M. -*TiPS* 6:109 (1985).
3. Caldirola P., Timmerman H., Mannhold R., and Zandberg P. -*8th Camerino-Noordwijkerhout Symp.* Poster presentation, abstr. book p. 74,(1991). Caldirola P., Monteil A, Mannhold R., Zandberg P., Timmerman H.Submitted.
4. Mannhold R., Caldirola P., and Timmerman H. -*XIth International Symposium on Medicinal Chemistry*-Poster presentation, Jerusalem (1990).
5. Means A.R., Van Berkum M.F.A., Bagchi I., Lu K.P., and Rasmussen C.D. -*Pharmacol. Ther.* 50: 255 (1991)
6. Weiss B., Prozialek W.C., and Wallace T.L. -*Biochem. Pharmacol.* 31: 2217 (1982).
7. Kaspersen F.M., Van Acquoy J., Van De Laar G.L.M., Wagenaars G.N., and Funke C.W. *Recl. Trav. Chim. Pays-Bas* 103: 32 (1984).
8. Hiskey R.G., and Harpold M.A. *Tetrahedron* 23: 3923 (1967).
9. Husbands M.G.E. *USP* 4, 745;191 (1988).
10. Moore M.L. in Adams R., Bachmann W.E., Blatt A.H., Fieser L.F., Johnson J.R., Snyder H.R., (Eds) *Organic Reactions* vol. 5, Wiley J, NY pp 301 (1969)