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Short communication

Synthesis and α-adrenergic and I_1-imidazoline activity of 3-phenylpiperidines dimethyl-substituted on the phenyl ring [1]

Bruno Macchia[a]*, David B. Bylund[c], Vincenzo Calderone[b], Gino Giannaccini[b], Antonio Lucacchini[b], Marco Macchia[a], Adriano Martinelli[a], Enrica Martinotti[b], Elisabetta Orlandini[a], Federico Romagnoli[a], Armando Rossello[a]

[a]*Dipartimento di Scienze Farmaceutiche, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy*
[b]*Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy*
[c]*Department of Pharmacology, University of Nebraska Medical Center, Omaha, NE 68198-6260, USA*

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Abstract – In a previous study we found that certain 3-phenylpiperidines (PPEs, **5**) display a good activity at α_2-adrenergic receptors (α_2-AR), whereas they are completely inactive at α_1-AR. The PPEs **5** are conformationally restricted analogs of the corresponding adrenergic drug with a phenylethylamine structure (PAEs, **4**) in which the benzylic hydroxyl group characteristic of the adrenergic catecholamines is not present. The most interesting of the PPEs proved to be the 3-(3,4-dimethylphenyl) substituted compound (**5a**) which had been found to be essentially inactive at β_1- and β_2-AR. The methyl groups present on the aromatic ring of **5a** are found, albeit in a different position, on the phenyl of α_2-adrenergic agonists with arylimidazolidine and arylimidazole structures. As such PPE **5a** provided a unique template for the design of α_2-AR ligands. On the basis of these premises, we synthesized all the possible dimethylphenyl-substituted isomers of PPE **5a**. Their activity on α_1- and α_2-AR and on I_1-imidazoline receptors (IR) was evaluated in vitro both by radioligand binding assays and by functional tests on isolated preparation. Two selected PPEs, **5a** and **5f**, were also tested for their affinity on α_2-AR subtypes. Conformational studies were carried out by means of theoretical calculations, in order to rationalise the results of pharmacological tests at the molecular level. © Elsevier, Paris

3-phenylpiperidine derivatives / α_1-adrenergic receptor / α_2-adrenergic receptor / imidazoline I_1 receptor / conformational analysis

1. Introduction

α_2-Adrenergic agonists [2] have therapeutic applications in a variety of disease states. α_2-Selective agonists are pharmacologically important not only as central antihypertensive agents, but also as drugs for the suppression of opiate withdrawal, anxiolysis, sedation, analgesia (including neoplastic diseases), glaucoma, diuresis, and thrombosis. The design of drugs selectively stimulating α_2-AR therefore represents a promising research field.

Most α_2-adrenergic agonists belong to the class of arylimidazoline and arylimidazole derivatives. The arylimidazoline compounds include both arylaminoimidazoline derivatives, like clonidine (**1**), and benzylimidazoline derivatives, like oxymetazoline (**2**).

Medetomidine (**3**) is one of the best known drugs belonging to the family of the arylimidazole derivatives; its S-(+)-enantiomer, dexmedetomidine, represents the active isomer [3, 4].

α_2-AR have been classically characterized by a high affinity for the antagonists rauwolscine, yohimbine and idazoxan and the agonists clonidine (**1**) and UK14304, and by a low affinity for the α_1-AR antagonist, prazosin. On the basis of the pharmacological profile determined by both radioligand binding (differences in ^{3}H-antagonist binding) and functional studies, as well as the identification of amino acid sequences and the chromosomal location of genes which code for them, α_2-AR

*Correspondence and reprints
[1] 20th paper in the series *Conformational effects on the activity of drugs*. For the preceding paper, see [1].

have been subdivided into three genetic or four pharmacological subtypes, namely α_{2A}, α_{2B}, α_{2C}, and α_{2D} [5].

Imidazoline receptors (IR) were identified as specific binding sites in various tissues for radiolabelled imidazolines. IR exhibit a high affinity for clonidine and idazoxan and their derivatives, but have low affinity for the endogenous catecholamine agonists, norepinephrine (NE) and epinephrine [6]. Radioligand binding and photoaffinity labeling studies indicate that imidazoline binding sites represent a heterogeneous family of proteins, currently grouped as I_1 and I_2 sites. The two groups of binding sites differ in their ligand recognition properties, in tissue distribution, and possibly in their localization within the cell.

Selective manipulation of relative α_2-adrenergic subtype/imidazoline binding properties should lead to the development of new compounds, with a high degree of selectivity for the biological target and with few side effects for use in therapeutics or experimental pharmacology [7] (see *figure 1*).

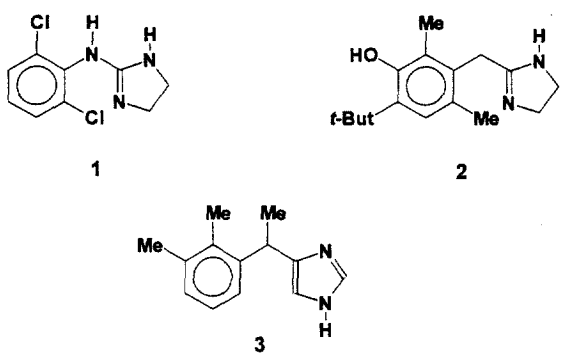

Figure 1. Structures of compounds **1–3**.

