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Semi-rigid analogues of the calcium antagonist verapamil: A molecular modelling study

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SUMMARY

In this work the rigid-analogue approach has been used to obtain information on the active conformation(s) of the calcium antagonist verapamil. A series of semi-rigid analogues of verapamil were synthesized and their biological activities evaluated on guinea-pig heart and aorta. These molecules were analysed by means of molecular modelling techniques.

On the basis of the pharmacological profile and conformational analysis of these compounds, two different models for negative inotropic and negative chronotropic activity are proposed. The two actions seem to be due to conformations of the molecules which differ in the orientation of their phenylethylamino groups.

INTRODUCTION

The rigid-analogue approach is a powerful tool for the medicinal chemist for clarifying the structural and conformational requirements for receptor selectivity. A rigid analogue is a blocked conformation of the conformationally flexible lead; the chemical manipulation of the molecule which is necessary to reduce its flexibility should be done in such a way that no other physicochemical properties are substantially altered apart from conformation. Thus, one can reasonably hypothesize that the lead and the analogue are able to reach and bind to the same receptor site. If the rigid analogue meets the spatial requirements of the pharmacophore, its biological activity will be equal to or higher than that of the lead, while if the analogue shows a wrong arrangement of the pharmacophore, the result will be a loss of biological activity.

It may happen, however, that because of the chemical manipulation the volume of the molecule is increased in a 'forbidden' region of space, hence producing a decrease in pharmacological

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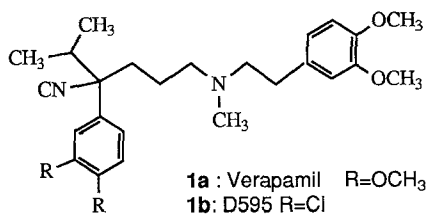
activity even if the spatial arrangement of the pharmacophore is the right one. In addition, the constraint introduced into the molecule could change the biological activity of the drug, shifting its action towards other receptors or subtypes of the same receptor. However, in all cases we will have information that will increase our knowledge of the system.

Tissue selectivity can therefore be one of the consequences of freezing conformations of a flexible molecule. In principle, tissue selectivity is due to a variety of reasons and in this sense there has been a recent report [1] on the existence of tissue-specific isoforms of the α_1 subunit of the calcium channel complex, which contains the binding sites of the calcium channel modulators. If a receptor is different in different tissues, a flexible molecule can interact with both receptors but with different conformations, while a rigid (or semi-rigid) analogue can interact only, or preferentially, with one of them.



1a : Verapamil R=OCH_3
1b: D595 R=Cl

For many years we have been studying the effects of structural modifications of the molecule verapamil to get information on its active conformation(s). Verapamil (**1a**) has been classified as a calcium entry blocker, since it antagonizes the influx of extracellular calcium into cells. This causes a number of biological responses including reduction of myocardial contractility, relaxation of vascular smooth muscle, reduction of SA node discharge and AV conduction rates [2]. It has also been reported that verapamil shows no tissue selectivity between myocardium (cardiac muscle and conduction tissue) and vascular smooth muscle [3], although Mannhold and coworkers [4] suggested a difference between cardiac and vascular smooth muscle receptors on the basis of different QSARs for verapamil congeners in these two tissues.

Structure–activity relationships for **1a** and analogues have been widely studied [5]. Its physicochemical properties [6,7] and its X-ray-derived structure [8] have already been reported, as have its theoretical and experimental conformational analysis [9,10]. In any case, verapamil appears to be a very flexible molecule that can assume several conformations. We have approached the problem of identifying the conformation(s) responsible for its pharmacological activities by synthesizing and studying several rigid analogues. These molecules, in which rigidity has been achieved by introducing cyclic moieties, may be seen as blocked conformations of the lead. Rigidity has been introduced either into the lipophilic part of the molecule (compounds **2–7**) or into the basic part of the molecule (compounds **8–10**).

The compounds studied are reported in Fig. 1. All compounds were tested for their negative inotropic and negative chronotropic activity on isolated guinea-pig atria, and for smooth-muscle relaxant activity on guinea-pig aortic strips. The binding affinities of compounds **4–10** for cat heart membranes were also measured. The results are reported in Table 1.

