Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 1861–1869

BIOORGANIC & MEDICINAL CHEMISTRY

Structure–Activity Relationships on Adrenoceptors and Imidazoline-Preferring Binding Sites ($I_{1,2}$-PBSs). Part 1: Weak Intramolecular H-bond and Conformational Flexibility in a New I_1-PBS-Selective Imidazoline Analogue, *trans*1-(4′,5′-Dihydro-1′*H*-imidazol-2′-yl)methyl-2-hydroxyindane (PMS 952)

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Received 1 December 1999; accepted 22 March 2000

Abstract—The highly selective I_1-PBS imidazoline analogue PMS 952 has been selected to study the incidence of intramolecular hydrogen bond and molecular ilexibility on its biological activity. On one hand, the weak energy difference between three calculated conformers does not support the stabilization of one conformer by an internal hydrogen bond. The 3-D electrostatic map confirms this feature and the solvent effect does not significantly modify the relative energy of these conformers. On the other hand, the conformational spaces of the neutral and ionized forms present a great number of equilibrium structures, in a short energetic range (20 Kcal). The results are representative of an exceptional conformational flexibility due to a cooperative effect between several parts of the molecule.

Introduction

In the past it has been established that many adrenergic agents can also interact with discrete imidazoline receptors I_1 and I_2 (for reviews, see Ref 1), named Imidazoline Preferring Binding Sites (I-PBS). The discussion is open about the nature of the I-PBSs. It was proved that adrenoreceptors and I_{1-2}-PBSs are biochemically different[2,3] and that the structure of isolated I_2-PBS depends on the animal origin, organs and cells.[4,5] Moreover I_1 and I_2-PBS may interconnect in human striatum.[6] In the present publication, we examine the binding affinity and the selectivity of a new imidazoline analogue *trans*-1-(4′,5′-dihydro-1′*H*-imidazol-2′-yl)methyl-2-hydroxyindane **5** (PMS 952) (see Scheme 1 and Fig. 1). This compound was selected into a large series of imidazoline derivatives which will be published later. Its intra and intermolecular H-bonds could influence the molecular flexibility and its interactions with the receptor(s).

In the present work, we analyze the geometry flexibility of PMS 952 depending on both main rotators defining the relative position of the imidazoline and the indane rings for the ionized and neutral forms. In fact, several cooperative conformational effects could also occur involving the imidazoline N-H inversion, the alcohol rotation and the relative distortion of the cyclopentane fragment. The OH and NH functions could also be involved in intermolecular H-bonds which will be modelized by a dimer of PMS 952.

The question of ionization of the imidazoline ring in the physiological medium was examined in our previous publications.[7] The short range of pK_a values around 8 units whatever the molecular skeleton of the analogue (here with an additional spacer that eliminates all electronic influences) nullifies a priori the influence of this factor as a determinative parameter on the variations of the biological activity in an homogeneous series. Two other factors can be taken into account: (i) the local pH inside the site of interaction is unknown and assumed to be constant and (ii) if the imidazoline nitrogen is protonated, the positive charge is probably counterbalanced by electrostatic interaction with the carboxylate of an

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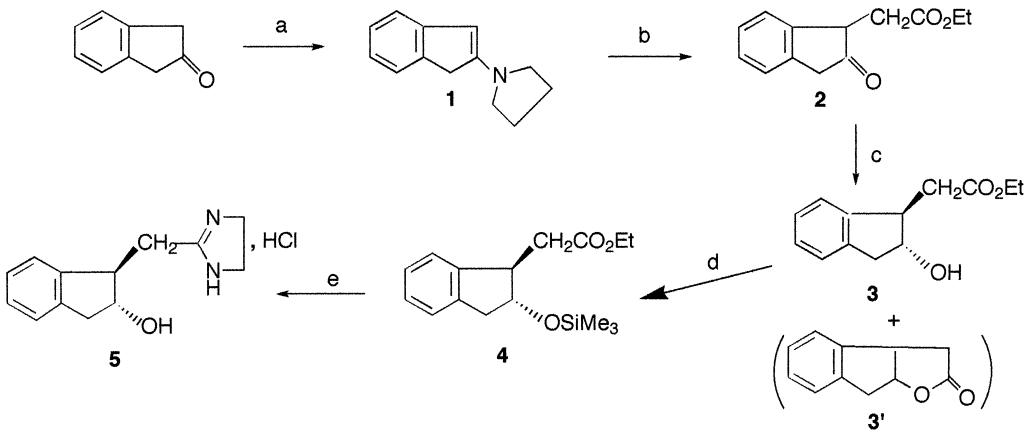
PII: S0968-0896(00)00115-2



Scheme 1. Reagents. (a) pyrrolidine, benzene, reflux; (b) i. $BrCH_2CO_2Et$, EtOH, 18 h; ii. H_2O; (c) $NaBH_4$, EtOH, −5 °C; (d) Me_3SiCl, Et_3N, THF, 0 °C; (e) i. DAE, Me_3Al, toluene, 80 °C; ii. HCl/MeOH.

