# Verapamil Analogues with Restricted Molecular Flexibility: Synthesis and Pharmacological Evaluation of the Four Isomers of α-[1-[3-[N-[1-[2-(3,4-Dimethoxyphenyl)ethyl]]-N-methylamino]cyclohexyl]]-α-isopropyl-3,4-dimethoxybenzeneacetonitrile

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The synthesis and pharmacological activities of the four isomeric racemates of α-[1-[3-[N-[1-[2-(3,4-dimethoxyphenyl)ethyl]]-N-methylamino]cyclohexyl]]-α-isopropyl-3,4-dimethoxybenzeneacetonitrile are reported (**2a–d**). The compounds are verapamil analogues with restricted molecular flexibility designed to gather information on the active conformation(s) of the parent drug. The relative stereochemistry of the four racemates was established by X-ray crystallography and by $^1$H NMR spectroscopy; conformational analysis was supported by theoretical calculations. Negative inotropic and chronotropic activities were evaluated on guinea pig atria, while vasodilatory activity on smooth muscle was tested on guinea pig aortic strips. Binding studies on cat ventricles were performed using (−)-[*N-methyl*-$^3$H]desmethoxyverapamil (D888) as a reference ligand. The results seem to support the hypothesis that cardiac depressant and vasorelaxant activities are due to different conformations of the verapamil molecule.

Verapamil (1) has many rotational degrees of freedom; as a consequence, it offers several opportunities for obtaining restricted analogues. In our previous papers dedicated to the search for the active conformations of this compound, we limited the free rotation of bonds a and b, leading to the fluorene derivative **3**;[1] bonds b, c, e, f, g leading to the cyclohexane derivatives **4a,b** and bonds b, c, e, f (g), h, leading to the piperidine derivative **5**[2] (see Chart I). In all cases the vasorelaxant activity on smooth muscles was lost; for compounds **4a,b** and **5** this was attributed to the fact that rotation around the quaternary carbon atom was completely impaired.[2] As a continuation of our research in this field, we thought it of interest to check this hypothesis and designed compounds **2a–d**, where bonds f and g are incorporated into a cyclohexane ring while the rotation around bonds a–e is maintained. Therefore, the four racemates of α-[1-[3-[N-[1-[2-(3,4-dimethoxyphenyl)ethyl]]-N-methylamino]cyclohexyl]]-α-isopropyl-3,4-dimethoxybenzeneacetonitrile (**2a–d**) were synthesized and studied. No attempt was made, at this stage of the research, to resolve the enantiomeric mixtures, which were tested as the racemates.

## Chemistry

The synthetic pathway used to obtain compounds **2a–d** is shown in Scheme I. The key step of the scheme is the Michael addition of α-isopropyl-3,4-dimethoxybenzeneacetonitrile[3] to 2-cyclohexen-1-one. Considerable time was spent finding the suitable reaction conditions; this was eventually performed using butyllithium at a low temperature. Even in this case side reactions could not be eliminated and the reaction of butyllithium with the CN group gives rise to the byproduct 2-methyl-3-(3,4-dimethoxyphenyl)-4-octanone. The two racemates obtained (**6a** and **6b**) were separated by crystallization and by column chromatography. Each of these products was reacted with homoveratrylamine to give the Schiff base; this was not isolated but was immediately reduced with $NaBH_4$ to the corresponding amine. Thus **6a** gave two isomeric racemates, **7a** and **7b**, in a 1:4 ratio, and under the same conditions, **6b** gave the racemates **7c** and **7d** in a 2:3 ratio. These results suggest that the two 1,3 substituents are trans in **7a** and **7c** and cis in **7b** and **7d**; $^1$H NMR spectroscopy (see next section) confirmed this attribution. The four secondary amines were methylated with formaldehyde/formic acid and gave the final products **2a–d** without isomerization as shown by TLC.[2]

## Stereochemistry and Conformational Analysis

$^1$H NMR spectroscopy at 600 MHz of **6a** and **6b** shows that, as expected, the bulky α-isopropyl-3,4-dimethoxybenzeneacetonitrile group is locked in an equatorial position. In fact the cyclohexane $C_1$ protons of both isomers have axial characteristics as can be deduced from the data reported in Table I. This feature is maintained in all the other compounds of the series.

X-ray crystallography of **6b** confirmed the equatorial location of the substituent and at the same time allowed us to establish the stereochemistry of the racemate, which is seen to be αR*,1S*[4] (see Figure 1 and Table II). As a consequence, **6a** has the αR*,1R* stereochemistry.

The relative stereochemistry of the substituents in position 1,3 was deduced by $^1$H NMR of the amines **7a–d**. Table I shows that the racemates obtained in lowest yield (**7a** and **7c**) have the $C_1$ proton with axial characteristics, while the $C_3$ proton shows equatorial characteristics. Moreover there is a clearcut deshielding effect of the axial nitrogen in position 3 on the axial proton in position 1, which points to the equatorial–axial relationships of the two groups in $C_1$ and $C_3$. On the other hand, the racemates obtained in the highest yield (**7b** and **7d**) show axial

