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Imidazoline Receptors: Qualitative Structure–Activity Relationships and Discovery of Tracizoline and Benazoline. Two Ligands with High Affinity and Unprecedented Selectivity†

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Abstract—The observation that all the attempts to characterize imidazoline (*I*) receptors have been carried out with non-selective or poorly selective ligands prompted us to undertake research aimed at developing selective ligand(s). In previous work using, as a starting point, cirazoline **1**, a potent α_1-adrenergic receptor agonist that also binds to *I* receptors, we showed that removal of the cyclopropyl ring (**2**) retains high affinity for I_2 receptors while reducing α_1-adrenergic agonist activity. However, it was felt that this residual, albeit modest, α_1-adrenergic agonist activity might diminish the usefulness of compound **2**, and we now report on our continuing efforts in this field. Starting from compound **2**, we first eliminated the α_1-agonist component by isosteric replacement and then, by means of conformational restrictions on compound **7**, succeeded in discovering tracizoline (**9**) and benazoline (**12**). These two new ligands with high affinity (pK_i value 8.74 and 9.07, respectively) and unprecedented selectivity with respect to both α_2- (I_2/α_2 7,762 and 18,621) and α_1- (I_2/α_1 2,344 and 2,691) adrenergic receptors, are valuable tools in the study of *I* receptor structure and function. In addition, the large number of derivatives studied has allowed us to establish congruent qualitative structure–activity relationships and identify some structural elements governing affinity and selectivity. © 1997 Elsevier Science Ltd.

Introduction

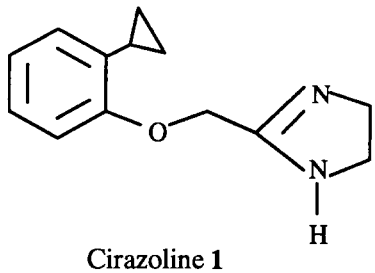
Some 10 years ago, Bousquet and co-workers,[1] studying the central cardiovascular effect of α-adrenergic drugs, demonstrated that clonidine-like substances may stimulate an imidazoline-preferring site in the nucleus reticularis lateralis region of the brainstem. This site differs from the classical α_2-adrenergic receptors in that it is not stimulated by catecholamines with high α_2-adrenergic affinity. Since then, the concept of imidazoline (*I*) receptors has been developed.

Recent studies have shown that *I* receptors are widely distributed in both the peripheral and central nervous system, and may participate in the regulation of various physiological effects.[2–5] Different rank orders of the affinity of ligands indicate the possible existence of at least two classes of *I* receptors, I_1 and I_2.[6] I_1 receptors preferentially bind [^{3}H]-pNH_2-clonidine and [^{3}H]-clonidine,[7–10] and appear to mediate the fall in arterial pressure elicited by imidazolines,[8] whereas I_2 ones preferentially bind [^{3}H]-idazoxan[11–15] and seem to be associated with the MAO enzyme.[16,17] Other *I* receptors that seem to differ from both I_1 and I_2 have also been identified.[18–20]

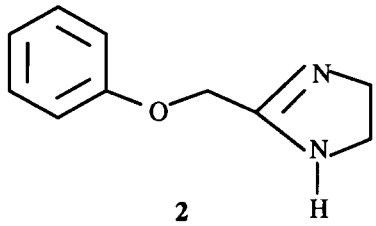
A definitive subclassification, with a statement of physiological function(s), has therefore yet to be decided on, and this could mainly be due to the fact that the ligands used for their characterization all suffer from lack of selectivity with respect to α-adrenergic receptors. Very recently, however, it has been reported that 2-(2-benzo-furanyl)-2-imidazoline (2-BFI) is a selective ligand for I_2 sites relative to α_2 sites.[21]

Despite the increasing number of reports published in this field, none of them had adopted a medicinal chemistry approach, until our group undertook a research project using as a starting point cirazoline **1**, a very potent α_1-adrenergic receptor agonist with moderate affinity for α_2-adrenergic receptors[22] and which also exhibits high affinity for *I* receptors in a variety of tissues.[10–14,23–26] We have already reported on a series of cirazoline derivatives,[27] showing that compound **2**, obtained by removal of the cyclopropyl ring,

†This work, in a preliminary form, was part of a lecture delivered at the 10th Camerino-Noordwijkerhout Symposium, held in Camerino (Italy), 10–14 September 1995.



Cirazoline 1



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retains high affinity for the *I* receptors while reducing affinity for the α_1-adrenergic receptors, thus proving to be the most selective one for *I* receptors versus α_1-adrenergic receptors. Nevertheless, compound **2** might be of limited value in view of the fact that it retains α_1 agonist activity, although lower than that of cirazoline (present study).[24]

In this paper we report on our subsequent research in this field, which has led to the discovery of two ligands, tracizoline and benazoline, with high affinity and unprecedented selectivity for I_2 receptors over α-adrenergic receptors.

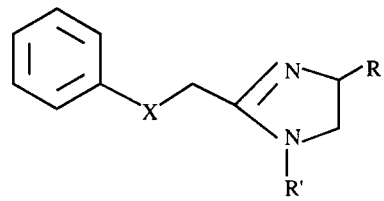
Results and Discussion

Our first aim was to eliminate the residual, albeit modest, α_1-adrenergic agonist activity of compound **2**, if possible without losing affinity for *I* receptors.

