Imidazoline Binding Sites (IBS) Profile Modulation: Key Role of the Bridge in Determining I_1-IBS or I_2-IBS Selectivity within a Series of 2-Phenoxymethylimidazoline Analogues

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The α- and *β*-methyl derivatives of 2-phenylethylimidazoline (compounds **7** and **8**) and the corresponding enantiomers were prepared and tested with the purpose of studying the role played by the ethylene bridge in modulating I_1- and I_2-IBS selectivity. The α-methylation appeared to be extremely critical regarding the affinity and selectivity for the I_1-IBS subtypes ($I_1/I_2 = 186$ for imidazoline **7**) and the stereospecificity of interaction (eudismic ratio (*S*)-(−)-**7**/(*R*)-(+)-**7** = 5888). Instead, even if in a more limited fashion, the *β*-methylation tended toward I_2-IBS selectivity ($I_2/I_1 = 50$ for imidazoline **8**). The unsubstituted compound **4** ($I_2/I_1 = 1479$) proved to be considerably more potent and selective with respect to I_2-IBS subtypes.

Introduction

The existence of imidazoline binding sites (IBS) is derived from studies on the activity of the α_2-adrenergic agonist clonidine. In fact, some 15 years ago, Bousquet and co-workers[1] discovered that microinjection of this ligand into the nucleus reticolaris lateralis of the brainstem caused a hypotensive response that was not equally evoked by catecholamines. Since then, many advances have been made in the knowledge of these sites, investigated above all with compounds bearing an imidazoline nucleus and defined as nonadrenergic, and substantial progress has been achieved in determining their pharmacological role.

The numerous studies, with radioligands and those of a functional type,[2] apart from confirming the existence of IBS have also revealed their heterogeneity. The current classification includes the I_1-IBS, which are preferentially recognized by [^{3}H]-*p*-NH_2-clonidine and [^{3}H]-clonidine, and the I_2-IBS subtypes, preferentially recognized by [^{3}H]-idazoxan.[3] In addition, the I_2-IBS subtypes have been further subdivided into I_2A-IBS and I_2B-IBS on the basis of their affinity, high and low, respectively, for Amiloride.[4] Still more recently, this classification has been updated with the proposed inclusion of another subtype to be known as I_3-IBS.[5]

The IBS interact with different ligands, are located subcellularly, and are widely distributed throughout the tissues of various species including man.[2] They are present in both the central and peripheral nervous systems and in various organs such as kidney, stomach, prostate, lung, and heart.[2]

The I_1-IBS subtypes are involved in the hypotensive activity produced by derivatives bearing an imidazoline nucleus and by correlated compounds.[2,6−9] Agmatine,[10] the substance displacing clonidine, or CDS,[11] and harmane[12] have been proposed as possible endogenous ligands. The transduction systems in PC 12 cells are associated with activation of PC-PLC and with a reduction of c-AMP.[13,14] The I_2-IBS subtypes, considered to be monoamino oxidase (MAO) allosteric sites,[15] are associated above all with inhibition of this species of enzyme in the central nervous system (CNS).[16]

Moreover, the IBS are involved in regulating intraocular pressure,[17] increasing the neuronal activity,[18] releasing insulin,[19−21] synthesizing proteins in astrocytes,[22] and inhibiting various neurotransmitter release systems.[23] Also studied was the role they play in food ingestion,[24,25] gastric secretion,[2,26] humor dysfunction,[2,27] tolerance to opioids and neuroprotection,[28] renal function,[2,29] and Alzheimer[22] and Parkinson's[30] diseases. This multiplicity of functions makes the IBS attractive targets for drugs but, at the same time, complicates their characterization also because the most commonly used ligands are generally scantily selective with respect to the two main subtypes I_1-IBS and I_2-IBS and to the various receptor systems, and particularly so in the case of α_2-adrenergic ones (ARs). Only recently have some new ligands with high affinity and selectivity toward the two main IBS subtypes been described.[31]

In some previous studies, we showed that affinity and selectivity for IBS with respect to the α_2-ARs can be modulated by slight changes in the 2-phenoxymethylimidazoline bridge (**1**) (Chart 1). In fact, while compound **1** recognized with high affinity the I_2-IBS subtypes ($pK_i(I_2) = 9.05$) and with good affinity the α_2-ARs ($pK_i = 7.28$), substitution of the ether oxygen atom with the NH group (compound **2**) ($pK_i(I_2) = 7.48$; $pK_{i(}\alpha_2) = 7.14$) or with the sulfur atom (compound **3**) ($pK_i(I_2) = 7.30$, $pK_i(\alpha_2) = 6.70$) gave rise to less selective ligands in that they have comparable affinity values for the I_2-IBS and

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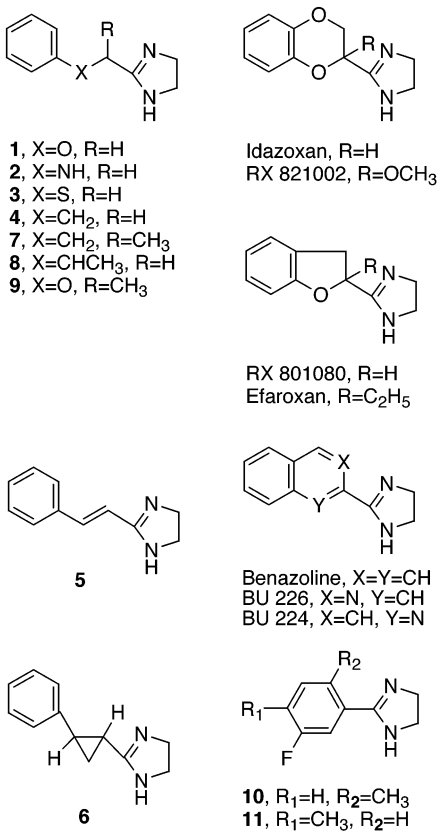
Chart 1