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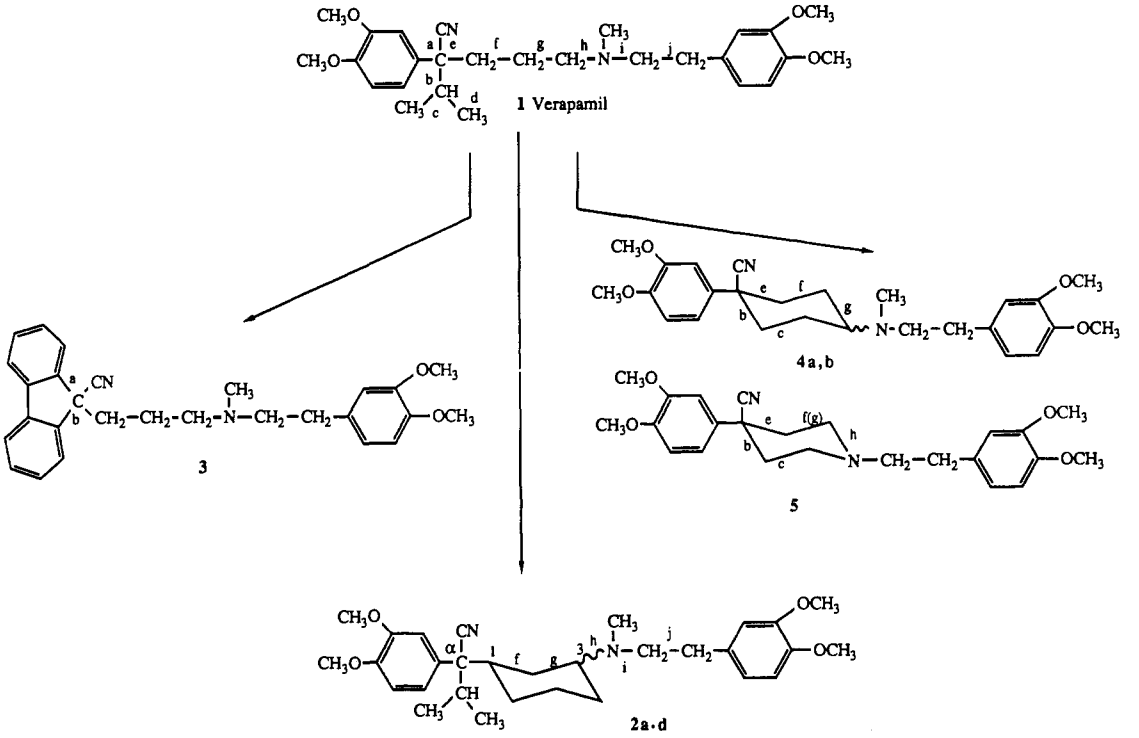
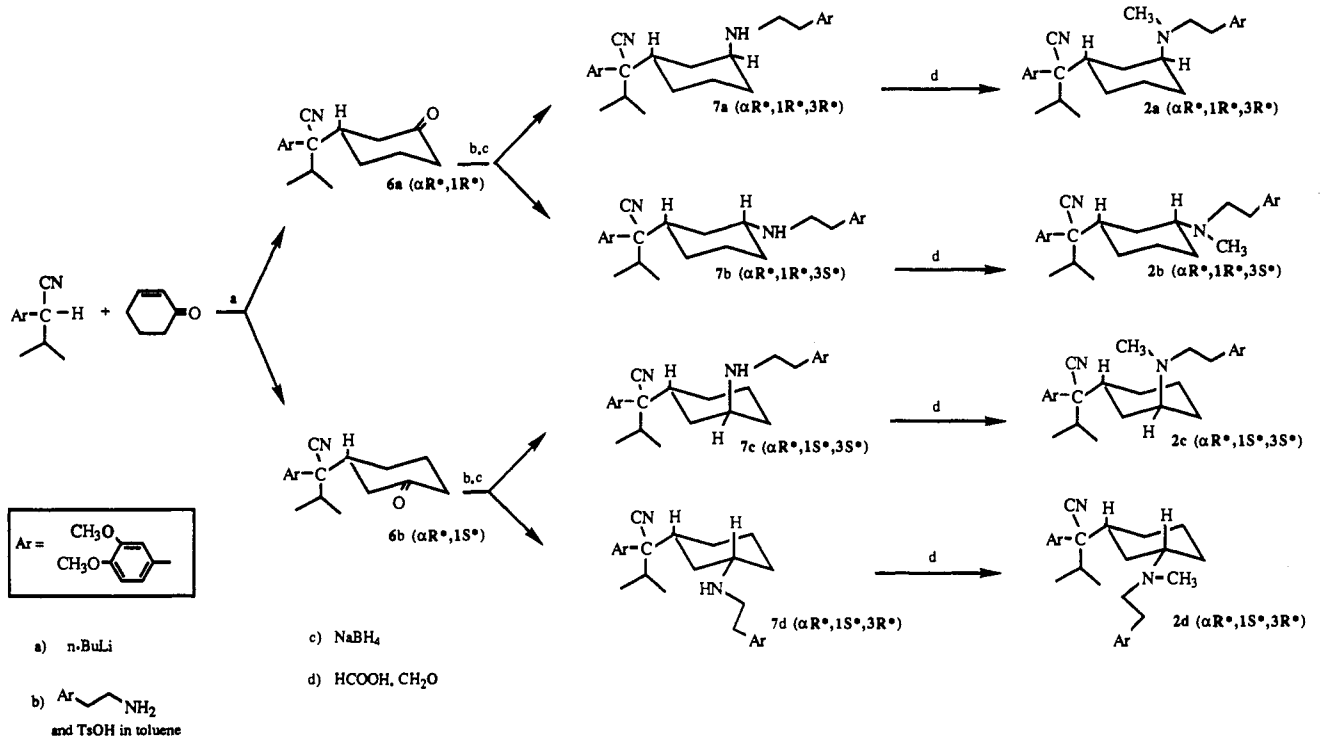
**Chart I**




**Scheme I**

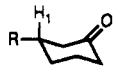
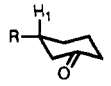
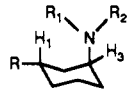
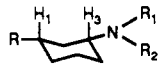
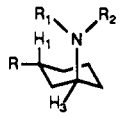
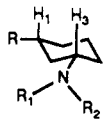


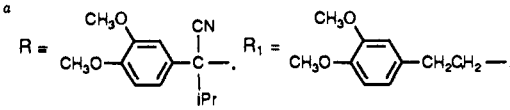

characteristics for both $C_1$ and $C_3$ protons, thus supporting the possibility of both the substituents being in an equatorial position. While for the $C_1$ protons it is usually possible to extract the $J_{aa}$ (and sometimes the $J_{ae}$) constants, the $C_3$ proton signal does not allow extraction of the coupling constants, even using such a high field instrument, probably because of the quadrupole effect of the nitrogen. However the chemical shift as well as the half-height amplitude of the signals ($w/2$)[5-7] allow confident attribution of their equatorial or axial natures.

The $^1$H NMR data regarding the *N*-methyl derivatives (**2a–d**) support the relative stereochemistry already attributed. Having established the relative stereochemistry of the groups in $C_1$ and $C_3$ and that of the two ketones (**6a** and **6b**) being known, the stereochemistry of the amino derivatives follows on in a straightforward way: $\alpha R^*$,-$1R^*$,$3R^*$ for **2a** and **7a**, $\alpha R^*$,$1R^*$,$3S^*$ for **2b** and **7b**, $\alpha R^*$,$1S^*$,$3S^*$ for **2c** and **7c**, and $\alpha R^*$,$1S^*$,$3R^*$ for **2d** and **7d**. Since conformers having both substituents in axial position would have a very high energy, it seems safe to assume that **7b**, **7d** and **2b**, **2d** exist only in the ee conformer.