In a previous paper investigating the role played by the benzylic hydroxyl group of adrenergic catecholamines in activating α-AR, we described certain 3-phenylpiperidines with the general formula **5** (PPEs), [8] which display a good activity on α_2-AR, but are completely inactive on α_1-AR.

The PPEs **5** are conformationally restricted analogs of the corresponding adrenergic drugs with phenylethylamine structure (PAEs, **4**), in which two of the active groups (the aromatic moiety and the amino group) are constrained into the pharmacophore spatial relationship, but the other active group (the benzylic hydroxyl group, characteristic of adrenergic catecholamines) is not present. The PPEs **5** represent a new class of α-agonists that are highly selective for α_2-AR, in addition to the well known class of arylimidazoline and arylimidazole derivatives mentioned above (see *figure 2*).

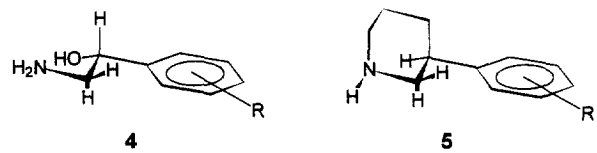

Figure 2. Structures of compounds **4** and **5**.

Unpublished results demonstrated that one of the PPEs the 3-(3,4-dimethylphenyl) substituted one (**5a**) not only lacks activity at α_1-AR, but has no (β_1-) or very low activity (β_2-) at AR. It is interesting to note that the two methyl groups present on the aromatic ring of **5a** are found, albeit in a different position, on the phenyl ring of the oxymetazoline (**2**) an arylimidazoline derivative, and of medetomidine (**3**), an arylimidazole derivative. As such, the PPE **5a** provided a unique template for the design of α_2-AR ligands.

In the light of these considerations, we synthesized the five possible dimethylphenyl-substituted isomers of the PPE **5a**, **5b–f** and, for the sake of comparison, the compound **5g** unsubstituted on the aromatic ring. Their activity on α_1- and α_2-AR and on I_1-IR was evaluated in vitro both by radioligand binding assays and by functional tests on isolated preparations. Two selected PPEs, **5a** and **5f**, were also tested for their affinity at the four α_2-AR subtypes, α_{2A}, α_{2B}, α_{2C}, and α_{2D} (see *figure 3*).

2. Chemistry

The (3,4-dimethylphenyl)-3-piperidine **5a** was prepared as previously described [8]. The dimethyl-substituted phenylpiperidines (**5b–f**) and the unsubstituted phenylpiperidine (**5g**) were synthesized as outlined in *figure 4*.

The cross-coupling reaction of the appropriate arylmagnesium bromide **6b–f** with 3-bromopyridine (**7**) catalyzed by dichlorobis-(triphenylphosphine)Ni(II) [9], yielded the corresponding 3-phenylpyridines **8b**, **8c** [10], **8d** [10], **8e** [11] and **8f**. Hydrogenation of **8b–f** in the presence of PtO_2 afforded the desired arylpiperidines **5b–f**.

The unsubstituted phenylpiperidine **5g** [12] was obtained directly by reduction of the 5-phenyl-2-piperidone **9** [12] with B_2H_6.

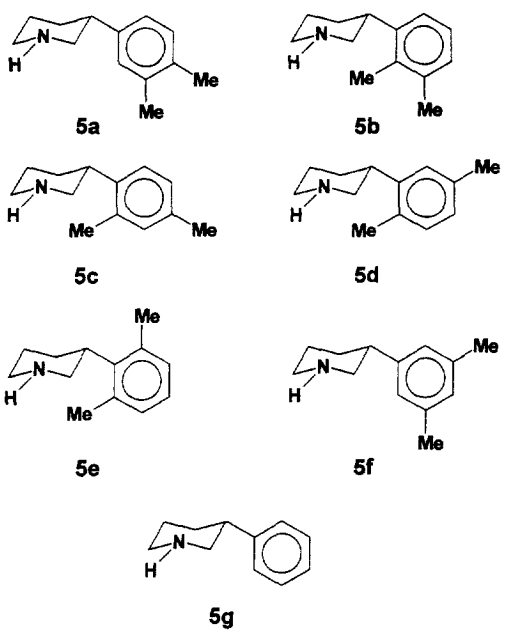

Figure 3. Structures of compounds **5a–g**.