α_2-ARs subtypes.[32] Instead, replacing the ether oxygen atom with a methylene group (compound **4**) allowed us to obtain a potent ligand that was really selective for the I_2-IBS subtypes with respect to the α_2-ARs with a $I_2/\alpha_2 = 794$ ($pK_i(I_2) = 8.60$; $pK_i(\alpha_2) = 5.70$). Limiting the flexibility of the carbon chain of **4** by introducing a double bond led to tracizoline (**5**), which proved to be even more selective toward I_2-IBS with respect to α_2-ARs ($I_2/\alpha_2 = 7413$) even if scarcely selective with respect to I_1-IBS subtypes ($I_2/I_1 = 10$).[32,33]

A significant improvement in selectivity between the two subtypes I_1-IBS and I_2-IBS was obtained by replacing the ethylene bridge of **4** with a cyclopropane ring, and the subsequent optical resolution of the *trans*-2-(2-phenylcyclopropyl)imidazoline (**6**).[33] In fact, whereas the (1*S*,2*S*)-(+)-**6** is more potent for the I_1-IBS subtypes ($pK_i(I_1) = 7.93$; $pK_i(I_2) = 6.91$), the (1*R*,2*R*)-(−)-**6** one is more potent for the I_2-IBS subtypes ($pK_i(I_1) = 6.46$; $pK_i(I_2) = 8.22$). This surprising result of reversed enantioselectivity highlighted some stereospecific requirements of the I_1-IBS and I_2-IBS subtypes and thus encouraged us to prepare other chiral agents as suitable tools for characterizing IBS.

Carrying forward our research with a view to clarifying which of the two chiral centers of compound **6** was decisive for I_1-IBS affinity and which for that of I_2-IBS, we designed and prepared the α- and *β*-methyl derivatives (**7** and **8**, respectively) of compound **4** (Chart 1) and their corresponding enantiomers. The affinity of these ligands for the α_2-ARs and for the subtypes I_1-IBS and I_2-IBS was assessed on rat cortex, on PC 12 cellular membranes, and on rabbit kidney. Furthermore, to carry out a more complete investigation of structure−affinity relationships, the pharmacological study on the I_1-IBS subtypes was also carried out on compounds **1**, **4**, and **9**, for which we had already reported the affinity values for the α_2-ARs and the I_2-IBS subtypes.[32,34] As a useful means of comparison, the I_1 antagonist efaroxan was included in the study.[35,36]

Chemistry

The imidazoline derivatives **7** and **8**[37] were prepared according to standard procedures by treatment of 2-methyl-3-phenylpropionic acid[38] and 3-phenylbutyric acid methyl esters,[39] respectively, with ethylenediamine in the presence of $Al(CH_3)_3$ (Scheme 1). By use of the same method starting from the optically active esters, we synthesized the enantiomers (*R*)-(+)-**7**, (*S*)-(−)-**7**, (*R*)-(−)-**8**, and (*S*)-(+)-**8**. In the first case, unfortunately, the reaction with (*R*)-(−) or (*S*)-(+)-2-methyl-3-phenylpropionic acid methyl esters[40] produced partial racemization. In fact, the ^{1}H NMR spectrometry analysis of the diastereomeric ureidic derivatives **12a** and **12b**, obtained by treatment of the respective final imidazolines (*R*)-(+)-**7** and (*S*)-(−)-**7** with (*S*)-(−)-α-methylbenzyl isocyanate, revealed an enantiomeric purity of <98%, since the signals of the two methyl groups $-CH_2-CH-CH_3$ and $-NH-CH-CH_3$ appeared as double doublets at δ 1.13 and δ 1.52, respectively. Consequently, the (*R*)-(+)-**7** and (*S*)-(−)-**7** imidazolines were subsequently resolved by fractional crystallization of (−)-di-*O*,*O*′-*p*-toluoyl-L-tartaric acid and (+)-di-*O*,*O*′-*p*-toluoyl-D-tartaric acid salts, respectively. In this case, the optical purity, evaluated in the same way, proved to be >98%, since the multiplicity of signals of the two methyl groups was reduced to two single doublets at δ 1.13 and δ 1.52 for **12a** and at δ 1.12 and δ 1.50 for **12b**.

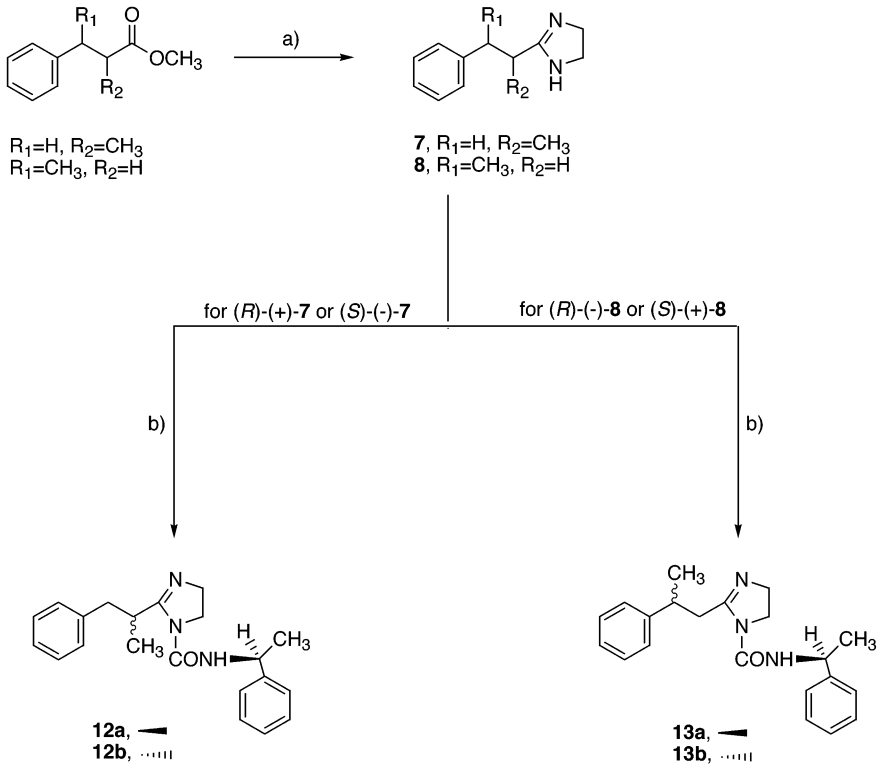
In the case of the reaction with (*R*)-(−)- or (*S*)-(+)-3-phenylbutyric acid methyl esters,[41] no racemization was observed and the final imidazoline, (*R*)-(−)-**8** or (*S*)-(+)-**8**, respectively, with the usual method displayed an optical purity of >98%. In fact, ^{1}H NMR spectrometry analysis of the diastereomeric ureidic derivatives **13a** and **13b** for the $-NH-CH-CH_3$ and $-NH-CH$-CH_3 groups showed a single doublet at δ 1.53 and a quintet at δ 5.04 for **13a** and at δ 1.52 and δ 5.03 for **13b**. The spectra thus proved to be clearly simplified compared with that of the ureidic analogue obtained from the racemate **8** in which the $-NH-CH-CH_3$ group displayed two doublets at δ 1.55, and the $-NH-CH-CH_3$ group had two overlapping quintets at δ 5.05.