It has been reported, for another series of imidazoline derivatives, that substitution on the imidazoline ring decreases α_1-adrenergic activity.[28] This was confirmed in our studies, but as can be seen in Table 1, although methylation of the imidazoline ring of compound **2** does eliminate α_1 agonist activity, it also sharply reduces (**3**) or even eliminates (**4**) imidazoline receptor affinity. We then turned our attention to the bridge linking the aromatic and the imidazoline rings. Isosteric substitution of the oxygen atom with the methylene group (**7**) eliminates α_1-adrenergic activity (i.a. = 0), while retaining I_2 receptor affinity. Affinity for α_2-adrenergic receptors is also decreased and, as a consequence, there is a 10-fold increase in selectivity with respect to the α_2-adrenergic receptors.

Having been successful in separating the two activities and in discovering the first really selective ligand for *I* receptors, we then set out to further improve potency and selectivity. Compound **7** has a substantial degree of flexibility resulting from rotation about the bridge bonds, as shown in Figure 1. Accordingly, we designed novel and selective compounds with restricted conformational flexibility. The results are reported in Table 2. The first attempt was not successful. In fact, bridging the carbon atom in ortho position of the phenyl ring with the distal carbon atom on the bridge (route *a* in Fig. 1) to obtain the chroman derivative **8**, resulted in a sharp decrease in selectivity due to a decrease in I_2 receptor affinity and an increase in α_2-adrenergic

Table 1. pD_2 (or pKb) values[a] in the rat vas deferens (α_1) and pK_i values[b] in rabbit kidney (I_2) and rat cortex (α_2) membranes



Compound	X	R	R′	α_1 pD_2 (pKb)	i.a.[c]	pK_i I_2	pK_i α_2	I_2/α_2[d]
				α_1				
1		Cirazoline		7.33 ± 0.18	1.00	8.41 ± 0.08	7.77 ± 0.09	4.4
2	O	H	H	5.26 ± 0.11	0.95	9.05 ± 0.15	7.28 ± 0.16	59
3	O	CH_3	H	(4.86 ± 0.21)	0	5.96 ± 0.08	4.72 ± 0.08	17
4	O	H	CH_3	(4.34 ± 0.20)	0	in[e]	in[e]	—
5	S	H	H	4.69 ± 0.10	0.60	7.30 ± 0.18	6.70 ± 0.07	4
6	NH	H	H	4.89 ± 0.23	1.00	7.48 ± 0.03	7.14 ± 0.16	2.2
7	CH_2	H	H	(4.51 ± 0.13)	0	8.60 ± 0.04	5.70 ± 0.06	794

[a]Values are the mean ±SE of four independent observations.
[b]Values are the mean of three or four experiments performed in triplicate.
[c]Intrinsic activity.
[d]Antilog of the difference between pK_i I_2 and pK_i α_2 values.
[e]Inactive at 10 mM.

Table 2. pK_i values[a] in rabbit kidney (I_2) and rat cortex (α_2 and α_1) membranes

Compound		pK_i I_2	pK_i α_2	pK_i α_1	I_2/α_2[b]	I_2/α_1[b]
1	Cirazoline	8.41 ± 0.08	7.77 ± 0.09	6.30 ± 0.05	4.4	129
2		9.05 ± 0.15	7.28 ± 0.16		59	
7		8.60 ± 0.04	5.70 ± 0.06		794	
8		7.80 ± 0.09	6.87 ± 0.13		8.5	
9	Tracizoline	8.74 ± 0.08	4.85 ± 0.09	5.37 ± 0.03	7,762	2,344
10		8.43 ± 0.04	6.15 ± 0.21		191	
11		7.95 ± 0.06	5.56 ± 0.07		245	
12	Benazoline	9.07 ± 0.25	4.80 ± 0.13	5.64 ± 0.05	18,621	2,691
13	Tolazoline	6.70 ± 0.11	6.70 ± 0.11		1	
14	Naphazoline	7.00 ± 0.14	8.56 ± 0.01		0.03	
15		8.72 ± 0.07	7.17 ± 0.20		35	
16		6.52 ± 0.04	7.03 ± 0.14		0.3	
17		8.40 ± 0.03	5.96 ± 0.14		275	
18		5.44 ± 0.02	5.39 ± 0.03		1.1	
19		7.66 ± 0.06	5.58 ± 0.27		120	
20		7.15 ± 0.18	5.55 ± 0.21		40	
21		6.00 ± 0.02	5.26 ± 0.21		5.5	
22		5.54 ± 0.04	6.15 ± 0.01		0.3	
23		7.62 ± 0.05	5.21 ± 0.24		257	

[a]Values are the mean of three or four experiments performed in triplicate.
[b]Antilog of the difference between pK_i I_2 and pK_i (α_2 or α_1) values.

receptor affinity. Another way of restricting conformational freedom is to insert a double bound in the bridge (route *b* in Fig. 1). This restriction gave very good results; compound **9**, in its *trans* form, which we have named tracizoline, with a pK_i of 8.74, maintains a high affinity for I_2 receptors, while at α_2-sites a sevenfold reduction is observed. This results in an I_2/α_2 selectivity of 7762, which is unprecedented. The I_2/α_1 selectivity of 2344 is also remarkable.

