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Synthesis and α-adrenergic activity of 2- and 6-methyl-substituted (3,4-dihydroxyphenyl)-3-piperidinols†

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Summary — Previous drug–receptor interaction mechanism studies at the molecular level of adrenergic drugs made it possible to construct two three-dimensional molecular models, A and B, using conformationally restrained cyclic analogues of natural catecholamines, including 3-(3,4-dihydroxyphenyl)-3-piperidinol (3-DPP, **3**); these models offer useful information about the steric requirements for the direct activation of α_1- and α_2-adrenergic receptors, respectively. In order to gain further knowledge about the steric requirements of these receptors, we also synthesized 3-(3,4-dihydroxyphenyl)-*c*-2-methyl-*r*-3-piperidinol (2-MDPP, **8**) and the 3-(3,4-dihydroxyphenyl)-*c*- and -*t*-6-methyl-*r*-3-piperidinols (6-MDPPs **9** and **10**); these differ from the 3-DPP **3** used for the construction of the molecular models exclusively in the presence of a methyl in the 2 or 6 position of the heterocyclic ring. The configuration and conformation of the MDPPs **8–10** were assigned by ^{1}H-NMR and IR studies, and confirmed by conformational analysis performed by means of theoretical calculations. The α_1- and α_2-adrenergic properties were evaluated in vitro both by radioligand binding assays and by functional tests on isolated preparations. The results obtained made it possible to obtain a more refined steric definition of the A and B models.

3-piperidinol derivative / adrenergic α-stimulating activity / conformational analysis / molecular model

Introduction

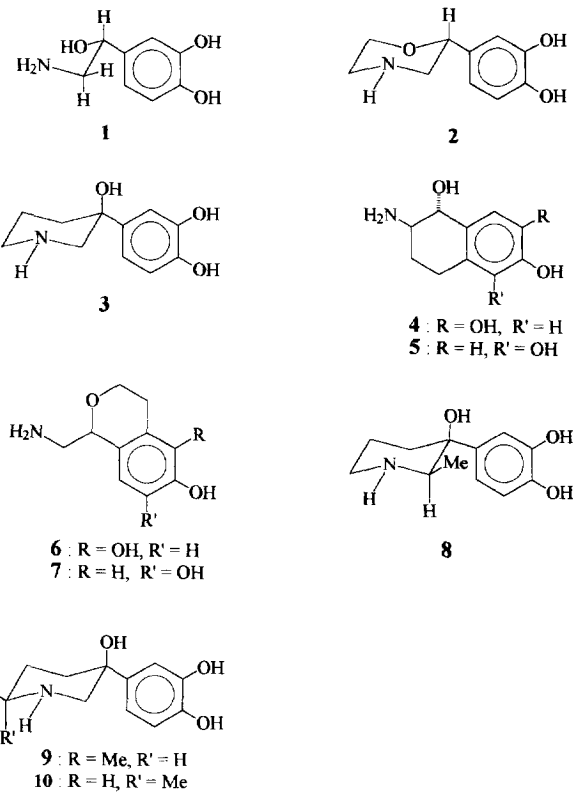
The adrenergic receptor [2] (AR) belongs to the sympathetic effector system and mediates many of the physiological actions of the endogenous catecholamines: norepinephrine (NE), the neurotransmitter of the adrenergic sympathetic nerves; and epinephrine, which is the principal hormone of the adrenal medullar.

AR is part of the G-protein coupled receptor superfamily [3, 4]. It crosses the cell membrane with seven putative α-helical segments, and in response to first messenger signals from the extracellular neurotransmitters, it modulates various intracellular biochemical processes via G-proteins and effector systems.

In the last few years, remarkable progress has been made towards both the isolation and the functional and structural characterisation of ARs (see, for example, [5–15]). A particular impulse to these studies has been supplied by the utilisation of the recombinant-DNA technique (see, for example, [3, 4, 9, 16, 17]) which has made possible the cloning, and therefore the partial determination, of the amino-acid sequence of multiple subtypes of α- and β-ARs. In spite of the development of these studies (see, for example, [5–15]), no AR site has been structurally determined, and it is still not possible to define the 3D-structure of this physiologically important receptor with certainty. Consequently, the design of AR ligands directly based on modelling is not yet possible. In the design of novel molecules capable of interacting with AR sites, it is therefore necessary to make use of indirect models, constructed on the basis of knowledge which can be obtained from the molecular features of both agonist and antagonist ligands or suitable derivatives (see [18, 19] and references therein).

In previous papers in this series [20–22], a comparative study was made of the stereostructures and of the α_1- and α_2-adrenergic stimulating activity of NE (**1**) and its morpholinic (2-DPM, **2**), piperidinic

*Correspondence and reprints
†Nineteenth paper in the series *Conformational effects on the activity of drugs*. For preceding paper, see reference [1].



(3-DPP, **3**), tetrahydronaphthalenic (2-ADTNs, **4** and **5**) and isochromanic (1-AMDICs, **6** and **7**) semirigid analogues. This study allowed us to propose the three-dimensional molecular models A and B (fig 1), as a result of the superimposition of the biologically most active molecules (NE and semirigid analogues **2** and **3** for model A, and NE and semirigid analogues **2–4** and **6** for model B) in the conformation which allows the spatial coincidence of the presumed active groups (aryl moiety, amine nitrogen, and alcoholic or ethereal benzylic oxygen) (see, for example, [22–28]). Models A and B suggest a spatial situation in which these pharmacophoric groups should interact at the receptor site. This pharmacophoric spatial situation corresponds to the one found in the preferred conformation of catecholamines [20, 26, 30–32]. These models thus provide information about the steric requirements for the direct activation of α_1- and α_2-ARs, respectively. Moreover, the models indicate the molecular portions that should not hinder a hypothetical 'fit' with the receptor.

In order to gain further knowledge about the steric requirements for the direct activation of α-ARs and, in particular, to evaluate the effect of the presence of a bulky group on the molecular models, we synthesized 3-(3,4-dihydroxyphenyl)-*c*-2-methyl-*r*-3-piperidinol (2-MDPP, **8**) and 3-(3,4-dihydroxyphenyl)-*c*- and -*t*-6-methyl-*r*-3-piperidinols (6-MDPPs, **9** and **10**), which differ from the 3-DPP **3** used for construction of the models A and B in the presence of a methyl group on the heterocyclic ring.