Pharmacology

The compounds, in the form of hydrogen oxalate or maleate salts, were evaluated for affinity at α_2-ARs and I_2-IBS using membranes of rat cortex and rabbit kidney, respectively, following already described procedures.[32] The I_1-IBS affinity was determined on rat pheochromocytoma cells (PC 12) according to the method of Separovic et al.[13] with slight modifications. The radioligands used were [^{3}H]-clonidine (2 nM, α_2), [^{3}H]-idazoxan (5 nM, I_2), and [^{125}I]-*p*-iodoclonidine (0.5 nM, I_1), and nonspecific binding was defined by inclusion of 10 μM phentolamine (α_2, 25%), 10 μM cirazoline (I_2, 10%), and 10 μM BDF 6143 (I_1, 35%).

In vivo studies were performed by measuring the effects of compound **7** on mean arterial blood pressure

Scheme 1[a]



[a] Reagents: (a) $H_2NCH_2CH_2NH_2/Al(CH_3)_3$/toluene; (b) (*S*)-(−)-$C_6H_5CH(CH_3)NCO$.

(MAP) after intracisternal administration in rabbits anesthetized according to methods described elsewhere.[42,43]

Results and Discussion

The capacity of the various classes of substances, including those with an imidazoline nucleus, to bind to the IBS and the α_2-ARs, led Hieble and Ruffolo to hypothesize that both receptor systems present significant analogies in the orientation of their critical binding functions and to suggest that the presence of a binding pocket of different steric hindrance allows the ligands, even with some modest structural modifications, to display altered affinity and therefore enhanced selectivity.[44] For example, it is well-known that idazoxan displays significant affinity both for the I_1-IBS and I_2-IBS subtypes and for α_2-ARs;[36] nevertheless, slight structural modifications are able to determine a decrease of affinity for the I_2-IBS in favor of the α_2-adrenergic one. In fact, introduction of a methoxy substituent on the carbon atom of the benzodioxane nucleus bearing the imidazoline ring markedly reduces I_2-IBS affinity, whereas it enhances that for the α_2-ARs. The RX 821002 thus obtained is widely used as a selective α_2-adrenergic antagonist and, if tritiated, as a radioligand for identifying α_2-ARs.[45] A similar conclusion is reached by examining the effects produced by introducing an ethyl group on the RX 801080 benzofurane moiety. In fact, while the latter has high affinity both for I_2-IBS and for α_2-ARs, the former, known as efaroxan, displays modest affinity for the I_2-IBS.[46]

In the case of benazoline, which is selective for IBS with respect to α_2-ARs, but unable to discriminate between the subtypes I_1-IBS and I_2-IBS,[32,47] substitution of the naphthyl nucleus with the isosteric quinoline and isoquinoline ones gave rise to the agents BU 224 and BU 226, which were selective for the I_2-IBS subtypes with respect to the I_1-IBS ones and to the α_2-ARs.[48] Moreover, in the series of 2-arylimidazoline derivatives, the ortho (compound **10**) or para (compound **11**) position of the methyl group on the aromatic ring favored selectivity for I_1-IBS or I_2-IBS, respectively.[31]

In our study, as well, the possibility of modulating the biological profile by means of slight chemical modifications (isosteric changes or introducing small substituents) with a view to obtaining selective I_1-IBS and I_2-IBS ligands devoid of the α_2-adrenergic component was significantly confirmed. The pharmacological results reported in Table 1 show in fact that compound **1** is selective for the I_2-IBS subtypes with respect to the I_1-IBS ones and to the α_2-ARs with I_2/I_1 and I_2/α_2 selectivity ratios equal to 794 and 59, respectively. The selectivity profile for the I_2-IBS is, however, greatly enhanced in the isosteric derivative **4**, in which the I_2/I_1 and I_2/α_2 selectivity ratios rise to 1479 and 794, respectively.

Introduction of a methyl group on the α-carbon atom of the ethylene bridge of **4** (compound **7**) makes the interaction with I_1-IBS subtypes particularly productive at the expense of that with I_2-IBS subtypes, with an I_1/I_2 selectivity ratio of 186 ($pK_i(I_1) = 8.3$; $pK_i(I_2) = 6.03$). In addition, in the same way as compound **4**, imidazoline **7** displays modest α_2-adrenergic affinity ($pK_i(\alpha_2) = 5.45$) with an I_1/α_2 selectivity ratio of 708. Thus, compound **7** displays high affinity and selectivity for the I_1-IBS subtypes, markedly higher than those of efaroxan, which, like idazoxan, also binds with high affinity to the α_2-ARs. Therefore, the α-methylation of the carbon chain of **4** makes the biological features of the ligands