Since the concept of conformational restriction worked so well, we decided to pursue this idea further, restricting the conformation of tracizoline by carrying out the bridging described above for the saturated compound **7** (route *a+b* in Fig. 1) to obtain the chromene and the dihydronaphthalene derivatives **10** and **11**. In both cases, there was a moderate reduction in affinity for I_2 receptors and an increase in affinity for α_2-adrenergic receptors, and therefore a significant decrease in selectivity. Interestingly, the dehydrogenation of compound **11** gave the best results; compound

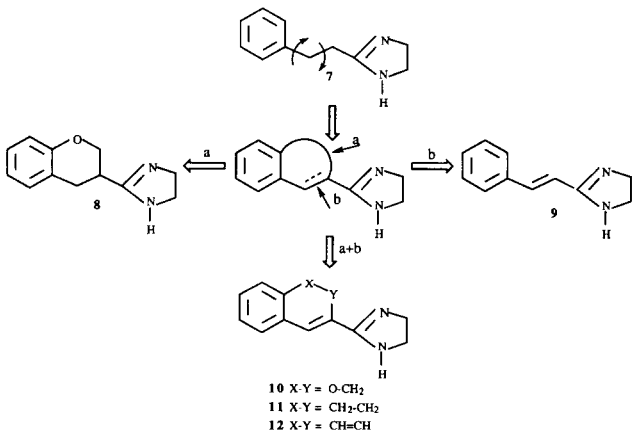


Figure 1. Design strategy to restrict conformation of compound **7**.

12, which we have named benazoline, displayed the highest affinity (pK_i = 9.07) and the highest selectivity with respect to both α_2- (I_2/α_2 = 18,621) and α_1-adrenergic receptors (I_2/α_1 = 2691). Thus, tracizoline and benazoline are two new I_2 receptor ligands with high affinity and high selectivity, higher than that reported for 2-BFI.[21]

In the course of our investigation into structure–activity restrictions, we were intrigued by the observation that compound **2** may be regarded as a close derivative of tolazoline (**13**), another imidazoline-based adrenergic drug in which the distance separating the phenyl and the imidazoline rings has been varied by the insertion of an oxygen atom. This variation increases affinity for I_2 receptors by more than two orders of magnitude, and also lead to a 60-fold increase in selectivity (Table 2). Accordingly, we set out to discover whether the same structural variation would work as well with naphazoline (**14**), another well-known, imidazoline-based adrenergic drug, and its β-isomer isonaphazoline (**16**). In doing so, we would be able to produce the two naphthoxy derivatives **15** and **17** (Fig. 2), which might be regarded as molecular yardsticks of the phenoxy derivative **2**, and are valuable probes useful in defining the spatial dimensions of the lipophilic region of the binding cleft.[29] The results reported in Table 2 show that the insertion of an oxygen atom in the bridges of naphazoline and isonaphazoline has a positive effect on I_2 receptor binding, while having a negative one on α_2-adrenergic receptor binding. As a consequence, there is a roughly 100-fold increase in selectivity for the former receptor. Comparison between the naphthoxy and phenoxy derivatives shows that a second phenyl group is admitted in the binding process with I_2 receptors, regardless of its position with respect to the ethereal bridge. Binding to the α_2-adrenergic receptors admits the second phenyl ring, but in this case its location with

respect to the bridge plays an important role, for the β-naphthoxy derivative **17** is 10 times less active than the α-naphthoxy derivative **15**. Within this series, compound **17** is the most selective for I_2 receptors, and it was chosen as the compound on which to carry out methylation of the oxymethylene bridge to obtain **18**. The binding data, reported in Table 2, show that this structural variation is detrimental to both affinity and selectivity and parallel the results obtained by methylating the corresponding carbon atom in the phenoxy derivatives series.[27] These results indicate that the two series of compounds bind in the same mode to the I_2 receptors.

To gain deeper insight into the topography of the binding site, compounds **19–23** were also studied (Table 2). Compound **19**, the regional isomer of benazoline **12**, displays lower affinity and selectivity than benazoline itself, which indicates that the relative positions of the aromatic and imidazoline rings play a crucial role in I_2 receptor binding. Compounds **20** and **21**, molecular yardsticks of benazoline **12** and of its α-isomer **19**, respectively, and compounds **22** and **23** also display low affinity and selectivity, which indicates that the region accommodating the aromatic moiety of the ligands is of limited size. Detailed 2-D and 3-D analyses in order to map I_2 receptors and to detect the molecular determinants responsible for high affinity and I_2/α_2 receptor selectivity are reported elsewhere in this issue.

Conclusions

In the last decade or so, since the hypothesis of Bousquet that the hypotensive action of clonidine and analogs could be related to their imidazoline structure rather than to their interaction with α_2-adrenergic receptors, the concept of *I* receptors has gained consensus. Investigations with radiolabeled ligands have shown the existence of imidazoline binding sites in several tissues and species, including humans. Furthermore, functional studies, although still limited in number, have demonstrated that these binding sites may represent functional receptors, and the discovery of agmatine[30] as a possible natural ligand seems to support this view. The observation that all the attempts to characterize this new receptor have been carried out with non- or poorly selective ligands, and that, despite the large number of reports in this field none had adopted a medicinal chemistry approach, prompted us to undertake research aimed at developing selective ligands.

