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NOVEL STEREOSELECTIVE CALCIUM CHANNEL LIGANDS OF THE DIPHENYLALKYLAMINE-TYPE

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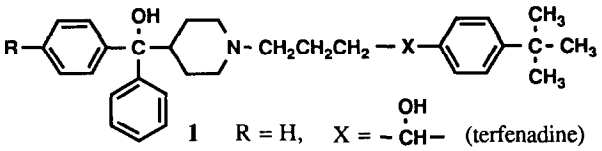
Abstract: The optically pure enantiomers of α-(4-chlorophenyl)-α-phenyl-1-[4-(4-*tert*-butylphenyl)butyl]-4-piperidinemethanol were synthesized and tested for calcium channel affinity on rat brain membranes. The *levo*-isomer (VUF4648) was found to exert a 190 times stronger inhibition of [^{3}H]nitrendipine binding than the *dextro*-isomer (VUF4647). Implications for the action mechanism of this type of compounds are discussed.

Calcium antagonists belong to structurally heterogeneous classes of organic compounds: 1). the 1,4-dihydropyridines (e.g., nifedipine), 2). phenylalkylamines (e.g., verapamil), 3). benzothiazepines (e.g., diltiazem) and 4). diphenylalkylamines (e.g., prenylamine).[1] Whereas the first three classes of compounds are all known to possess pronounced stereoselective activity,[2,3] the stereoselectivity of the fourth class, the diphenylalkylamines, has so far not been reported. Binding studies indicate that the binding sites for the dihydropyridines, phenylalkylamines and benzothiazepines are linked allosterically one to the other, so that occupancy at one site modulates the interaction of drugs at the other sites.[4,5] Murphy *et al* observed that the inhibition of [^{3}H]nitrendipine binding by prenylamine, lidoflazine, or flunarizine was reversed by D600, a verapamil analogue, suggesting that the diphenylalkylamines act at the same site as the phenylalkylamines.[6] To check the validity of this assumption we decided to study the role of chirality for the calcium channel affinity of the diphenylalkylamine-type calcium antagonists.



1 R = H, X = –CH(OH)– (terfenadine)
2 R = H, X = $-CH_2-$ (VUF4591)
3 R = Cl, X = $-CH_2-$

Figure 1. Structures of terfenadine and its analogues

Initially we used the optically pure enantiomers of terfenadine (**1**), a histamine H_1-receptor antagonist, as the probes to study the stereoselectivity of the diphenylalkylamine calcium antagonists.[7] It was found that both terfenadine enantiomers exhibited same potency in the inhibition of [^{3}H]nitrendipine binding. Moreover when the chiral center was abrogated, the affinity of the corresponding compound VUF4591 (**2**) to calcium channels was more than 6-fold increased. We thus concluded that the binding site linked to the calcium channel is not sterically restricted towards the phenylbutanol part of the terfenadine molecule. In the present study, we take VUF4591 as our prototype and introduce a chiral center in the diphenylmethanol part of the molecule by replacing one phenyl ring with a *p*-chlorophenyl ring. The enantiomers of the resulting compound **3** were tested for calcium channel affinity by a binding assay on rat cerebral cortex membranes. In this paper we describe the synthesis, resolution and binding results of the enantiomers of compound **3**.