Table 1. Binding Affinities (pK_i[a]) of Compounds **1**, **4**−**9**, and Enantiomers (*R*)-(+)-**7**, (*S*)-(−)-**7**, (*R*)-(−)-**8**, and (*S*)−(+)-**8**

compd	$pK_i(I_1)$	Eu[b]	$pK_i(I_2)$	Eu[b]	$pK_i(\alpha_2)$	I_1/I_2[c]	I_2/I_1[c]	I_1/α_2[c]	I_2/α_2[c]
1	6.15 ± 0.11		9.05 ± 0.15		7.28 ± 0.16	0.0013	794	0.074	58.90
4	5.43 ± 0.13		8.60 ± 0.04		5.70 ± 0.06	0.0007	1479	0.54	794
5	7.72 ± 0.14		8.72 ± 0.13		4.85 ± 0.15	0.10	10	741	7413
6	7.76 ± 0.10		7.64 ± 0.05		6.40 ± 0.03	1.32	0.80	23	17
(1*R*,2*R*)-(−)-**6**	6.46 ± 0.05		8.22 ± 0.02	20[d]	6.92 ± 0.12	0.017	58	0.35	20
(1*S*,2*S*)-(+)-**6**	7.93 ± 0.14	29[e]	6.91 ± 0.10		6.62 ± 0.07	10.50	0.10	20.40	2
7	8.30 ± 0.09		6.03 ± 0.11		5.45 ± 0.07	186	0.005	708	4
(*R*)-(+)-**7**	5.20 ± 0.08		4.90 ± 0.12		5.40 ± 0.15	2	0.50	0.60	0.30
(*S*)-(−)-**7**	8.97 ± 0.16	5888[f]	6.84 ± 0.13	87[f]	5.30 ± 0.06	135	0.007	4667	35
8	5.22 ± 0.07		6.91 ± 0.19		5.93 ± 0.15	0.02	49	0.20	10
(*R*)-(−)-**8**	6.23 ± 0.05	12[g]	5.60 ± 0.12		5.90 ± 0.06	4.30	0.23	2	0.50
(*S*)-(+)-**8**	5.14 ± 0.13		7.00 ± 0.17	25[h]	5.80 ± 0.09	0.025	40	0.22	16
9	6.51 ± 0.17		5.57 ± 0.11		7.01 ± 0.08	8.71	0.11	0.32	0.04
efaroxan	7.14 ± 0.15				7.29[i]			0.70	

[a] pK_i affinity values for I_1-IBS, I_2-IBS, and α_2-ARs were assessed by measuring the ability of the test compounds to displace [^{125}I]-*p*-iodoclonidine (rat pheochromocytoma cells, PC 12), [^{3}H]-idazoxan (rabbit kidney membranes), and [^{3}H]-clonidine (rat cortex membranes), respectively. Values are the mean ± SEM of, in each case, a minimum of six experiments. [b] Eudismic ratio. [c] Antilog of the difference between $pK_i(I_1)$ and $pK_i(I_2)$ (I_1/I_2), $pK_i(I_2)$ and $pK_i(I_1)$ (I_2/I_1), $pK_i(I_1)$ and $pK_i(\alpha_2)$ (I_1/α_2), and $pK_i(I_2)$ and $pK_i(\alpha_2)$ (I_2/α_2) values. [d] (1*R*,2*R*)-(−)-**6**/(1*S*,2*S*)-(+)-**6**. [e] (1*S*,2*S*)-(+)-**6**/(1*R*,2*R*)-(−)-**6**. [f] *S*-(−)-**7**/*R*-(+)-**7**. [g] *R*-(−)-**8**/*S*-(+)-**8**. [h] *S*-(+)-**8**/*R*-(−)-**8**. [i] pK_i affinity value determined by measuring the ability of the compound to displace [^{3}H]-RX 821002 (rabbit white fat cell membranes).[44]

deviate significantly from I_2-IBS selectivity (compound **4**) to an I_1-IBS one (compound **7**). A change in the biological profile can also be observed in **1** and **9**. In the case of compound **9**, which is different from **1** because of the presence of a methyl group in the oxymethylene bridge, a drastic decrease in affinity is recorded for I_2-IBS[34] and a slight increase in affinity is recorded for I_1-IBS. However, in both compounds, unlike in the corresponding carbon derivatives, there remains good affinity toward the α_2-ARs. This is probably ascribable to the presence of the oxygen atom in the bridge.

Following the optical resolution of compound **7**, the enantiomer (*S*)-(−)-**7** proved to be the eutomer with respect to the I_1-IBS ($pK_i(I_1) = 8.97$; $pK_i(I_2) = 6.84$) with an I_1/α_2 selectivity ratio of 4667. The distomer (*R*)-(+)-**7** bound only with very low affinity to the IBS ($pK_i(I_1) = 5.2$; $pK_i(I_2) = 4.9$). The high (*S*)-(−)-**7**/(*R*)-(+)-**7** eudismic ratio observed, equal to 5888, shows the importance of the chiral center on C-1 with a view to I_1-IBS affinity. As was to be expected, the configuration of the eutomer (*S*)-(−)-**7** was, with regard to the C-1 carbon atom, in agreement with that of the already described I_1-selective compound (1*S*,2*S*)-(+)-**6**.[33]

Even though with lower values, compound **8**, in which the methyl substituent was bound to the β carbon atom of the ethylene bridge of **4**, displayed greater affinity for the I_2-IBS subtypes ($pK_i(I_1) = 5.22$; $pK_i(I_2) = 6.91$; $pK_i(\alpha_2) = 5.93$) with I_2/I_1 selectivity ratios equal to 49 and I_2/α_2 ratios equal to 10. Analogous to what had already occurred with the cyclopropane derivative **6**, the resolution of **8** in the corresponding enantiomers allowed us to confirm the reversed enantioselectivity in the interaction with the IBS, whereby the (*R*)-(−)-**8** enantiomer becomes the eutomer for the I_1-IBS subtypes ($pK_i(I_1) = 6.23$; $pK_i(I_2) = 5.60$; $pK_i(\alpha_2) = 5.90$) and the (*S*)-(+)-**8** enantiomer becomes the eutomer for the I_2-IBS subtypes ($pK_i(I_1) = 5.14$; $pK_i(I_2) = 7.0$; $pK_i(\alpha_2) = 5.80$). The fact that the eudismic ratio value obtained was higher, even though only modestly so, for the I_2-IBS subtypes (Eu = 25) compared with the I_1-IBS ones (Eu = 12) led us to think that a stereospecific interaction at the level of the carbon atom in position 2 of the bridge enhanced the affinity for the I_2-IBS.