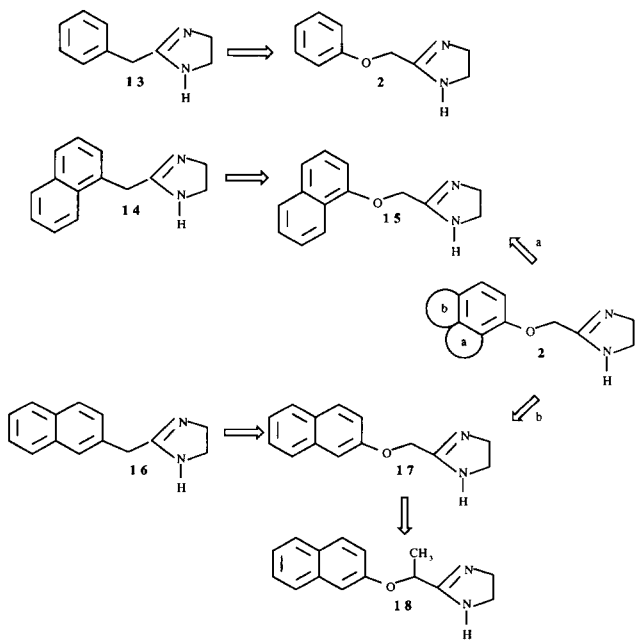


Figure 2. Comparison of tolazoline **13** and compound **2**, and strategy to design compounds **15**, **17**, and **18**.

While this manuscript was in preparation, Munk et al.[31] described a selective ligand for I_1 receptors over α-adrenergic receptors. This compound does not possess imidazoline structure and is devoided of both agonist and antagonist activity.

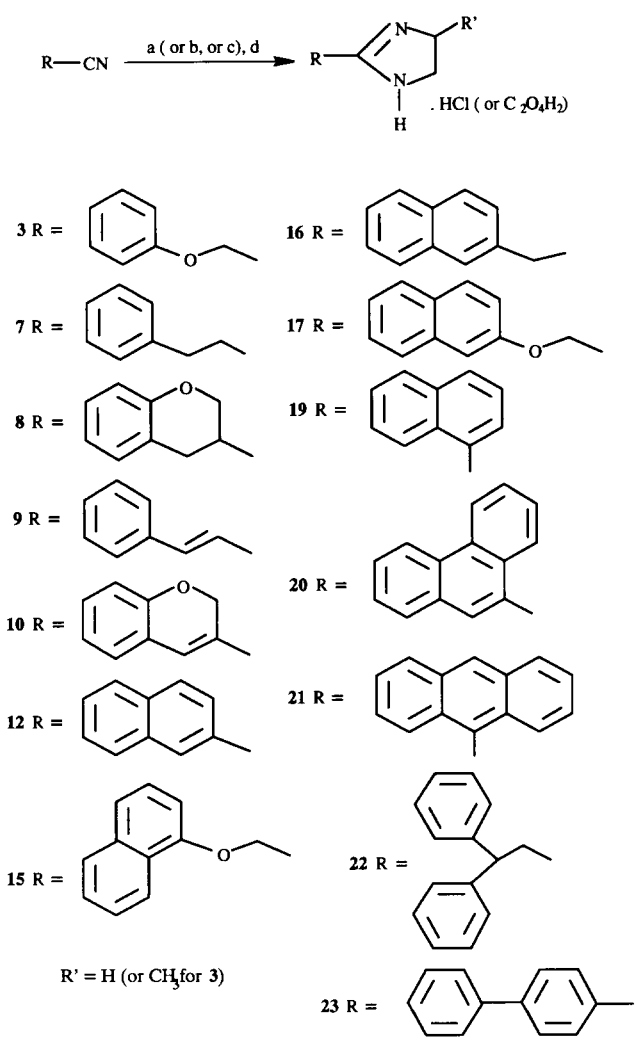
In this paper we have reported on several interesting imidazoline-based structures, two of which, tracizoline and benazoline, are outstanding for their affinities and unprecedented selectivities. These two compounds will contribute to a better understanding of *I* receptor characterization and function.

Experimental

Chemistry

The compounds were synthesized by standard procedures as outlined in Schemes 1–4. Melting points were taken in glass capillary tubes on a Buchi SMP-20 apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Varian EM-390 spectrometer, and peak position are given in parts per million (δ), downfield from tetramethylsilane as the internal standard, and spin multiplicities are given as s (singlet), d (doublet), t (triplet), or m (multiplet). The microanalyses were performed in the Microanalytical Laboratory of our department (Università di Camerino), and, where they are indicated using symbols, the results are within ±0.4% of the theoretical values. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash chromatography. Reactions and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F_{254} Merck plates.