The case of the two substituents in e/a relationships (**7a**, **7c** and **2a**, **2c**, respectively) is less obvious. In this

**Table I.** $^1$H NMR Signals Due to C1 and C3 Protons at 600 MHz

| structure[a] | $H_1$ | $H_1$ ($R_2$ = H) | $H_3$ ($R_2$ = H) | $H_1$ ($R_2$ = $CH_3$) | $H_3$ ($R_2$ = $CH_3$) |
|---|---|---|---|---|---|
|  | **6a**<br>$\delta$ = 2.49 ppm<br>$J_{aa}$ = 11.6 Hz<br>$J_{ae}$ = 3.2 Hz | | | | |
|  | **6b**<br>$\delta$ = 2.46 ppm<br>$J_{aa}$ = 13.1 Hz<br>$J_{ae}$ = 4.4 Hz | | | | |
|  | | **7a**<br>$\delta$ = 2.60 ppm<br>$w/2^b$ = 40 Hz | **7a**<br>$\delta$ = 2.97 ppm<br>$w/2^b$ = 18 Hz | **2a**<br>$\delta$ = 2.58 ppm<br>$J_{aa}$ = 11.82 Hz | **2a**<br>$\delta$ = 2.50 ppm<br>$w/2^b$ = 10 Hz |
|  | | **7b**<br>$\delta$ = 2.06 ppm<br>$J_{aa}$ = 13.5 Hz | **7b**<br>$\delta$ = 2.55 ppm<br>$w/2^b$ = 29.7 Hz | **2b**<br>$\delta$ = 2.07 ppm<br>$J_{aa}$ = 12.0 Hz | **2b**<br>$\delta$ = 2.54 ppm<br>$w/2^b$ = 32 Hz |
|  | | **7c**<br>$\delta$ = 2.42 ppm[c] | **7c**<br>$\delta$ = 3.04 ppm<br>$w/2^b$ = 15 Hz | **2c**<br>$\delta$ = 2.53 ppm<br>$J_{aa}$ = 12.18 Hz<br>$J_{ae}$ = 3.0 Hz[d] | **2c**<br>$\delta$ = 2.51 ppm<br>$w/2^b$ = 8 Hz |
|  | | **7d**<br>$\delta$ = 2.07 ppm<br>$J_{aa}$ = 12.8 Hz | **7d**<br>$\delta$ = 2.59 ppm<br>$w/2^b$ = 28.2 Hz | **2d**<br>$\delta$ = 2.07 ppm<br>$J_{aa}$ = 11.80 Hz<br>$J_{ae}$ = 2.60 Hz[d] | **2d**<br>$\delta$ = 2.59 ppm<br>$J_{aa}$ = 11.58 Hz |

[a] 

[b] The use of $w/2$ (half-height width) to estimate the equatorial or axial properties of cyclohexane protons is a simple way of evaluating the size of coupling constants when the exact value cannot be obtained.[5-7] [c] Signal obscured. [d] Estimated.

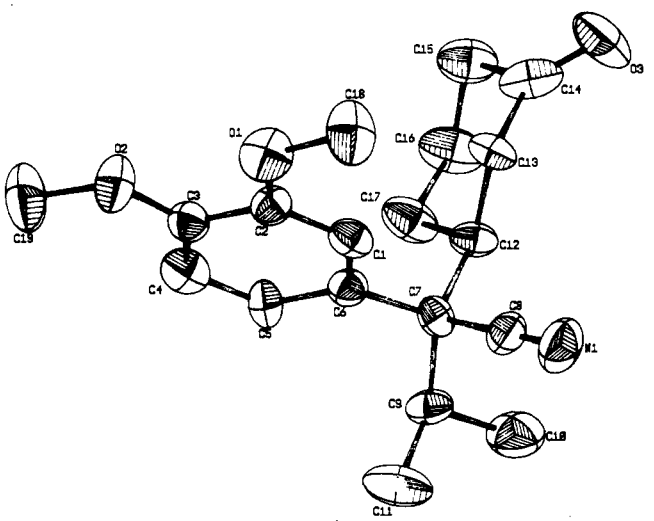



**Figure 1.** Computer generated ORTEP drawing of X-ray-derived crystal structure of **6b**.

**Table II.** Crystal Data of **6b**

| | |
|---|---|
| compound | $C_{19}H_{25}NO_3$ |
| fw | 315.4 |
| space group | $P_{bca}$ No. 61 |
| $a$, Å | 21.292 (2) |
| $b$, Å | 18.223 (2) |
| $c$, Å | 9.051 (1) |
| $V$, Å$^3$ | 3511.8 (6) |
| $Z^a$ | 8 |
| $d_m$ (by flotation), g cm$^{-3}$ | 1.17 |
| $d_{calcd}$, g cm$^{-3}$ | 1.19 |
| $\mu$ (Mo K$\alpha$), cm$^{-1}$ | 0.46 |
| radiation | Mo K$\alpha$ |
| scan mode | $\nu$–$2\nu$ |
| scan width, deg in $\omega$ | 1.0 |
| scan speed, deg (in $\omega$/min) | 1.2 (spe 0.02) |
| $2\theta$ range, deg | 56 |
| no. unique reflections | 3988 |
| max shift of parameters | 0.58 |
| $R$ | 0.056 |
| no. of reflections for $R$ | 1464 |

[a] Number of formula units in the unit cell.

case the bulkier substituent should dictate the prevalent conformation and this seems to be confirmed by the $^1$H NMR data. In fact the protons in position 1 have in all cases clearcut axial characteristics as compared to those in position 3. Therefore, even if a small amount of 1a/3e conformers cannot be ruled out, the prevailing conformers indeed seem to be the 1e/3a ones.

Molecular mechanics calculation lends further support for the $^1$H NMR-based conformational analysis. The conformational behavior of compounds **2a–d** was calculated by means of molecular mechanics, using MMPMI[8] as force-field. Calculations on ketone **6a** show that the equatorial position of the bulky substituent is highly favored ($\Delta E$ = 4.4 kcal/mol), in accordance with the results of $^1$H NMR and X-ray crystallography. However, although the possibility of rotation around bond e (Chart I) is maintained, the crowding of the substituents makes this rotation not completely free, as shown in Figure 2, where the results for compound **6a** are reported.

Once the lipophilic head ($\alpha$ carbon and its substituents) had been fixed in the low-energy conformation, the possibility of rotation of the amino group of compounds **2a–d** was examined. The 27 possible conformers arising from 60° increments of bonds h, i, and j, corresponding to the positions ±gauche and extended (Chart I), showed that when the amino group is in the equatorial position, the energy difference between conformers is definitely lower than with the axial configurations.