Table 2. Hemodynamic Measurements[a]

ic pretreatment 15 min before clonidine[b]	MAP[c] (mmHg)		HR[d] (beats/min)	
	before clonidine	after clonidine	before clonidine	after clonidine
vehicle ($n = 5$)	100 ± 6	79 ± 7[e]	290 ± 10	254 ± 9[e]
7[f] ($n = 4$)	99 ± 5	91 ± 6 n.s[g]	363 ± 7	355 ± 7 n.s[g]

[a] Data are given as the mean ± standard error to the mean. Results were then compared using an ANOVA with repeated measurements followed by a post hoc test. *P* values of less than 0.05 were used as the criterion of significance, and *n* was the number of experiments. The calculations were made by computer-assisted analyses with Statview (Abacus concepts). [b] Clonidine hydrocloride (RBI) was administered iv at a dose of 10 μg kg^{-1} in a group of five animals receiving 100 μL of saline intracisternally. [c] Mean arterial pressure. [d] Heart rate. [e] $p < 0.01$ vs basal values. [f] Compound **7** was administered ic at the total dose of 300 μg kg^{-1} in another group of four animals. [g] n.s means not significant.

In in vivo studies, the selected compound **7** had no significant cardiovascular effects. In fact, cumulative doses of 10, 30, 100, and 300 μg kg^{-1} given intracisternally (ic) did not modify mean arterial pressure (MAP) by itself (98 ± 3 vs 99 ± 5 mmHg). Pretreatment with compound **7**, given ic at a dose of 300 μg kg^{-1} 15 min before a standard dose of clonidine injected intravenously (10 μg kg^{-1}), prevented the hypotensive and bradycardic effects of clonidine. In fact, while in the control experiments in which clonidine was given 15 min after the vehicle, MAP significantly decreased from 100 ± 6 to 79 ± 7 mmHg ($p < 0.01$) and heart rate (HR) decreased from 290 ± 10 to 254 ± 9 mmHg ($p < 0.01$). In animals pretreated with **7**, MAP and HR did not vary significantly after injection of clonidine (from 99 ± 5 to 91 ± 6 mmHg and from 363 ± 7 to 355 ± 7 beats/min, respectively) (Table 2).

Conclusions

As a result of slight modifications to the 2-phenoxymethylimidazoline structure (**1**), we showed, on one hand, the key role played by the bridge joining the presumed pharmacophores (the aromatic ring and the imidazoline nucleus) in the interaction with the IBS and, on the other, the stereospecific nature of such binding sites. Moreover, we verified that the wholly carbon chain of the bridge tended to favor selectivity

for the IBS with respect to the α_2-ARs. It can therefore be hypothesized that in the less selective compounds (**1**, **2**, **3**, **9**), the bridge heteroatom, by setting up hydrogen bonds with the receptor protein and/or intramolecular bonds with the NH group of imidazoline nucleus, allow the ligands to assume conformations that are suitable for recognizing the α_2-ARs.

Interestingly, this study led to two potent ligands with high selectivity for the I_2-IBS subtypes or for the I_1-IBS subtypes. They are compound **4** and (*S*)-(−)-**7**, both of which had poor affinity for the α_2-ARs.

Since Parini's group demonstrated that different I_2-IBS subtypes were associated with the monamino oxidase B (MAO B)[16,49] and since García-Sevilla's group attributed distinct binding proteins to such subtypes,[50] it can be hypothesized that, on account of this multiplicity of species, particularly selective ligands such as compound **4** can give valuable help in characterizing the pharmacological properties of the I_2-IBS (antidepressant effects in man, increase in the level of biogenic monoamines in rat brain, increase in food ingestion in rat, neuroprotection in stroke models, and modulation of antinociceptive responses).[28,51−54]

Finally, compound **7**, showing modest affinities at I_2-IBS and α_2-ARs and high affinity for I_1-IBS, does not behave as an agonist at I_1-IBS but is able to prevent the hypotensive effects of clonidine. Owing to its high I_1-IBS/α_2-ARs selectivity ratio, comparable to that of S23757[43] and markedly better than that of efaroxan, it might be considered a new I_1-IBS antagonist useful for characterizing the pharmacological responses of the I_1-IBS and in particular for determining the role played by them in modulating hypotensive effects.

Experimental Protocols

Chemistry. Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin-Elmer 297 and Varian EM-390 instruments, respectively. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quintet), or m (multiplet). Although the IR spectral data are not included (because of the lack of unusual features), they were obtained for all compounds reported and are consistent with the assigned structures. Optical activity was measured at 20 °C with a Perkin-Elmer 241 polarimeter. HPLC analyses were recorded on an HP series 1090 I chromatograph with an RP-18 (5 μm) 125-3 mm stainless steel column Purospher (Merck). The mobile phase was $(NH_4)_2HPO_4$ (pH 7.25)/CH_3CN (6:4). The flow rate was set at 1.0 mL/min. The microanalyses were performed by the Microanalytical Laboratory of our department, and the elemental compositions of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040−0.063 mm, Merck) by flash chromatography. The term "dried" refers to the use of anhydrous sodium sulfate. Compounds were named following IUPAC rules as applied by Beilstein-Institut AutoNom (version 2.1), a software for systematic names in organic chemistry.