The preferred conformations and the configurations in solution of MDPPs **8–10** were assigned by means of ^{1}H-NMR and IR studies and confirmed by means of conformational analysis performed throught molecular mechanics calculations. The activity of **8–10** on α_1- and α_2-ARs was assessed in vitro by means both of radioligand binding assays and of functional tests.

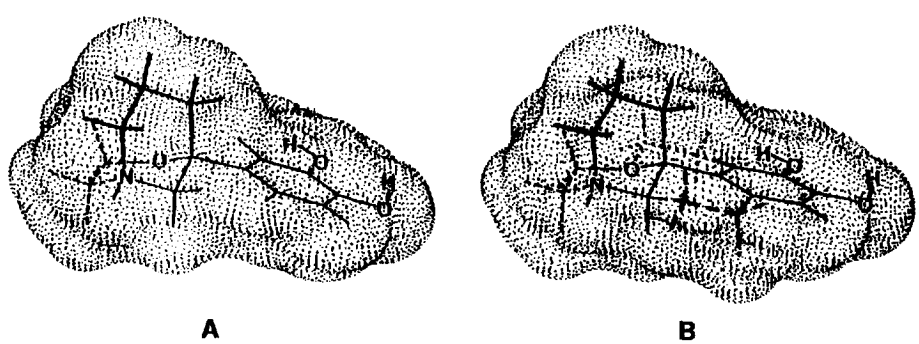


Fig 1. Molecular models arising from the superimposition of the pharmacophoric groups (aryl moiety, aminic nitrogen, alcoholic or ethereal oxygen) of drugs **1–7** in the conformation in which they should interact with the α_1- (A) and α_2-adrenergic receptor (B). The common arylethanolaminic portion is represented as a thin solid line, the portion arising from morpholine **2** as a thin dashed line, the portion arising from piperidine **3** as a thick solid line, the portion arising from the tetrahydronaphthalene **4** as a thick dashed line and the portion arising from isochromane **6** as a thick dotted line. The dot clouds indicate the molecular volumes; these volumes correspond to steric hindrances that arise from atoms present in drugs **1–6**, used in turn for the construction of the models.

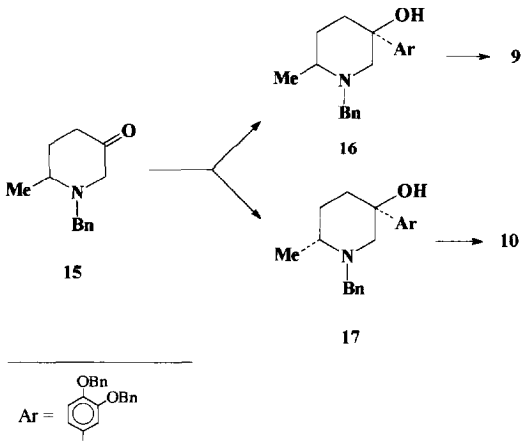
Chemistry

The 2-methyl-piperidinol **8** (2-MDPP) was synthesized as indicated in scheme 1. The Dieckmann condensation of diester **11** [33, 34] with sodium ethoxide [33] gave the ketoester **12** [34] as hydrochloride; by subsequent decarboxylation with hydrochloric acid 6 N the piperidone **13** [34] was obtained as hydrochloride. The reaction of **13** with 3,4-(dibenzyloxy)phenylmagnesium bromide [35] yielded exclusively the isomer 3-(3,4-dibenzyloxyphenyl)-*c*-2-methyl-*r*-3-(*N*-benzyl)piperidinol **14** which by catalytic hydrogenolysis yielded the 2-MDPP **8**.

Attempts to transform the *cis*-piperidinol **14** into its *trans* isomer by means of the transformation of **14** into the corresponding 3-chlorine derivative and subsequent solvolysis [36, 37] were unsuccessful due to a lack of reactivity of alcohol **14** to thionyl chloride, used as a halogenating agent, under the usual conditions for the substitution of an alcoholic hydroxyl with chlorine.

The MDPPs **9** and **10** were synthesized as reported in scheme 2. The reaction of *N*-benzyl-6-methyl-3-piperidone **15** [38] with 3,4-(dibenzyloxy)phenylmagnesium bromide [35] yielded the 3-(3,4-dibenzyloxyphenyl)-*c*- and 3-(3,4-dibenzyloxyphenyl)-*t*-6-methyl-*r*-3-(*N*-benzyl)piperidinols **16** and **17** in a ratio of about 60:40, which were subsequently separated by column chromatography. Catalytic hydrogenolysis of **16** and **17** yielded **9** and **10**, respectively. All the catecholic derivatives **8–10** were isolated as hydrochlorides, because of their instability as free bases.

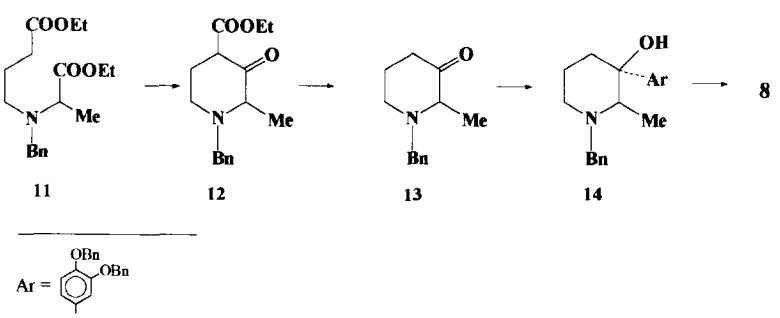
The conformation and the configuration of MDPPs **8–10** were assigned on the basis both of IR studies in the 3 μ range in a dilute solution and of the analysis of the ^{1}H-NMR spectra of their corresponding precursors protected on the catecholic hydroxyls by a benzylic group (**14**, **16** and **17**). The instability of free catecholic nuclei in solution unfortunatly does not make it possible to carry out these studies on MDPPs **8–10** as free bases.



