Communications to the Editor

2-(2-Phenylcyclopropyl)imidazolines: Reversed Enantioselective Interaction at I_1 and I_2 Imidazoline Receptors

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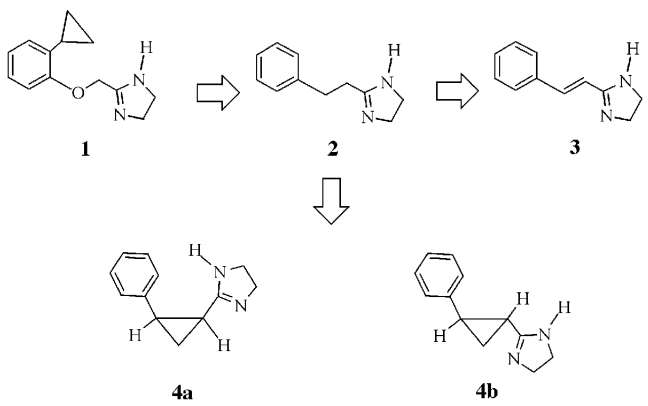
Introduction. During the past decade, the concept of imidazoline (I) receptors has been developed and gained consensus. Findings from different laboratories have shown that they are widely distributed in different tissues and species and may participate in the regulation of various physiological functions. Moreover, recent evidence suggests their involvement in diverse pathologies, and therefore a more definite knowledge of the structure and function of this receptor system could help in the search for therapeutic agents useful for treating efficaciously a variety of disorders such as hypertension, diabetes mellitus, gastric ulcer, endogenous depression, and stroke. Different rank order of the affinity of ligands indicates the existence of at least two major classes: I_1 and I_2; however, this seems an oversimplification since additional categories appear to exist (non-I_1, non-I_2).[1−5]

Despite the intense efforts in the field, a conclusive (sub)classification and some physiological roles of the I receptors have yet to be assessed, mainly because the ligands used for their characterization often suffered from lack of selectivity with respect to α-adrenoceptors. In this context, a few years ago we began a systematic study aimed at the discovery of selective I receptor ligands. Using as a starting lead Cirazoline (**1**) (Chart 1), a potent α_1-adrenergic agonist that also binds to I receptors, we showed that by removing the *o*-cyclopropyl substituent[6] and substituting the oxygen atom with an isosteric methylene unit,[7,8] high affinity for I_2 receptors was maintained, while the α_1-adrenergic agonist activity was abolished (compound **2**). Conformational restriction of **2**, carried out by inserting a double bond in the bridge, led to the discovery of Tracizoline (**3**), a ligand with high affinity and unprecedented selectivity for I_2 receptors.[7,8]

Chart 1



Another way of blocking the free rotation around the central C−C bond of compound **2** is the insertion of a rigid cyclopropyl ring, which incorporates the ethylenic unit between the phenyl and imidazoline moieties. This way of restricting the conformational freedom, which implies a minimal additional molecular bulk, generates the *cis* (**4a**) and *trans* (**4b**) isomers, each of which may be resolved in the two enantiomers, thus offering the opportunity of studying the interaction of this type of molecules with I receptors also from a stereospecific point of view. This idea was further supported by our COMFA model, recently developed for I_2 receptors using a large number of compounds,[9,10] which predicted the *trans* isomer to be more active than the *cis* isomer, with the two enantiomers of the former showing a significant eudismic ratio for I_2 receptors. Furthermore, considering that eudismic analysis is one of the means to discriminate between receptor subtypes, the pharmacological testing was performed on rabbit kidney and PC12 cell membranes for evaluating the affinities at I_2 and I_1 imidazoline receptors, respectively.

Chemistry. The synthesis of geometric isomers of compound **4**, whose stereochemistry was not defined in its *cis*/*trans* ratio, was reported in 1962 within a study of new MAO inhibitors.[11] In our work pure *cis* and *trans* isomers **4a** and **4b** were prepared.

Commercially available *trans*-2-phenylcyclopropanecarboxylic acid (**5b**) was converted into the methyl ester **6b** by a conventional method (CH_3OH and H_2SO_4) and then condensed with ethylenediamine in the presence of $Al(CH_3)_3$ to give the *trans*-imidazoline **4b**.[12] The *cis*-isomer **4a**[12] was similarly prepared starting from the corresponding acid **5a**[13] (Scheme 1).