No attempt was made to correlate binding affinities and functional data, as has been done for ring-varied verapamil congeners [11], because the biological tests were performed on different tissues: cat ventricles were used for binding studies while negative inotropic and chronotropic

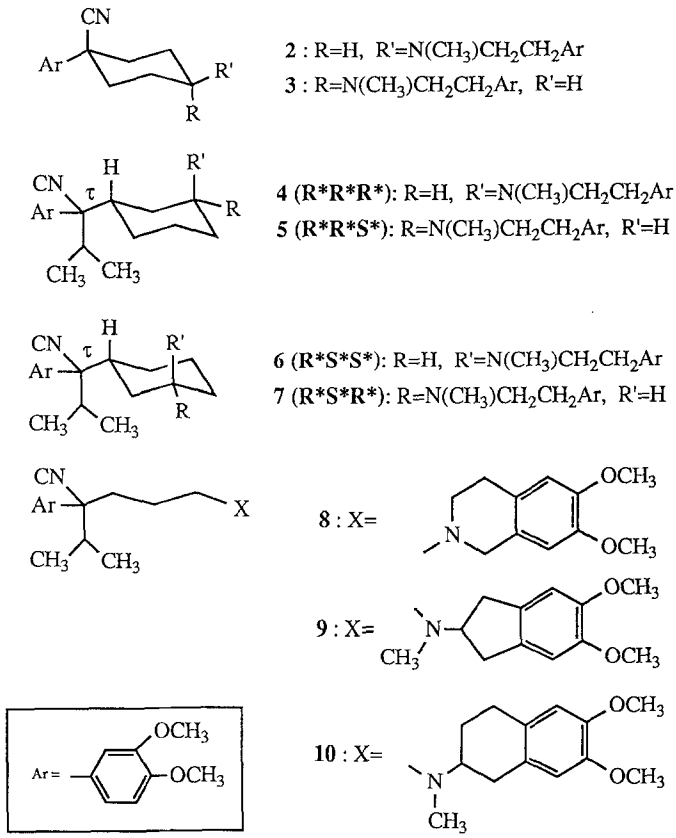


Fig. 1. The compounds studied.

activities were measured on isolated guinea-pig atria. Although other pharmacological models for inotropy are available, the left atrium has been used because of its simplicity and it is also used for testing other verapamil-like compounds [12]. It must be pointed out that negative chronotropic activity may not be solely due to calcium antagonistic properties (Na^+- and K^+-channels may also be involved); nevertheless, the binding affinities of compounds **4–10**, in the same range as that of verapamil, confirm that these compounds interact with the cardiac calcium channel.

While the biological activities of compounds **2** and **3** [13] are two orders of magnitude lower than that of verapamil both on heart and aorta, compounds **4–7** [14] show potent negative inotropic and negative chronotropic activities. Some of them (**4,6**) are in fact more potent than verapamil in decreasing left atrial contractility; compound **4** is as potent as verapamil in decreasing the right atrial rate. On the other hand, for all compounds the ability to relax vascular smooth muscle is poor or undetectable. It seems therefore that some selectivity between myocardium and vascular smooth muscle has been reached, at least in in vitro functional studies. This selectivity, which is absent in the parent compound, seems to be due to the constraint imposed on the molecule. The possibility that this may reflect a change in the ability of the molecule to reach the corresponding binding site has already been discussed [14].

If instead we consider negative inotropic or negative chronotropic activity, the rank order of potency of compounds **4–7** is different. In addition, while compounds **9** and **10** show good potency for both actions, compound **8** has good negative chronotropic activity but is unable to decrease the developed tension on guinea-pig isolated left atria. These findings suggest that the requirements for negative inotropic and negative chronotropic activities are different. Taking as model our rigid compounds, we now try to clarify these requirements by using molecular modelling studies.