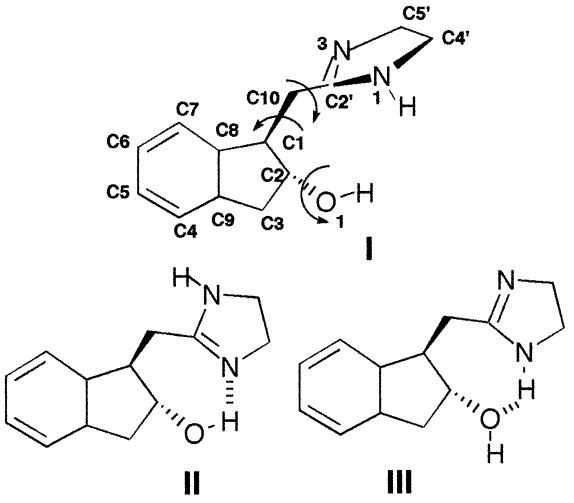


Figure 1. Conformers of PMS 952 without (**I**) and with intramolecular H-bond (**II** and **III**).

aspartate or glutamate moiety in the receptor site. Nevertheless, all energetic data have been calculated in both neutral and ionized forms.

Chemistry

The enamine **1** (see Scheme 1) was prepared by the condensation of pyrrolidine with indan-2-one. Alkylation of the enamine with ethyl bromoacetate led to the γ-ceto ester **2** after hydrolysis.[8] Reduction of **2** with sodium borohydride at −5 °C in ethanol[9] provided a mixture of *trans* alcohol **3** and lactone **3′**, which resulted from a cyclisation of the *cis* alcohol. The hydroxyl group was protected with trimethylsilyle before introducing the imidazoline group.[10] The deprotection has been occured during the treatment of the reaction from ester to imidazoline.[7]

Results and Discussion

Biological results

PMS 952 has a high affinity for the I_1-PBS (22 nM) and a high binding selectivity versus adrenoreceptors (ratio/α_2: 145.5 and /α_1: 250). PMS 952 presents a moderate affinity on the I_2-PBS (5.4 times less) and lower selectivities concerning adrenoreceptors (Table 1).

Conformational analysis of the free base

The molecule presents three rotatable bonds involved in the conformation of the alcohol and the imidazoline group. Their mutual incidence could generate at least three types of conformers: (i) without any intramolecular H-bond **I**, (ii) with one H-bond between the O-H group and the imidazoline N sp^2 **II**, and (iii) between the O-H and the imidazoline H-N sp^3 **III** (Fig. 1). These conformers have been located by full geometry optimization at the ab initio level involving the MINI-1′ minimal basis

Table 1. Binding assays and selectivity of PMS 952 on adrenoreceptors and IPBs[a]

K_i (nM)				Selectivity				
I_1	I_2	α_1	α_2	I_1/α_2	I_1/α_1	I_2/α_2	I_2/α_1	I_1/I_2
22 (±2.0)	120 (±11.5)	5500 (±430)	3200 (±220)	145.5	250	26.6	45.8	5.4

[a]Binding assays: see Experimental.

Table 2. Relative energy values versus the most stable conformer **II**, $\varepsilon = 1$[a]

	MINI-1// MINI-1		6-31G// 6-31G		6-31G*// 6-31G*		B3Lyp 6-31G* // 6-31G	B3Lyp 6-31G*// 6-31G*
Conformers	ΔE	ΔG	ΔE	ΔG	ΔE	ΔG	ΔE	ΔE
I	5.760	4.393	6.241	5.514	4.244	3.935	5.610	5.040
II	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
III	3.240	3.340	5.475	5.274	4.562	4.140	5.012	4.698

[a]All the values are expressed in Kcal/mol. ΔG is calculated at 298.15° K and at 1 atmosphere.

set,[11] the double ζ 6-31G without[12] and with additional polarization functions 6-31G*.[13]

Totally in accordance with the X-ray structure of the free base,[14] the conformer **II**, the most stable one, presents an intramolecular H-bond of 1.92, 1.94, and 1.99 Å in the three basis sets. In the conformation **III**, with the other H-bond type, the distance remains in the same range: 1.85, 2.01, and 2.08 Å.

Nevertheless, the energy difference between the three conformers does not significantly support a high stabilization of one of them by an internal H-bond. Indeed, conformers **I**, without H-bond, and **III**, with the other type of H-bond, are only approximatively 4.5 Kcal less stable than conformer **II** (Table 2). These results are also confirmed by the higher level calculation B3Lyp either at the optimized 6-31G or 6-31G* geometries.

In the conformation **II**, the 3-D electrostatic potential map clearly shows that both nitrogens are surrounded by a negative envelope at −20 Kcal/mol with the same shape (Fig. 2), the absolute minimum (−74.3 Kcal/mol) lying near the oxygen. As previously discussed,[15] a negative envelop also appears over the aromatic part of the indane.

Solvent effects

For the three main representative conformers, the incidence of the solvent has been investigated by a complete reoptimization at the 6-31G level using the Onsager's model.[16] For CCl_4, $CHCl_3$ and acetonitrile, the dielectric constant ε is 2.2, 4.8, and 38, respectively. The energetic results (Table 3) are not significantly modified for the conformer **III** as the difference between conformers **II** and **III** remains in the range of 5.475 ($\varepsilon = 1$) to 5.876 ($\varepsilon = 38$) Kcal/mol as if the intramolecular H-bond was not influenced by the solvent. This difference is slightly increased for conformer **I** going from 6.241 ($\varepsilon = 1$) to 7.307 ($\varepsilon = 38$) Kcal/mol.