The two enantiomers of the most active *trans* isomer, (1*R*,2*R*)-(−)-**4b**[14] and (1*S*,2*S*)-(+)-**4b,**[14] were obtained as depicted in Scheme 2, from the corresponding *trans* forms of 2-phenylcyclopropanecarboxylic acids (**5b**), resolved by dehydroabietylamine and quinine as previously described in the literature (Scheme 2).[15,16] The absolute configuration of the two enantiomeric acids (−)-**5a** and (+)-**5b** has been reported in ref 16.

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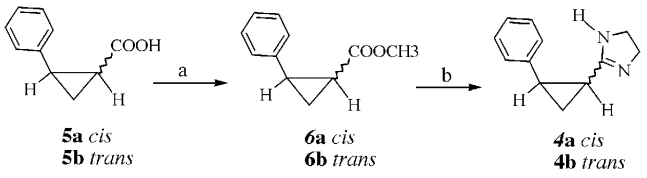
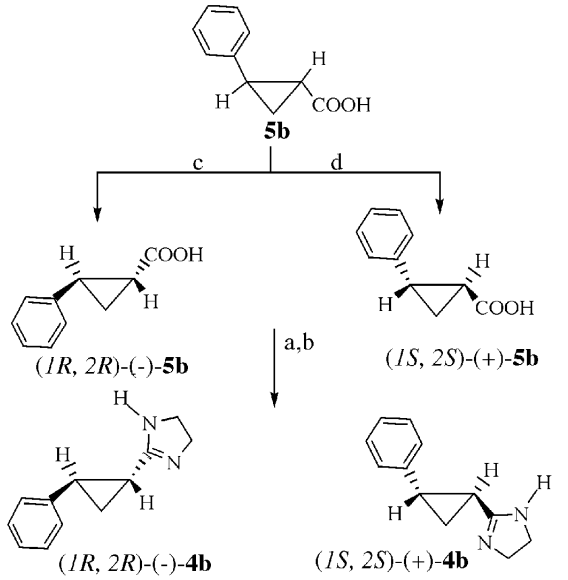
Scheme 1[a]



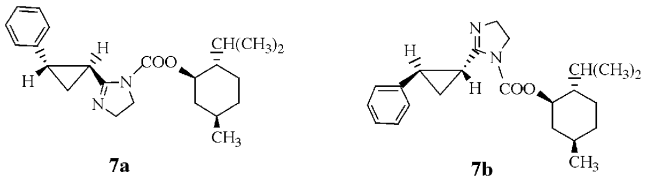
[a] (a) CH_3OH/H_2SO_4; (b) $CH_2NH_2)_2/Al(CH_3)_3$.

Scheme 2[a]



[a] (c) Dehydroabietilamine, NaOH, HCl; (d) quinine, NaOH, HCl; (a) CH_3OH/H_2SO_4; (b) $(CH_2NH_2)_2/Al(CH_3)_3$.

Enantiomeric purity of compounds (+)-**4b** and (−)-**4b** was indirectly measured by ^{1}H NMR spectroscopy of the corresponding diastereomeric carbamates **7a** and **7b**, obtained by reacting (+)-**4b** and (−)-**4b** with (−)-menthyl chloroformate, and found to be >98% (detection limit) for both enantiomers. The difference of chemical shifts[17] of the cyclopropyl protons at C-1 and C-2 was quite evident and of diagnostic value: compound **7a** showed two multiplets at δ 2.39 and 2.79 for protons at C-2 and C-1, respectively, whereas for compound **7b** the corresponding signals were at δ 2.49 and 2.94.



Pharmacology. The new compounds, in the form of hydrogen oxalate salts, were evaluated for affinity at α_2-adrenergic receptors and I_2 imidazoline receptors using membranes of rat cortex and rabbit kidney, respectively, following already described procedures.[8] The I_1-imidazoline receptor affinity was determined on rat pheochromocytoma cells (PC12) according to the methods of Separovic et al.[18] with slight modifications.[19]

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%). IC_{50} 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[20] and reported as pK_i ± SEM.