TABLE 1
PHARMACOLOGICAL ACTIVITIES OF COMPOUNDS **2–10**

Compound	Negative inotropic activity[a]	ED_{50} (μM) of negative inotropic activity[d]	Negative chronotropic activity[e]	ED_{30} (μM) of negative chronotropic activity[d]	Vasorelaxant activity[h]	IC_{50} (μM) of vasorelaxant activity[d]	IC_{50} (μM) of displacement of [^{3}H] D888[j]
2	56 ± 5.8[b]	-	67 ± 3.4[b]	-	66 ± 2.3[i]	-	3.94 ± 0.257 (1.00)
3	52 ± 2.9[b]	-	65 ± 5.5[b]	-	59 ± 3.6[i]	-	-[k]
4 (*R*R*R**)	84 ± 1.2[c]	0.10 (0.07–0.14)	78 ± 1.5[f]	0.075 (0.06–0.09)	37 ± 2.5[b]	-	0.39 ± 0.032 (1.06)
5 (*R*R*S**)	75 ± 3.2[c]	1.30 (0.70–1.70)	93 ± 2.7[g]	0.23 (0.18–0.30)	30 ± 2.1[b]	-	0.27 ± 0.018 (1.13)
6 (*R*S*S**)	86 ± 1.5[c]	0.06 (0.04–0.08)	89 ± 4.1[c]	1.30 (1.10–1.60)	49 ± 2.8[b]	18 (13–24)	0.50 ± 0.033 (1.05)
7 (*R*S*R**)	74 ± 3.3[c]	0.20 (0.16–0.23)	75 ± 3.6[g]	0.42 (0.30–0.56)	58 ± 3.5[b]	10 (8–13)	0.26 ± 0.014 (0.99)
8	21 ± 1.2[b]	-	67 ± 5.4[f]	0.52 (0.46–0.60)	37 ± 2.6[b]	-	0.35 ± 0.025 (0.90)
9	85 ± 2.7[b]	0.68 (0.62–0.80)	64 ± 3.8[f]	0.19 (0.16–0.23)	34 ± 1.5[b]	-	0.32 ± 0.015 (0.94)
10	87 ± 2.6[b]	0.70 (0.60–0.77)	54 ± 3.7[f]	0.56 (0.50–0.65)	32 ± 2.4[b]	-	-[k]
Verapamil	84 ± 2.1[c]	0.61 (0.40–0.80)	94 ± 3.4[f]	0.07 (0.05–0.10)	95 ± 1.7[c]	0.38 (0.2–0.7)	0.15 ± 0.014 (1.04)

[a] Decrease in the developed tension in isolated guinea-pig left atrium, expressed as percentage change from controls ± S.E.M. (n = 5,6). The left atria were driven at 1 Hz.

[b] At 10^{-4} M.

[c] At 10^{-5} M.

[d] Calculated from log[conc.]/response curves (probit analysis according to Litchfield and Wilcoxon). 95% confidence limits are given in parentheses.

[e] Decrease in atrial rate in guinea-pig spontaneously beating isolated right atrium, expressed as percentage change from controls ± S.E.M. (n = 7,8). Pretreatments ranged between 165–195 beats/min.

[f] At 10^{-6} M; at this concentration verapamil produces a complete standstill in spontaneously beating right atria (five out of seven experiments).

[g] At 5×10^{-6} M.

[h] Percentage inhibition of calcium-induced contractions on KCl-depolarized guinea-pig aortic strips. Values are means ± S.E.M. (n = 6,7).

[i] At 5×10^{-4} M.

[j] Displacement of [^{3}H]D888 from cat ventricle membranes. Values are means ± S.E.M. (n = 9). Apparent Hill coefficients are given in parentheses.

[k] Not tested.

METHODS

Conformational analysis

Sterically allowed low-energy conformations for the compounds in the protonated form were searched for with Discover (Biosym), run on a Personal IRIS (Silicon Graphics). The input geometries for each dihedral were 60°, 180° and 300°. All possible conformers were retained, regardless of their energy value. Analysis of the resulting conformations was made with Insight II on a Personal IRIS. Superimpositions of the molecules were performed only with minimized conformations.

The conformational analysis of compounds **8–10** was done only on the conformers showing the most suitable arrangement of the three-methylene chain, i.e., those generated by overlapping the substituents on the quaternary carbon and the nitrogens of compounds **8–10** with those of **4** or **6**, by means of the 'torsion' option of Insight II, and then minimizing the resulting geometries. The input geometry for the methoxy groups was the same in all compounds; conformational analysis on this part of the molecules was not further explored. As far as chirality is concerned, the quaternary carbon atom for compounds **4–10** was built with the same configuration as *S*-(−)-verapamil.

The synthesis and detailed conformational analysis of compounds **2–7**, based on ^{1}H NMR data and on molecular mechanics calculations (MMPMI), have already been reported [13,14].