2-(1-Methyl-2-phenylethyl)-4,5-dihydro-1*H*-imidazole Oxalate [7]. A solution of ethylenediamine (0.9 mL, 13.40 mmol) in dry toluene (4.0 mL) was added dropwise to a mechanically stirred solution of 2 M trimethylaluminum (6.7 mL, 13.40 mmol) in dry toluene (11.0 mL) at 0 °C in a nitrogen atmosphere. After being stirred at room temperature for 1 h, the solution was cooled to 0 °C and a solution of 2-methyl-3-phenylpropionic acid methyl ester[38] (1.2 g, 6.73 mmol) in dry toluene (10 mL) was added dropwise. The reaction mixture was heated to 100 °C for 3 h, cooled to 0 °C, and quenched cautiously with MeOH (1.9 mL) followed by water (0.37 mL). After addition of $CHCl_3$ (15.0 mL), the mixture was left for 15 min at room temperature to ensure the precipitation of the aluminum salts. The mixture was filtered, and the organic layer was extracted with 2 N HCl. The aqueous layer was made basic with 10% NaOH and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered, and evaporated in vacuo to give the free base as an oil, which was purified through flash chromatography using cyclohexane/AcOEt/MeOH/33% NH_4OH (4:3:1:0.1) as eluent. The free base (0.4 g, yield 31.5%) was transformed into the oxalate salt, which was recrystallized from CH_3CN (mp 151−152 °C): ^{1}H NMR (DMSO) δ 1.19 (d, 3, CH_3), 2.73−3.15 (m, 3, CH_2CH), 3.77 (s, 4, NCH_2CH_2N), 7.19−7.40 (m, 5, ArH), 9.69 (br s, 1, NH exchangeable with D_2O). Anal. ($C_{12}H_{16}N_2 \cdot H_2C_2O_4$) C, H, N.

(*R*)-(+)-2-(1-Methyl-2-phenylethyl)-4,5-dihydro-1*H*-imidazole Oxalate [(*R*)-(+)-7]. Compound (*R*)-(+)-**7** was prepared following the same procedure described for **7** starting from (*R*)-(+)-2-methyl-3-phenylpropionic acid methyl ester.[40]

The free base (*R*)-(+)-**7** was purified through flash chromatography using cyclohexane/AcOEt/MeOH/33% NH_4OH (4:3:1:0.1) as eluent (1.77 g, 30% yield). The enantiomeric purity, determined by ^{1}H NMR of the corresponding diastereomeric ureidic derivative obtained by reacting (*R*)-(+)-**7** with (*S*)-(−)-α-methylbenzyl isocyanate, was 85%. To increase this enantiomeric purity, the free base was then further purified by fractional crystallization of its (−)-*O*,*O*′-di-*p*-toluoyl-L-tartaric acid salt.

A solution of (−)-*O*,*O*′-di-*p*-toluoyl-L-tartaric acid (2.44 g, 6.8 mmol) in hot EtOH was added to a stirred solution of (*R*)-(+)-**7** (1.54 g, 8.0 mmol) in hot EtOH (100 mL). After the mixture was stirred for 3 days at room temperature, the white solid was filtered. The salt was dissolved in water, and the ice-cooled solution was made basic with 10% NaOH. The resulting mixture was extracted with $CHCl_3$. Removal of dried solvent gave (*R*)-(+)-**7** (0.83 g, 4.40 mmol). The above procedure was repeated once more to obtain optically pure (*R*)-(+)-**7**: 0.5 g; 32% yield; $[\alpha]^{20}_D$ +43.94 (*c* 1, $CHCl_3$). The optically pure free base was transformed into the oxalate salt: mp 148−149 °C; $[\alpha]^{20}_D$ +53.39 (*c* 1, MeOH); ^{1}H NMR (DMSO) δ 1.19 (d, 3, CH_3), 2.73−3.15 (m, 3, CH_2CH), 3.77 (s, 4, NCH_2CH_2N), 7.19−7.40 (m, 5, ArH), 9.58 (br s, 1, NH exchangeable with D_2O). Anal. ($C_{12}H_{16}N_2 \cdot H_2C_2O_4$) C, H, N. The enantiomeric purity of the free base was determined by HPLC and ^{1}H NMR spectrometry of its corresponding diastereomeric ureidic derivative **12a**, which was prepared as follows.

A solution of (*R*)-(+)-**7** (0.1 g, 0.53 mmol) and (*S*)-(−)-α-methylbenzyl isocyanate (0.077 g, 0.32 mmol) in dry CH_2Cl_2 (2 mL) was stirred at room temperature for 4 h. After evaporation of the solvent, the residue was purified through flash chromatography using cyclohexane/AcOEt (8:2) as eluent: ^{1}H NMR ($CDCl_3$) δ 1.13 (d, 3, $CH_3CHC{=}N$), 1.52 (d, 3, CH_3CHN), 2.62 (dd, 1, $ArCH_2$), 3.16 (dd, 1, $ArCH_2$), 3.59−3.83 (m, 4, NCH_2CH_2N), 3.9 (m, 1, $CHC{=}N$), 4.76 (d, 1, NH exchangeable with D_2O), 5.05 (q, 1, CHN), 7.14−7.42 (m, 10, ArH). Enantiomeric purity, determined by HPLC, was >98%.

(*S*)-(−)-2-(1-Methyl-2-phenylethyl)-4,5-dihydro-1*H*-imidazole Oxalate [(*S*)-(−)-7]. Compound (*S*)-(−)-**7** was prepared following the same procedure described for **7** starting from (*S*)-(+)-2-methyl-3-phenylpropionic acid methyl ester.[40] The free base (*S*)-(−)-**7** was purified through flash chromatography using cyclohexane/AcOEt/MeOH/33% NH_4OH (4:3:1:0.1) (1.55 g, yield 28%) as eluent. The enantiomeric purity, determined by ^{1}H NMR of the corresponding diastereomeric ureidic derivative obtained by reacting (*S*)-(−)-**7** with (*S*)-(−)-α-methylbenzyl isocyanate, was 80%. To increase this enantiomeric purity, the free base was then further purified by fractional crystallization of its (+)-*O*,*O*′-di-*p*-toluoyl-D-tartaric acid salt following the procedure described for (*R*)-(+)-**7**. The optically pure free base {0.4 g; 26% yield; $[\alpha]^{20}_D$ −42.1 (*c* 1, $CHCl_3$)} was transformed into the oxalate salt: mp 150−152 °C; $[\alpha]^{20}_D$ −51.3 (*c* 1, MeOH); ^{1}H NMR (DMSO) δ 1.19

(d, 3, CH_3), 2.73−3.15 (m, 3, CH_2CH), 3.77 (s, 4, NCH_2CH_2N), 7.19−7.40 (m, 5, ArH), 9.36 (br s, 1, NH exchangeable with D_2O). Anal. ($C_{12}H_{16}N_2 \cdot H_2C_2O_4$) C, H, N. The enantiomeric purity of the free base was determined by HPLC and ^{1}H NMR spectrometry of its corresponding diastereomeric ureidic derivative **12b**, which was prepared following the procedure described for **12a**.