Results and Discussion. From the binding data reported in Table 1, it can be seen that Tracizoline, first described as an I_2/α_2-selective ligand, was found to be I_2-selective, although to a lesser extent, also when compared to the I_1 subtype. In addition, it can be observed that the compounds studied bind to the two I subtypes in a different manner. In fact, while at the I_2 subtype a single binding site was revealed, at the I_1 subtype high- and low-affinity binding sites were detected (Table 1 and Figure 1). The resolution of I_1 binding in two components has been previously observed and related to the presence of two interconvertible forms of the same receptor.[21,22]

Furthermore, the pharmacological results clearly show that conformational restriction of compound **2**, resulting from the insertion of a cyclopropyl ring, was detrimental, with respect to Tracizoline (**3**), for both I_2 receptor affinity and I_2/α_2 selectivity. However, the decreased I_2 receptor affinity of the *cis* isomer **4a** was consistently larger than that of the *trans* isomer **4b** (about 468- vs 12-fold) resulting in a *trans*/*cis* affinity ratio close to 40. This difference indicates that the distance between the phenyl and imidazoline rings plays a crucial role for high affinity. Although compound **4b** retained a relatively good affinity for I_2 receptors (pK_i = 7.64), overall our findings parallel those obtained with another class of imidazolines, namely, 2-(phenoxymethyl)imidazolines, in which the introduction of a methyl group on the carbon atom of the oxymethylene bridge caused an even larger decrease of affinity for I_2 receptors.[6−8] However, it is worth noting that the lower affinity of cyclopropyl derivatives of formula **4**, compared to **3**, might be due not only to the additional steric hindrance arising from the cyclopropyl ring but also to the mutual spatial arrangement of the two terminal rings. Indeed, the structure of **3** is nearly planar, whereas in compounds **4** the two rings lie in two distinct planes. Conformational analyses have shown that in the minimum-energy conformer the angle between the planes containing the phenyl and imidazoline rings is equal to 12.5° for compound **3** and equal to 107° and 105°, respectively, for (1*S*,2*S*)-(+)-**4b** and (1*R*,2*R*)-(−)-**4b**.[23]

It is interesting to note that the rank order of binding affinities of the enantiomers of the *trans* ligand **4b** can be correctly predicted by our recently refined three-field (steric, electrostatic, and lipophilic) CoMFA model.[10] Taking into account that this model was derived from relatively flexible or planar ligands, the predicted pK_i for very rigid, nonplanar compounds, such as the title imidazolines, may be considered quite satisfactory. The different binding modes of two low-energy conformers of the *trans*-enantiomers **4b** can be observed in the CoMFA coefficient contour maps drawn in Figure 2. With its phenyl group, the most active ligand (1*R*,2*R*)-(−)-**4a** is able to establish favorable contacts with electrostatic (magenta), lipophilic (yellow), and steric (green) regions, unlike its (1*S*,2*S*)-(+)-**4b** enantiomer whose phenyl ring may reach sterically hindered regions.

Table 1. I_1-, I_2-, and α_2-Receptor Binding Affinities of Compounds **3** and **4**

compound	pK_i I_1[a]	Eu[b]	pK_i I_2 pred[c]	pK_i I_2[a]	Eu[b]	pK_i α_2[a]	Eu[b]	I_2/I_1[d]	I_2/α_2[d]
Tracizoline (**3**)	7.72 ± 0.14 (35%) 5.42 ± 0.04 (65%)			8.72 ± 0.13		4.85 ± 0.15		10	7413
(±)-**4a**	5.24 ± 0.07			6.05 ± 0.11		4.53 ± 0.04		7	33
(1*S*,2*R*)-**4a**			6.48						
(1*R*,2*S*)-**4a**			6.14						
(±)-**4b**	7.76 ± 0.10 67%) 5.26 ± 0.37 (33%)			7.64 ± 0.05		6.40 ± 0.03		0.8	17
(1*R*,2*R*)-(−)-**4b**	6.46 ± 0.05	29	7.64	8.22 ± 0.02	20	6.92 ± 0.12	2	58	20
(1*S*,2*S*)-(+)-**4b**	7.93 ± 0.14 (66%) 4.51 ± 0.10 (34%)		6.60	6.91 ± 0.10		6.62 ± 0.07		0.1	2

[a] The affinity values (pK_i) for I_1 and I_2 imidazoline and α_2-adrenergic receptors were assessed by measuring the ability of the test compounds to displace [^{125}I]*p*-iodoclonidine (PC12 membranes), [^{3}H]idazoxan (rabbit kidney membranes), and [^{3}H]clonidine (rat cortex membranes), respectively. [b] Eudismic ratio is the antilog of the difference between the pK_i values for the eutomer and the distomer. [c] Predicted values by a three-field CoMFA model.[10] [d] Antilog of the difference between pK_i I_2 and pK_i I_1 (or pK_i α_2) values.