These first results suggest that PMS 952 could interact with Imidazoline Binding Sites via expected intermolecular H-bonds. Such interactions could be related with the exceptional flexibility of this molecule, in spite of the putative intramolecular H-bond which is rather weak. Remarkably this molecule can adopt several conformations significantly different in their geometry, but lying in a very small energetic range. It is the reason why a more extensive investigation of the conformational adaptibility has to be performed.

2-D Conformational analysis

A complete scan of the conformational space has been performed using the conformer **II** as starting point. A two dimensional map has been calculated following both dihedral angles of the methylene group linking the imidazoline to the cyclic part of the molecule. At each step of the 15 degrees regular mesh for these two degrees of freedom (dof), all the other 3N-8 ones have been re-optimized at the MINI-1′ ab initio level.[17]

The 2-D isoenergetic contour map (Fig. 3) highlights the complex ways that the molecular system could follow to go from one conformer to another within a maximum 15 Kcal range. Depending on the considered edges of the surface quite as much as 21 equilibrium structures have been located on this map (Table 4). In a second step, these conformers: 13 minima, 5 transition state structures and 3 second order critical points have been fully optimized and characterized by analytical frequency

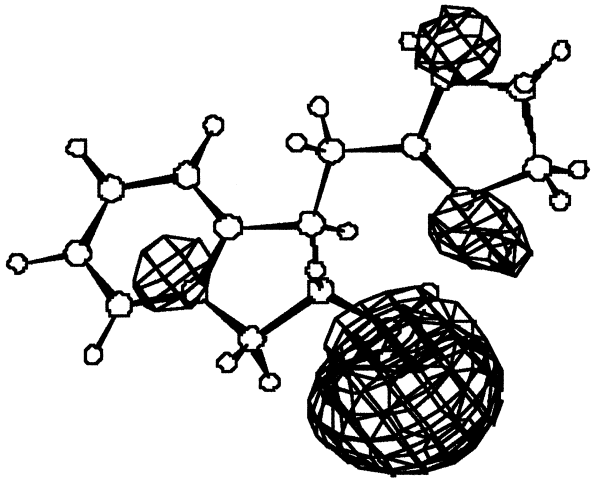


Figure 2. Ab initio MINI-1′ 3-D electrostatic potential isocountoured at −20 Kcal/mol for the MINI-1′ optimized conformation **II**.

Table 3. Solvent effect on relative energy values

	6-31G//6-31G					
Conformers	ΔE (Kcal/mol)			ΔG (Kcal/mol)		
	CCl_4	$CHCl_3$	CH_3CN	CCl_4	$CHCl_3$	CH_3CN
I	6.723	7.030	7.307	5.851	6.209	6.645
II	0.000	0.000	0.000	0.000	0.000	0.000
III	5.675	5.788	5.876	5.398	5.462	5.504

calculation. With regard to the conformer **II**, the less stable in energy structure is the second order point **XIX**.

Remarkably, three other minima (**IV**, **VI** and **XVI**) more stable than conformer **I** have also been localized. They confirm the very high geometric flexibility of this molecule due to a combination of effects as the rotation of the alcohol and the inversion of the NH imidazoline nitrogen. The two second order critical points (P.C. 2) are characterized by two negative hessian eigenvalues corresponding to energy maxima for the two dihedral angles and all the other positive ones to energy minima in all

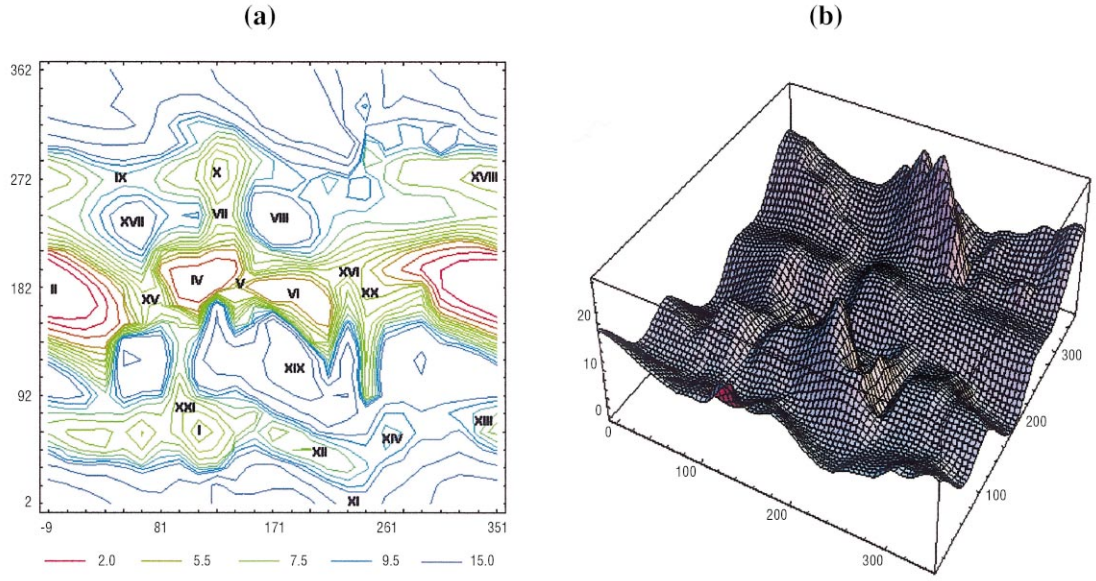


Figure 3. (a) Neutral molecule: energetic isocontours from 2 Kcal (red) to 15 Kcal (blue) including isocontour 7.5 Kcal (green). Horizontal axis: p_2; vertical axis: p_1. (b) Perspective view of the 2-D conformational map showing the valleys joining one conformer to another. Going up axis: p_1 going down axis: p_2.