Chemistry

Melting points were measured on a Buchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin–Elmer 681 spectrophotometer. ^{1}H NMR spectra were measured on a Varian Gemini 200. Flash chromatographic separations were performed on silica gel (Kieselgel 40, 0.040–0.063 mm, Merck).

General procedure for the synthesis of compounds **8–10**

α-[1-(3-Chloropropyl)]-α-isopropyl-3,4-dimethoxybenzeneacetonitrile [15] was prepared according to a previously reported procedure [16], using butyllithium instead of LDA. The chloroderivative (1 equivalent), the suitable amine [17] (1 equivalent) and an excess of triethylamine were heated under reflux for 24 h. The mixture was then treated with $CHCl_3$ and washed with water and a solution of 10% NaOH. After anhydrification and evaporation of the solvent, the residue was purified by flash chromatography. Other experimental details and the physicochemical characteristics of compounds **8–10** are reported in Table 2.

Pharmacology

Negative inotropic and chronotropic activities were tested on isolated guinea-pig atria preparations, and vasodilator activity was tested on guinea-pig aortic strip preparations following standard procedures, details of which have been reported in Ref. 18. Binding experiments were performed according to the procedure reported in Ref. 14.

RESULTS AND DISCUSSION

From a structural point of view, as can be seen from Fig. 1, a three-carbon chain between the

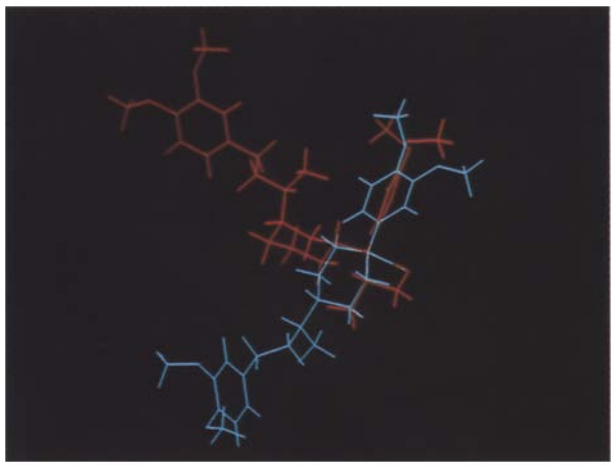


Fig. 2. Overlap of **6** (red) and **2** (light blue). Phenyl ring, nitrile and quaternary carbon of both molecules are superimposed; the protonated nitrogens and the second aromatic rings lie in very different positions.

basic nitrogen and the quaternary carbon atom was maintained in all compounds, but compounds **2–7** represent different arrangements of the methylene chain of verapamil. In compounds **2** and **3** the quaternary carbon atom was included in a cyclohexane ring. The phenyl ring and nitrile can easily achieve coplanarity, a feature thought to be important for activity [19], but this lipophilic part of the molecule is not free to move with respect to the basic nitrogen. Compounds **4–7** can be seen as folded conformations of verapamil; in these compounds the lipophilic part of the molecule can rotate with respect to the basic nitrogen. However, rotation is not completely free, since the energy barrier for rotation of the dihedral angle CN–C–C–H (τ) (see Fig. 1) is about

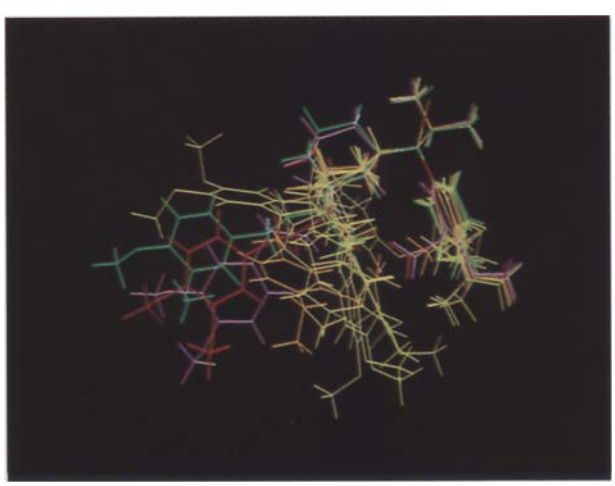


Fig. 3. Model for negative inotropic activity. Compound **8** (yellow) is shown in the six low-energy conformations arising from rotation of the exocyclic C–N bond, considering the substituent on the nitrogen both axial and equatorial. Compounds **4** (green), **5** (orange), **6** (red) and **7** (violet) are shown in conformations where the quaternary carbon atom and the protonated nitrogens overlap with **8**, but where the phenylethyl groups occupy a different area of space from **8**.