12b: ^{1}H NMR ($CDCl_3$) δ 1.12 (d, 3, C*H*$_3$CHC═N), 1.50 (d, 3, C*H*$_3$CHN), 2.55 (dd, 1, $ArCH_2$), 3.15 (dd, 1, $ArCH_2$), 3.58−3.90 (m, 4, NCH_2CH_2N), 3.82 (m, 1, CHC═N), 4.75 (d, 1, NH exchangeable with D_2O), 5.05 (q, 1, CHN), 7.10−7.40 (m, 10, ArH). Enantiomeric purity, determined by HPLC, was >98%.

(*R*)-(−)-2-(2-Phenylpropyl)-4,5-dihydro-1*H*-imidazole Maleate [(*R*)-(−)-8]. Compound (*R*)-(−)-**8** was prepared following the same procedure described for **7** starting from (*R*)-(−)-3-phenylbutyric acid methyl ester.[41] The free base (*R*)-(−)-**8** was purified through flash chromatography using cyclohexane/AcOEt/MeOH/33% NH_4OH (4:4:1:0.1) as eluent (0.85 g, yield 77%). The free base was transformed into the maleate salt: mp 110−112 °C; $[\alpha]^{20}_D$ −46.38 (*c* 1, MeOH); ^{1}H NMR (DMSO) δ 1.28 (d, 3, CH_3), 2.75 (d, 2, *C*H_2CH), 3.22 (m, 1, CH_2*C*H), 3.78 (s, 4, NCH_2CH_2N), 6.02 (s, 2, CH═CH), 7.21−7.42 (m, 5, ArH), 9.95 (br s, 1, NH exchangeable with D_2O). Anal. ($C_{12}H_{16}N_2 \cdot H_4C_4O_4 \cdot 0.25H_2O$) C, H, N. The enantiomeric purity, determined by ^{1}H NMR of the corresponding diastereomeric ureidic derivative **13a** that was prepared following the procedure described for **12a** by reacting the free base of (*R*)-(−)-**8** with (*S*)-(−)-α-methylbenzyl isocyanate, was >98%.

13a: ^{1}H NMR ($CDCl_3$) δ 1.31 (d, 3, C*H*$_3$CHCH$_2$), 1.53 (d, 3, C*H*$_3$CHN), 3.10 (m, 2, CH_2C═N), 3.35 (m, 1, C*H*CH$_2$), 3.53−3.81 (m, 4, NCH_2CH_2N), 4.85 (d, 1, NH, exchangeable with D_2O), 5.04 (q, 1, CHN), 7.10−7.42 (m, 10, ArH).

(*S*)-(+)-2-(2-Phenylpropyl)-4,5-dihydro-1*H*-imidazole Maleate [(*S*)-(+)-8]. Compound (*S*)-(+)-**8** was prepared following the same procedure described for **7** starting from (*S*)-(+)-3-phenylbutyric acid methyl ester.[41] The free base (*S*)-(+)-**8** was purified through flash chromatography using cyclohexane/AcOEt/MeOH/33% NH_4OH (4:4:1:0.1) as eluent (0.56 g, yield 75%). The free base was transformed into the maleate salt: mp 110−112 °C; $[\alpha]^{20}_D$ +45.53 (*c* 1, MeOH); ^{1}H NMR (DMSO) δ 1.28 (d, 3, CH_3), 2.75 (d, 2, *C*H_2CH), 3.22 (m, 1, CH_2*C*H), 3.78 (s, 4, NCH_2CH_2N), 6.02 (s, 2, CH═CH), 7.20−7.40 (m, 5, ArH), 9.86 (br s, 1, NH exchangeable with D_2O). Anal. ($C_{12}H_{16}N_2 \cdot H_4C_4O_4$) C, H, N. The enantiomeric purity, determined by ^{1}H NMR of the corresponding diastereomeric ureidic derivative **13b**, which was prepared following the procedure described for **12a** by reacting the free base of (*S*)-(+)-**8** with (*S*)-(−)-α-methylbenzyl isocyanate, was >98%.

13b: ^{1}H NMR ($CDCl_3$) δ 1.30 (d, 3, C*H*$_3$CHCH$_2$), 1.52 (d, 3, C*H*$_3$CHN), 3.08 (m, 2, CH_2CN), 3.32 (m, 1, C*H*CH$_2$), 3.52−3.85 (m, 4, NCH_2CH_2N), 4.75 (d, 1, NH, exchangeable with D_2O), 5.03 (q, 1, CHN), 7.10−7.40 (m, 10, ArH).