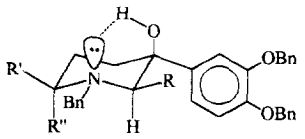
Scheme 2.

The IR spectra in a dilute solution of the benzyl derivatives **14**, **16** and **17** contain a strong band between 3505 and 3450 cm^{-1} (see *Experimental protocols*) which can be attributed to an OH···N interaction. This interaction can take place only in one of the two possible chair conformers, that is to say, in the one where the hydroxyl is in the axial position [39–42] and the possibility thus exists of the formation of an intramolecular hydrogen bond between the hydroxyl and the nitrogen of the piperidinic ring. These data thus indicate that the preferential conformation of piperidinols **14**, **16** and **17** in solution is the one where the hydroxyl is axial and the aryl group is situated in the more favourable equatorial position (fig 2).

The equatorial or axial position of the methyl group was assigned on the basis of the values of the differences in chemical shift (Δδ) generated by the methylenic protons of the benzylic group linked to the nitrogen, observed in ^{1}H-NMR spectra of the compounds **14**, **16** and **17**, as reported in literature for other 2- and



Scheme 1.



14: R = Me, R' = R" =H
16: R' = Me, R = R" =H
17: R" = Me, R = R' =H

Fig 2. Preferred chair conformers of piperidinols **14**, **16** and **17**. The broken line indicates the intramolecular hydrogen bond.

6-methyl-substituted piperidines [43, 44]. The equatorial position of the methyl linked to the C(2) in **14** and to the C(6) in **16**, and therefore the *trans*-diequatorial relationship between these groups and the aryl, derives from the high $\Delta\delta$ value of the AB quartet of the methylenic protons of the benzyl group (0.92 and 0.95 ppm, respectively). The axial position of the methyl in position 6 in **17**, and therefore the *cis* axial–equatorial relationship between this group and the aryl, derives from the low $\Delta\delta$ value (0.21 ppm) found for the same protons. These results are in agreement with those found for analogous studies previously carried out on other 3-arylpiperidinols differently substituted on the aromatic ring [1, 38, 45]. The conformations thus deduced for MDPPs **8–10** as free bases were subsequently confirmed by theoretical studies carried out on MDPPs **8–10** as cationic forms, that is to say, in the form in which these compounds should exist in aqueous physiological medium.

Theoretical calculations

Conformational analysis was carried out in vacuo on the 3-DPP **3**, the 2-MDPP **8**, and the 6-MDPPs **9** and **10** by means of the Discover [46] molecular mechanics program; all compounds were considered as cationic forms which should be more stable at physiological pH.

The results of the calculations indicate that the conformation of the piperidinic ring with the phenyl group in an equatorial position is preferred by 3.2 kcal/mol for the 3-DPP **3**, by 11.8 kcal/mol for the 2-MDPP **8**, and by 6.6 kcal/mol for the 6-MDPP **9**; in the other 6-MDPP **10**, the two possible chair conformations are energetically equivalent, seeing that the one in which the phenyl ring is in the axial position is preferred by only 0.2 kcal/mol. The conformation found for **3** is in agreement with the one previously reported [20]. The conformations found for **8** and **9** are in agreement with those found experimentally by IR and ^{1}H-NMR measurements for **14** and **16**. Also in the case of compound **10**, there is no contrast with the experimental data found for **17**, because the two conformations have very low energy differences, and therefore are equally probable.

The conformational energy of the 3-DPP **3** and the MDPPs **8–10** was also studied as a function of the τ torsion angle [C_β-C_α-C(3)-C(2)] which defines the rotameric position of the aryl ring with respect to the piperidinic ring.

The conformation with the phenyl ring in an equatorial position was considered for all compounds. This conformation was found to be largely preferred for **3**, **8** and **9** and possible for **10**. The choice of this conformation also for **10** is justified by the fact that in it, the active groups (aryl moiety, amine nitrogen and alcoholic benzylic oxygen) are in a spatial position which corresponds to the one the same groups have in the preferred conformation of NE [20, 26, 30–32], which is considered to be the pharmacophoric one (see steric models A and B).

As shown in figure 3, all compounds considered (**3** and **8–10**) have two energy minima, the first one corresponding to a τ value of $-60°$, and the second one less than 1 kcal/mol higher, corresponding to a τ value of $+110°$. The latter minimum energy conformation roughly corresponds to the value which the τ angle has in the steric models A and B. These results therefore indicate that the presence of the methyl on the C(6) in **9** and **10** does not influence in any manner the conformational energy trend with respect to that of the unsubstituted 3-DPP **3**. The methyl substitution on the C(2) in **8** only has the effect of raising the values of the energy barriers; the conformational energy trend is not influenced in this case, either.

Radioligand binding assays

The affinity of MDPPs **8–10** for α-adrenergic receptors was determined by binding tests carried out on rat brain membrane preparations (table I). [^{3}H]Prazosin and [^{3}H]rauwolscine were used as specific tritiated ligands for α_1- and α_2-receptors, respectively.

Rat brain α_1-receptors

The 6-MDPP **10** showed an inhibitory activity in the [^{3}H]prazosin labelled binding assays, which was considerably lower (about 70 times) than that of NE, but only twice as low as that of the 3-DPP **3**. The 2-MDPP **8** and the 6-MDPP **9** were found to be inactive.

Rat brain α_2-receptors

The 6-MDPPs **9** and **10** showed a very similar activity in inhibiting [^{3}H]rauwolscine binding, which was about three orders of magnitude lower than that of NE and about one order of magnitude lower than that of