The effect of inversion of the configuration at $C_1$ and $C_3$ was also examined. When the two substituents are cis to each other (compound **2b** and **2d**), the ee conformers have a much lower energy than the aa ones, where the cyclohexane ring is actually forced into a boat conformation

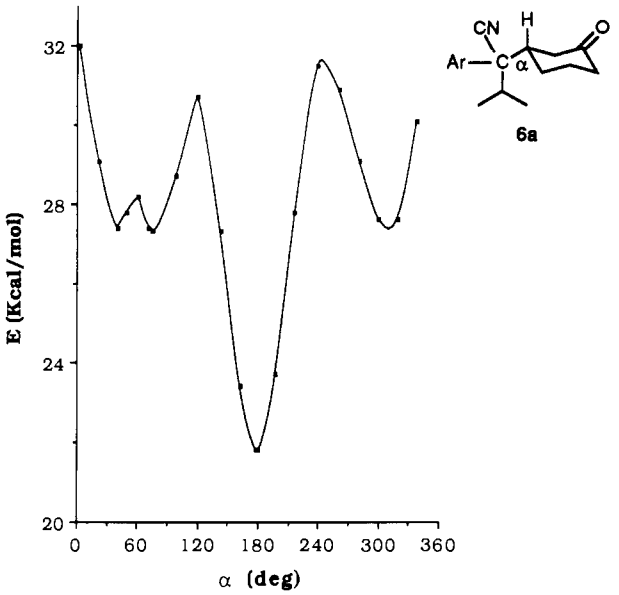



**Figure 2.** Plot of energy (kcal/mol) vs the values of the dihedral angle ($\alpha$) CN–C–C–H for compound **6a**, obtained by rotating the bond e.

to minimize steric interactions between the two groups. When the groups on $C_1$ and $C_3$ are trans to each other (compound **2a** and **2c**), the conformers having the bulky quaternary C atom equatorial and the amino group axial are more stable than the corresponding ae isomers ($\Delta E$ = 4–7 kcal/mol), thus confirming the results obtained by $^1$H NMR that the most populated conformations are the 1e/3a ones.

## Pharmacological Results and Discussion

The inotropic, chronotropic, and vasodilator activities of compounds **2a–d** and of their desmethyl analogues **7a–d** are reported in Tables III and IV in comparison with those of verapamil. Our compounds are definitely less potent than verapamil as calcium antagonists on smooth muscle (aorta strips); the most effective compound of this series (**2d**) is indeed some 30 times less potent than the reference compound. Thus, restriction of molecular flexibility results once again in a strong reduction of calcium antagonist action, at least on smooth muscle. It is to be noted that the desmethyl derivative **7d** has the highest vasodilator activity of the compounds studied, even if is some 10 times less potent than verapamil.

On the other hand, as far as negative inotropic activity is concerned, all the racemates show a comparable potency to that of verapamil, the most potent being **2c**, which is about 10 times more potent than the reference compound. Also negative chronotropic activity is maintained, and in this case compound **2a** appears to be the most potent, with an identical $ED_{30}$ to that of verapamil. The desmethyl derivatives appear to be equipotent or slightly less potent as far as negative inotropic and chronotropic activity are concerned.

Considering also the compounds synthesized and studied in the previous papers[1,2] of this series, it clearly emerges that any limitation of molecular flexibility so far attempted has resulted in a large drop in calcium antagonistic activity on smooth muscles like aorta. On the other hand compounds **2** and **7** maintain remarkable negative inotropic and chronotropic activity, which in some cases exceeds that of the reference compound.

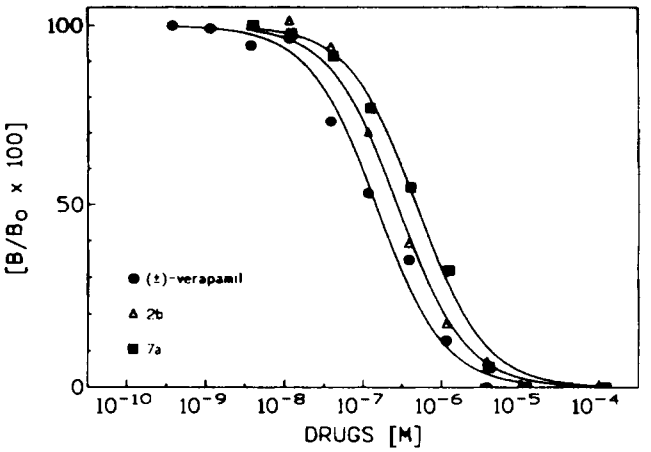



**Figure 3.** Inhibition of (–)-[$^3$H]D-888 binding to kitten heart ventricle membranes by a selection of test compounds. (–)-[$^3$H]D-888 (1.5–2.2 nM) was incubated in presence of the indicated drug concentration in an assay volume of 78 $\mu$L in Tris buffer for 15 min at 37 °C. Nonspecific binding (blank) was defined with 10 $\mu$M unlabeled (–)-D600. $B_0$ represents specifically bound (–)-[$^3$H]D-888 and B indicates specific binding in the presence of above defined blank definition. The data for binding inhibition were pooled and computer-fitted to the general dose–response equation according to De Lean et al.[16]

These results suggest that if the mechanism of action of **2** and **7** remains that of calcium antagonism, a separation of verapamil biological activities (vasodilator/cardiac depressant) has been obtained. To clarify this aspect of the problem we studied the binding of compounds **2** and **7** on cat ventricle preparations; the results are reported in Table V and Figure 3. Even if there is no clearcut correlation between binding and negative inotropic and chronotropic activities, all compounds do bind to cat ventricle calcium channels with a comparable affinity to that of verapamil. This finding suggests that the potent heart depressant action of compounds **2** and **7** is due to the same mechanism of verapamil: antagonism of slow calcium channels. On this basis it seems safe to accept that their potent cardiac depressant action as compared to the very low activity on aorta is due to a remarkable tissue selectivity. Accordingly, it can be hypothesized that inotropic and chronotropic activities of verapamil and like compounds may be due to different conformers from those responsible for calcium antagonistic activity on smooth muscle and that the active cardiodepressant conformers correspond closely to the conformation of **2a** and **2c**, the most potent and selective isomers.

On the other hand, the influence of the additional bulk used to restrict conformation has to be taken into careful account; it might play some role, as suggested by the fact that the desmethyl compounds (**7a–d**), unlike verapamil,[9] maintain remarkable pharmacological properties. For example, a variation in lipophilicity is easily predictable and this could influence the availability of the drug at the receptor site according to the model recently proposed by Herbette.[10] Finally, preliminary calculations (more details of which will be given elsewhere) show that compounds **2a** and **2c** are most closely related to the model proposed by Hoeltje for D595.[11]

## Experimental Section

**Chemistry.** All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 681 spectrophotometer in a Nujol mull for solids and neat for liquids. Mass spectra were measured on a