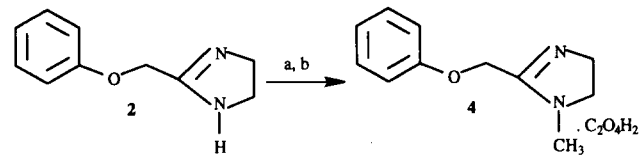
5-Methyl-2-phenoxymethyl-4,5-dihydro-1H-imidazole oxalate (3). HCl was bubbled through a stirred and cooled (0 °C) solution of phenoxyacetonitrile[32] (0.161 g, 1.21 mmol) and MeOH (0.1 mL, 2.42 mmol) in dry $CHCl_3$ (2 mL) for 45 min. After 12 h at 0 °C, dry ether was added to the reaction mixture to give the intermediate imidate, which was filtered. This solid (0.035 g, 0.21 mmol) was added to a cooled (0 °C) and stirred solution of 1,2-propanediamine (0.022 mL, 0.26



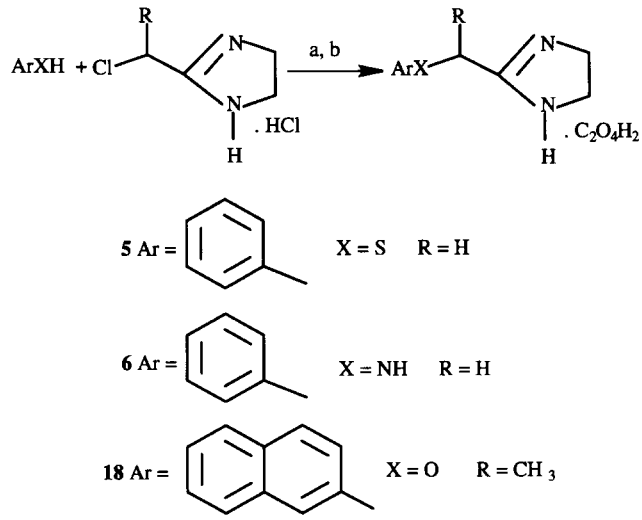
Scheme 1. Reagents and conditions: (a) HCl(g)/CH_3OH, $H_2NCH_2CH_2NH_2$ [or $H_2NCH(CH_3)CH_2NH_2$], 2 N NaOH; (b) CH_3ONa/CH_3OH, $H_2NCH_2CH_2NH_2$, HCl(g)/CH_3OH, 2 N NaOH; (c) $H_2NCH_2CH_2NH_2$.PTSA, 2 N NaOH; (d) 2 N HCl (or $C_2O_4H_2$).

mmol) in abs EtOH (1 mL). After 1 h, concentrated HCl (a few drops) in abs EtOH (0.5 mL) was added to the reaction mixture, which was stored overnight in the refrigerator. It was then diluted with abs EtOH (1 mL) and heated at 75 °C for 5 h. After cooling, the solid was collected and discarded and the filtrate was concentrated and filtered again. The filtrate was evaporated to dryness to give a residue which was taken up in $CHCl_3$ (10 mL), washed with 2 N NaOH and dried over Na_2SO_4. Removal of the solvent gave **3**, which was transformed into the oxalate salt and recrystallized from EtOH. Yield 85%, mp 176–177 °C, ^{1}H NMR (DMSO-d_6) δ 1.65 (d, 3H, CH_3), 2.76 (m, 1H, $CHCH_3$), 4.04 and 4.22 (2m, 2H, CH_2N), 4.56 (s, 2H, CH_2O), 6.50–6.75 (m, 5H, ArH). Anal. ($C_{13}H_{16}N_2O_5$) C, H, N.

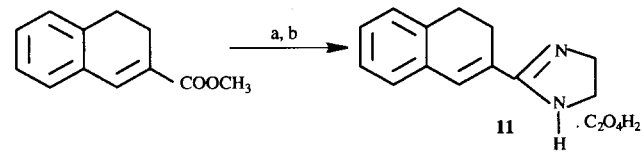
Similarly, compounds **7**,[33] **8**,[34] **10**,[34] **12**,[34] **15**,[35] and **17**[35] were obtained from the appropriate nitriles, either commercially available or prepared as previously described.[36–38]



Scheme 2. Reagents and conditions: (a) HCOOH/HCHO, NaOH (pellets); (b) $C_2O_4H_2$.



Scheme 3. Reagents and conditions: (a) Na/C_2H_5OH, 20% NaOH; (b) $C_2O_4H_2$.



Scheme 4. Reagents and conditions: (a) $H_2NCH_2CH_2NH_2$, 2 M $Al(CH_3)_3$, 2 N NaOH, 2 N HCl; (b) $C_2O_4H_2$.

2-Phenethyl-4,5-dihydro-1H-imidazole oxalate (7). ^{1}H NMR ($CDCl_3$) δ 2.60 (m, 2H, $CH_2C{=}N$), 2.98 (m, 2H, CH_2Ar), 3.58 (s, 4H, NCH_2CH_2N), 7.08–7.47 (m, 5H, ArH). The free base was transformed into the oxalate salt and recrystallized from EtOH. Yield 44%, mp 144–147 °C. Anal. ($C_{13}H_{16}N_2O_4$) C, H, N.