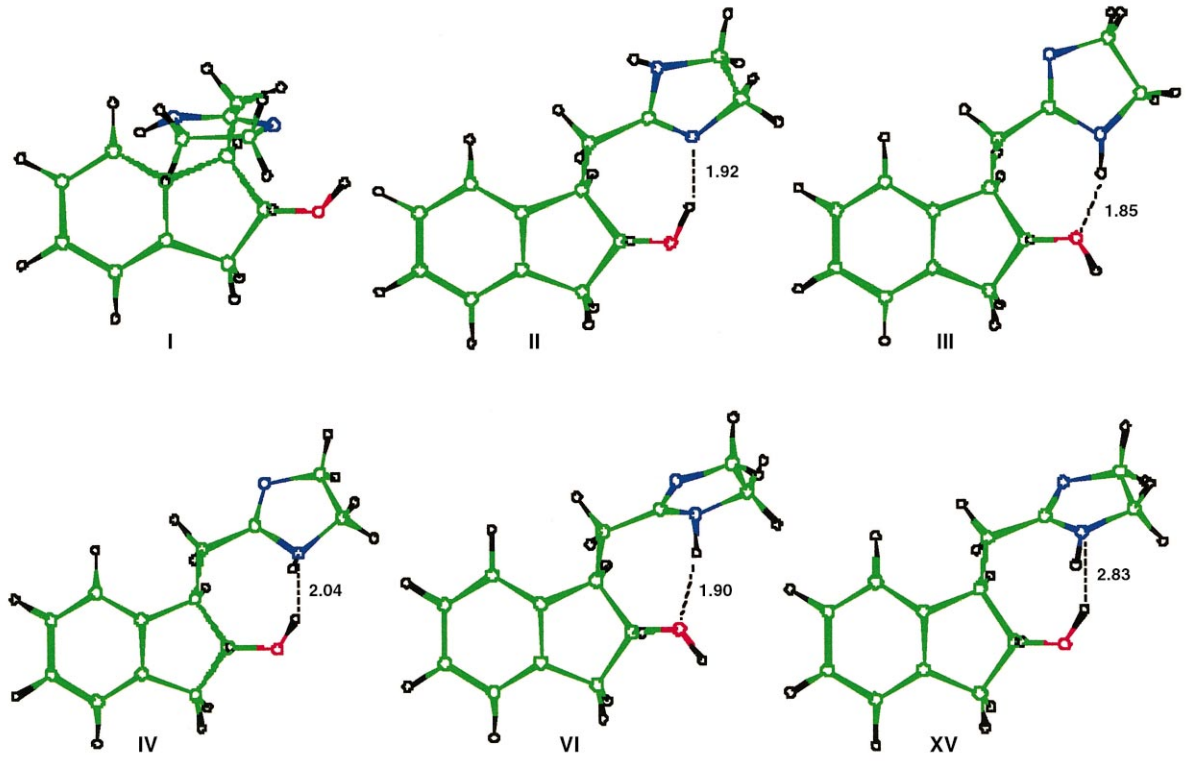


Figure 4. Selection of six equilibrium structures of the neutral form. The TS **XV** structure connects the minima **II** and **IV**. The interatomic H-bond distances are expressed in Å.

Table 4. Dihedral angles and energy values of 21 equilibrium structures of the neutral form[a]

Conformation	Dihedral angles[b]				
	p_1	p_2	Entropy	ΔE	ΔG
I min	64.55	100.38	118.430	5.760	4.394
II min	181.95	−9.21	115.132	0.000	0.000
III min	−165.31	129.40	114.780	3.240	3.340
IV min	−174.90	106.51	114.013	3.005	3.423
V TS	−159.51	153.38	112.302	7.236	7.314
VI min	176.32	−158.98	116.201	3.910	3.494
VII TS	−117.21	131.58	111.689	7.542	7.592
VIII P.C. 2	−122.51	175.75	107.463	11.489	12.184
IX TS	−88.31	53.51	112.130	8.848	8.694
X min	−82.71	114.93	117.749	6.027	4.836
XI TS	−2.72	−99.75	112.881	12.348	12.006
XII min	43.20	−150.72	117.889	7.968	6.802
XIII min	56.64	23.75	120.481	7.321	5.336
XIV min	56.54	−100.85	118.214	8.761	7.457
XV TS	179.20	82.09	119.453	7.758	5.272
XVI min	181.42	−115.59	120.553	5.529	3.523
XVII P.C.2	−129.02	77.59	108.890	11.207	11.323
XVIII min	−85.61	−14.00	119.359	7.186	5.404
XIX PC 2	128.33	190.02	108.379	21.224	21.724
XX min	−179.30	−104.44	119.529	5.932	4.185
XXI min	67.51	111.92	118.876	6.839	5.249

[a]Min: zero order critical point, true minimum; TS: first order critical point, transition state structure; P.C. 2: second order critical point: energy maximum in both directions p_1, p_2.
[b]Entropy in cal/mol. ΔE and ΔG in Kcal/mol. Dihedral angles in degrees. p_1: C2′-C10-C1-C8, p_2: N3-C2′-C10-C1, after re-optimization of all the 3N-6 degrees of freedom.

the other directions. It can be noted that, on the same surface, two P.C. 2 have a ΔE lower than the first order critical point TS **XI**. The incidence of the entropy term significantly varies on the free energy value depending on the nature of the critical point. In fact, this term is lower for the first and second order points when compared to the minima. Six equilibrium structures are drawn on the Figure 4. As illustrated on the map (Fig. 3), the TS **XV** connects the minima **II** and **IV** mainly via the maximum energy path following the dihedral angle p_2. Having the same type of intramolecular H-bond, minima **III** and **VI** differ from one another in their p_2 value and mainly in the sp^3 nitrogen inversion while they remain in the same energetic range of 3.5 Kcal/mol in ΔG.