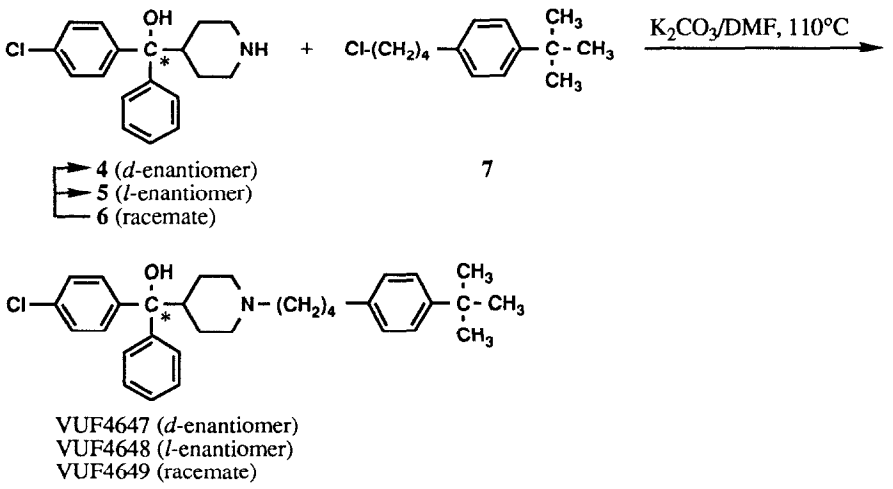


Figure 2. Synthesis of the enantiomers of the novel calcium channel ligand VUF4649

As illustrated in Figure 2, the enantiomers of compound **3** were prepared by the alkylation of the corresponding pure enantiomers of α-(4-chlorophenyl)-α-phenyl-4-piperidinemethanol (**4**, **5**) with 4-chloro-1-[4-(4-*tert*-butylphenyl)]butane (**7**) in the presence of K_2CO_3. Thus, to the solution of the chlorobutane **7** (79 mg, 0.33 mmol) in 25 ml dry acetone was added NaI (100 mg, 0.66 mmol) and the solution was refluxed for 4 hours. After filtration, the solvent was removed by evaporation at room temperature. To the residue was then added the enantiomerically pure 4-piperidinemethanol **4** or **5** (100 mg, 0.33 mmol), K_2CO_3 (46 mg, 0.33 mmol) and 50 ml dry DMF. The mixture was stirred at 110°C overnight. After evaporation to dryness, the residue was taken up with CH_2Cl_2. Purification by a 14 cm x 1 cm silica gel column (Merck, Kieselgel 60, Korngröβe 0.063 - 0.100 mm) with 200 ml eluent (EtAc/Et_2O/Et_3N 1:1:0.1; Rf = 0.49 for the product) furnished a thick colourless oil (yield: 12.5%). The alkylation of **4** yielded the *dextro*-enantiomer VUF 4647

(oxalate $[\alpha]_D^{25}$ + 26.0°, *c* = 1, EtOH) and the alkylation of **5** afforded the *levo*-enantiomer VUF4648 (oxalate $[\alpha]_D^{25}$ - 26.1°, *c* = 1, EtOH).[8] The optical purity of VUF4647 and VUF4648 was more than 98% examined on a cellulose-type HPLC column.