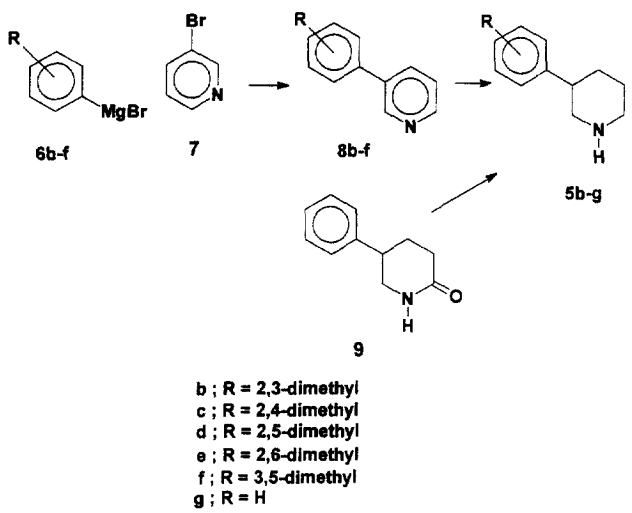

Figure 4.

3. Radioligand binding assays

The affinities of PPEs **5a–g** for α-AR and I_1-IR were determined by radioligand binding assays carried out on rat brain membrane preparations (*table I*). The antagonists [^{3}H]prazosin and [^{3}H]rauwolscine were used as specific radioligands for α_1- and α_2-AR, respectively. [^{3}H]clonidine was used as an agonist radioligand for α_2-AR and as a radioligand for I_1-IR. In order to evaluate the affinity of PPEs only for I_1-IR, binding tests were carried out on rat brain membrane preparations which had been pretreated with an excess of rauwolscine, so as to selectively block the α_2-AR.

3.1. Rat brain α_1-adrenergic receptors

The PPEs **5a**, **5c**, and **5g** showed an affinity against [^{3}H]prazosin binding about 4–5 times lower than that of NE [1]. The other PPEs **5b, 5d, 5e** and **5f** were found to be practically inactive.

3.2. Rat brain α_2-adrenergic receptors

PPE **5g** had the highest affinity in inhibiting [^{3}H]rauwolscine binding (252 nM), which was two orders of magnitude higher than that of NE, [1] clonidine (**1**), and rauwolscine which all had the similar K_i values in this assay system. The PPEs **5a–d** showed a slightly lower affinity with K_i values between 325 and 655 nM. The PPEs **5e** and **5f** had an extremely low affinity.

3.3. Rat brain α_2-adrenergic receptors and I_1-imidazoline receptors

The PPEs **5a, 5b** and **5g** showed a similar affinity (K_i values of 463, 702, and 656 nM respectively) in inhibiting [^{3}H]clonidine binding, which was about two orders of magnitude lower than that of NE and clonidine (**1**) and about one order of magnitude lower than that of rauwolscine. The PPEs **5c–f** showed an affinity lower than that of **5a, b** and **g.** All compounds had similar affinities against the antagonist [^{3}H]rauwolscine and the agonist [^{3}H]clonidine (within a factor of 3), with the exception of rauwolscine which was about 6-fold more potent against [^{3}H]rauwolscine as compared to [^{3}H]clonidine.

3.4. Rat brain I_1-imidazoline receptors

PPE **5a** had the highest affinity in inhibiting [^{3}H]clonidine binding in the presence of an excess of rauwolscine with a K_i value of 562 nM, about 50 times lower than that of clonidine (**1**). The PPEs **5b**, **c**, **d** and **g** showed an affinity which was half that of **5a**, with K_i values between 921 and 1118 nM. The PPEs **5e** and **5f** were completely inactive. In agreement with literature data NE and rauwolscine has low affinity for the I_1-receptor [13].

3.5. α_2-Adrenergic receptor subtypes

Compounds **5a** and **5f** were assayed using [^{3}H]RX821002 in systems known to contain a single

Table I. Radioligand α-adrenergic and I_1-imidazolinic binding affinities of PPEs **5a–g**.

Compound	K_i nM [a]			
	Rat brain (α_1) [b]	Rat brain (α_2) [c]	Rat brain (α_2 and I_1) [d]	Rat brain (I_1) [e]
5a	2210 (1590–2930)	325 (207–442)	463 (379–545)	562 (450–670)
5b	– [f]	655 (515–770)	702 (618–785)	954 (800–1105)
5c	2600 (1770–3430)	617 (490–730)	1000 (854–1145)	921(775–1066)
5d	– [f]	434 (355–510)	1270 (1138–1406)	960 (814–1105)
5e	– [f]	3840 (2730–4950)	2930 (2100–3760)	– [g]
5f	– [f]	1248 (840–1660)	1915 (1150–2670)	– [g]
5g	1570 (1280–1860)	252 (176–327)	656 (570–740)	1118 (830–1410)
NE	450 (390–520) [h]	4.8 (4.5–7.1) [h]	6.8 (5.4–8.1)	– [g]
RAU	– [f]	4.5 (3.4–5.6)	26.3 (19–32)	– [g]
CLO	– [f]	4.5 (3.3–5.7)	5.2 (2.8–7.6)	9.5 (6.6–12.4)