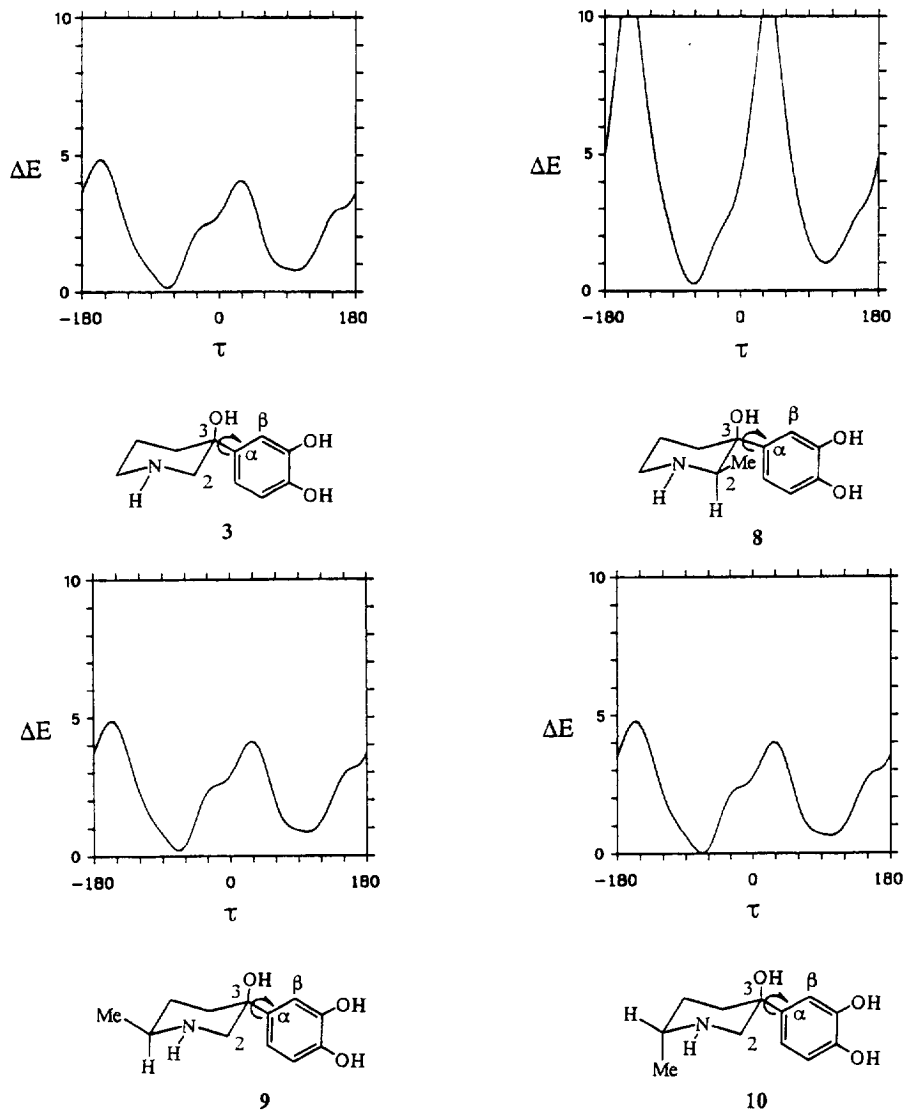


Fig 3. Plot of the relative conformational energy ΔE (each unit of the ordinate scale corresponds to 1 kcal/mol) of compounds **3** and **8–10** against the torsion angle τ [Cβ-Cα-C(3)-C(2)].

the 3-DPP **3**. The 2-MDPP **8** showed an affinity much lower than that of **9** and **10**.

Functional tests

The MDPPs **8–10** were tested on isolated rat vas-deferens for their activity on α_1-receptors and on isolated guinea-pig ileum for their activity on α_2-receptors (table II).

Rat vas-deferens α_1-receptors

The 2-MDPP **8** and the 6-MDPP **10** exhibited a stimulating activity, with pD_2 values which were 0.5 lower than that of the 3-DPP **3** and one order of magnitude lower than that of NE. The 2-MDPP **8**, however, unlike **10**, showed a very modest intrinsic activity (ia). The 6-MDPP **9** was practically inactive.

Guinea-pig ileum α_2-receptors

The 6-MDPPs **9** and **10** exhibited a stimulating effect on the α_2-AR located on cholinergic fibres of the guinea-pig similar to that of the 3-DPP **3**; the 6-MDPP **10** displayed a pD_2 value one order of magnitude lower than that of NE (**1**). The 2-MDPP **8** showed only a weak activity on this receptor subtype.

Table I. Radioligand adrenergic binding affinities of compounds **1**, **3** and **8–10**.

Compound	*α-Adrenergic binding affinity (K_i, nM)*[a]	
	Rat brain (α_1)	*Rat brain (α_2)*
1	450 (390–520)[b]	4.8 (4.5–7.1)[b]
3	15 000 (12 500–17 500)[b]	500 (410–580)[b]
8	>100 000	12 000 (11 200–13 800)
9	>100 000	4600 (4100–5100)
10	33 000 (30 400–35 600)	4500 (4100–4900)

[a]Geometric means with confidence limits shown in parentheses; [b]reference [21].

Discussion

An examination of the data presented in tables I and II indicates that the results of the functional tests show an activity trend that is substantially similar to the affinity trend revealed in the binding tests. Quantitative differences between the responses of the functional tests and those of the binding tests might depend on differences between the animal species and the kinds of tissues used; in addition, for an agonist, both differences in spare receptors or receptor reserves and amplification factors may differentiate the curve of the functional response from that of the occupancy [47].

α_1-Adrenergic activity

An examination of the data presented in tables I and II indicates that the presence of a methyl group on the heterocyclic ring of the 3-DPP **3** leads to a drop in the activity when it is in the equatorial position and is linked to the C(2) or the C(6), as in the MDPPs **8** and **9**, respectively. When, on the contrary, the methyl linked to the C(6) is in the axial position, as in the 6-MDPP **10**, it allows the expression of the activity similar to that of the unsubstituted piperidinol (3-DPP, **3**).

α_2-Adrenergic activity

The activity data shown in tables I and II indicate that the presence of the methyl on the C(2) in the equatorial position (2-MDPP, **8**), determines, also in this case, a drop in the activity. The methyl on the C(6), either in the equatorial or in the axial position, as in the 6-MDPPs **9** and **10**, respectively, allows, on the contrary, the expression of a good activity, similar to that of the unsubstituted piperidinol (3-DPP, **3**).

Theoretical calculations

The results indicate that the introduction of the methyl group on the piperidinic ring did not influence the conformational trend of the phenyl ring observed for the unsubstituted 3-DPP **3**. Furthermore, even if the methyl group can modify the relative stability of the two possible chair conformers of the piperidinic ring, the conformer in which the active groups are in the spatial relationship corresponding to the one the same groups have in the steric models A and B, was found to be largely preferred in **8** and **9** and probable in **10**

Table II. α-Adrenoceptor agonistic activities of compounds **1**, 3-DPP (**3**), 2-MDPP (**8**) and 6-MDPPs (**9** and **10**) on isolated preparations.