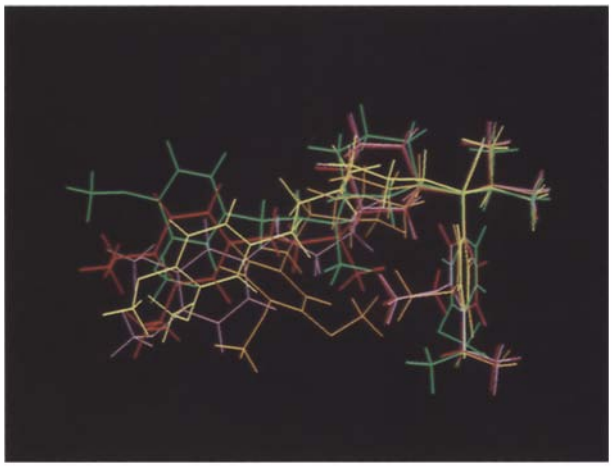


Fig. 4. Overlap of **4** (green), **5** (orange), **6** (red) and **7** (violet) in the putative active conformations that induce negative inotropic activity. D595 (**1b**, yellow), in the active conformation proposed by Höltje [20], fits this model well.

11 kcal/mol [14]. The basic part of the molecule is free to rotate, and it can assume several low-energy conformations.

As far as inotropic activity is concerned, the most potent compound is **6**, which shows a 10-fold higher activity than verapamil. At the same time its negative chronotropic activity is some 20 times less. It seems therefore safe to use **6** as the template to build a model for negative inotropic action. However, since the lipophilic head of the molecule (i.e., the substituents on the quaternary carbon) possesses some rotational freedom, we have no information about the exact conformation of this group. As a starting point we therefore fixed the quaternary carbon in the same arrangement as found by Höltje for D595 (**1b**) [20]. It must be pointed out that the conformation

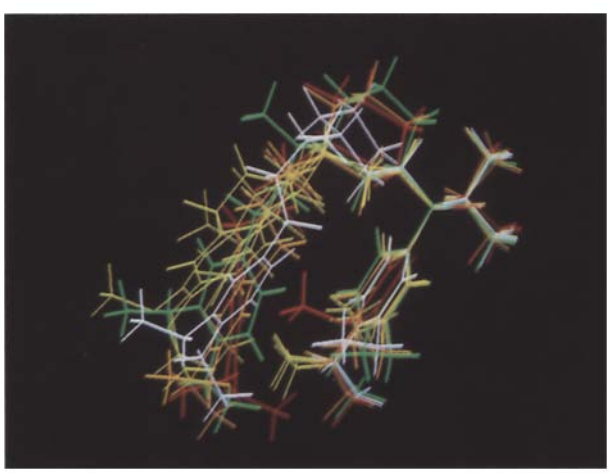


Fig. 5. Overlap of the putative active conformations of compounds **4**–**7** that produce negative chronotropic activity. Compound **8** (yellow) is shown in the three low-energy conformations arising from rotation of the exocyclic C–N bond. Compounds **4** (green), **5** (orange), **6** (red) and **7** (light violet) are shown in conformations where the quaternary carbon atoms and protonated nitrogens overlap with **8** and the phenylethylamino groups occupy a common area of space with **8**.

proposed by Höltje as the active one fits well with **6** when the dihedral angle τ is −60° and with **4** when τ is 180°, as far as the quaternary carbon and basic nitrogen are concerned. These two values of τ correspond to two of the three energy minima found for the rotation of the lipophilic head with respect to the cyclohexane ring [14].

