In 1984 Bousquet and co-workers discovered non-adrenoceptor binding sites and proposed the existence of imidazoline receptors (IR) which specifically recognize compounds containing imidazoline moiety [1]. Since then, the concept of imidazoline receptors gained consensus, and they have been classified into three groups; I₁, which are thought to play a role in controlling systemic blood pressure [2]; I₂ considered to be allosteric sites on monoamino oxidase and involved in eating behavior, psychiatric disorders and opiate withdrawal [3]; I₃ sites, which are involved in the control of KᵢATP-channels located within the pancreas, which are potential target for the treatment of diabetes [4].

The heterogeneity of the imidazoline receptors and their implication in various physiological and pathological processes necessitate the development of new chiral agents as suitable tools for characterizing the targets connected to each biological effect. Stereospecific requirements for α₂ and IR was discussed by Hieble and Ruffolo [5] and in previous studies it has been shown that affinity for I₁ with respect to α₂ receptors can be modulated by changing chirality of ligands [6–8]. Recently, structure-activity relationships on adrenoceptors and imidazoline receptors have led to discovery of I₁ selective imidazoline compound trans-1-(4’,5’-dihydro-1’H-imidazol-2’-yl)methyl-2-hydroxyindane (PMS 952) [9] (Figure 1).

The above results encouraged us to prepare chiral analogues of PMS 952 containing imino-bridge in place of methylene group. Compound 3a exhibited moderate almost equal activity at both imidazoline I₁ receptors and α₂-adrenoceptors, while enantiomer 3b was found to be selective for α₂-adrenoceptors (α₂/I₁ selectivity ratio = 10.4).

**KEYWORDS:** 1-[(4,5-Dihydroimidazolidin-2-yl)imino]indan-2-ols; synthesis; structure; I₂, imidazoline and α₂-adrenergic receptors binding affinities
N for all new compounds were within ±0.4% of the theoretical values. The starting 2-chloro-4,5-dihydroimidazole (1) was obtained according to the method described by Trani and Bellasio [14]. (1R, 2S)-(+)-cis- and (1S, 2R)-(-)-cis-1-amino-2-indanols (2a-b) were purchased from Aldrich Chemical Co.

(1R, 2S)- (+)-CIS- AND (1S, 2R)- (-)-CIS-1-[(4,5-DIHYDROIMIDAZOLIDIN-2-YL)IMINO]INDAN-2-OL HYDROCHLORIDES (3a-b) General method
To a solution of 2-chloro-4,5-dihydroimidazole (1) (12.3 mol) in chloroform (30 mL) the appropriate 1-amino-2-indanol 2 (1.22 g, 8.2 mmol) was added, and the reaction mixture was stirred under reflux for 2 h. Then, the solvent was evaporated under reduced pressure and the oily residue was treated with anhydrous acetone (20 mL). The precipitate thus obtained was filtered off, washed with acetone and recrystallized from suitable solvent. Physical and analytical data for compounds 3a-b are presented in Table 1.

(1R, 2S)- (+)-CIS-1-[(4,5-DIHYDROIMIDAZOLIDIN-2-YL)AMINO]INDAN-2-OL HYDROCHLORIDES (3a-b)

A suspension of hydrochloride 3a (0.55 g, 2.2 mmol) in anhydrous methanol (10 mL) was treated with 5% methanolic NaOH solution (2.2 mL) for 0.5 h. Then, the solvent was evaporated under reduced pressure and the viscous residue was extracted with methylene chloride (40 mL). The organic layer was dried over MgSO4 and evaporated under vacuum. The crude product thus obtained was purified by crystallization from ethanol to give free base 4a. Physical and analytical data for compound 4a are presented in Table 1.

### Competition binding
Crude P2 brain membranes were prepared as follows. All procedures were carried out at 4°C unless otherwise stated, rat brains (male, Wistars,
Preparation, structure and affinity for imidazoline...

250-300 g) were taken and homogenized in 10 volumes of ice cold buffer (50 mM Tris-HCl, 1 mM MgCl₂ and 320 mM sucrose, pH 7.4). The homogenate was centrifuged (1000 ◊ g for 10 min) and the precipitate was discarded. The supernatant was centrifuged for a second time (3200 ◊ g for 20 min) and then discarded, with the remaining precipitate making up the crude P₂ membrane preparation. This was washed twice in excess buffer (50 mM Tris-HCl, 1 mM MgCl₂) at room temperature, 30 mL were added, the precipitate was re-suspended and centrifuged (3200 × g for 20 min). The washed

Scheme 1. Synthesis of compounds 3a-b and 4a.

Figure 2. AM1 optimized conformations of free base 4a (top left and bottom left) and hydrochloride 3a (top right and bottom right) [18].
Non-specific binding was determined using 10 \( \mu \)M BU224, \( I_2 \) binding and 10 \( \mu \)M rauwolscine, \( \alpha_2 \)-adrenoceptor binding. Each incubation was performed in triplicate, at room temperature and allowed to reach equilibrium (45 min). Bound and free radioactivity were separated by rapid filtration through pre-soaked (0.5% polyethyleneimine) glass-fibre filters (Whatman GF/B). Filters were then washed twice with 5 mL of ice-cold buffer and membrane bound radioactivity remaining on the filters was determined by liquid scintillation counting.

RESULTS AND DISCUSSION

The two enantiomers of cis isomer 1R, 2S-(+)(3a) and 1S, 2R-(−)(3b) were obtained as depicted in Scheme 1 from 2-chloroimidazoline (1) and the corresponding commercially available aminoindanols 2a and 2b. The reactions carried out in chloroform at reflux for two hours gave the hydrochlorides 3a and 3b in 52% and 41% yields, respectively. The hydrochloride 3a, upon treatment with 5% methanolic NaOH solution, was converted into the corresponding free base 4a (Scheme 1).