Pharmacology. Radioligand Binding Assays. α_2-Adrenoreceptors Binding Assays. Affinity for α_2-ARs in the rat brain was assessed by measuring the ability of the test compounds to displace [^{3}H]-clonidine from these receptors. Although [^{3}H]-clonidine may bind to the I_1 site, there is no indication of any I_1 binding in rat cortex. It was previously shown that when bound to rat cortical membranes, [^{3}H]-clonidine is completely displaced by noradrenaline in a monophasic manner.[55] In this assay, the cerebral cortex of rat brain was homogenized in 20 volumes of Tris buffer (50 mM, pH 7.4) and 5 mM EDTA with a 30 s burst from a PT10 Polytron homogenizer set at 6. The homogenate was centrifuged at 500*g* for 10 min. The supernatant obtained was then centrifuged at 65000*g* for 25 min, and the resulting pellet was washed twice with Tris-HCl (50 mM) without EDTA. The final pellet was resuspended in the same buffer and stored at −80 °C until required. Competition binding assays were performed by incubating washed rat cerebral membranes (200 μg of protein) with 5 nM [^{3}H]-clonidine (NEN, 60−63 Ci/mmol) in the absence or presence of a range of 10−12 concentrations of the competing ligand in a total volume of 400 μL of Tris assay buffer (50 mM Tris-HCl, pH 7.4). Nonspecific binding was defined as the concentration of bound ligand in the presence of 10 μM phentolamine. Specific binding represented about 75% of the total binding at 5 nM [^{3}H]-clonidine. Following equilibrium (45 min at 25 °C), bound radioactivity was separated from free by filtration through a GF/B filter with a Brandel cell harvester. Bound radioactivity on the glass fiber filter was determined by liquid scintillation counting. Each point was performed in triplicate.

I_1 Imidazoline Binding Sites Binding Assays. PC 12 cells were obtained from Dr. G. Rebel (IRCAD, Strasbourg, France). They were cultured in 75 cm^2 flasks at 37 °C with 10% CO_2 in Dulbecco's modified Eagle's medium (DMEM, 1000 mg/mL glucose) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. When the cells reached confluence (3−4 days after plating), they were harvested by 1 min exposure to 0.25% trypsin at 37 °C. For binding assays, after removal of the medium, cells at confluence were frozen in the flasks at −20 °C until they were used for membrane preparation. Frozen PC 12 cells were scraped into cold Tris-HEPES buffer (5 mM Tris-HEPES, pH 7.7, 0.5 mM EDTA, 0.5 mM EGTA, and 0.5 mM $MgCl_2$) and homogenized with a Potter homogenizer. After centrifugation at 75000*g* for 20 min, the pellet was washed in cold Tris-HEPES buffer at 2−4 mg of protein/mL and used immediately for binding assays. Binding assays on PC 12 cell membranes were performed with [^{125}I]-paraiodoclonidine (PIC). Incubation was initiated by the addition of membranes (200 μg of protein/400 μL final volume) and carried out at 25 °C for 30 min. For saturation experiments, concentrations of [^{125}I]-PIC ranging from 0.05 to 5 nM were used, while for competition experiments, increasing concentrations of drugs (10^{-10}−10^{-4} M) were added with 0.5 nM [^{125}I]-PIC (corresponding to the K_D value of the radioligand). Nonspecific binding was defined with 10 μM BDF6143 according to Separovic et al.[13] To stop the incubation, samples were filtered very quickly through GF/B glass fiber filters and incubated for 3 h in 0.03% polyethylenimine with a Brandel harvester. Filters were washed twice with 3 mL of 50 mM cold Tris-HCl buffer, pH 7.7. Radioactivity retained on the dried filters was determined in a Minaxi γ-counter (Packard, Meriden, CT).

I_2 Imidazoline Binding Sites Binding Assays. Rabbit kidney was homogenized in 10 volumes of Tris-HCl buffer (50 mM, pH 7.4) and 250 mM sucrose and centrifuged at 500*g* for 10 min. The supernatant was centrifuged at 28000*g* for 30 min, and the resulting pellet was washed twice with the same buffer without sucrose. The final pellet was resuspended in Tris-HCl buffer (50 mM, pH 7.4) and stored at −80 °C until use. Rabbit kidney membranes (200 μg of protein) were incubated with 5 nM [^{3}H]-idazoxan (Amersham, 43 Ci/mmol) in the absence or presence of a range of 10−12 concentrations of competing ligand drug in a total volume of 400 μL of assay buffer. To mask adrenoreceptors, 10 μM (−)-norepinephrine (in the presence of 0.005% ascorbic acid) was added to all tubes. Nonspecific binding was determined with 10 μM of cirazoline. Specific binding represented about 90% of the total binding at 5 nM [^{3}H]-idazoxan. Following equilibrium (45 min at 25 °C), bound radioactivity was separated from free by filtration as described above. Each point was performed in triplicate.

Computer Analysis of Binding Data. IC_{50} values were determined by nonlinear regression analysis of binding data with the aid of the Graphpad program. K_i values were calculated by the equation of Cheng and Prusoff.[56] Each curve was repeated at least three times, and the results are given as the mean ± SEM.

Cardiovascular Experiments. Cardiovascular evaluations of compound **7** were performed according to published procedures.[42,43] Normotensive male rabbits (Zika strain) weighing 2.5−3.5 kg were anesthetized with sodium pentobarbitone (Sanofi, Libourne, France) (40 mg kg^{-1}) injected through the marginal vein of the ear. Rectal temperature was maintained at 38° ± 0.5° with the aid of a warming blanket as soon as anesthesia was established (Harvard apparatus LTD, Millis, MA). The animals were tracheotomized, immobilized with pancuronium bromide (Pavulon, Organon) (1 mg kg^{-1}, iv), and

artificially ventilated with room air (Hugo Sachs electronic model 6025, March-Hugstetten, Germany). The ventilation parameters were adjusted to maintain O_2 at approximately 100 mmHg and CO_2 below 40 mmHg. The right femoral vein was catheterized to allow iv injections, and the instantaneous arterial pressure was measured through a catheter placed in the abdominal aorta via the right femoral artery, connected to a pressure processor and recorder (Gould Electronics model BS-272, Longjumeau, France). Mean arterial pressure (MAP) was calculated as the diastolic pressure plus one-third of the differential pressure. Heart rate (HR) was also continuously monitored from the pressure signal with a Gould Biotach amplifier (model 13-4615-66). In experiments during which the drug was injected intracisternally, an amount of 0.2 mL of drug solutions was injected after removal of the same volume of cerebrospinal fluid. In control experiments, vehicle alone, injected in the same way and in the same volume, never modified MAP significantly.

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