The O-H position in the conformer **III** on one hand and the observed crystal packing on the other hand suggest that an intermolecular H-bond could also appear between the oxygen of the alcohol of one molecule and the N-H of the neighbouring one.[14]

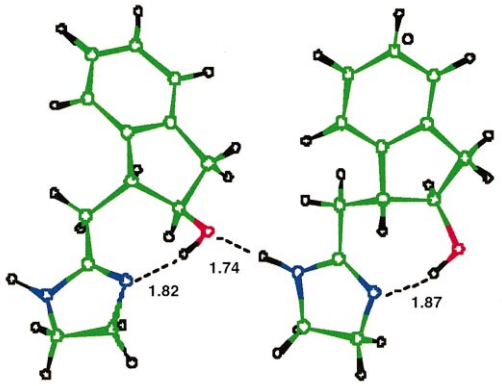


Figure 5. MINI-1′ optimized conformation of the dimer. The interatomic H-bond distances are expressed in Å.

Intermolecular model

As a model of intermolecular interaction capability of this compound, a dimer has been fully optimized using MINI-1′ and the two extended basis sets 6-31G and 6-31G*.

Depending on the basis set size, the intramolecular H-bond is slighly shorter in the dimer than in the isolated form. The intermolecular H-bond is of the same order between 1.745 Å (MINI-1′) and 2.104 Å (6-31G*). The MINI-1′ conformer looks like the X-ray conformation whereas the optimized structures in double ζ are significantly twisted (Fig. 5). The energetic results are given in Table 5. With 544 basis functions, the analytical frequency calculation could not be run for the 6-31G* dimer. Two results are remarkable: the entropy factor of the dimer is significantly smaller than twice the entropy term of each monomer thus leading to a nonstabilizing ΔG at 298.15 °K and 1 atm. The second point concerns the very weak deformation energy by comparison between the interaction and complexation energy. At their geometry in the dimer, each monomer does exhibit only a small energy difference by reference to the isolated optimized geometry as given by the complexation energy.

Conformational analysis of imidazoline protonated compound

A priori, the local symmetry of the protonated imidazoline reduces the number of equilibrium structures which can be located in the 2-D conformational map (Fig. 6). It has been computed following the same procedure that was applied for the neutral molecule. Nine conformers have been characterized as critical points at

Table 5. Energy values of the dimer[a]

	Entropy (calories)		Complexation Energy		Interaction Energy
	2×Monomer//Monomer	Dimer//Dimer	ΔE (Kcal/mol)	ΔG (Kcal/mol)	ΔE (Kcal/mol)
MINI-1′	230.264	201.764	8.523	−2.338	9.306
6-31G	227.802	194.608	10.161	−2.151	10.783
6-31G*	—	—	7.612	—	7.998

[a]See text and experimental.

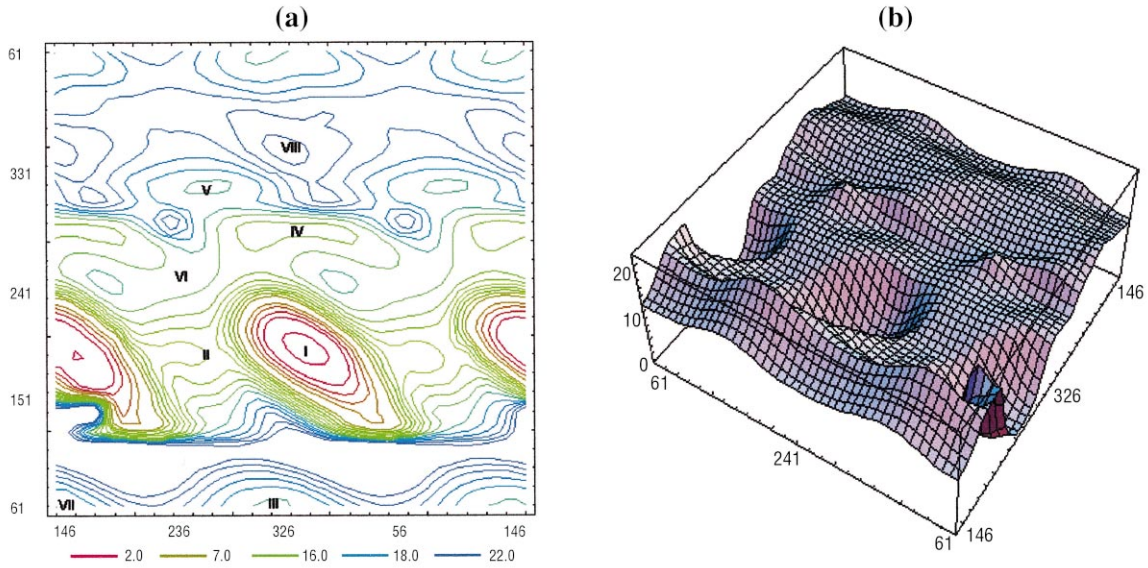


Figure 6. (a) Protonated molecule: energetic isocontours from 2 Kcal (red) to 15 Kcal (blue) including isocontour 7 Kcal (green). Horizontal axis: p_2; vertical axis: p_1. (b) Perspective view of the 2-D conformational map showing the valleys joining one conformer to another. Going up axis: p_1; going down axis: p_2.