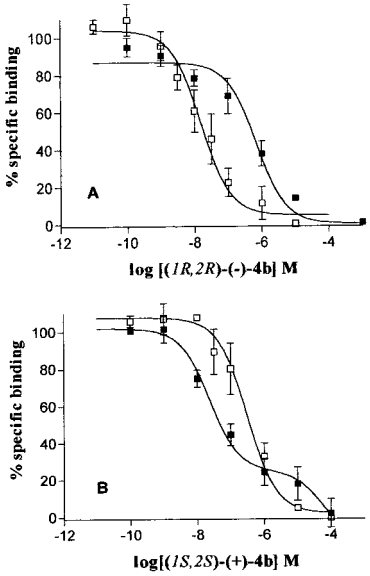


Figure 1. Competition binding curves of (*1R,2R*)-(−)-**4b** (A) and (1*S*,2*S*)-(+)-**4b** (B) on I_1 (filled squares) and I_2 (open squares) binding sites on PC12 cell membranes or rabbit kidney membranes, respectively.

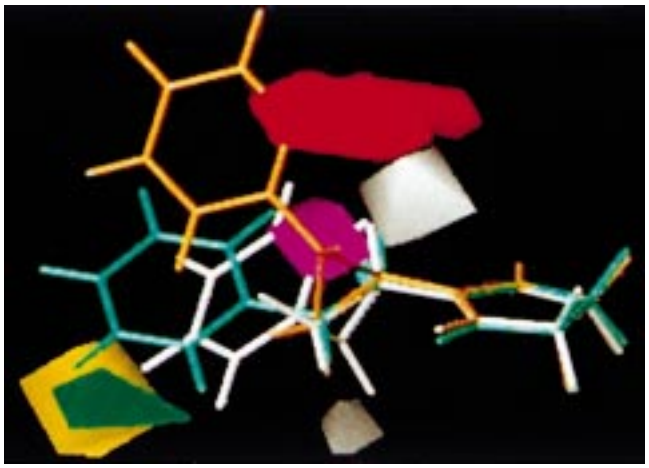


Figure 2. Isocontour maps from a previously published steric, electrostatic, and lipophilic CoMFA model.[10] Color code is as follows: green and red, favorable and unfavorable steric zones, respectively; magenta and white, favorable and unfavorable electrostatic zones for electron-rich groups, respectively; yellow and cyan, favorable and unfavorable zones for lipophilic moieties, respectively. The two *trans*-enantiomers (−)-**4b** (white) and (+)-**4b** (orange) show a different binding mode. Tracizoline (**3**) (green) has been added to the color maps for comparison.

At I_1 sites, the same diastereoselectivity seen for I_2 sites is also observed, with an even larger (330-fold) *trans*/*cis* ratio. However, in this case the *trans* isomer **4b** displays the same affinity (pK_i = 7.76) as Tracizoline (pK_i = 7.72), indicating that the cyclopropyl ring provides a good replacement for the double bond of **3.** Therefore, the *trans* geometry appears to be an essential requirement for optimum affinity, and steric hindrance, and deviation from coplanarity of the two ring systems seems not to have any effects on it, unlike what seems to be the case at the I_2 subtype.

The most important results have been obtained by resolving the racemate of the *trans* isomer **4b**, the most active of the two diastereoisomers, to give the enantiomers (1*R*,2*R*)-(−)-**4b** and (1*S*,2*S*)-(+)-**4b** which were tested in order to study further the role of the stereochemistry on binding to both I_1 and I_2 receptors. At I_1 sites, the dextrorotatory enantiomer (1*S*,2*S*)-(+)-**4b,** with a pK_i of 7.93, was more potent than the corresponding optical antipode (1*R*,2*R*)-(−)-**4b** (pK_i = 6.46), and the resulting eudismic ratio (Eu) of 29 was indicative of a reasonable enantiospecific interaction. At I_2 sites, enantioselective binding was also observed, the Eu being 20, but in this case the eutomer was the levorotatory enantiomer (1*R*,2*R*)-(−)-**4b**, with a pK_i of 8.22. There was clearly a reversal of enantioselectivity which is very peculiar and unprecedented. Owing to this differential interaction at the two I subtypes, the enantiomer (1*S*,2*S*)-(+)-**4b** proved to be 10-fold selective for I_1 receptors while the enantiomer (1*R*,2*R*)-(−)-**4b** was about 60-fold selective for I_2 receptors. Therefore, depending on the stereochemistry, replacement of the double bond in the Tracizoline structure, which is 10-fold selective for the I_2 subtype, by a cyclopropyl ring provides ligands selective for one or the other subtype, with a 6-fold increase of I_2 selectivity and a 100-fold increase (reversed) of I_1 selectivity.