In any case, we can assume that the best spatial arrangement of the quaternary carbon atom with respect to the basic nitrogen is the one derived from the arrangement of the three-carbon chain of **6**, in which the two chiral centers on the cyclohexane have the *S*S** configuration, and the distance between the quaternary carbon and the nitrogen is 4.5 Å. The negative inotropic potency of all compounds can be related to the degree of fitting to this model. Compound **4** is almost equiactive with **6**: although the configuration on the cyclohexane chiral centers is *R*R**, the above-mentioned distance is the same. Nitrogen and the substituents on the quaternary carbon of both **4** and **6** can be overlapped by simply rotating τ. However, the receptor seems to have the possibility of accommodating distances greater than 4.5 Å since the equatorial isomer **7**, whose N–C distance is 5.1 Å, is still more active than verapamil. Compound **5**, the other equatorial isomer, obviously shows the same distance of 5.1 Å and can be superimposed on **7** simply by rotating τ. So, by overlapping the lipophilic head of the molecules and suitably rotating τ, the

TABLE 2
CHEMICAL AND PHYSICAL CHARACTERISTICS OF COMPOUNDS **8–10**[a]

Compound	Yield (%)	Chromatographic solvent	m.p. (°C)	IR (neat) (cm^{-1})	^1H NMR[f] ($CDCl_3$,δ) (ppm)
8	25	$CHCl_3$/MeOH 98:2	130–132[c]	2260[e]	0.80 (d,3) and 1.19 (d,3) (CH_3CCH_3); 1.50–1.70 (m,1,$CHMe_2$); 1.82–2.28 (m,4,$CNCCH_2CH_2$); 2.40–2.50 (m,2); 2.54–2.64 (m,2); 2.70–2.82 (m,2); 3.41 (s,2,NCH_2Ar); 3.83 (s,6), 3.87 (s,3) and 3.89 (s,3) ($4OCH_3$); 6.47 (s,1), 6.57 (s,1) and 6.82–6.94 (m,3) (aromatics).
9	36	$CHCl_3$/MeOH 95:5	58–60[c]	2260[e]	0.80 (d,3) and 1.20 (d,3) (CH_3CCH_3); 1.45–1.65 (m,1,$CHMe_2$); 1.73–2.15 (m,4,CH_2CH_2CCN); 2.11 (s,3,NCH_3); 2.38 (t,2,CH_2N); 2.68–3.01 (m,4); 3.20–3.35 (m,1,CHN); 3.83 (s,3), 3.84 (s,3), 3.88 (s,3) and 3.89 (s,3) ($4OCH_3$); 6.71–6.95 (m,5) (aromatics).
10	26	[b]	118–120[d]	2260[e]	0.80 (d,3) and 1.19 (d,3) (CH_3CCH_3); 1.42–1.66 (m,3,$CHMe_2$ and CH_2CCN); 1.79–2.15 (m,5,CHN and CH_2CH_2Ar); 2.19 (s,3,NCH_3); 2.43–2.50 (m,2,CH_2Ar); 2.72– 2.76 (m,4,CH_2N and CH_2CN); 3.82 (s,6) and 3.83 (s,6) ($4OCH_3$); 6.55 (bs,2) and 6.79–6.94 (m,3) (aromatics).

[a] All compounds were analyzed for C, H, N; their elemental analyses were within 0.4% of the theoretical.
[b] Petroleum ether/abs. ethanol/$CHCl_3$/diethyl ether/NH_3 30:6:12:12:0.33.
[c] As hydrochloride, crystallized from abs. ethanol/anhydrous ether.
[d] As dibenzoyl tartrate, recrystallized from abs. ethanol/anhydrous ether.
[e] CN.
[f] As free bases.

axial nitrogen and the equatorial nitrogen are at a distance of 2.2 Å from each other, the same range of distance as between the two ends of a 'bidentate' carboxylate anion [21].

As far as compound **2** is concerned, the distance between the quaternary carbon and the nitrogen is the required one, but the lipophilic head of the molecule does not fit with **6**. In fact, there is only one possible arrangement of the three important groups (phenyl, nitrile and basic nitrogen) that are actually lying on a plane passing through the 1,4 positions of the cyclohexane ring. For compound **3**, the three groups are arranged similarly and in addition, the quaternary carbon–basic nitrogen distance is shorter than the one in **2**. The difference between compounds **2** and **3** is the distance between the quaternary carbon and the nitrogen, which is 4.1 Å when the substituent is axial and 4.4 Å when the substituent is equatorial. The inability of the nitrogen and the phenylethyl chain to reach the exact position would explain the loss of activity of these two compounds (Fig. 2).