the MINI-1′ level (Table 6). The energy range connecting all these structures is rather similar at the results derived from the neutral analysis except that the energy difference between the minima is more significant. The most striking feature is given by the comparison between the two more stable conformers in the neutral (**II**) and protonated molecule (**I**) (Fig. 7). The relative position of the imidazoline ring is quite similar, the main difference between these conformers being the alcohol rotation.

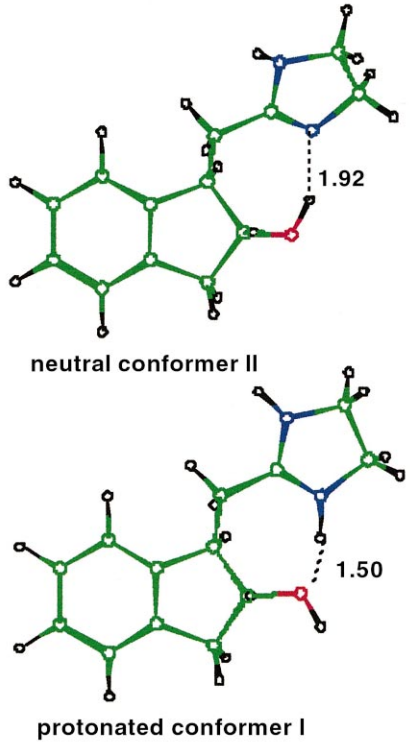


Figure 7. Most stable conformers in the neutral (**II**) and in the protonated (**I**) molecules. The interatomic H-bond distances are expressed in Å.

Conclusion

The molecular flexibility of PMS 952 has been investigated by a 2-D regular 15 degrees conformational map in which all the 3N-8 degrees of freedom of the 625 points were fully optimized at the MINI-1′ ab initio level. No more stable conformer than the previously described one **II** has been found but new stable minima and their related transition state structures have been located. Remarkably, one second order critical point, with a maximum energy following the two investigated dihedral angles, lies in energy lower than a first order critical point. It can be concluded that PMS 952 presents a significant conformational flexibility which could have a great incidence on its affinity towards the I-PBS

Table 6. Dihedral angles and energy values of nine structures calculated at the equilibrium for the protonated form[a]

Conformation	Dihedral angles[b]		Entropy	ΔE	ΔG
	p_1	p_2			
I min	−174.06	−22.39	113.350	0.000	0.000
II TS	−172.05	−88.55	113.381	11.950	11.322
III min	61.81	48.77	117.604	12.188	10.851
IV min	−80.68	−57.69	119.503	13.387	11.486
V min	−65.45	−84.34	120.358	14.458	12.237
VI TS	−121.27	−125.38	114.488	15.039	14.075
VII min	60.96	146.43	114.376	15.737	14.522
VIII P.C. 2	−14.89	−38.97	106.828	21.090	21.684
IX min	123.54	−90.73	113.317	22.490	21.709

[a]For definitions, see Table 4.
[b]After re-optimization of all the 3N-6 dof.

receptors. Moreover the conformational similarities between the most stable conformer in neutral and protonated forms has to be pointed out. As the hydroxyl group rotation is relatively free, an intermolecular H-bond with the receptor may be expected in both forms, but at least, many other factors would determine selectivity between I-PBS and adrenoceptors binding.

Experimental

Calculations

All the calculations were performed with GAUSSIAN 94[17] on two computers, a Dec Alpha 8400 8 processor and a Dec Alpha 4100 4 processor. For all the systems, a full geometry optimization was done at the Hartree-Fock level with the minimal MINI-1′ basis set.[11] The nature of the critical points was determined by the frequency analytical calculation. The free energies were computed from the analytical frequencies through the usual statistical mechanics formulas at 298.15 °K and 1 atmosphere.[18]

Chemistry

The purity of each compound was checked by thin-layer chromatography on TLC plastic sheets (silica gel $60F_{254}$, layer thickness 0.2 mm) from Merck. Column chromatography purification was carried out on silica gel 60 (particle size 0.063–0.200 mm) from Merck, without any special treatment. All melting points were determined in a digital melting point apparatus (Electrothermal) and are uncorrected. The structures of all compounds were confirmed by IR and ^{1}H NMR spectra. IR spectra were obtained with a ATI Mattson Genesis Series FTIR infrared spectrometer, and ^{1}H NMR spectra were recorded in $CDCl_3$ on a BRUCKER AC 200 spectrometer using hexamethyldisiloxane (HMDS) as an internal standard.

Pyrrolidine enamine of 2-indanone (1). A solution of 2-indanone (30 g, 0.23 mol) and pyrrolidine (24 g, 0.35 mol) in 200 mL of benzene was refluxed for 3 h under nitrogen using a Dean–Stark apparatus to eliminate water. After removal of the solvents under reduced pressure, 42 g (100%) of crude enamine (**1**) were obtained: R_f 0.66 (ether:petroleum ether, 50:50, v/v).