Compound	*α-Adrenoceptor activity*[a]			
	Isolated rat vas deferens (α_1)		*Isolated guinea pig ileum (α_2)*	
	pD_2	*ia*[b]	*pD_2*	*ia*[b]
1	5.20 (± 0.09)[c]	1.00[c]	6.60 (± 0.10)[c]	1.00[c]
3	4.80 (± 0.18)[c]	0.96[c]	5.49 (± 0.10)[c]	1.00[c]
8	4.19 (± 0.07)	0.40	4.15 (± 0.17)	0.69
9	<3.50		5.29 (± 0.20)	1.00
10	4.23 (± 0.20)	1.08	5.70 (± 0.05)	1.00

[a]The agonist activity of each compound on the receptors was evaluated by means of pD_2 value, ie, the negative logarithm of the drug molar concentration that produces 50% of the maximal effect; the values represent the mean of four to six experiments for each drug ± standard error; [b]intrinsic activity, ie, the ratio between the maximal response elicited by the compound under test and that elicited by the full agonist, namely NE; [c]reference [1].

(see fig 3). Therefore, the effect on the pharmacological properties due to the introduction of a methyl group into the 3-DPP would seem not to be attributable to conformational effects, but rather explainable in terms of steric hindrance.

Molecular models

Figure 4 shows the MDPPs **8–10** in their preferred conformations deduced by IR and ^{1}H-NMR studies. For **8** and **9** this conformation corresponds to the one determined by theoretical studies; in the case of **10**, it corresponds to one of the two relative minimum conformations calculated, which are equally probable. As may be observed (see fig 4), the MDPPs in this conformation present their active groups in a spatial situation which corresponds to that of the same groups in the models A and B, and therefore they are superimposable on these steric models.

The MDPPs **8–10** highlight the steric hindrance areas generated, respectively, by the methyl group on the C(2) in the equatorial position (H_A), on the C(6) in the equatorial position (H_B), and on the C(6) in the axial position (H_C).

Furthermore, figure 4 shows the skeletons of the steric models A and B, highlighting the three possible steric hindrance areas, H_A, H_B and H_C, corresponding to the ones generated by the methyl of MDPPs **8–10**, respectively.

The lack of any α_1- or α_2-activity in the 2-MDPP **8** shows that the presence of the H_A does not allow the drug to fit with either of the receptors. The good activity of the 6-MDPP **10** on both kinds of α-ARs shows that the H_C hindrance does not impede the fit with these receptors. The good activity shown by the 6-MDPP **9** on the α_2-AR, and its limited activity on the α_1-AR show that the presence of the H_B hindrance allows the drug to fit with the α_2-receptor, whereas it is an obstacle to its fit with the α_1-receptor. The presence of the H_B hindrance would therefore appear to produce a certain selectivity for α_2-ARs.

Figure 5 shows the refined models A_1 and B_1 for the activation of α_1- and α_2-receptors, respectively, obtained by adding to the hindrance present in the A and B models those hindrances whose presence is not an obstacle to interaction with the receptors: H_C due to the C(6) axial methyl in both A and B models; and H_B due to the C(6) equatorial methyl group in model B.

The fact that the H_A hindrance present on the same side of the models as the aminic nitrogen and the ethereal oxygen (see fig 4) hinders the interaction with the receptors would seem to suggest the involvement of this side of the hypothetical models in the interaction with the α-ARs.

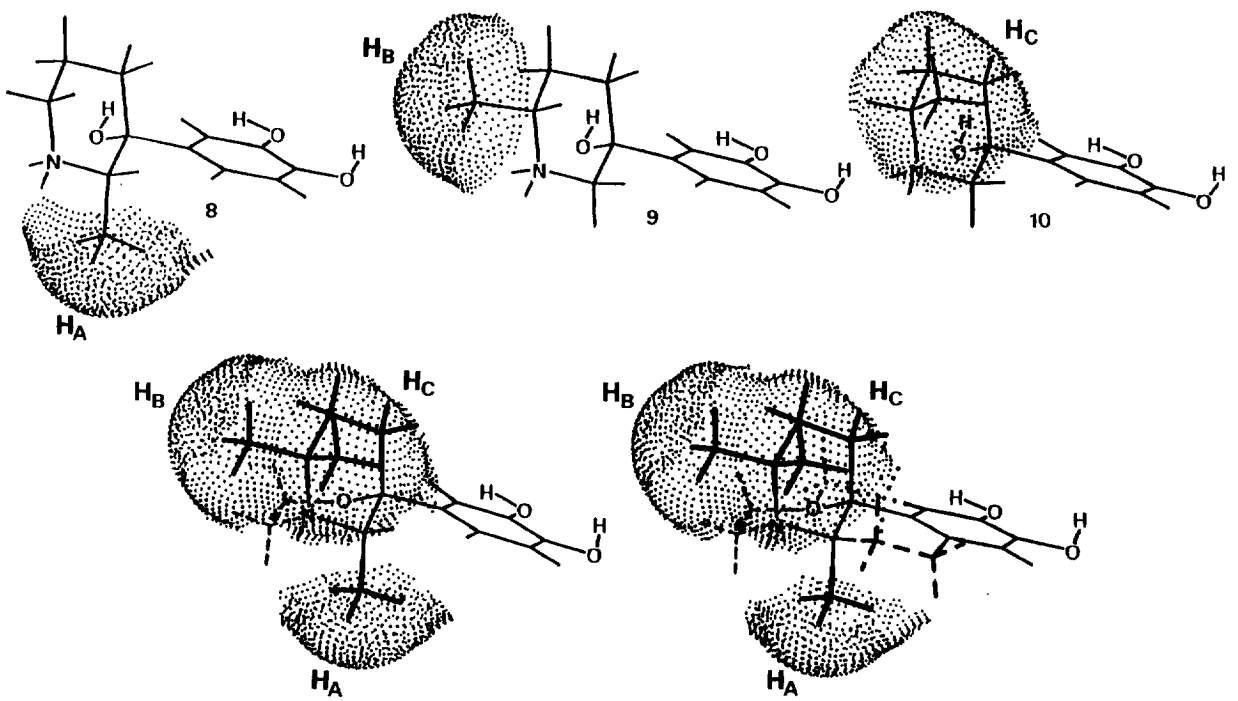


Fig 4. MDPPs **8–10** in which the molecular volume: (H_A, H_B and H_C, shown as dot clouds) of the corresponding methyl group is shown. The skeletons of models A and B show the molecular volumes H_A, H_B and H_C, which would arise from the introduction of the methyl groups of MDPPs **8–10** into the models.