[a] The values of the inhibition costants (K_i nM) are calculated from the respective IC_{50} values by applying the Cheng–Prusoff equation. [b] [^{3}H] prazosin was used as the radioligand at a concentration of 0.5 nM. [c] [^{3}H] rauwolscine was used as the radioligand at a concentration of 2.0 nM. [d] [^{3}H] clonidine was used as the radioligand at a concentration of 2.0 nM. [e] [^{3}H] clonidine was used as the radioligand at a concentration of 2.0 nM in rat membranes pretreated with an excess of rauwolscine at 300 nM. [f] In the screening carried out at a concentration of 10 μM, these compounds proved not to possess any appreciable capacity to inhibit the binding of [^{3}H] prazosin. [g] In the screening carried out at a concentration of 10 μM, these compounds proved not to possess any appreciable capacity to inhibit the binding of [^{3}H] clonidine in the presence of rauwolscine 300 nM. [h] See [1].

α_2-AR subtype (*table II*). Compound **5a** had similar affinities for the four pharmacological subtypes of the α_2-AR, and the values were also similar to the K_i value determined in the rat brain (*table I*). **5f** was five-fold more potent at the α_{2C}-subtype as compared to the α_{2D}-subtype, and thus has a slight selectivity for the α_{2C}-AR. The affinities of NE, rauwolscine and clonidine are also given in *table II* for purposes of comparison.

4. Functional tests

The PPEs **5a–g** were tested on isolated rat vas deferens for their activity on α_1-AR and on isolated guinea pig ileum for their activity on α_2-AR (*table III*).

4.1. Rat vas-deferens α_1-receptors

None of the PPEs **5a–g** tested on α_1-AR showed any agonistic activity.

4.2. Guinea-pig ileum α_2-receptors

All the PPEs **5a–g** showed a significant α_2-agonistic activity, but with various degrees of potency. The most active PPEs were **5a** and **5f,** which showed pD_2 values (5.12 and 5.75 respectively) about one order of magnitude lower than that of NE. The others PPEs showed pD_2 values that varied between 4.98 and 4.28. All the compounds possessed an intrinsic activity (i.a. = 1.00) of full agonists.

Table II. Affinities of PPEs **5a** and **5f** for the four pharmacological α_2-adrenergic receptor subtypes.

Compound	K_i, nM [a]			
	α_{2A}-AR [b]	α_{2B}-AR [c]	α_{2C}-AR [d]	α_{2D}-AR [e]
5a	677 ± 22	231 ± 8	270 ± 15	278 ± 17
5f	2200 ± 290	1260 ± 170	726 ± 192	3490 ± 360
NE [f]	116	244	24	209
RAU [g]	0.32	0.37	0.13	12
CLO [f]	15	27	48	53

[a] Inhibition constants (K_i, nM) were calculated from the respective IC_{50} values by applying the Cheng–Prusoff equation. The values are means ± SEM for three experiments for **5a** and two experiments for **5f**. [^{3}H]RX821002 was used as the radioligand at a concentration of 0.35 nM for the α_{2A}, α_{2C}, α_{2D} subytpes and 0.88 nM for the α_{2B} subtype. [b] CHO cells transfected with the human α_{2A} receptor. [c] CHO cells transfected with the rat α_{2B} receptor. [d] CHO cells transfected with the opossum α_{2C} receptor. [e] Bovine neurosensory retina. [f] Values are from reference 33. [g] Values are from [22].

Table III. α-Adrenoceptor agonistic activities of PPEs **5a–g**, on isolated preparations.

Compound	α Adrenoceptor activity [a]			
	Isolated rat vas deferens (α_1)		Isolated guinea pig ileum (α_2)	
	pD_2	i.a. [b]	pD_2	i.a. [b]
5a	– [c]		5.12 (± 0.12) [c]	1.00 [c]
5b	–		4.75 (± 0.028)	1.00
5c	–		4.81 (± 0.06)	1.00
5d	–		4.98 (± 0.05)	1.00
5e	–		4.28 (± 0.06)	1.00
5f	–		5.75 (± 0.12)	1.00
5g	< 3.50		4.33 (± 0.05)	1.00
NE	5.20 (± 0.09) [c]	1.00 [c]	6.60 (± 0.10) [c]	1.00 [c]

[a] The agonist activity of each compound on the receptors was evaluated by means of the pD_2 value, i.e. the negative logarithm of the drug molar concentration, that produces 50% of the maximal effect; the values represent the means of 4–6 experiments for each drug ± standard error. [b] Intrinsic activity, i.e. the ratio between the maximal response elicited by the compound tested and that elicited by the full agonist, namely NE. [c] See [8].

The three most active PPEs in functional tests (**5a**, **5d** and **5f**) were also tested for their activity on β_1- and β_2-receptors. None of the three PPEs showed any agonistic activity on guinea-pig atria β_1-receptors. PPEs **5a** ($pD_2 = 3.8$, i.a. 0.83) and **5d** ($pD_2 = 4.09$, i.a. 1.00) showed an extremely limited activity on the guinea-pig trachea β_2-receptors, whereas the PPe **5f** exhibited a higher pD_2 value (5.75), which, however was accompanied by a lower ia value (0.66).