2-Chroman-3-yl-4,5-dihydro-1H-imidazole hydrochloride (8). ^{1}H NMR ($CDCl_3$) δ 2.58–3.16 (m, 3H, CH_2Ar and $CHC{=}$), 3.60 (s, 4H, NCH_2CH_2N), 3.85–4.55 (m, 2H, CH_2O), 6.70–7.25 (m, 4H, ArH). The free base was transformed into the hydrochloride salt and recrystallized from EtOH/Et_2O. Yield 75%, mp 204–205 °C. Anal. ($C_{12}H_{15}ClN_2O$) C, H, N.

2-(2H-Chromen-3-yl)-4,5-dihydro-1H-imidazole hydrochloride (10). The free base was transformed into the hydrochloride salt and recrystallized from EtOH/Et_2O. Yield 62%, mp 242–244 °C. ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 4H, NCH_2CH_2N), 5.10 (s, 2H, CH_2O), 6.90–7.90 (m, 5H, $CH{=}C$ and ArH). Anal. ($C_{12}H_{13}ClN_2O$) C, H, N.

2-Naphthalen-2-yl-4,5-dihydro-1H-imidazole hydrochloride (12). ^{1}H NMR ($CDCl_3$) δ 3.86 (s, 4H, NCH_2CH_2N), 7.48–8.25 (m, 7H, ArH). The free base was transformed into the hydrochloride salt and recrystallized from EtOH. Yield 60%, mp 280–281 °C. Anal. ($C_{13}H_{13}ClN_2$) C, H, N.

2-(Naphthalen-1-yloxymethyl)-4,5-dihydro-1H-imidazole oxalate (15). ^{1}H NMR ($CDCl_3$) δ 3.72 (s, 4H, NCH_2CH_2N), 4.97 (s, 2H, OCH_2), 6.84–8.27 (m, 7H, ArH). The free base was transformed into the oxalate salt and recrystallized from EtOH. Yield 25%, mp 217–218 °C. Anal. ($C_{16}H_{16}N_2O_5$) C, H, N.

2-(Naphthalen-2-yloxymethyl)-4,5-dihydro-1H-imidazole oxalate (17). ^{1}H NMR ($CDCl_3$) δ 3.68 (s, 4H, NCH_2CH_2N), 4.88 (s, 2H, OCH_2), 7.13–7.83 (m, 7H, ArH). The free base was transformed into the oxalate salt and recrystallized from EtOH. Yield 10%, mp 209–210 °C. Anal. ($C_{13}H_{16}N_2O_5$) C, H, N.

1-Methyl-2-phenoxymethyl-4,5-dihydro-1H-imidazole oxalate (4).[39] A solution of 2-(phenoxymethyl)-4,5-dihydro-1H-imidazole (**2**)[32] (0.75 g, 4.26 mmol), HCOOH (5 mL), and 40% HCHO (5 mL) was refluxed overnight. The solution was basified with NaOH (pellets) and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4. The evaporation of the solvent gave an oil (0.6 g, yield 74%), which was transformed into the oxalate salt and recrystallized from EtOH. Yield 85%, mp 119–121 °C, ^{1}H NMR (DMSO-d_6) δ 2.75 (s, 3H, NCH_3), 3.11 (t, 2H, NCH_2), 3.48 (t, 2H, $=NCH_2$), 4.52 (s, 2H, CH_2O), 6.88–7.38 (m, 5H, ArH). Anal. ($C_{12}H_{14}N_2O_5$) C, H, N.

2-Phenylsulfanylmethyl-4,5-dihydro-1H-imidazole oxalate (5).[39–41] Thiophenol (1.21 mL, 11.8 mmol) was added to a solution of Na (0.54 g, 23.6 mmol) in abs EtOH (15 mL). After 1 h, 2-(chloromethyl)-imidazoline hydrochloride[42] (0.92 g, 5.9 mmol) was added to the reaction mixture, which was heated to reflux for 8 h under vigorous stirring. The solution was evaporated to dryness to give a residue which was taken up in $CHCl_3$ (100 mL) and washed with water (20 mL), 20% NaOH (5 × 20 mL) and water (20 mL). Removal of the solvent gave the free base as an oil, which was transformed into the oxalate salt, and then recrystallized from EtOH. Yield 21%, mp 120–122 °C, ^{1}H NMR (DMSO-d_6) δ 3.78 s, 4H, NCH_2CH_2N), 4.14 (s, 2H, SCH_2), 7.19–7.58 (m, 5H, ArH). Anal. ($C_{12}H_{14}N_2O_4S$) C, H, N.

Similarly, compound **18**[43] was obtained by reacting suitable phenols and 2-(2-chloroethyl)-imidazoline hydrochloride.[42]

2-[1-(Naphthalen-2-yloxy)-ethyl]-4,5-dihydro-1H-imidazole oxalate (18). ^{1}H NMR ($CDCl_3$) δ 1.70 (d, 3H, CH_3), 3.66 (m, 4H, NCH_2CH_2N), 5.25 (q, 1H, OCH), 7.11–7.80 (m, 7H, ArH). The free base was transformed into the oxalate salt and recrystallized from EtOH. Yield 25%, mp 216–218 °C. Anal. ($C_{17}H_{18}N_2O_5$) C, H, N.