Compound **8** does not show any negative inotropic activity and should not fit the template **6**. In fact, its phenylethyl chain is not free to move and the active conformation of **6** is not accessible to **8**. Since it is possible to rotate the three-methylene chain of **8** to overlap both the lipophilic head and the basic nitrogen with **6** (and consequently with **4**) and with their equatorial isomers **5** and **7**, the difference must be due to the phenylethyl chain. So, for the conformation showing the quaternary carbon and the nitrogen in the right arrangement, the three possible conformations of the dihedral C–C–N–C, with the amino substituent in either equatorial or axial position, were minimized and overlapped with **6**. A systematic search was then performed on the phenylethyl chain to look for conformational spaces other than those occupied by **8** but which were common to the four isomers **4–7**. From this search, conformations were selected which gave the best fitting of the phenyl rings, taking into account both the distances between the rings and the possibility for them to overlap (Fig. 3). Remarkably, the resulting model fits well the one proposed by Höltje [20] who, besides the verapamil analogue D595 (**1b**), also considered other calcium antagonists (diphenylalkyl amines and diphenylbutyl piperidines) with similar chemical structures to phen-

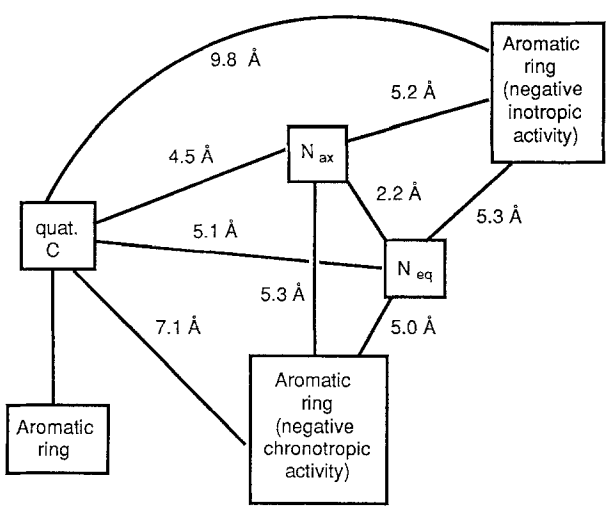
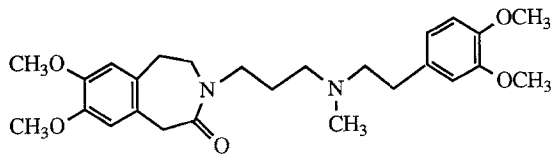


Fig. 6. Distances between groups which are necessary to produce negative inotropic and negative chronotropic activities. The distances come from superimpositions of the putative active conformations (see text).



11 : UL-FS 49

ylalkylamines but which show a different pharmacological profile from them and which, for these reasons, have been placed in other classes of calcium antagonists [22] (Fig. 4).

To build a model for negative chronotropic activity, we can apply the reverse of the approach used above, because the aromatic rings of the phenylethyl chains must be in a similar position to **8** which, as previously reported, shows good negative chronotropic activity but lacks negative inotropic action and which can be used as the template for the corresponding model. An important role can be played in this case by **4**, which has good negative inotropic and chronotropic activities. Its chiral carbons in the cyclohexane ring have the configuration *R*R**. The minimized conformation of **4** is overlapped with the previously found conformation of **8**, searching for conformations of the phenylethyl chain that bring the aromatic ring close to that of **8**. The search was then extended to the other isomers **5–7**. Again, the choice was made on the basis of the possibility for the rings to overlap. The resulting conformations are reported in Fig. 5.

According to this model, negative chronotropic activity is produced when the molecules assume conformations that bring the aromatic ring of the phenylethylamino chain to a distance of 5.3 Å from the protonated nitrogen (axial) and at 7.1 Å from the quaternary carbon, while for the negative inotropic activity the latter distance is 9.8 Å (Fig. 6). As mentioned before, the model for negative inotropic activity is then very similar to that found by Höltje [20]. However, it must be stressed that, for the rigid molecules so far available, we have no information on the exact position of the phenyl ring on the quaternary carbon and cannot therefore say at what distance the two aromatic rings must be. In this respect it is important to note that the potent bradycardic benzolactam derivative UL-FS 49 (**11**) [12] is reported to adopt a U-shaped conformation in the crystalline state, and this is very similar to our putative active conformation for the negative chronotropic activity. Preliminary calculations show a good overlap between **4** and **11**.