Table III. Negative Inotropic and Chronotropic Activities of Compounds 2a–d and 7a–d

| compd | negative inotropic activity[a] | $ED_{50}$ of negative inotropic activity[b] | 95% conf lim ($\times 10^{-6}$) | negative chronotropic activity[c] | $ED_{30}$ of negative chronotropic activity[b] | 95% conf lim ($\times 10^{-6}$) |
|---|---|---|---|---|---|---|
| **2a** | 84 ± 1.2 | 0.10 | 0.07–0.14 | 78 ± 1.5[d] | 0.075 | 0.06–0.09 |
| **2b** | 75 ± 3.2 | 1.30 | 0.70–1.70 | 93 ± 2.7[e] | 0.23 | 0.18–0.30 |
| **2c** | 86 ± 1.5 | 0.06 | 0.04–0.08 | 89 ± 4.1 | 1.30 | 1.10–1.60 |
| **2d** | 74 ± 3.3 | 0.20 | 0.16–0.23 | 75 ± 3.6[e] | 0.42 | 0.30–0.56 |
| **7a** | 82 ± 3.1 | 0.90 | 0.60–1.30 | 78 ± 5.1 | 2.40 | 2.10–2.80 |
| **7b** | 74 ± 3.5 | 0.11 | 0.08–0.14 | 66 ± 3.5[e] | 0.06 | 0.04–0.09 |
| **7c** | 70 ± 3.2 | 0.66 | 0.49–0.85 | 64 ± 3.6 | 1.40 | 1.10–1.90 |
| **7d** | 83 ± 1.2 | 1.10 | 0.80–1.30 | 84 ± 1.6 | 0.37 | 0.28–0.49 |
| verapamil | 84 ± 2.1 | 0.61 | 0.40–0.80 | 94 ± 3.4[d] | 0.07 | 0.05–0.10 |

[a] Decrease in developed tension in isolated guinea pig left atrium at $10^{-5}$ M, expressed as percent changes from the control ±SEM ($n$ = 5–6). The left atria were driven at 1 Hz. The $10^{-5}$ M concentration gave the maximum effect for most compounds. [b] Calculated from log concentration/response curves (probit analysis according to Litchfield and Wilcoxon[17] with $n$ = 6–8). Expressed as μM. [c] Decrease in atrial rate on guinea pig spontaneously beating isolated right atrium at $10^{-5}$ M, expressed as percent changes from the control ±SEM ($n$ = 7–8). Pretreatment ranged from 165–195 beats/min. The $5 \times 10^{-6}$ M concentration gave the maximum effect for most compounds. [d] At $10^{-6}$ M. At this concentration 2a and verapamil produce a complete standstill of spontaneously beating right atria (five out of seven experiments). [e] At $5 \times 10^{-6}$ M.

Table IV. Vasodilation of Compounds 2a–d and 7a–d

| compound | vasodilation[a] | $IC_{50}$ of vasodilation[b] | 95% conf lim ($\times 10^{-6}$) |
|---|---|---|---|
| **2a** | 37 ± 2.5 | | |
| **2b** | 30 ± 2.1 | | |
| **2c** | 49 ± 2.8 | 18 | 13–24 |
| **2d** | 58 ± 3.5 | 10 | 8–13 |
| **7a** | 62 ± 3.5 | 17 | 14–21 |
| **7b** | 35 ± 2.7 | | |
| **7c** | 40 ± 2.5 | | |
| **7d** | 56 ± 2.8 | 3.8 | 3.1–4.3 |
| verapamil | 95 ± 1.7[c] | 0.38 | 0.20–0.70 |

[a] Percent inhibition of calcium-induced contraction on $K^+$-depolarized guinea pig aortic strips at $10^{-4}$ M. Values are means ±SEM ($n$ = 6–7). The $10^{-4}$ M concentration gave the maximum effect for most compounds. [b] Calculated from log concentration/response curves (probit analysis according to Litchfield and Wilcoxon[17] with $n$ = 6–8). Expressed as μM. [c] At $10^{-5}$ M.

Table V. (−)-[$^3$H]D888 Competition[a]

| compound | $IC_{50}$ (μM)[b] | $n_H$[c] |
|---|---|---|
| **2a** | 0.39 ± 0.032 | 1.06 |
| **2b** | 0.27 ± 0.018 | 1.13 |
| **2c** | 0.50 ± 0.033 | 1.05 |
| **2d** | 0.26 ± 0.014 | 0.99 |
| **7a** | 0.48 ± 0.032 | 1.07 |
| **7b** | 0.46 ± 0.010 | 1.04 |
| **7c** | 0.54 ± 0.044 | 1.01 |
| **7d** | 0.41 ± 0.025 | 1.08 |
| verapamil | 0.15 ± 0.014 | 1.04 |

[a] The pharmacological profile of verapamil analogues was evaluated by employing nine drug concentrations (each in quadruplicate) under standard assay conditions with 1.5–2.2 nM (−)-[$^3$H]D888 and 17.9–26.5 μg/75 μL of protein. Data from three independent experiments were computer-fitted separately to the general dose–response equation according to De Lean et al.[16] [b] $IC_{50}$ values are given as means ± asymptotic standard deviation. [c] $n_H$ = apparent Hill coefficient.

Perkin-Elmer 8420 capillary gas chromatograph connected to a Perkin-Elmer Ion Trap detector. Unless otherwise stated, NMR spectra were measured on a Gemini 200 spectrometer; a 600-MHz instrument was also used when necessary. Chromatographic separations were performed on a silica gel column by gravity chromatography (Kieselgel 40, 0.063–0.200 mm, Merck) or flash chromatography (Kieselgel 40, 0.040–0.063 mm, Merck). Yields are given after purification, unless otherwise stated. Where analyses are indicated by symbols, the analytical results are within ±0.4% of the theoretical values.

**(α*R**,3*R**)-α-Isopropyl-α-[3-(1-oxocyclohexyl)]-3,4-dimethoxybenzeneacetonitrile (6a) and (α*R**,3*S**)-α-Isopropyl-α-[3-(1-oxocyclohexyl)]-3,4-dimethoxybenzeneacetonitrile (6b).** A solution of butyllithium (50 mL of a 1.6 M solution in hexane, 80 mmol) was added to a solution of α-isopropyl-3,4-dimethoxybenzeneacetonitrile[3] (13.5 g, 61.6 mmol) in anhydrous diethyl ether at −78 °C under $N_2$. The mixture turned yellow and was kept at −78 °C for 3 h. Then 5.94 g (61.8 mmol) of the commercially available 2-cyclohexen-1-one were added, and the reaction was left to reach room temperature overnight. To the crude reaction mixture a saturated aqueous $NH_4Cl$ solution was added, the layers were separated, and the organic extracts were washed with water and dried over $Na_2SO_4$. The solvent was removed in vacuo, and the crude products were purified by flash chromatography on silica gel, using cyclohexane/ethyl acetate (50/50) as the eluent, to remove a small amount of the side product 2-methyl-3-(3,4-dimethoxyphenyl)-4-octanone (15%). MS–GC showed the presence of two peaks corresponding to the two diastereomeric racemates. The mixture (7.2 g, 37% yield) was recrystallized three times from absolute ethanol, giving 1.32 g of **6b**. The mother liquors were evaporated under vacuum, and the mixture was separated by column chromatography on alumina gel (aluminum oxide 90, 0.063–0.200 mm, Merck) using chloroform/petroleum ether (50/50) as the eluting system. This separation gave 2.70 g of **6a** (white solid, mp 120–123 °C) and 2.58 g of **6b** (white solid, mp 138–140 °C): yield (overall) 40.9% of **6a**, 59.1% of **6b**, respectively. **6a**: IR (Nujol) ν 2240 (CN), 1720 (CO) cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 0.91 (d, $J$ = 6.7 Hz, 3 H) and 1.07 (d, $J$ = 6.7 Hz, 3 H) ($CH_3CCH_3$), 1.34 (m, 1 H, cyclohexane proton), 1.70 (m, 1 H, cyclohexane proton), 2.05–2.55 (m, 8 H, cyclohexane protons and $CH_3CH$), 3.89 (s, 6 H, 2 $OCH_3$), 6.87 (s, 3 H, aromatics) ppm; $^{13}$C NMR ($CDCl_3$) δ 210.31, 149.27, 149.21, 126.25, 121.93, 120.55, 111.98, 111.40, 57.35, 56.56, 56.39, 43.47, 41.44, 32.44, 28.17, 25.01, 19.10, 18.30 ppm; MS $m/e$ 315 ($M^+$), 298, 272, 218. Anal. ($C_{19}H_{25}NO_3$) C, H, N.