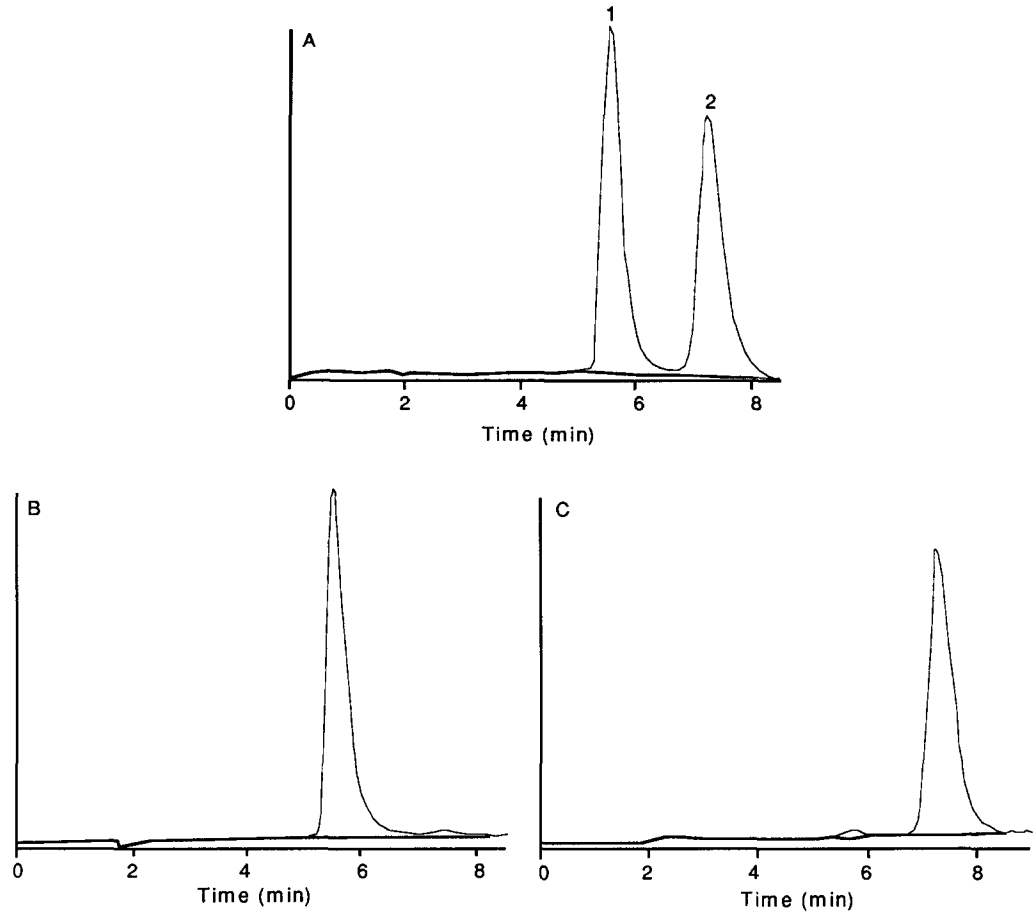


Figure 3. Chiral HPLC separation of α-(4-chlorophenyl)-α-phenyl-4-piperidinomethanol (**6**). A: Racemic mixture; B: (-)-enantiomer; C: (+)-enantiomer. Peaks: 1 = (-)-enantiomer; 2 = (+)-enantiomer. Amount injected: 8-9 mg of each compound in 1 ml of the mobile solvent.

Enantiomerically pure 4-piperidinemethanols **4** and **5** were obtained by a preparative chiral HPLC separation of the racemate **6** which was synthesized according to the literature method.[9] The chromatogram for the enantiomeric separation of **6** is shown in Figure 3. The chromatographic column used for the separation was a prepacked 50 cm x 2 cm chiralcel OJ (Daicel Chemical Industries, Ltd., Japan) operated with a mixture of

hexane/ethanol/isopropanol/diethylamine (90:7:3:0.2, v/v) as the mobile phase. Retention times of about 5.5 and 7.3 min. were obtained for **5** and **4**, respectively. Analyses of the individual enantiomers **5** (mp. 163.8 - 166.0°C; $[\alpha]_D^{25}$ - 5.8°, *c* = 1, $CHCl_3$) and **4** (mp. 148.3 - 150.3°C; $[\alpha]_D^{25}$ + 5.8°, *c* = 1, $CHCl_3$) are also shown in the same figure. Analytical results indicated an enantiomeric excess of 98.1 and 98.4% for **5** and **4** respectively whilst the synthetic racemate **6** shows a typical 50:50 area ratio.

VUF4647, VUF4648 and VUF4649 were tested for their inhibitory potency *vs* [^{3}H]nitrendipine binding on the rat cerebral cortex membranes. The preparation of the membranes was based on that described in the literature.[10] In the binding assay, all compounds were used as oxalate and were dissolved in 99% (v/v) DMSO and diluted with 50 mM TRIS-HCl (pH 7.4) buffer to the desired concentrations. The membrane suspension of the rat cerebral cortex (180 µg protein/ml) was then incubated with the indicated compounds for 60 min. at 37°C in 50 mM TRIS-HCl buffer (pH 7.4 at 37°C). Incubation volume was 0.5 ml and [^{3}H]nitrendipine concentration was 0.25 nM. The reaction was stopped by the addition of 4 ml ice-cold TRIS-HCl buffer (pH 7.4 at 0°C), followed by immediate filtration under reduced pressure onto Whatman GF/C filters. The filters were washed twice with 4 ml cold buffer. The retained radioactivity was counted with a Packard liquid scintillation counter after addition of 5 ml scintillation fluid to the filters. Each experiment was performed in triplicate. As a reference, prenylamine was also tested under the same conditions. In the saturation experiment 1 µM nifedipine was used for non-specific binding. Because of photolability of dihydropyridines, binding experiments were performed under a sodium lamp. The binding data were evaluated by the non-linear curve-fitting program LIGAND.[11]