A constraint was also introduced into the phenylethylamino chain in compounds **9** and **10**, but with different results than for **8**, because the shifting of the nitrogen from an endocyclic position to an exocyclic one resulted in a loss of selectivity between the two cardiodepressive actions. In fact, while in compound **8** only rotation around the C-heterocycle bond can affect the position of the aromatic ring of the basic part of the molecule, in **9** and **10** there are two rotatable bonds. The position of the aromatic ring can therefore vary to a greater extent than in **8** and, as a consequence, we can find conformations of compounds **9** and **10** that fit the model for both negative chronotropic and negative inotropic activity, which is in accordance with their dual biological action (Figs. 7 and 8).

As far as smooth-muscle relaxation is concerned, nothing can be said about the 'active conformation' of verapamil except that it must be different from that identified with our compounds, or that, as mentioned in the Introduction, the bulk of the carbon chain used to freeze verapamil completely impairs the interaction with the smooth-muscle channel. In fact, despite the high number of compounds synthesized, we did not obtain any compound with good activity on vascular smooth muscle, and in the literature we found only one example of a semi-rigid ana-

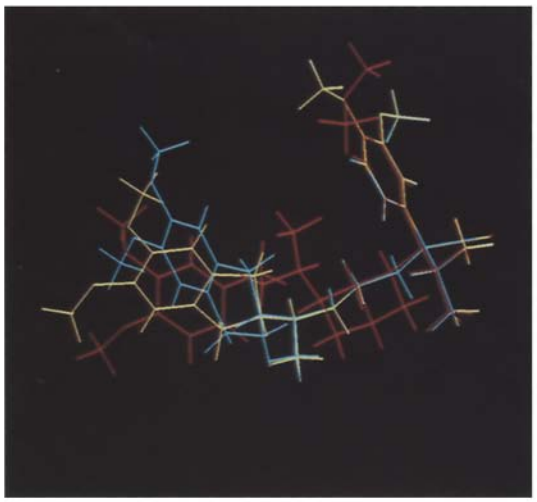


Fig. 7. Overlap of **9** (yellow) and **10** (light blue) with **6** (red) in the putative active conformations that induce negative inotropic activity.

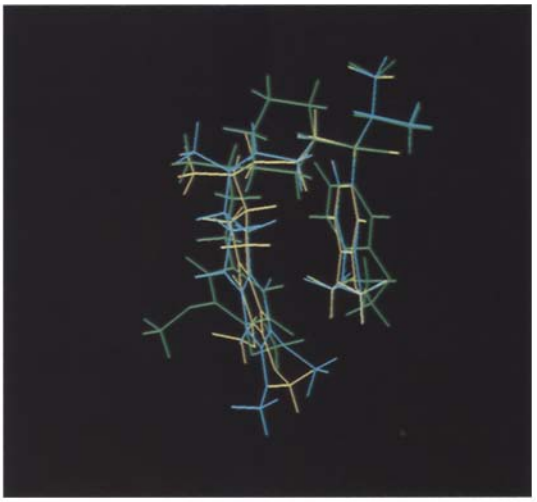
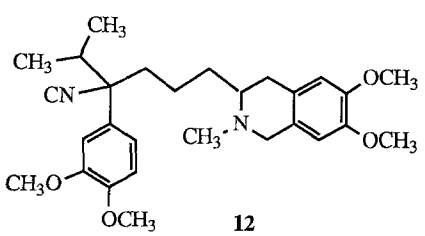


Fig. 8. Overlap of **9** (yellow) and **10** (light blue) with **4** (green) in the putative active conformations that produce negative chronotropic activity.

logue, namely the Syntex compound **12** [23], with high potency on smooth muscle. In this compound, rigidity was brought into the phenylethylamino chain, which was constrained into a tetrahydroisoquinoline ring, but in a different way from compound **8**. While **8** is devoid of calcium antagonistic activity on KCl-contracted guinea-pig aortic strips, the *SS* enantiomer of **12** is reported to be twice as potent as racemic verapamil in relaxing the $BaCl_2$-contracted rat aortic strips. Besides differences due to animal species, we can suppose that a different arrangement of the quaternary carbon and the nitrogen groups, and of the phenylethylamino chain, are responsible for the good calcium antagonistic activity on smooth muscle.



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In conclusion, for verapamil-like compounds we have defined two different models for negative inotropic and negative chronotropic activities. These two models explain the two pharmacological actions on the basis of different orientations of the phenylethylamino groups. The synthesis of other rigid analogues will help us to refine our models, as well as to propose another one for the interaction with the smooth-muscle calcium channel.

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