**6b**: IR (Nujol) ν 2240 (CN), 1720 (CO) cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 0.91 (d, $J$ = 6.6 Hz, 3 H) and 1.00 (d, $J$ = 6.7 Hz, 3 H) ($CH_3CCH_3$), 1.40–1.84 (m, 3 H, cyclohexane protons), 2.04–2.70 (m, 7 H, cyclohexane protons and $CHCH_3$), 3.89 (s, 6 H, 2 $OCH_3$), 7.80–7.95 (m, 3 H, aromatics) ppm; $^{13}$C NMR δ 210.26, 149.23, 149.14, 126.69, 121.70, 120.64, 112.00, 111.34, 57.33, 56.56, 56.38, 44.91, 43.43, 41.54, 32.94, 26.91, 25.04, 19.29, 17.91 ppm; MS $m/e$ 315 ($M^+$), 298, 272, 218. Anal. ($C_{19}H_{25}NO_3$) C, H, N.

**(α*R**,1*R**,3*R**)-α-[1-[3-[*N*-[1-[2-(3,4-Dimethoxyphenyl)ethyl]]amino]cyclohexyl]]-α-isopropyl-3,4-dimethoxybenzeneacetonitrile (7a) and (α*R**,1*R**,3*S**)-α-[1-[3-[*N*-[1-[2-(3,4-Dimethoxyphenyl)ethyl]]amino]cyclohexyl]]-α-isopropyl-3,4-dimethoxybenzeneacetonitrile (7b).** A solution of 2.70 g (8.6 mmol) of **6a** in anhydrous benzene, homoveratrylamine (1.55 g, 8.6 mmol) and $p$-toluenesulfonic acid monohydrate (0.6 g) was heated under reflux for 36 h, water being removed from the reaction with a Dean–Stark trap. The solvent was distilled off and the residue dissolved in $CHCl_3$, washed with a $NaHCO_3$ solution, and dried. Evaporation of the solvent gave 5.23 g of an oil that is quite unstable and was used as such without purification in the next reaction: IR (neat) ν 2240 (CN), 1670 (C=N) cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 0.90 (d, $J$ = 6.5 Hz, 3 H) and 1.12 (d, $J$ = 6.6 Hz, 3 H) ($CH_3CCH_3$), 1.50–2.50 (m, 10 H, cyclohexane protons and $CH_3CHCH_3$), 2.68 (t, $J$ = 6.6 Hz, 2 H, $CH_2N$), 2.92 (t, $J$ = 7.0 Hz, 2 H, $CH_2Ph$), 3.84, 3.86, 3.88 (3 s, 12 H, 4 $OCH_3$), 6.68–6.90 (m, 6 H, aromatics) ppm. Then $NaBH_4$ (0.5 g) was cautiously added to a solution of the crude Schiff base in hot methanol (50 mL) over 0.5 h. The mixture was then heated under reflux for 2 h. After cooling, the mixture was treated with a few drops of water and the excess solvent removed. The residue was dissolved

in $CH_2Cl_2$, washed with an aqueous solution of $Na_2CO_3$ (10%, p/v) and dried. Evaporation of the solvent gave 4.30 g of an oil which was a mixture of two isomers (TLC). They were separated by column chromatography on silica gel using chloroform/methanol/petroleum ether (55/15/30) as eluent. This separation gave 2.52 g of the mixture of the two isomers (yield 59%). **7a** was eluted first and was 28.2% of the mixture of the two isomers (0.71 g): IR (neat) $\nu$ 2240 (CN) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.90 (d, $J$ = 6.2 Hz, 3 H) and 0.95 (d, $J$ = 6.6 Hz, 3 H) ($CH_3CCH_3$), 1.14–2.04 (m, 9 H, cyclohexane protons), 2.41 (m, 1 H, $CH_3CH$), 2.66–2.80 (m, 4 H, $NCH_2CH_2$), 2.96 (bs, 1 H, cyclohexane proton), 3.86 (s, 3 H, $OCH_3$), 3.87 (s, 3 H, $OCH_3$), 3.89 (s, 3 H, $OCH_3$), 3.90 (s, 3 H, $OCH_3$), 6.70–6.95 (m, 6 H, aromatics) ppm. The hydrochloride recrystallized from absolute ethanol and melted at 232–235 °C. Anal. ($C_{29}H_{41}ClN_2O_4$) C, H, N.

The second isomer **7b** constitutes about 71.8% of the mixture of the two isomers (1.81 g): IR (neat) $\nu$ 2240 (CN) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.86 (d, $J$ = 6.6 Hz, 3 H) and 1.05 (d, $J$ = 6.6 Hz, 3 H) ($CH_3CCH_3$), 0.80–1.10 (m, 3 H, cyclohexane protons), 1.31 (m, 1 H, cyclohexane proton), 1.64–2.14 (m, 5 H, cyclohexane protons), 2.38–2.62 (m, 2 H, cyclohexane proton and $CH_3CH$), 2.74–2.93 (m, 4 H, $NCH_2CH_2$), 3.85 (s, 6 H, 2 $OCH_3$), 3.87 (s, 3 H, $OCH_3$), 3.89 (s, 3 H, $OCH_3$), 6.67–6.88 (m, 6 H, aromatics) ppm. The hydrochloride recrystallized from absolute ethanol/anhydrous diethyl ether and melted at 115–118 °C. Anal. ($C_{29}H_{41}ClN_2O_4$) C, H, N.