Compound **6** was prepared as previously described.[39,44]

(4,5-Dihydro-1H-imidazol-2-ylmethyl)-phenyl-amine oxalate (6). The free base was transformed into the oxalate salt and recrystallized from EtOH. Yield 52%, mp 165–168 °C. ^{1}H NMR (DMSO-d_6) δ 3.84 (s, 4H, NCH_2CH_2N), 4.22 (d, 2H, CH_2NH), 6.40 (t, 1H, ArNH), 6.58–7.28 (m, 5H, ArH). Anal. ($C_{12}H_{15}N_3O_4$) C, H, N.

2-Styryl-4,5-dihydro-1H-imidazole oxalate (9).[39,45] A mixture of *trans*-cinnamonitrile (2.1 g, 15.9 mmol), sodium methoxide (0.074 g, 1.37 mmol), and MeOH (11 mL) was stirred for 18 h. On cooling to 0–10 °C, a solution of ethylenediamine (1.1 g, 17.8 mmol) in MeOH (5 mL) was added dropwise with stirring. After a few minutes a solution of HCl in MeOH (2.11 mL of 7.856 N solution, 16.58 mmol) was added dropwise and the mixture was allowed to warm to room temperature. After 78 h the mixture was made slightly acid with methanolic HCl and filtered. The filtrate was evaporated and the residue was taken up in H_2O. The solution was basified with 2 N NaOH and extracted with Et_2O. Evaporation in vacuo gave a white solid that was washed with Et_2O (0.3 g, mp 158–163 °C). ^{1}H NMR ($CDCl_3$) δ 3.73 (m, 4H, NCH_2CH_2N), 6.72 (d, 1H, CHC=N), 7.08 (d, 1H, CHAr), 7.30–7.52 (m, 5H, ArH). The free base was then transformed into the oxalate salt, which was recrystallized from EtOH. Yield 11%, mp 183–185 °C. Anal. ($C_{13}H_{14}N_2O_4$) C, H, N.

The same procedure was adopted to prepare compound **16**.[46]

2-Naphthalen-2-ylmethyl-4,5-dihydro-1H-imidazole hydrochloride (16). The free base was transformed into the hydrochloride salt and recrystallized from EtOH. Yield 40%, mp 277–278 °C. ^{1}H NMR (DMSO-d_6) δ 3.84 (s, 4H, NCH_2CH_2N), 4.06 (s, 2H, CH_2C), 7.49–8.00 (m, 7H, ArH). Anal. ($C_{14}H_{15}ClN_2O$) C, H, N.

2-(3,4-Dihydro-naphthalen-2-yl)-4,5-dihydro-1H-imidazole oxalate (11).[47] A solution of ethylenediamine (0.71 mL, 10.6 mmol) in dry toluene (3.7 mL) was added dropwise to a mechanically stirred solution of 2 M trimethylaluminium (5.3 mL, 10.6 mmol) in dry toluene (8.8 mL) at 0 °C and in a nitrogen atmosphere. After being stirred at room temperature for 1 h, the solution was recooled to 0 °C and a solution of methyl 3,4-dihydronaphthalene-2-carboxilate[48] (1 g, 5.3 mmol) in dry toluene (6.3 mL) was added dropwise. The reaction mixture was refluxed for 45 min, cooled to 0 °C, and quenched cautiously with MeOH (2.5 mL) followed by water (0.5 mL). After addition of $CHCl_3$ (20 mL), the mixture was refluxed for 1 h at 100 °C to ensure the precipitation of the aluminium salts. The mixture was dried over Na_2SO_4, filtered, diluted with $CHCl_3$, and washed twice with water. The organic layer was extracted with 2 N HCl. The aqueous layer was basified with 2 N NaOH and extracted with Et_2O. The organic layer was dried over Na_2SO_4, filtered, and evaporated in vacuo to give the free base as a white

solid (0.65 g, yield 63%): ^{1}H NMR ($CDCl_3$) δ 2.73 (m, 2H, CH_2CH_2), 2.91 (m, 2H, CH_2CH_2), 3.72 (s, 4H, NCH_2CH_2N), 6.91 (s, 1H, CH=C), 7.09–7.25 (m, 4H, ArH). The free base was then transformed into the oxalate salt which was recrystallized from EtOH. Yield 64%, mp 167–169 °C. Anal. ($C_{15}H_{16}N_2O_4$) C, H, N.

2-Anthracen-9-yl-4,5-dihydro-1H-imidazole oxalate (21). A solution of 2-aminoethylammonium toluene-*p*-sulfonate[49] (12.26 g, 49 mmol) and 9-anthracenecarbonitrile (1 g, 4.9 mmol) was heated at 200 °C for 3 h. After cooling and addition of H_2O, the solution was basified with 2 N NaOH and extracted with $CHCl_3$. The organic layer was washed with H_2O, dried over Na_2SO_4, and evaporated to give a residue that was purified on silica gel column using as eluent Cyclohexane/AcOEt/MeOH/NH_4OH 33% (5.8/2.1/2.1/0.025). The free base was obtained as a solid (0.63 g, mp 220 °C dec): ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 4H, NCH_2CH_2N), 7.43–8.50 (m, 9H, ArH). The free base was then transformed into the oxalate salt, which was recrystallized from EtOH. Yield 52%, mp 249–250 °C. Anal. ($C_{19}H_{16}N_2O_4$) C, H, N.