5. Theoretical calculations

Conformational analysis was carried out in vacuo on compounds **5a–g** by means of the Discover [14] molecular mechanics program. All compounds were considered as cationic forms which should be more stable at physiological pH. The *S*-enantiomer was chosen for the chirality of the carbon (3) of the PPEs [C(3)].

The results of calculations indicate that these compounds exist in the conformation in which the piperidine ring is in a chair conformation and the aromatic ring in the equatorial position, as shown in *figure 5*. The other chair conformation of the piperidine ring, in which the aromatic ring is in an axial position, was found to be less favoured in all compounds by about 4 kcal/mol, in agreement with previous findings for analogous compounds on the basis of theoretical [15] and experimental [16] studies. All compounds **5a–g** have two equivalent rotational minima corresponding to τ values of about 60° and −120° (see *figure 5*).

The conformational trend is practically identical in compounds **5a, f,** and **g,** which do not possess methyl groups in the *ortho* position. The presence of a methyl group in the *ortho* position (compounds **5b–d**) makes the conformer with this substituent on the same side as the hydrogen linked to the C(3) ($\tau = 60°$) less stable by about 2 kcal/mol. In compound **5e** with a methyl substituent in both the *ortho* positions, the two minima ($\tau = 60°$ and $\tau = -120°$) are energetically equivalent. In both these conformations, the C(3)–H bond is practically coplanar with the phenyl ring. The *ortho* substitution reduces the rotational freedom of the phenyl ring, making the rotational barriers higher (about 13 kcal/mol in **5b** and **c** against about 6 kcal/mol in **5a, f** and **g**).

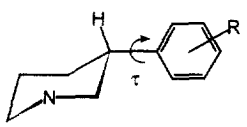

Figure 5. Structure of compound **5** in its preferred conformation.

6. Discussion

The results of the binding assays on α_1- and α_2-AR shown in *table I* demonstrate that the dimethyl-substituted PPEs **5a–f** display a marked selectivity towards α_2-AR, confirming the selectivity already revealed by PPE **5a** for this receptor in functional tests. The binding assays also show that all the PPEs **5a–f** have a lower affinity at I_1-IR compared with their affinity at α_2-AR. In particular, the PPEs **5e** and **5f** bind exclusively at the α_2-AR, albeit not with high affinity. PPE **5a** did not

show any selectivity in the binding assays with the four pharmacological subtypes of the α_2-AR, whereas **5f** was slightly selective for the α_{2C} as compared to the α_{2D} (*table II*). Because the α_{2A} and α_{2D} subtypes are species orthologues, this selectivity would only be apparent in α_{2D} species such as rat, mouse and cow, and not in α_{2A} species including human, pig and rabbit.

The results of the functional tests listed in *table III* show that the new dimethyl-substituted PPEs **5b–f** all display an appreciable activity on α_2-AR, whereas they are completely inactive at α_1-AR, thus confirming the pharmacological characteristics previously observed for the PPE **5a** at α-AR [8]. Also the tests carried out on β-AR showed for **5d** and **5f** a behaviour analogous to that of **5a**. The new PPEs **5d** and **5f** did not display any stimulating activity on β_1-AR and only a very limited activity towards β_2-AR, as found for **5a**.

An examination of the data presented in *tables I, II* and *III* shows that the results of the functional tests are substantially in agreement with the results of the binding assays, even if the activity trend shown by the functional tests does not always agree with the affinity trend revealed in the binding tests. Differences between the potency values obtained in the functional studies ($pD_2 = -\log EC_{50}$) and those obtained in radioligand binding assays (K_i) are not surprising because, even if the EC_{50} of an agonist is linked to its affinity for the receptor, the parameter is also markedly influenced by many other factors, such as transduction pathway, receptor density, spare receptors, etc. Large differences between the values of EC_{50} and K_i are widely reported in literature, even in the same tissues of the same animal species [17].

The PPEs **5a** and **5f** proved to be particularly interesting. PPE **5a** has a good affinity for α_2-AR and I_1-IR, even though it also has some affinity for α_1-AR (see *table I*). The PPE **5f** was found in binding assays to be very selective for α_2-AR even though it does not have a particularly high affinity for these receptors (see *table I*). **5f** also shows a certain subtype selectivity (see *table II*). Subsequently, the functional tests showed that PPEs **5a** and **5f** are essentially inactive at α_1-AR and β_1-AR, have a weak activity on β_2-AR, but have a good activity at α_2-AR, as shown by their pD_2 and ia values (see *table III*). The interest of PPE **5a** is justified by the fact that this compound is a derivative with a phenylethylamine structure which, contrary to expectations for this kind of derivative, displays a good affinity for the imidazoline receptor. The interest of PPE **5f** derives from the fact that this compound is selective for α_2-AR which is a characteristic that might be interesting as regards its therapeutic usefulness. PPEs are probably completely protonated at physiological pH and thus might not be able to cross the blood–brain barrier. The potential therapeutic interest of PPEs, and in particular of PPE **5f** might therefore be limited to their use for peripherally acting compounds.