**($\alpha R^*$,1$S^*$,3$S^*$)-$\alpha$-[1-[3-[*N*-[1-[2-(3,4-Dimethoxyphenyl)ethyl]]-amino]cyclohexyl]]-$\alpha$-isopropyl-3,4-dimethoxybenzeneacetonitrile (7c) and ($\alpha R^*$,1$S^*$,3$R^*$)-$\alpha$-[1-[3-[*N*-[1-[2-(3,4-Dimethoxyphenyl)ethyl]]amino]cyclohexyl]]-$\alpha$-isopropyl-3,4-dimethoxybenzeneacetonitrile (7d).** Following the same procedure as described before and starting from 3.90 g (12.4 mmol) of **6b** and 2.24 g (12.4 mmol) of homoveratrylamine, 6.10 g of Schiff base were obtained as an oil that was not further purified: IR (neat) $\nu$ 2240 (CN), 1670 (C=N) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.92 (d, $J$ = 6.5 Hz, 3 H) and 1.02 (d, $J$ = 6.7 Hz, 3 H) ($CH_3CCH_3$), 1.40–2.55 (m, 10 H, cyclohexane protons and $CHCH_3$), 2.71 (t, $J$ = 6.6 Hz, 2 H, $CH_2N$), 2.98 (t, $J$ = 6.6 Hz, 2 H, $CH_2Ph$), 3.85 (s, 6 H, 2 $OCH_3$) and 3.90 (s, 6 H, 2 $OCH_3$), 6.70–6.90 (m, 6 H, aromatics) ppm.

Following the same procedure as described for **7a** and **7b** and starting from 6.10 g of the Schiff base described above, 6.20 g of a mixture of the two isomers (TLC) were obtained. The two diastereomeric racemates were separated by column chromatography on silica gel using chloroform/methanol/petroleum ether (55/15/30) as the eluting system. This separation gave 3.91 g of the two isomers (yield 63%). The isomer **7c** was eluted first (1.56 g, 39.9% of the mixture of the two isomers): IR (neat) $\nu$ 2240 (CN) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.85 (d, $J$ = 6.2 Hz, 3 H) and 0.99 (d, $J$ = 6.6 Hz, 3 H) ($CH_3CCH_3$), 1.05–1.80 (m, 7 H, cyclohexane protons), 2.01 (m, 1 H, cyclohexane proton), 2.32–2.52 (m, 2 H, cyclohexane proton and $CH_3CHCH_3$), 2.76–2.96 (m, 4 H, $NCH_2CH_2$), 3.04 (bs, 1 H, cyclohexane proton), 3.87 (s, 3 H, $OCH_3$), 3.88 (s, 3 H, $OCH_3$), 3.89 (s, 3 H, $OCH_3$), 3.90 (s, 3 H, $OCH_3$), 6.75–6.90 (m, 6 H, aromatics) ppm. The hydrochloride recrystallized from absolute ethanol/anhydrous diethyl ether and melted at 201–203 °C. Anal. ($C_{29}H_{41}ClN_2O_4$) C, H, N.

The isomer **7d** was eluted second (2.35 g, 60.1% of the mixture): IR (neat) $\nu$ 2240 (CN) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.88 (d, $J$ = 6.6 Hz, 3 H) and 1.05 (d, $J$ = 6.4 Hz, 3 H) ($CH_3CCH_3$), 1.56–2.24 (m, 9 H, cyclohexane protons), 2.41–2.68 (m, 2 H, cyclohexane proton and $CH_3CHCH_3$), 2.78 (t, $J$ = 6.4 Hz, 2 H, $CH_2N$), 2.92 (t, $J$ = 7.0 Hz, 2 H, $CH_2Ph$), 3.87 (s, 6 H, 2 $OCH_3$) and 3.88 (s, 6 H, 2 $OCH_3$), 6.74–6.84 (m, 6 H, aromatics) ppm. The hydrochloride recrystallized from absolute ethanol/anhydrous diethyl ether and melted at 112–115 °C. Anal. ($C_{29}H_{41}ClN_2O_4$) C, H, N.

**General Procedure for the Preparation of the Methylated Amines 2a–d.** A solution of the suitable secondary amine in absolute ethanol was refluxed for 2 h with an excess of HCOOH (85% solution) and formaline. After removal of the solvent, the residue was dissolved in chloroform, washed with NaOH (10% p/v solution), and dried ($Na_2SO_4$). Evaporation of the solvent gave an oil that was purified of minor side products by column chromatography on silica gel (chloroform/methanol, 90/10) or by conversion into the hydrochloride.

**($\alpha R^*$,1$R^*$,3$R^*$)-$\alpha$-[1-[3-[*N*-[1-[2-(3,4-Dimethoxyphenyl)ethyl]]-*N*-methylamino]cyclohexyl]]-$\alpha$-isopropyl-3,4-dimethoxybenzeneacetonitrile (2a).** Starting from 0.31 g of **7a**, 0.2 g of **2a** were obtained as an oil: IR (neat) $\nu$ 2240 (CN) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.82 (d, $J$ = 6.5 Hz, 3 H) and 0.91 (d, $J$ = 6.5 Hz, 3 H) ($CH_3CCH_3$), 1.14–1.40 (m, 3 H, cyclohexane protons), 1.50–1.80 (m, 3 H, cyclohexane protons), 1.90–2.06 (m, 2 H, cyclohexane protons), 2.23 (s, 3 H, $NCH_3$), 2.33–2.80 (m, 7 H, $NCH_2CH_2$, $CH_3CHCH_3$, $H_1$ and $H_3$ cyclohexane protons), 3.85 (s, 12 H, 4 $OCH_3$), 6.45–6.95 (m, 6 H, aromatics) ppm. The hydrochloride recrystallized from absolute ethanol and melted at 141–144 °C. Anal. ($C_{30}H_{43}ClN_2O_4$) C, H, N.

**($\alpha R^*$,1$R^*$,3$S^*$)-$\alpha$-[1-[3-[*N*-[1-[2-(3,4-Dimethoxyphenyl)ethyl]]-*N*-methylamino]cyclohexyl]]-$\alpha$-isopropyl-3,4-dimethoxybenzeneacetonitrile (2b).** Starting from 0.52 g of **7b**, 0.52 g of **2b** were obtained as an oil: IR (neat) $\nu$ 2240 (CN) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.88 (d, $J$ = 6.8 Hz, 3 H) and 1.05 (d, $J$ = 6.4 Hz, 3 H) ($CH_3CCH_3$), 1.14–1.38 (m, 2 H, cyclohexane protons), 1.70–2.14 (m, 7 H, cyclohexane protons), 2.26 (s, 3 H, $NCH_3$), 2.40–2.76 (m, 6 H, $NCH_2CH_2$, $CH_3CHCH_3$ and $H_3$), 3.85 (s, 3 H, $OCH_3$), 3.86 (s, 3 H, $OCH_3$), 3.88 (s, 3 H, $OCH_3$), 3.89 (s, 3 H, $OCH_3$), 6.65–6.90 (m, 6 H, aromatics) ppm. The hydrochloride recrystallized from absolute ethanol and melted at 94–96 °C. Anal. ($C_{30}H_{43}ClN_2O_4$) C, H, N.