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra for comparison between compounds were recorded with a Perkin-Elmer Infracord Model 137 instrument, as Nujol mulls in the case of solid substances, or as liquid film in the case of liquids. IR spectra for the determination of OH···N stretching bands were taken in CCl_4 solution with a Perkin-Elmer Model 257 double-beam grating spectrophotometer for compounds **14**, **16** and **17**, using the indene band at 3110 cm^{-1} as a calibration standard; a quartz cell of 1 cm optical length was employed and the concentration of the solution was 5 x 10^{-3} M or lower, to prevent intermolecular association. ^{1}H-NMR spectra were routinely recorded with a Varian EM 360 A instrument in a ca 5% solution of $CDCl_3$ (for the neutral compounds or the free bases) or D_2O (for the salts), using Me_4Si or $Me_3Si(CH_2)_3SO_3Na$ as the internal standard, respectively. ^{1}H-NMR spectra were also measured for compound **14** on a Varian CFT-20 spectrometer operating at 80 MHz, and for compounds **16** and **17** on a Varian VXR 300 instrument. Column chromatography for the separation of **16** and **17** was carried out on 70-230 mesh silica gel; glc analyses were performed on a Carlo Erba model 4200 apparatus with a flame ionisation detector and using a column 1.8 m x 3.5 mm OV1 3% on chromosorb W silanised 80/100 mesh (column temperature 230 °C; evaporator and detector 260 °C; nitrogen flow 30 mL/min). Evaporations were made in vacuo (rotating evaporator). $MgSO_4$ was always used as the drying agent. Elemental analyses were performed by our analytical laboratory and agreed with theoretical values to within 0.4%.

Ethyl 4-[N-benzyl-N-(1-ethoxycarbonylethyl)]aminobutirrate ***11***

The *N*-benzyl derivative (**11**) was prepared following the method described previously [33]. **11**: bp 118–120 °C (0.3 mmHg) [lit bp [34] 123–128 °C (0.4 mmHg)].

N-Benzyl-2-methyl-4-carbethoxy-3-piperidone hydrochloride ***12•HCl***

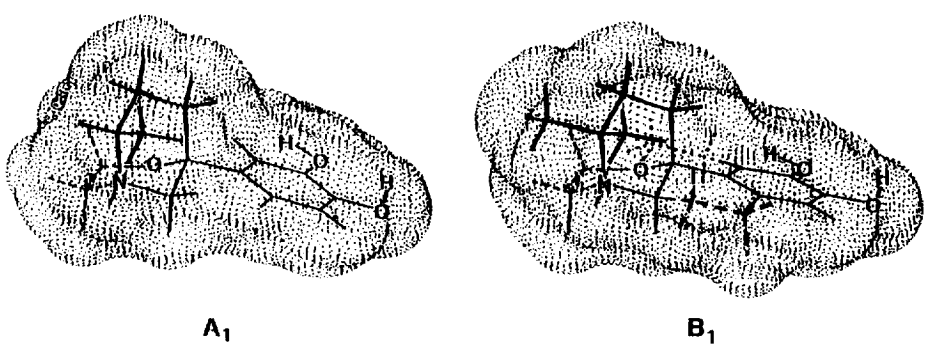
Pure **11** (5.0 g, 0.01 mol), was added dropwise to a suspension of sodium ethoxide (prepared from 0.015 gatom of Na and anhydrous EtOH (10 mL)) [33] and the temperature was gradually raised to 140 °C until the complete elimination of EtOH. After 30 min at this temperature, the mixture was cooled. The vitrous product was treated with H_2O and the resulting mixture was acidified to pH 3, maintaining the temperature under 10 °C by external cooling. The aqueous solution was neutralised with solid K_2CO_3 and extracted with Et_2O until the organic layer no longer gave a red colour with $FeCl_3$. The evaporation of the dried and filtered organic extracts yielded **12** (lit [34] physical constants unreported) as an oil (2.5 g, 80%) practically pure (glc). The crude oil (0.80 g) was dissolved in anhydrous Et_2O and then treated with Et_2O•HCl to yield a solid precipitate which was filtered and crystallized from EtOH/Et_2O to give pure **12•HCl** (0.66 g) mp 90–93 °C; ^{1}H-NMR δ 1.33 (t, 3H, *J* = 7.0 Hz, CH_2CH_3), 1.76 (d, 3H, $CHCH_3$), 4.23 (q, 2H, *J* = 7.0 Hz, CH_2CH_3). Anal for $C_{16}H_{22}ClNO_3$ (C, H, N).

N-Benzyl-2-methyl-3-piperidone ***13***

A solution of **12•HCl** (10.0 g, 0.036 mol) in 6 N HCl (30 mL) was heated as reported in the literature [34] over a steam-bath for 3 h. The excess of HCl was removed by distillation and the oily residue was crystallized using EtOH/Et_2O to give **13•HCl** (7.0 g) as an amorphous hygroscopic solid. **13•HCl** was converted into the free base by treating an aqueous solution of the salt with solid KOH and extracting with $CHCl_3$. The organic layer was washed (H_2O), filtered and evaporated to give pure piperidone **13** as an oil. **13**: bp 90–93 °C (0.4 mmHg); [lit bp [34] 90–95 °C (0.5 mmHg)]. ^{1}H-NMR δ 1.20 (d, 3H, $CHCH_3$), 3.45 and 3.85 (2d, 2H, *J* = 12.0 Hz, NCH_2Ph), 7.33 (s, 5H, Ph).