The effects of the methyl substitution on the affinity of PPEs towards both α_2-AR and I_1-IR (see *table I*), might be explained by simply taking into account the hindrance of the substituted phenyl ring: the best affinity is possessed by the unsubstituted PPE **5g**. Moreover, conformational data helped us to hypothesize a possible rationalisation of the influence of the methyl substitution on the α_2-agonist activity of the phenylpiperidines (shown in *table III*). Only the substitutions in the *meta* and *para* positions seem to have a direct positive effect, as suggested by the highest activities of **5f** and **5a**; these methyl substituents could improve the interaction of the phenyl moiety with a lipophilic pocket of the α_2-receptor. On the contrary the *ortho* substitution seems not to influence the α_2-agonist activity, as a result of the very similar activity of the *o,o*-dimethyl-substituted PPE **5e** and the unsubstituted PPE **5g**; the *ortho* substitution could only have a certain conformational effect. The higher agonist activity of the *m,m*-dimethyl-substituted PPE **5f**, compared with that of the *m,p*-dimethyl-substituted PPE **5a,** suggests that the *meta* position is the most favourable one for the agonist activity. Moreover, considering PPEs **5b–d**, in which the presence of the methyl group in the *ortho* position reduces the rotational freedom of the phenyl ring, the better agonist activity is shown by PPE **5d,** in which the other methyl is in a *meta* position, on the same side as the hydrogen linked to the C(3), as shown in *figure 6*. It would therefore appear that the presence of a methyl in this position is best for the expression of the activity of the PPEs.

All the PPEs display a good agonist activity for α_2-AR as shown by the results of functional tests. The different methyl substitutions in the PPEs modulate the affinity towards I_1-IR, as shown by the results of binding studies. For this reason, the PPEs could be a good starting point for understanding of the structural requirements that could differentiate the selective agonism towards α_2-AR and I_1-IR.

7. Experimental protocols

7.1. Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra for comparisons between compounds were taken with a FT-IR Mattson 1000 Unicam spectrometer, as Nujol mulls in the case of solid substances, or as liquid film in the case of liquids. ^{1}H NMR spectra were routinely detected with a Varian EM 360 A instrument or with Varian CFT-20 spectrometer operating at 80 MHz in ca. 5% solution of $CDCl_3$ (for the neutral

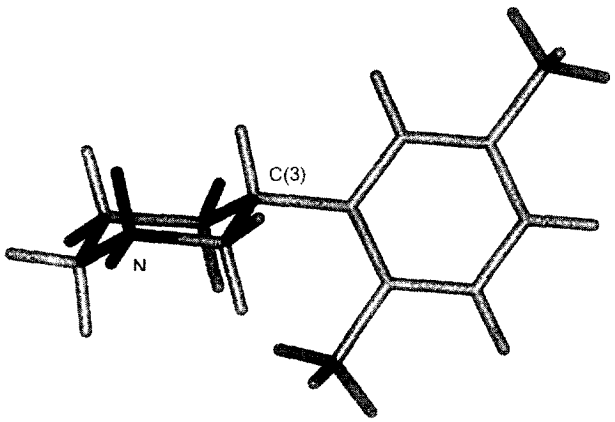

Figure 6. Compound **5d** in its preferred conformation.

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. The electron impact mass spectra were recorded on a Hewlett Packard 5988A spectrometer by direct introduction at a nominal electron energy of 70 eV and a source temperature of 350 °C. Evaporations were made in vacuo (rotating evaporator). Column chromatographies were carried out on 70–230 mesh silica gel. $MgSO_4$ was always used as the drying agent. Elemental analyses were performed by our analytical laboratory and agreed with theoretical values to within ± 0.4%.

*7.1.1. General procedure for the synthesis of 3-(2,3-dimethylphenyl)- (**8b**•HCl), 3-(2,4-dimethylphenyl)- (**8c**•HCl) [10], 3-(2,5-dimethylphenyl)- (**8d**•HCl) [10], 3-(2,6-dimethylphenyl)- (**8e**•HCl) [11], 3-(3,5-dimethylphenyl)-pyridine hydrochloride (**8f**•HCl)*

A stirred solution of the appropriate arylmagnesium bromide **6b–f** [(prepared from the corresponding aryl bromide (60.0 mmol) and Mg (1.5 g, 61.7 mmol) in anhydrous THF (150 mL)] was treated dropwise, at 10 °C with a solution of 3-bromopyridine (**7**) (9.48 g, 60.4 mmol) and dichlorobis(triphenylphosphine) Ni (II) [9], (0.70 g, 1.1 mmol) in anhydrous THF (135 mL). When the addition was complete the reaction mixture was stirred at room temperature under nitrogen for 24 h and then poured into ice-cold 5% aqueous HCl and washed with Et_2O. The aqueous layer was basified to pH 8.0 with solid K_2CO_3 and extracted with Et_2O. Evaporation of the washed (H_2O) and dried extracts yielded a crude residue which was purified by chromatography on silica gel column, eluting with 3:7 AcOEt/Hexane. Compounds **8b–f** were converted into hydrochlorides by dissolving the bases in Et_2O and treated with an excess of Et_2O•HCl. The solid precipitate was filtered and crystallized from MeOH/Et_2O in the case of **8b**, **8c**, **8f**•HCl or from EtOH/Et_2O in the case of **8e**•HCl.