Ethyl (indan-2-on-1-yl)-acetate (2). To a solution of enamine (**1**) (39 g, 0.21 mol) in 200 mL of dry methanol was added dropwise ethyl bromoacetate (36.3 g, 0.21 mol). The solution was refluxed for 18 h, and after addition of 9 mL of water, refluxing was continued for another hour. Addition of water (200 mL) and extraction with ether gave, after drying and distillation, 22.2 g (48.24%) of γ-keto ester (**2**) as a white oil: $bp_{0.2mmHg}$ 151–152 °C; mp 47–47.5 °C; R_f 0.30 (ether:petroleum ether, 20:80, v/v): IR (film) ν 1750 (C=O), 1735 (C=O ester); ^{1}H NMR δ 7.25 (4H, m, ArH), 4.05 (2H, m, CO_2CH_2), 3.7 (1H, t, J = 5.5 Hz, CH), 3.55 (2H, s, CH_2CO), 2.95 (2H, d, J = 5.5 Hz, CH_2CO_2), 1.15 (3H, t, J = 7.5 Hz, CH_3).

***trans*-1-(Ethoxycarbonylmethyl)-2-hydroxyindane (3).** $NaBH_4$ (3.8 g, 0.1 mol) was added in portions to a stirred solution of keto ester (**2**) (21.8 g, 0.1 mol) in 150 mL of 95% EtOH at −5 °C. After the addition was complete, the reaction was maintained in the same conditions (−5 °C) with stirring for 3 h. Then it was quenched with water (200 mL) and the mixture was extracted with ether three times. The extracts were combined, washed with H_2O and brine, dried ($MgSO_4$), and evaporated. Purification of the residue by column chromatography on silica gel using ether:petroleum ether (10:90, v/v) as eluent gave 14.3 g (65%) of the alcohol (**3**): R_f 0.15 (ether: petroleum ether, 50:50, v/v); IR (film) ν 3440 (O-H), 1731 (C=O); ^{1}H NMR δ 7.12 (4H, m, ArH), 4.31 (1H, ddd, J = 9.7, 7.4 and 2.5 Hz, CH-O), 4.15 (2H, q, J = 2.5 Hz, CO_2CH_2), 3.79 (1H, d, J = 2.5 Hz, D_2O exchangeable, OH), 3.33 (1H, ddd, J = 11.5, 7.2 and 6.3 Hz, CH-Ph), 3.21 (1H, dd, J = 15.9 and 7.3 Hz, one H of CH_2-Ph), 2.94 (1H, dd, J = 16.8 and 4.4 Hz, one H of CH_2CO_2), 2.87 (1H, J = 15.8 and 7.4 Hz, one H of CH_2-Ph), 2.46 (1H, dd, J = 16.8 and 10.3 Hz, one H of CH_2CO_2), 1.24 (3H, t, J = 7.1 Hz, CH_3).

***trans*-1-(Ethoxycarbonylmethyl)-2-(trimethylsilyloxy)-indane (4).** To a stirred solution of the alcohol (**3**) (14 g, 64 mmol) and 20 mL of Et_3N in 100 mL of THF at 0 °C was added dropwise chlorotrimethylsilane (10.4 g, 96 mmol). After the addition, the stirring was continued at room temperature for 8 h. The mixture was evaporated in vacuo and the residue was dissolved in water (30 mL) and extracted three times with ether. The combined ether extracts were dried ($MgSO_4$) and evaporated under vacuum to yield 18.1 g (96.8%) of (**4**) as a white oil: R_f 0.59 (ether:petroleum ether, 50:50, v/v); IR (film) ν 1737 (C=O); ^{1}H NMR δ 7.09 (4H, m, ArH), 4.18 (1H, dd, J = 13.4 and 6.7 Hz, CH-OSi), 4.11 (2H, q, J = 7.1 Hz, CO_2CH_2), 3.40 (1H, dd, J = 13.6 and 6.7 Hz, CH-Ph), 3.05 (1H, dd, J = 15.4 and 6.7 Hz, one H of CH_2-Ph), 2.76 (1H, dd, J = 15.4 and 6.9 Hz, one H of CH_2-Ph), 2.56 (1H, d, J = 2.0, one H of CH_2CO_2), 2.52 (1H, d, J = 2.7 Hz, one H of CH_2CO_2), 1.21 (3H, t, J = 7.4 Hz, CH_2CH_3), 0.90 (9H, s, $Si(CH_3)_3$).

***trans*-1-(4′,5′-Dihydro-1′*H*-imidazol-2′-ylmethyl)-2-hydroxyindane (5).** Trimethylaluminium (90 mL of a 2 M solution in toluene, 0.18 mol) was added dropwise to a stirred solution of ester (**4**) (17.5 g, 0.06 mol) in 120 mL of toluene at 0 °C, followed by ethylenediamine (8 mL, 0.12 mol). After the addition, the reaction mixture was heated at 80 °C for 6 h, then cooled with an ice bath and treated dropwise with water (30 mL). After decantation, the aqueous layer was extracted two times with toluene. The organic phases were combined, washed with H_2O and brine, dried over $MgSO_4$, and filtered. The solvent was evaporated in vacuo and the residue, taken up in 100 mL of HCl saturated methanol, evaporated again under vacuum, was crystallized in CH_3OH:CH_2Cl_2: ether (5:80:20, v/v/v) giving 13.5 g (88.8%) of (**5**, HCl), as white crystals: mp 176–177 °C; R_f 0.21 (CH_3OH: $CHCl_3$, 10:90, v/v) on Al_2O_3. IR (paraffin) ν 3250 (OH), 3198 (NH); ^{1}H NMR δ 7.11 (4H, m, ArH), 5.90 (2H, br s, D_2O exchangeable, NH and OH), 4.26 (1H, dd, J = 16.8 and 7.8 Hz, CH-O), 3.58 (4H, s, two N-CH_2), 3.29 (1H, m, CH-Ph), 3.21 (1H, dd, J = 15.7 and 7.7 Hz, one H of CH_2-Ph), 2.95 (1H, dd, J = 17.0 and 2.9 Hz, one