**($\alpha R^*$,1$S^*$,3$S^*$)-$\alpha$-[1-[3-[*N*-[1-[2-(3,4-Dimethoxyphenyl)ethyl]]-*N*-methylamino]cyclohexyl]]-$\alpha$-isopropyl-3,4-dimethoxybenzeneacetonitrile (2c).** Starting from 0.6 g of **7c**, 0.5 g of **2c** were obtained as an oil: IR (neat) $\nu$ 2240 (CN) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.87 (d, $J$ = 6.8 Hz, 3 H) and 1.11 (d, $J$ = 6.4 Hz, 3 H) ($CH_3CCH_3$), 0.88 (m, 1 H, cyclohexane proton), 1.00–1.15 (m, 2 H, cyclohexane protons), 1.40–1.76 (m, 3 H, cyclohexane protons), 1.92 (m, 1 H, cyclohexane proton), 2.21 (m, 1 H, cyclohexane proton), 2.37 (s, 3 H, $NCH_3$), 2.44–2.56 (m, 3 H, $H_1$, $H_3$ and $CH_3CHCH_3$), 2.67–2.83 (m, 4 H, $NCH_2CH_2$), 3.86 (s, 3 H, $OCH_3$), 3.89 (s, 6 H, 2 $OCH_3$), 3.91 (s, 3 H, $OCH_3$), 6.77–6.87 (m, 6 H, aromatics) ppm. The hydrochloride recrystallized from absolute ethanol and melted at 152–155 °C. Anal. ($C_{30}H_{43}ClN_2O_4$) C, H, N.

**($\alpha R^*$,1$S^*$,3$R^*$)-$\alpha$-[1-[3-[*N*-[1-[2-(3,4-Dimethoxyphenyl)ethyl]]-*N*-methylamino]cyclohexyl]]-$\alpha$-isopropyl-3,4-dimethoxybenzeneacetonitrile (2d).** Starting from 0.8 g of **7d**, 0.72 g of **2d** were obtained as an oil: IR (neat) $\nu$ 2240 (CN) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.90 (d, $J$ = 6.8 Hz, 3 H) and 1.00 (d, $J$ = 6.6 Hz, 3 H) ($CH_3CCH_3$), 1.10–1.30 (m, 4 H, cyclohexane protons), 1.50–2.13 (m, 5 H, cyclohexane protons), 2.32 (s, 3 H, $NCH_3$), 2.47 (m, 1 H, $CH_3CHCH_3$), 2.59 (m, 1 H, $H_3$), 2.62–2.74 (m, 4 H, $NCH_2CH_2$), 3.85 (s, 6 H, 2 $OCH_3$), 3.87 (s, 6 H, 2 $OCH_3$), 6.70–6.92 (m, 6 H, aromatics) ppm. The hydrochloride recrystallized from absolute ethanol and melted at 86–89 °C. Anal. ($C_{30}H_{43}ClN_2O_4$) C, H, N.

**In Vitro Assays.** Inotropic and chronotropic activities were tested on guinea pig isolated atria preparations, and vasodilator activity was tested on guinea pig aortic strip preparations following standard procedures, details of which have already been reported.[12]

**Binding Assays.** (−)-[$^3H$]D-888 {(−)-desmethoxyverapamil} with a specific activity of 74 Ci/mmol was obtained from Amersham Radiochemical Centre, U.K. Unlabeled (−)-D-600 was a gift from Knoll AG, Ludwigshafen, FRG.

Partially-purified plasma membranes from kitten heart ventricle were prepared according to a standard procedure already described.[13] Tissue slices were placed in an ice-cold solution containing 20 mmol/L $NaHCO_3$ and 0.1 mmol/L phenylmethanesulfonyl fluoride (PMSF), pH 7.4. The tissue was homogenized by means of three 20-s disruptions with an Ultraturrax. The crude homogenate was centrifuged at 1500$g$ for 15 min. The pellet was resuspended and centrifuged again under identical conditions. The supernatant of both runs was spun at 45000$g$ for 15 min. The pellets were suspended in a Tris-buffer (50 mmol/L tris(hydroxymethyl)aminomethane, 0.1 mmol/L PMSF, pH 7.4) and sedimented twice at 45000$g$ for 15 min. The final membrane pellet was suspended in the Tris-buffer at a protein concentration of 2–3 mg/mL and stored in liquid nitrogen until use. The protein was determined by the method of Lowry,[14] using bovine serum albumin as standard.

The receptor binding assay was performed in a total volume of 78 μL at 37 °C. Serial dilutions of unlabeled compounds were performed in pure DMSO in Eppendorff cups. A 3-μL portion of these dilutions was placed in the incubation tube, and the reaction was started by adding 75 μL of a protein/radioligand mixture (15–22 μg/75 μL of protein, 1.5–2.2 nM (−)-[$^3$H]D-888); a final DMSO concentration (v/v) of 4% was not exceeded. Specific binding was defined as the difference between total binding and binding in presence of 10 M nonlabeled (−)-D-600;[13] nonspecific binding amounted to 30–55% of total binding.

Competition studies with the phenylalkylamine receptor were performed according to Glossmann and Ferry.[15]

Separation of bound and free radioligand was performed by rapid vacuum filtration using glass fiber filters (Whatman GF/C) as previously described.[15] Filter-retained radioactivity was quantified by liquid scintillation counting with 60% counting efficiency.

Radioligand binding data were analyzed by means of the general dose–response equation as described by De Lean et al.[16]

**Crystallographic Work.** Crystals of **6b** were grown from absolute ethanol. The structure is shown in Figure 1. Measurements of diffraction were carried out on a Philips PW 1100 diffractometer, using graphite-monochromated, Mo Kα radiation (λ = 0.7107 Å). The unit cell dimensions were obtained from least squares of 25, 2θ values between 14° and 28°. Three reference reflections monitored showed no significant (10%) deterioration during data collection. Corrections were made for Lorentz and polarization factors, but not for absorption. Only reflections with $F \geq 7\sigma(F)$ were considered. The crystal data and experimental details are summarized in Table II. The structure was resolved with Shel X 86 direct methods. The atomic parameters of non-hydrogen atoms were refined anisotropically by blocked full-matrix least-squares with $w = 1$. The hydrogen atoms were located on a DF map and isotropically refined. The final atomic parameters are given in the supplementary material.

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**Supplementary Material Available**: Tables of X-ray parameters for **6b** (4 pages). Ordering information is given on any current masthead page.

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