***8b**•HCl* (20%): m.p. 183–184 °C; ^{1}H NMR δ 2.27, 2,33 (2s, 6H, CH_3), 7.23–7.46 (m, 3H, Ar), 7.6–9.6 (m, 4H, Py); MS *m/z* 183 $(M–HCl)^+$; Anal. ($C_{13}H_{14}$ NCl) C, H, N.

***8c**•HCl* (20%): m.p. 170–172 °C; ^{1}H NMR δ 2.30, 2.42 (2s, 6H, CH_3), 7.23 (m, 3H, Ar), 8.03–9.17 (m, 4H, Py); MS *m/z* 183 $(M–HCl)^+$; Anal. ($C_{13}H_{14}$NCl) C, H, N.

***8d**•HCl* (12%): m.p. 170–173 °C; ^{1}H NMR δ 2.25, 2.37 (2s, 6H, CH_3), 7.30, 7.18 (m, 3H, Ar), 8.06–9.17 (m, 4H, Py); MS *m/z* 183 $(M–HCl)^+$; Anal. ($C_{13}H_{14}$ NCl) C, H, N.

***8e**•HCl* (10%): m.p. 169–171 °C; ^{1}H NMR δ 2.05 (m, 6H, CH_3), 7.30 (m, 3H, Ar), 7.90–9.20 (m, 4H, Py); MS *m/z* 183 $(M–HCl)^+$; Anal. ($C_{13}H_{14}$ NCl) C, H, N.

***8f**•HCl* (15%): m.p. 163–164 °C; ^{1}H NMR δ 2.43 (m, 6H, CH_3), 7.23 (m, 3H, Ar), 7.83–9.17 (m, 4H, Py); MS *m/z* 183 $(M–HCl)^+$; Anal. ($C_{13}H_{14}$ NCl) C, H, N.

*7.1.2. General procedure for the synthesis of 3-(2,3-dimethylphenyl)- (**5b**•HCl), 3-(2,4-dimethylphenyl)- (**5c**•HCl), 3-(2,5-dimethylphenyl)- (**5d**•HCl), 3-(2,6-dimethylphenyl)- (**5e**•HCl), 3-(3,5-dimethylphenyl)-piperidine hydrochloride (**5f**•HCl)*

A mixture of the appropriate pyridine derivative **8b–f**•HCl (5.95 mmol) and PtO_2 (0.75 g) in MeOH (150 mL) was shaken at room temperature under hydrogen at atmospheric pressure. When the hydrogen absorption stopped, the catalyst was filtered off, and the solvent was evaporated to yield a solid residue which was crystallized from MeOH/Et_2O to give the hydrochloride salt of **5b–f**.

***5b**•HCl* (60%): m.p. 148–150 °C; ^{1}H NMR δ 1.98–2.6 (m, 4H), 2.27, 2.29 (2s, 6H, CH_3), 2.8–3.75 (m, 5H), 7.15 (m, 3H, Ar); MS *m/z* 189 $(M–HCl)^+$; Anal. ($C_{13}H_{20}NCl$) C, H. N.

***5c**•HCl* (85%): m.p. 198–200 °C; ^{1}H NMR δ 1.66–2.26 (m, 4H), 2.36, 2.40 (2s, 6H, CH_3), 2.90–3.73 (m, 5H), 7.26 (m, 3H, Ar); MS *m/z* 189 $(M–HCl)^+$; Anal. ($C_{13}H_{20}NCl$) C, H, N.

***5d**•HCl* (80%): m.p. 189–190 °C; ^{1}H NMR δ 1.70–2.20 (m, 4H), 2.35, (s, 6H, CH_3), 3.0–3.73 (m, 5H), 7.15 (m, 3H, Ar); MS *m/z* 189 $(M–HCl)^+$; Anal. ($C_{13}H_{20}NCl$) C,H,N.

***5e**•HCl* (50%): m.p. 274–276 °C; ^{1}H NMR δ 1.55–2.15 (m, 4H), 2.41 (s, 6H, CH_3), 3.0–3.81 (m, 5H), 7.10 (m, 3H, Ar); MS *m/z* 189 $(M–HCl)^+$; Anal. ($C_{13}H_{20}NCl$) C,H,N.

***5f**•HCl* (80%): m.p. 123–124 °C; ^{1}H NMR δ 1.73–2.22 (m, 4H), 2.37 (s, 6H, CH_3), 2.88–3.70 (m, 5H), 7.11 (m, 3H); MS *m/z* 189 $(M–HCl)^+$; Anal. ($C_{13}H_{20}NCl$) C,H,N.

