Polish Pharmaceutical Society

Imidazoline-containing agents acting at $\alpha_2$-adrenoceptors exhibit important pharmacological effects including hypotension, bradycardia, analgesia, sedation, mydriasis, organ-protection, stimulation of growth hormone secretion and decreased output of endocrine and exocrine secretory glands, such as decreased insulin secretion and decreased saliva (1-11). On the other hand, the therapeutic potential of agents which selectively interact with $\alpha_1$-adrenoceptors includes nasal congestion, urinary incontinence as well as sexual, CNS and eating dysfunctions (12-16).

It is well established that imidazoline derivatives of type A with methylene bridge between the imidazoline and the aryl ring (Figure 1, $X = \text{CH}_2$) such as xylometazoline, oxymetazoline and naphazoline induce an increase in blood pressure due to peripheral $\alpha_1$-adrenergic receptor stimulation, while $\alpha_2$-adrenoceptors exhibit hypotensive effect. If $X = \text{CF}_2$, the resulting compounds are a series of centrally-acting imidazolines with moderate hypotensive effect (12-15). These agents also have some effects on the cardiovascular system, such as increased heart rate and decreased blood pressure (16-19). Figure 1. Imidazoline derivatives with circulatory activity

**Abstract:** N-[(Imidazolin-2-yl)amino]indolines and N-[(imidazolin-2-yl)amino]-1,2,3,4-tetrahydroquinolines, previously described in patent literature as hypertensive agents, were synthesized and tested in vitro for their affinities to $\alpha_2$- and $\alpha_1$-adrenoceptors as well as imidazoline $I_1$ and $I_2$ receptors. The compounds most potent at either $\alpha_2$- or $\alpha_1$-adrenoceptors were administered intravenously to normotensive Wistar rats to determine their effects on mean arterial blood pressure and heart rate. Upon intravenous administration at dose of 0.1 mg/kg to normotensive male Wistar rats, the initial transient pressor effect was followed by long-lasting hypotension and bradycardia. In view of the above results the 1-[(imidazolin-2-yl)amino]indolines and [(imidazolin-2-yl)amino]-1,2,3,4-tetrahydroquinolines are now found to possess circulatory profile characteristic of the centrally acting clonidine-like hypotensive imidazolines.

**Keywords:** imidazolines, indolines, 1,2,3,4-tetrahydroisoquinolines, $\alpha$-adrenoceptors, imidazoline receptors, hypertensive effect, hypotensive effect

**Figure 1.** Imidazoline derivatives with circulatory activity

---

R

X

H

N

A. $R = \text{alkyl, halogen}$

$X = \text{NH (hypotensive)}$

$X = \text{CH}_2$ (hypertensive)

---

R

X

Y

N

B. $R = \text{alkyl, halogen}$

$Y = \text{N, CH}$

$X = \text{NH, CH}_2$ (hypertensive)

---

R

X

Y

N

C. $R = \text{alkyl, halogen}$

$Y = (\text{CH}_2)_n$

$X = \text{NH (hypotensive?)}$

---

ref. (36,46) – marسانidine, 7-Me-mارسانidine

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the analogues related to clonidine with amino bridge (Figure 1, X = NH) cause a secondary long lasting decrease in blood pressure caused by central \( \alpha_2 \)-adrenoceptor activation (17). Our previous investigations on \( \alpha \)-adrenoceptor ligands led to the discovery of imidazoline-containing indoles and indazoles (Figure 1, structure B) which, regardless the structure of bridging moiety X (either NH or CH\(_2\)), exhibited hypotensive activity due to either \( \alpha_2 \)-adrenoceptor agonist or \( \alpha_1 \)-adrenoceptor antagonist activity (18-22). However, a comprehensive survey of patent literature revealed that imidazolines connected to the partially hydrogenated indole and quinoline rings via NH bridge (Figure 1, structure C) were described as hypertensive agents when administered to rats at doses as low as 0.02-0.5 \( \mu \)g/kg (23, 24). Although imidazolines represent rather peculiar class of adrenergic agents because small structural modifications may result in altering the balance between agonist and antagonist activity (25), from the point of view of structure-activity relationships (SAR), the results of biological tests presented in aforementioned patents seemed to be rather dubious, since the overall structure of compounds C bears resemblance to the hypotensive amine-bridged imidazolines of type B (Fig. 2).

Molecular modeling studies with use of Spartan 08 program v. 1.2 indicate that the imidazoline N1 nitrogen atoms in C and B are situated 5.89 Å and 6.07 Å, respectively, apart from phenyl ring centroid and lie at the distance of 1.27 Å and 1.69 Å, respectively, from the phenyl ring best plane. Therefore, in the present work, the influence of partial hydrogenation of B leading to indolines and tetrahydroquinoline analogues of type C on \( \alpha \)-adrenoceptor affinity and selectivity has been explored. The hemodynamic effects of such ligand modification in anesthetized rats were also reinvestigated.