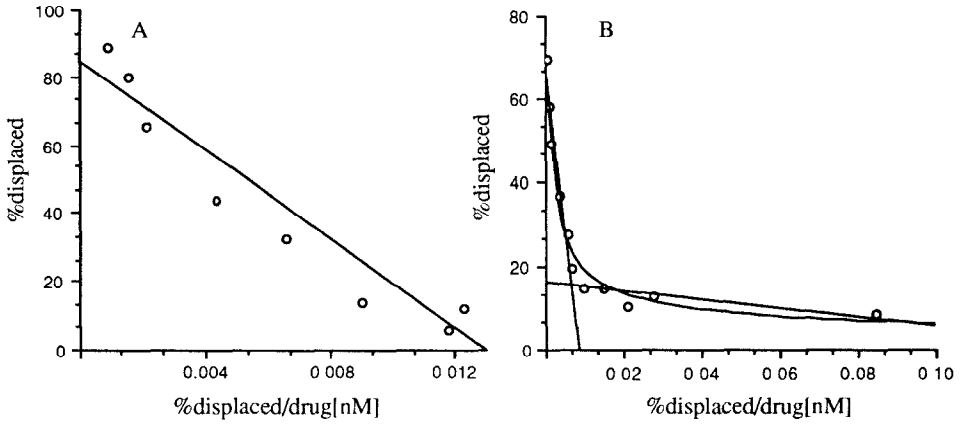


Figure 4. Hofstee plots of the displacement data from A: VUF4647 and B: VUF4648. The amount of the displaced [^{3}H]nitrendipine is plotted *vs* this value divided by the drug concentration (nanomolar). Each point is the mean of three independent determinations performed in triplicate.

The Hofstee transformation of the displacement data from VUF4648 generates curvilinear plots (Figure 4B). The best fit of this plot assuming a one-site model resulted large residual variances. The runs test of this fit

indicated that the points were not randomly distributed around the line. It is obvious that the one-site model is inadequate for the interpretation of the displacement data of VUF4648. Linear regressions were then performed for the two portions of the curve assuming that VUF4648 may be binding to two sites at the channel. The dissociation constants (K_D) for the high and low affinity sites, obtained when the values cease to change on repeated iterations, are $4.68 \pm 0.18 \times 10^{-8}$ mol/L and $8.81 \pm 0.09 \times 10^{-6}$ mol/L, respectively. The affinity ratio for the two sites is 188. However, the second binding site was not detectable for VUF4647 apparently because of the compound's lower affinity. Hofstee analysis of the displacement data from this compound gives a K_D value of $8.90 \pm 0.14 \times 10^{-6}$ mol/L (Figure 4A). VUF4649 and prenylamine showed the K_D values of $1.15 \pm 0.11 \times 10^{-7}$ mol/L and $2.09 \pm 0.10 \times 10^{-7}$ mol/L, respectively. The racemate VUF4649 has at least three different kinds of binding behavior (if the low affinity binding site were not sterically discriminative). The complication diffuses the actual displacement data so that a statistically significantly better fit for the two-site model than the one-site model cannot be generated by the LIGAND program although the graphic of the Hofstee plot may look curvilinear (figure not shown). Thus the K_D value of VUF4649 for the one-site model is intermediate between the "true" K_{D1} and K_{D2} for the two-site model but much closer to the high-affinity value or that twice as much as the K_{D1} value of VUF4648 (9.36×10^{-8} mol/L).