The same procedure was adopted to prepare compounds **19**,[49] **20**,[50] **22**,[51] and **23**,[52] starting from the appropriate nitriles, either commercially available or prepared as previously described.[53]

2-Naphthalen-1-yl-4,5-dihydro-1H-imidazole oxalate (19). ^{1}H NMR ($CDCl_3$) δ 3.76 (s, 4H, NCH_2CH_2N), 7.38-8.68 (m, 7H, ArH). The free base was then transformed into the oxalate salt, which was recrystallized from EtOH. Yield 45%, mp 190–192 °C. Anal. ($C_{15}H_{14}N_2O_4$) C, H, N.

2-Phenanthren-9-yl-4,5-dihydro-1H-imidazole oxalate (20). ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 4H, NCH_2CH_2N), 7.58–8.78 (m, 9H, ArH). The free base was then transformed into the oxalate salt, which was recrystallized from EtOH. Yield 70%, mp 231–232 °C. Anal. ($C_{19}H_{16}N_2O_4$) C, H, N.

2-(2,2-Diphenyl-ethyl)-4,5-dihydro-1H-imidazole oxalate (22). ^{1}H NMR ($CDCl_3$) δ 3.00 (d, 2H, CH_2), 3.43 (s, 4H, NCH_2CH_2N), 4.38 (t, 1H, CH), 7.15–7.39 (m, 10H, ArH). The free base was then transformed into the oxalate salt, which was recrystallized from EtOH. Yield 51%, mp 179–182 °C. Anal. ($C_{19}H_{20}N_2O_4$) C, H, N.

2-Biphenyl-4-yl-4,5-dihydro-1H-imidazole oxalate (23). The free base was transformed into the oxalate salt, which was recrystallized from EtOH. Yield 69%, mp 235–236 °C. ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 4H, NCH_2CH_2N), 7.09–8.03 (m, 9H, ArH). Anal. ($C_{17}H_{16}N_2O_4$) C, H, N.

Pharmacology

Rat vas deferens. Male albino rats (175–200 g) were killed and both vasa deferentia were carefully removed, freed from adhering connective tissue and transversally bisected. The epididymal portion was mounted in 20 mL organ baths containing a physiological salt solution (PSS) of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.1. The initial loading tension was set at 1 g. The medium was maintained at 37 °C and aerated with 95% O_2–5% CO_2. The tissues were allowed to equilibrate for at least 1 h before the addition of any drug. Cirazoline concentration-response (contracture) curves were obtained cumulatively and taken as a control. The tissues were washed for 1 h and then challenged with the agonist under study. Responses were expressed as a percentage of the maximal response obtained in the control curve. It was verified in parallel experiments that the second cirazoline concentration–response curve was identical to the first, since the change in ratio of equi-effective concentrations was less than two, which usually entails a minimal correction. The results are expressed in terms of $-\log ED_{50}$ (pD_2), which is the concentration of agonist required to produce 50% of the maximum contraction. When the compound under study did not show any agonist activity, it was studied as an antagonist, and the second dose-response curve of cirazoline was obtained after incubation of the tissue for 30 min. In this case the results are expressed in terms of pK_b, calculated according to Van Rossum,[54] at only one concentration (30 mM).

α_1- and α_2-adrenergic binding affinity. Affinity for α_1- and α_2-adrenergic receptors in the rat brain was assessed by measuring the ability of the test compounds to displace [^{3}H]-prazosine or [^{3}H]-clonidine from these receptors. Although [^{3}H]-clonidine may bind to I_1 site, there is no indication of any I_1 binding in rat cortex. It was previously showed that [^{3}H]-clonidine bound to rat cortical membranes 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) 5 mm EDTA with a 30 s burst from PT10 Polytron homogeniser set at 6. The homogenate was centrifuged at 500 *g* for 10 min. The supernatant obtained was then centrifuged at 43,000 *g* for 25 min and the resulting pellet washed twice with Tris-HCl 50 mM without EDTA. The final pellet was resuspended in the same buffer and stored under liquid nitrogen until required. Competition binding assays were performed by incubating washed rat cerebral membranes (200 μg of protein) with 0.2 nM [^{3}H]-prazosine (NEN, 78 Ci/mmol) or with 2 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 mL of Tris assay buffer (50 mM Tris-HCl, pH 7.4). Non-specific binding was defined as the concentration of bound ligand in the presence of 10 μM phentolamine. Specific binding represented about 80–90% of total binding at 0.2 nM [^{3}H]-prazosine and 75% of the total binding at 2 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-fibre filter was determined by liquid scintillation counting. Each point was performed in triplicate.

Imidazoline (I_2) receptor binding affinity. Rabbit kidney were homogenized in 10 vol of Tris-HCl buffer (50 mM, pH 7.4), 250 mM sucrose and centrifuged at 500 *g* for 10 min. The supernatant was centrifuged at 28,000 *g* for 30 min and the resulting pellet washed twice with the same buffer without sucrose. The final pellet was resuspended in Tris-HCl buffer (50 mM, pH 7.4) and stored in liquid nitrogen 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 mL of assay buffer. To mask adrenoceptors, 10 μM (−)-norepinephrine was added to all tubes. Non-specific 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} were determined by non-linear 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.

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