H of CH_2-C=N), 2.89 (1H, dd, $J = 16.8$ and 9.8 Hz, one H of CH_2-Ph), 2.26 (1H, dd, $J = 16.9$ and 11.7 Hz, one H of CH_2-C=). Anal. calcd for $C_{13}H_{16}N_2O$.HCl: C, 61.78; H, 6.78; N, 11.08. Found: C, 61.46; H, 6.60; N, 10.87.

Pharmacology

General procedure. Tissue and membrane preparation. Cerebral cortex was obtained from whole bovine brains and homogenized in 20 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.4). The homogenate was centrifuged twice at 48 000 *g* for 25 min at 4 °C. The pellet (used for α_2-binding assays) was resuspended in a phosphate buffer (pH 7.4), flash-frozen and stored at −80 °C until α_2-binding assays.

Reticular nucleus from calf's bulbis was homogenized in ice-cold 50 mM Tris–HCl buffer (pH 7.7) containing 5 mM EDTA. The homogenate was centrifuged at 500 *g* for 10 min at 4 °C. The pellet (P1) was resuspended in the same buffer and centrifuged again. The combined supernatants were centrifuged at 50 000 *g* for 25 min at 4 °C. The resulting pellet (P2) was resuspended in 50 mM Tris–HCl buffer (pH 7.7) containing 0.1 mM paramethylsulfonylfluoride and incubated for 30 min at 25 °C, then centrifuged again in the same conditions and resuspended in 50 mM Tris–HCl (pH 7.7), flash-frozen and stored at −80 °C until I_1-binding assays.

Renal cortex was obtained from male New Zealand white rabbits and homogenized in ice-cold preparation buffer (20 mM $NaHCO_3$). The homogenate was centrifuged at 40 000 *g* for 30 min at 4 °C. The pellet was resuspended in 50 mM Tris–HCl buffer containing 0.5 mM EDTA (pH 7.4), centrifuged again and resuspended in the same buffer, flash-frozen and stored at −80 °C until I_2-binding assays.

Binding assays. [^{3}H]RX 821002 (48 Ci/mmol), [^{3}H]clonidine (50 Ci/mmol), and [^{3}H]idazoxan (42 Ci/mmol) were obtained from Amersham (Buckinghamshire, UK), and phentolamine, guanabenz, tolazoline, idazoxan, yohimbine, clonidine, naphazoline and amiloride from Sigma (St Louis, MO). Radioligand binding assays with [^{3}H]prazosine, [^{3}H]RX 821002, [^{3}H]clonidine, or [^{3}H]Idazoxan for determination of specific binding to α_1 and α_2-adrenoceptors and affinity for I_1 and I_2-Preferring Binding Sites, were respectively evaluated by a modification of methods previously described (see Ref 7). Membranes were slowly thawed and diluted to a concentration of 0.4 mg protein/mL for renal and cerebral cortex and 0.7 mg protein/mL for calf's bulbis. Assays were conducted in a total volume of 525 µL in polypropylene tubes, and each tube contained 500 µL of membrane suspension, 20 µL of radioligand and 5 µL of drug. Incubation was initiated by the addition of membrane and was carried out for 45 min at 25 °C. Nonspecific binding was defined in the presence of yohimbine (10 µM) in [^{3}H]RX 821002 binding assays, either phentolamine (10 µM) or guanabenz (5 µM) in [^{3}H]clonidine binding assays and either tolazoline (10 µM) and amiloride (10 µM) in [^{3}H]idazoxan binding assays. For each drug, 12 concentrations from 10^{-4} to 10^{-11} M were used in triplicate. Incubations were terminated by vacuum filtration over Whatman GF/B glass fiberfilters using a cell harvester. The filters were washed three times with the buffer, placed in scintillation vials, covered with 2 mL of scintillation cocktail (Pico-Fluor, Packard Instrument) and counted (Packard 2000 CA). Protein concentration was evaluated by the Bradford method.

Binding results were analysed using the iterative non linear least square fitting with GRAPH PAD software (Institut for Scientific Information, Philadelphia, PA). Results are expressed as $K_i \pm$SEM.

K_i were determined using Cheng-Prusoff method.

Acknowledgements

This work was in part supported by ADIR-Servier and we acknowledge Doctors Pierre Renard and Bruno Pfeiffer for their helpful discussions. This work was also supported by the Belgian program on Interuniversity Poles of Attraction initiated by the Belgian State, Prime Minister's Office, "Service fédéraux des affaires scientifiques, techniques et culturelles (PAI no P4/03)" the "Fonds de la Recherche Scientifique Médicale Belge (FRSM, contrat no 3.4531.92)". GD is "chercheur qualifié" of the FNRS, Brussels.

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