*7.1.3. 3-Phenyl-piperidine hydrochloride **5g**•HCl [12]*

A stirred solution of $NaBH_4$ (0.77 g, 20 mmol) in anhydrous THF (20 mL) was cooled at 0 °C and treated, under external cooling, dropwise, with a solution of BF_3•Et_2O (3.36 mL, 26.6 mmol) in anhydrous THF (20 mL) and then with a solution of **9** (0.630 g, 3.6 mmol) in anhydrous THF (20 mL). After completion of the addition, the reaction mixture was stirred at room temperature for 2 h and then refluxed for 2 h. The cooled mixture, was teated with HCl 10% (30 mL) and stirred for 1 h. After evaporation of THF, the aqueous solution was extracted with Et_2O. Evaporation of the washed (H_2O) and dried extracts gave a residue which was converted into the crude hydrochloride of **5g** for treatment with an excess of Et_2O•HCl. The solid precipitate was filtered and crystallized from MeOH/Et_2O to yield pure **5g**•HCl [16] (0.50 g, 70%), m.p. 142–143 °C (literature [12]: m.p. 143–144 °C (EtOH/Et_2O)); ^{1}H NMR δ 1.73–2.30 (m, 4H), 2.90–3.76 (m, 5H), 7.56 (s, 5H, Ar); MS *m/e* 161 $(M–HCl)^+$.

7.2. Radioligand binding methods

7.2.1. α_1- And α_2-adrenergic receptors

α_1- and α_2-receptor binding assays were performed in rat cerebral cortex membranes, as elsewhere reported [18, 19]. Simi-

larly, the selective radioligand binding assays for the four pharmacological subtypes were performed with membrane preparations and [^{3}H]RX821002 (Amersham, Arlington Heights, IL) essentially as previously described [20–22]. The receptor sources were CHO cells transfected with the human α_{2A} receptor, [23] the rat α_{2B} receptor [24] and the opossum α_{2C} receptor [25], and bovine neurosensory retina [22].

7.2.2. Rat brain I_1-IR

IR binding was determined in rat cerebral cortex membranes using [^{3}H]clonidine as the ligand (Du Pont, New England). The radioligand binding assay with [^{3}H]clonidine was performed by a modification of the methods previously described [26–31]. Cerebral cortex membranes were suspended in 50 mM Tris HCl pH 7.7 (= 0.4 mg of proteins), and incubated with 2 nM [^{3}H]clonidine in the presence of 300 nM rauwolscine. After incubation at 25 °C for 60 min, the samples were filtered with 3 × 5 mL of 50 mM Tris HCl pH 7.7 buffer, dried, and 4 mL of Ready Protein Beckman Scintillation cocktail was added. Non-specific binding was measured in the presence of 1 μM clonidine. The concentrations inhibiting the specific binding by 50% (IC_{50} values) were calculated from the inhibition curves by log probit analysis. The dissociation constant (K_i) was derived from the equation of Cheng and Prusoff [31]. The ligand affinity (K_d) of [^{3}H]clonidine was 2.5 nM.

7.3. Pharmacological methods

The assays were conducted in accordance with the legislation of the Italian Authorities (D.L. 27/01/92, n. 116) concerning animal experimentation. The animals under Et_2O anaesthesia were killed by cervical dislocation and bled, and then the abdominal cavity was opened by a midline incision. The organs were immediately placed in cold Tyrode solution [composition (mM): NaCl (136.8); KCl (2.95); $CaCl_2$ (1.80); $MgSO_4 \bullet 7H_2O$ (1.05); NaH_2PO_4 (0.41); $NaHCO_3$ (11.9); Glucose (5.5)] gassed with carbogen (95% O_2:5% CO_2).

7.3.1. 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).

7.3.2. 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 × 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 the beginning of 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-AR 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 (as bitartrate); **5a–b** (as hydrochlorides).

7.4. Theoretical calculation

Conformational analyses were performed by molecular mechanics calculations using the Discover programme [14] with the CVFF force field in which partial atomic changes are also defined. The starting geometries of compounds **5a–g**, considered in their protonated form, were built by using those of other phenylpiperidines previously studied, [8, 15, 16,] the two conformers corresponding to the two possible chair conformation of the piperidine ring were built separately and then fully minimized. The minimizations were performed by using Newton–Raphson with 10^{-4} Kcal/mol Å on RMS derivatives as convergence criteria. The dielectric constant was fixed at 1, independently of the distance; test calculations indicated that results are not appreciably influenced by this choice.

In the case of systematic conformational search studies, the torsion angles were varied by 5° steps while all the other freedom degrees of the molecule were fully optimized; the starting geometry of the piperidine ring of **5a–g** was the one with the phenyl ring in an equatorial position as explained above and it remained unchanged during optimization. In previous studies, it had been found that the Discover programme gave relative conformational energies for compounds similar to those considered here in agreement with those obtained by ab initio MO calculations ([32] and cited references).

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