As expected, both isomers exhibited identical physico-chemical and spectroscopic properties. For example, in 1H NMR spectrum of hydrochlorides 3a and 3b, imidazoline CH 2-CH2 protons appear as a singlet at 3.64 ppm; methylene H a-C3-Hb protons of the indane ring are nonequivalent and appear as two separate signals with geminal coupling constant \( J_{\text{gem}} = 16 \text{ Hz} \) and the signal of H a-C3 proton being split into doublet of doublets due to additional coupling with C2-H (\( J_3 = 4.4 \text{ Hz} \)); protons C1-H and C2-H are found as multiplets at 5.02 and 4.52 ppm, respectively; multiplet in the range of 7.21-7.27 ppm corresponds to four aromatic protons. Another four exchangeable protons are found at 5.53 ppm (OH), 7.6 ppm (endocyclic NH, br singlet), 8.34 ppm (exocyclic NH, doublet, \( J_3 = 7.8 \text{ Hz} \)) and 9.08 ppm (endocyclic NH, br singlet).

Table 2. Binding affinities and \( I/\alpha \) selectivity of compounds 3a-b

<table>
<thead>
<tr>
<th>Compound</th>
<th>( K_i ) binding site Mean ± SD (nM)</th>
<th>( K_i ) ( \alpha_2 )-adrenoceptors Mean ± SD (nM)</th>
<th>Selectivity ratio ( I/\alpha )</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>152.8 ± 79.2</td>
<td>177.2 ± 45.9</td>
<td>1.1</td>
</tr>
<tr>
<td>3b</td>
<td>5694.0 ± 1655.9</td>
<td>548.1 ± 78.8</td>
<td>0.09</td>
</tr>
<tr>
<td>PMS 952*</td>
<td>120 ± 11.5</td>
<td>3200 ± 220</td>
<td>26.6</td>
</tr>
</tbody>
</table>

* H.F. Ye, et al. (ref. 9).

Figure 3. Competition binding curves of 3a (top) and 3b (bottom) on I2 (filled squares) and \( \alpha_2 \) (filled triangles) on whole brain membranes.

Figure 3. Competition binding curves of 3a (top) and 3b (bottom) on I2 (filled squares) and \( \alpha_2 \) (filled triangles) on whole brain membranes.

It has been well recognized that biological activity of compounds, especially those acting at...
receptors located in central nervous system, is a function of physico-chemical properties and it is the free base that passes the blood-brain barrier. We therefore determined \( pK_a \) value of the newly synthesized compounds.

2-Iminoimidazolidines are known to possess \( pK_a \) values around 8 units [15, 16]. To our surprise, the determined \( pK_a \) value for free base 4a was 6.3, i.e. about two orders of magnitude lower than those found for other iminoimidazolidines. According to Henderson-Hasselbalch equation:

\[
\text{pH} = pK_a + \log\left(\frac{[\text{unionized}]}{[\text{ionized}]})\right)
\]

under physiological conditions (pH = 7.4) the indane derivatives exist about 92% in the free base form and about 8% in the protonated form 3. We suggest that charge-dipole interactions between the protonated guanidine moiety and polar OH substituent in 3 may contribute to lowering base strength of these compounds. A similar “stereochemical substituent effect” has been observed for a series of piperidines containing various polar groups [17].

In order to gain a closer insight into the three-dimensional structures of the compounds obtained, the conformational properties of model compounds 3a and 4a (these possessing the 1R, 2S(+)-configuration) were studied using the molecular modeling program Spartan [18].

As shown in Figure 2, significant differences in spatial arrangements were observed between free base 4a and the protonated form 3a. Free base 4a has the hydroxyl group axial with intramolecular OH-N= hydrogen bond, while in the protonated guanidinium compound 3a N=OH intramolecular hydrogen bond in observed with the hydroxyl substituent placed equatorial.

These findings are in contrast to the three-dimensional structures of PMS 952 and its protonated conformer, in which the relative position of the imidazoline rings is similar, and the main difference between the two conformers being the alcohol group rotation [9].

Imidazoline \( \alpha_2 \) and \( \alpha_3 \)-adrenergic receptor binding studies

The affinity of the compounds 3a and 3b to the imidazoline \( \alpha_2 \)-receptors and \( \alpha_3 \)-adrenoceptors was determined by radioligand binding assay performed on \( \beta_2 \) membrane preparations obtained from rat whole brains. As shown in Table 2 and Figure 3, enantiomer 1R, 2S(+)-3a exhibited moderate affinities at both imidazoline binding sites (\( K_i = 152.8 \) nM) and \( \alpha_3 \)-adrenoceptors (\( K_i = 177.2 \) nM). In contrast, compound 3b with 1S, 2R(-) configuration exhibited decreased affinity for \( \alpha_2 \) receptors (\( K_i = 548.1 \) nM) and activity at \( \alpha_2 \)-receptors was almost abolished (\( K_i = 5694.0 \) nM) (Figure 3).

From the above data one may conclude that 3b displays selectivity for the \( \alpha_3 \)-adrenergic receptors with respect to the imidazoline \( \alpha_2 \)-receptors (selectivity ratio 10.4) and none of the compounds 3 can act as a bioisoster of PMS 952 agent which exhibited preference for imidazoline \( \alpha_2 \)-receptors (\( K_i = 120 \) nM) versus \( \alpha_3 \)-adrenoceptors (\( K_i = 3200 \) nM) [9].

Acknowledgements

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18. The geometries of 3a and 4a were optimised by semiepirical AM1 method using the Spartan 5.0 program package, 1997, Wavefunction, Inc., Irvine, CA 92715, installed on a Silicon Graphics O2 workstation.

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