Calcium channels may be considered as pharmacologic receptors.[3] As conventional receptors, calcium channels possess specific drug binding sites with defined structure-activity relationships including stereoselectivity. Before this report, three of the four major chemical classes of calcium antagonists were known to exhibit stereoselectivity at calcium channels. In this study, we demonstrated that the fourth class, the diphenylalkylamine-type calcium antagonists also show stereoselectivity at the channel. The 190 time difference in affinity to calcium channels between VUF4648 and its enantiomeric analogue VUF4647 supports the previous hypothesis that the diphenylalkylamines act at the same site of calcium channels as the phenylalkylamines. An additional binding site available for the diphenylalkylamines, as suggested by the Hofstee analysis[12] of the VUF4648 data (Figure 4B), might explain why these compounds generally exert greater inhibition of [^{3}H]nitrendipine binding than the phenylalkylamines. Together with the fact that the terfenadine enantiomers possess the same affinity to calcium channels, the present study probably indicates that the α-(4-chlorophenyl)-α-phenyl-4-piperidinemethanol moiety in VUF4648 and analogues corresponds to the chiral part of the verapamil molecule. This conclusion offers further opportunity for the future discovery of novel calcium antagonists belonging to either phenylalkylamines or diphenylalkylamines.

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References and Notes

1. Godfraind, T.; Miller, R.; Wibo, M. *Pharmacol. Rev.* **1986**, *38*, 321.
2. Triggle, D.J.; Janis, R.A. *Structure and Physiology of the Slow Inward Calcium Channel*; Venter, J.C. and Triggle, D.J., Eds.; Alan R. Liss, Inc.: New York, 1987; pp. 29-50.
3. Kwon, Y.-W.; Triggle, D.J. *Chirality* **1991**, *3*, 393.
4. Schwartz, A. *Am. J. Cardiol.* **1989**, *64*, 3I.
5. Triggle, D.J. *Comprehensive Medicinal Chemistry*; Emmett, J.C., Ed.; Pergamon Press: Oxford, 1990; Vol 3, pp. 1047-1100.
6. Murphy, K.M.M.; Gould, R.J.; Snyder, S.H. *Nitrendipine*; Urban and Schwarzenberg, Eds.; Baltimore-Munich, 1984, pp. 107-131.
7. Zhang, M.Q.; Caldirola, P.; Timmerman, H. *Pharm. Weekbl. [Sci]* **1992**, *14* (Suppl.B), 10.
8. The following ^{1}H-NMR spectra were recorded on a Bruker AC 200 spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane and coupling constants are in Hz. HRMS spectral data were registered on a Finnigan MAT 90 mass spectrometer with electron impact (EI) ionization. Melting points were determined on a Mettler FP5 melting point apparatus and were uncorrected.
   VUF4647 oxalate: mp. 148.3-150.3°C; HRMS (*m/e*, relative intensity, %): 489 (M^+, 17), 471 (M^+-H_2O, 16), 314 (M^+- 175, 100); ^{1}H-NMR (free base in $CDCl_3$): δ 1.32 (s, 9H, CH_3), 1.47-1.63 (m, 8H, piperidine $C_{3'}$,$_{5'}$-H & -CH_2CH_2-), 1.94 (m, 2H, piperidine $C_{2'}$,$_{6'}$-H_{ax}), 2.33 (t, 2H, J = 7, NCH_2), 2.47 (m, 1H, piperidine $C_{4'}$-H), 2.59 (t, 2H, J = 7, -CH_2Ph), 2.97 (m, 2H, piperidine $C_{2'}$,$_{6'}$-H_{eq}), 7.09-7.50 (m, 13H, aromatic H).
   VUF4648 oxalate: mp. 175.3-177.4°C; HRMS (*m/e*, relative intensity, %): 489 (M^+, 21), 471 (M^+-H_2O, 7), 314 (M^+- 175, 100); ^{1}H-NMR spectrum is identical to that of VUF4647.
9. McCarty, F.J.; Tilford, C.H.; Van Campen, M.G.,Jr. *J. Org. Chem.* **1961**, *26*, 4084.
10. Bast, A.; Leurs, R.; Timmerman, H. *Drugs* **1987**, *33*(Suppl.2), 64.
11. Munson, P.J.; Rodbard, D. *Anal. Biochem.* **1980**, *107*, 220.
12. Molinoff, P.B.; Wolfe, B.B.; Weiland, G.A. *Life Sci.* **1981**, *29*, 427.