3-(3,4-Dibenzyloxyphenyl)-c-2-methyl-r-3-(N-benzyl)piperidinol hydrochloride ***14•HCl***

A solution of **13** (5.10 g, 0.024 mol) in anhydrous Et_2O (30 mL) was added at room temperature to a stirred solution of 3,4-(dibenzyloxy)phenylmagnesium bromide [35] prepared from 3,4-(dibenzyloxy)bromobenzene (10.0 g, 0.027 mol) and Mg (0.028 gatom) in anhydrous THF (30 mL). The mixture was stirred for 12 h at room temperature, hydrolyzed and acidified with solid NH_4Cl, and washed with Et_2O. The aqueous phase was alkalinized and extracted with Et_2O. Evaporation of the dried organic layers gave an oil (3.20 g) which was dissolved in anhydrous Et_2O and treated with an



A$_1$ **B$_1$**

Fig 5. Molecular models A_1 and B_1 obtained by adding to the molecular volumes of models A and B (see fig 1) the molecular volumes arising from the corresponding methyl groups which are not an obstacle to interaction with the receptors: H_C in both A and B models, and H_B only in model B. The dot clouds indicate the molecular volumes which correspond to steric hindrances that arise from atoms present in the models. These regions of bulk should not therefore hinder a hypothetical 'fit' of models A_1 and B_1 with the α_1- and α_2-AR, respectively.

excess of Et_2O•HCl to yield a solid precipitate which was filtered and crystallized from AcOEt to give pure **14•HCl** (1.5 g, 12%) mp 118–120 °C dec. Anal for $C_{33}H_{36}ClNO_3$ (C, H, N).

The hydrochloride salt of **14** was converted into the free base by treating an aqueous solution of **14•HCl** with solid KOH and extracting the free base with $CHCl_3$. The organic layer was washed (H_2O), dried and evaporated to give pure **14** as a solid: mp 94–96 °C; ^{1}H-NMR (80 MHz) δ 0.81 (d, 3H, $CHCH_3$), 4.09 and 3.16 (2d, 2H, J = 13.5 Hz, NCH_2Ph); IR (CCl_4) ν 3505 cm^{-1} (OH···N).

14•HCl (0.40 g, 0.74 mmol) was treated with $SOCl_2$ (1.41 mmol) in CH_2Cl_2 (50 mL) at reflux temperature for 4 h. Evaporation of reaction mixture gave the starting compound unaltered.

3-(3,4-Dihydroxyphenyl)-c-2-methyl-r-3-piperidinol hydrochloride ***8•HCl***

A solution of the hydrochloride salt of **14** (0.300 g, 0.56 mmol) in EtOH (10.0 mL) was stirred under hydrogen at 50 °C, and atmospheric pressure in the presence of 10% Pd on charcoal (0.10 g). When the absorption stopped, the catalyst was filtered off and the solution was evaporated to yield a solid residue, which was crystallized from MeOH/Et_2O to afford the pure hydrochloride of **8** (0.08 g, 55%): mp 154 °C dec; ^{1}H-NMR δ 1.03 (d, 3H, J =7.0 Hz, $CHCH_3$), 3.58 and 3.85 (m, 1H, J = 7.0 Hz, $CHCH_3$), 7.06 (m, 3H, Ar). Anal for $C_{12}H_{18}ClNO_3$ (C, H, N).

N-Benzyl-6-methyl-3-piperidone ***15***

Compound **15** was obtained following the synthetic route described previously [38].

3-(3,4-Dibenzyloxyphenyl)-c- ***16•HCl*** *and 3-(3,4-dibenzyloxyphenyl)-t-6-methyl-r-3-(N-benzyl)piperidinol hydrochloride* ***17•HCl***

A solution of **15** (5.00 g, 0.024 mol) in anhydrous Et_2O (20 mL) was added to a stirred solution of 3,4-(dibenzyloxy)-phenyl magnesium bromide [35] (prepared from Mg (0.695 g 0.028 gatom) and 3,4-(dibenzyloxy)bromobenzene (10.0g 0.027 mol)) in anhydrous THF (26.0 mL). The reaction mixture was stirred at room temperature overnight, and hydrolyzed with cold 25% aqueous NH_4Cl until the pH was 5. The solid precipitate (4.0 g) was filtered and crystallized using $CHCl_3$/Et_2O to yield a crude mixture of **16** and **17** as a hydrochlorides (3.30 g) in a ratio of 60:40 (^{1}H-NMR).

The mixture of **16** and **17** as salts (0.700 g) was converted into the mixture of free bases by treating an aqueous suspension of the salts with solid KOH and extracting with $CHCl_3$. The $CHCl_3$ layer was washed (H_2O), dried and evaporated to give the crude mixture of **16** and **17** (0.40 g), which was purified by column chromatography on silica gel cooled at 18 °C, eluting with a 90:10:2 hexane/AcOEt/Et_3N mixture and collecting 10 mL fractions.

The evaporation of fractions 15–30 yielded 3-(3,4-dibenzyl-oxyphenyl)-*c*-6-methyl-*r*-3-(*N*-benzyl)piperidinol (**16**) as a solid (0.092 g): mp 113–115 °C; ^{1}H-NMR (300 MHz) δ 1.27 (d, 3H, J = 6.0 Hz, $CHCH_3$), 2.34 (m, 1H, $CHCH_3$), 2.63 and 2.18 (2d, 2H, J = 11.2 Hz, $BnNCH_2$), 3.22 and 4.14 (2d, 2H, J = 13.5 Hz, NCH_2Ph), 6.83–7.45 (m, 18H, Ar); IR (CCl_4) ν 3470 cm^{-1} (OH···N). Compound **16** was dissolved in Et_2O and treated with an excess of Et_2O•HCl to yield a solid, which after trituration with Et_2O, gave pure **16•HCl**: mp 180 °C dec. Anal for $C_{33}H_{36}ClNO_3$ (C, H, N).