In conclusion, we have shown that the imidazoline I_1 and I_2 receptor binding sites have stereospecific requirements, the two subtypes showing a reversal of enatioselectivity, which may offer leads to the development of selective imidazoline receptor ligands. The couple of imidazoline enantiomers (1*R*,2*R*)-(−)-**4b** and (1*S*,2*S*)-(+)-**4b** reported herein, together with other properly designed chiral ligands, may be considered as valid tools for a more extensive eudismic analysis aimed at a

necessary subtyping of the I receptors. Work along this line is in progress.

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(12) Compound **4a**: ^{1}H NMR ($CDCl_3$) δ 1.29 (1H, m, 3-CH_2); 1.60 (1H, m, 3-CH_2); 1.70 (1H, m, CHC═N); 2.45 (1H, m, CH−Ar); 3.60 (4H, s, NCH_2CH_2N); 3.95 (1H, br s, NH, exchangeable with D_2O); 7.0−7.32 (5H, m, Ar−H). It was characterized as the hydrogen oxalate salts: fusion started at 90 °C and was complete at 119−120 °C (from 2-PrOH). Anal. ($C_{12}H_{14}N_2 \cdot H_2C_2O_4$) C, H, N. Compound **4b**: mp 133−135 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (1H, m, 3-CH_2); 1.61 (1H, m, 3-CH_2); 2.13 (1H, m, CHC═N); 2.50 (1H, m, CH−Ar); 3.29 (4H, m, NCH_2CH_2N); 3.73 (1H, br s, NH, exchangeable with D_2O); 7.11−7.33 (5H, m, Ar−H). It was characterized as the hydrogen oxalate salt: mp 185−186 °C (from EtOH). Anal. ($C_{12}H_{14}N_2 \cdot H_2C_2O_4$) C, H, N.

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(14) Compound (1*R*,2*R*)-(−)-**4b:** mp 114−116 °C; $[\alpha]^{22}_D = -320.72$ (c = 1, $CHCl_3$). It was characterized as the hydrogen oxalate salt: mp 178−179 °C (from 2-PrOH/EtOH). Anal. ($C_{12}H_{14}N_2 \cdot H_2C_2O_4$) C, H, N. Compound (1*S*,2*S*)-(+)-**4b**: mp 114−116 °C; $[\alpha]^{22}_D = +318.80$ (c = 1, $CHCl_3$). It was characterized as hydrogen oxalate salt: mp 177−179 °C (from 2-PrOH/EtOH). Anal. ($C_{12}H_{14}N_2 \cdot H_2C_2O_4$) C, H, N.

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(17) Compound **7a**: ^{1}H NMR ($CDCl_3$) δ 0.52−2.01 (20H, m, 3-CH_2 and menthyl H); 2.39 (1H, m, CH−Ar); 2.79 (1H, m, CHC═N); 3.77 (4H, m, NCH_2CH_2N); 4.57 (1H, m, COOCH); 7.08−7.29 (5H, m, Ar−H). Compound **7b**: ^{1}H NMR ($CDCl_3$) δ 0.72−2.10 (20H, m, 3-CH_2 and H-menthyl); 2.49 (1H, m, CH−Ar); 2.94 (1H, m, CHC═N); 3.78 (4H, m, NCH_2CH_2N); 4.65 (1H, m, COOCH); 7.09−7.31 (5H, m, Ar−H).

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(19) Binding experiments on I_1 receptors were performed as described in ref 18, although crude membrane preparations were used. Briefly, PC12 cells were homogenized with a Potter homogenizer in ice-cold Tris-Hepes buffer (5 mM, pH 7.7) containing 0.5 mM EDTA, 0.5 mM EGTA, and 0.5 mM $MgCl_2$ and centrifuged twice during 20 min at 75000*g*. P2 pellets were resuspended in the same buffer at 2−4 mg of protein/mL and frozen at −80 °C until used in binding experiments. Competition experiments were carried out with 0.5 nM [^{125}I]*p*-iodoclonidine (NEN), 50 μg of protein, and incresing concentrations of drugs (10^{-10}−10^{-4} M). Incubations were performed for 30 min at 25 °C. Nonspecific binding was determined with 10 μM BDF 6143.

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(23) In the conformations selected for CoMFA studies, however, the angle values were 29.5°, 114°, and 132° for **3,** (1*S*,2*S*)-(+)-**4b**, and (1*R*,2*R*)-(−)-**4b,** which imply energy differences of 0.50, 1.09, and 0.60 kcal/mol, respectively.

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