The evaporation of fractions 35–65 yielded: 3-(3,4-dibenzyl-oxyphenyl)-*t*-6-methyl-*r*-3-(*N*-benzyl)piperidinol (**17**) as an oil (0.076 mg); ^{1}H-NMR (300 MHz) δ 1.06 and 1.08 (d, 3H, J = 6.6 Hz, $CHCH_3$), 2.14 (m, 1H, $CHCH_3$), 3.54 and 3.66 (2d, 2H, J = 13.3 Hz, NCH_2Ph), 6.87–7.47 (m, 18H, Ar); IR (CCl_4) ν 3450 cm^{-1} (OH···N).

Compound **17** was dissolved in Et_2O and treated with an excess of Et_2O•HCl to yield a solid, which after trituration with Et_2O gave pure **17•HCl**: mp 172–175 °C dec. Anal for $C_{33}H_{36}ClNO_3$ (C, H, N).

3-(3,4-Dihydroxyphenyl)-c-6-methyl-r-3-piperidinol hydrochloride ***9•HCl***

A solution of **16•HCl** (0.35 g, 0.66 mmol) in a 5:2 EtOH/CH_2Cl_2 mixture (10 mL) was shaken under hydrogen at 50 °C and atmospheric pressure in the presence of 10% Pd on charcoal (0.175 g). When the absorption stopped, the catalyst was filtered off and the solution was evaporated to give a solid residue (0.28 g) which was crystallized with MeOH/Et_2O to yield the hydrochloride salt of **9** (0.12 g): mp 185–187 °C; ^{1}H-NMR δ 1.42 (d, 3H, $CHCH_3$), 2.02 (br, 4H), 3.37 (br, 3H), 7.03 (m, 3H, Ar). Anal for $C_{12}H_{18}ClNO_3$ (C, H, N).

3-(3,4-Dihydroxyphenyl)-t-6-methyl-r-3-piperidinol hydrochloride ***10•HCl***

A solution of **17•HCl** (0.300 g, 0.56 mmol) in a 5:2 EtOH/CH_2Cl_2 mixture (8 mL) was hydrogenated in the presence of Pd on charcoal under the conditions described above for the preparation of **9•HCl**. The evaporation of the filtered mixture yielded a solid residue which was crystallized using EtOH/Et_2O to yield **10•HCl** as an hygroscopic solid (0.11 g); ^{1}H-NMR δ 1.50 (d, 3H, $CHCH_3$), 2.53 (br, 4H), 3.35 (br, 3H), 7.00 (m, 3H, Ar). Anal for $C_{12}H_{18}ClNO_3$ (C, H, N).

Theoretical calculations

Conformational analyses were performed by molecular mechanics calculations using the Discover program [46] with the CVFF force field [48] in which the atomic partial changes are also defined. Starting geometries were built using the Builder module of the Insight II molecular modelling program [46] which uses standard band angles and lengths; for each molecule, the two conformers corresponding to the two possible chair conformation of the piperidine ring were separately built and then fully minimized using Newton–Raphson with 10^{-4} kcal/mol•Å on RMS derivatives as convergence criteria. The dielectric constant was fixed to 1, independently of the distance; test calculation indicated that results are not appreciablely influenced by this choice.

In the case of the conformational study of the aromatic ring, the torsion angle τ was varied with 5° steps while all other freedom degrees of the molecule were fully optimized; the starting geometry of the piperidine ring was that with the phenyl ring in an equatorial position as explained above and it remained unchanged during the optimization. In previous works it had been found that the Discover program was able to give relative conformational energies for compounds similar to those considered here in agreement with those obtained by ab initio MO calculations ([49] and references cited therein).

Radioligand binding methods. Rat brain α_1- and α_2-receptors

The α_1- and α_2-receptors binding were determined in rat cerebral cortex membranes as elsewhere reported [50].

Pharmacological methods

The assays were conducted in accordance with the legislation of the Italian Authorities (DL 27/01/92, no 116) concerning

animal experimentation. The animals under Et_2O anesthesia were killed by cervical dislocation and bled, then the abdominal cavity was opened by a midline incision. The organs were immediately explanted and placed in cold Tyrode solution (composition (mM): NaCl (136.8); KCl (2.95); $CaCl_2$ (1.80); $MgSO_4 \cdot 7H_2O$ (1.05); NaH_2PO_4 (0.41); $NaHCO_3$ (11.9); Glucose (5.5)) gassed with carbogen (95% O_2/5% CO_2).

Isolated rat vas deferens

The α_1-adrenoceptor activity was assayed on isolated vas deferens taken from Sprague–Dawley male albino rats (200–250 g body weight). Both vasa deferentia were carefully removed without stretching from the epididymis to the prostatic urethra, after moving the intestine to one side. The intact duct was carefully separated from extraneous surrounding tissues and placed in a 10 mL organ bath containing Tyrode solution (pH 7.4) at 37 °C, bubbled with carbogen. The preparation was suspended longitudinally between the organ holder and a force displacement transducer (Basile Model 7006) loaded with 0.5 *g*, connected to a microdynamometer (Basile Model 7050).

Isolated guinea-pig ileum

Dunkin–Hartley male guinea pigs weighing 250–300 g were deprived of food intake for 24 h before the experiments. Portions of ileum 2–3 cm in length, about 10 cm distal to the ileocecal valve, were carefully dissected, freed from the surrounding mesenteric tissue, attached with a thread to the organ holder and to the recording system by opposite sides of their open ends, and suspended in a 10 mL organ bath containing Tyrode solution at 37 °C gassed with carbogen. The ileum preparations were placed between two platinum electrodes (4 x 45 mm) set at a distance of 7 mm in the bath. The tissues were preloaded with a tension of 0.5 *g* and left to stabilize for 45–60 min before beginning electrical stimulation, which was carried out with a digit stimulator (Biomedica Mangoni Model BM-ST3) using the following parameters: single rectangular pulses, 0.1 Hz frequency, 0.3 ms pulse width, 12 V supramaximal voltage. The activity of the tested drugs on α_2-adrenoceptors was evaluated as their ability to inhibit acetylcholine release evoked by electrical stimulation of nerve fibres. The effects of the released mediator on intestinal smooth muscle were recorded as longitudinal contractions by an isotonic transducer (Basile Model 7006) connected with a unirecord microdynamometer (Basile Model 7050).

The following drugs were used as salts: *l*-NE (**1**) (as bitartrate); **8**, **9** and **10** (as hydrochlorides).

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