**EXPERIMENTAL**

Melting points were determined on a Boetius apparatus and are uncorrected. FT-IR spectra were measured on Nicolet 380 apparatus. Results of C, H, N elemental analyses were within ±0.4% of theoretical values. \( ^1 \)H- and \( ^13 \)C-NMR spectra were recorded on Varian Gemini 200 or Varian Unity 500 apparatus. \( ^1 \)H and \( ^13 \)C chemical shifts were measured relative to the residual solvent signal at 2.50 ppm and 39.5 ppm (DMSO-d\(_6\)) or 7.26 and 77.2 (CDCl\(_3\)). The following compounds were obtained according to previously described procedures: 8-methyl-1,2,3,4-tetrahydroquinoline (26), 4-chloroindoline (27), N-amino-indolines and N-amino-1,2,3,4-tetrahydroquinolines (28), 2-chloro-4,5-dihydro-1\( \text{H} \)-imidazole (29), N-tert-butoxycarbonyl-2-methylthio-4,5-dihydro-1\( \text{H} \)-imidazole (30). Structure optimization of indole (B) and indoline (C) was carried out using Spartan program v. 8.0 (Wavefunction Inc., Irvine, CA, USA).

**N-nitroso-indolines 2a-d and N-nitroso-1,2,3,4-tetrahydroquinolines 2a,b**

To the appropriate cyclic amine (20 mmol) in hexane (15 mL) amyl nitrite (60 mmol, 7 g, 8 mL) was added in one portion. The resulting mixture was

![Figure 2. Minimum energy conformations of indazole derivative B (left) and indoline derivative C (right) optimized at density functional level of theory (B3LYP) with the 6-31G* basis set.](image-url)
stirred for 30 min at room temperature. The precipitated solid was collected by filtration, washed with hexane, dried and used for preparation of corresponding N-aminoindolines 3a-d and N-amino-1,2,3,4-tetrahydroquinolines 8a,b without purification.

N-aminoindolines 3a-d and N-amino-1,2,3,4-tetrahydroquinolines 8a,b

To a suspension of LiAlH₄ (32 mmol, 1.12 g) in THF or anhydrous diethyl ether (50 mL) was added dropwise a solution of appropriate N-nitrosoamine (21.2 mmol) in anhydrous diethyl ether/THF (9 : 1, v/v) (100 mL) and the resulting mixture was stirred at room temperature for 30 min and then at reflux for 2 h. Upon cooling to 0°C water was added dropwise to destroy excess LiAlH₄. The reaction mixture was filtered under reduced pressure and the filter cake was washed with dichloromethane. The combined filtrates were dried over MgSO₄ and evaporated to dryness to obtain the corresponding hydrochlorides, while free bases 3a-c and 8a were used for further reaction without purification.

N-amino-4-chloroindoline hydrochloride (3d)

Yield: 3.5 g (80%); m.p. 205–208°C. IR (KBr, cm⁻¹): 3169, 2930, 2872, 1628, 1595, 1496, 1284, 750. ¹H NMR (200 MHz, DMSO-d₆, δ, ppm): 7.89 (d, J = 8.2 Hz, 1H), 7.00–7.13 (m, 2H), 6.77 (t, 1H), 5.99 (s, 2H, NH), 3.91 (t, 2H, CH₂). ¹³C NMR (50 MHz, DMSO-d₆, δ, ppm): 159.08, 144.81, 130.72, 126.99, 124.52, 120.46, 113.75, 48.60 (2C), 27.46.

N-[(imidazolin-2-yl)amino]indoline (4a)

Yield 49%, m.p. 97–99°C. IR (KBr, cm⁻¹): 3169, 2930, 2872, 1628, 1595, 1496, 1284, 750. ¹H NMR (200 MHz, DMSO-d₆, δ, ppm): 7.89 (d, J = 8.2 Hz, 1H), 7.00–7.13 (m, 2H), 6.77 (t, 1H), 5.99 (s, 2H, NH), 3.91 (t, 2H, CH₂). ¹³C NMR (50 MHz, DMSO-d₆, δ, ppm): 159.08, 144.81, 130.72, 126.99, 124.52, 120.46, 113.75, 48.60 (2C), 27.46.

N-[(imidazolin-2-yl)amino]indoline hydrochloride (5a)

Yield 98%, m.p. 323–326°C. IR (KBr, cm⁻¹): 3227, 3095, 1640, 1591, 1550, 1496, 1287, 1067, 761. ¹H NMR (200 MHz, DMSO-d₆, δ, ppm): 8.79 (s, 2H, NH), 7.24–7.42 (m, 3H), 7.12 (t, 1H), 4.09 (t, 2H, CH₂). ¹³C NMR (50 MHz, DMSO-d₆, δ, ppm): 135.69, 131.34, 126.18, 112.49, 49.64 (2C), 22.85.

N-[(imidazolin-2-yl)amino]-2-methylindoline (4b)

Yield 30%; m.p. 53–55°C. IR (KBr, cm⁻¹): 3411, 3165, 3044; 2960, 2862; 1656 (C=N), 1604 (δ N–H), 1473, 1458, 1279, 1248, 750. ¹H NMR (200 MHz, CDCl₃, δ, ppm): 9.43 (s, 1H, NH), 7.14–7.16 (m, 2H), 6.52–6.56 (m, 1H), 6.28 (br, s, 2H, NH≈N), 4.32 (t, 2H, CH₂), 3.75 (s, 3H, CH₃), 3.22 (2H, CH₂).

N-[(imidazolin-2-yl)amino]-2-methylindoline hydrochloride (5b)

Yield: 100%; m.p. 234–236°C (lit. (23) m.p. 226–228°C); IR (KBr, cm⁻¹): 3400, 3250, 3142,
3.15 (dd, 1H, J = 15.6 Hz, J2 = 7.8 Hz), 6.95 (d, 1H, J1 = 8.0 Hz), 3.80–3.50 (m, 3H), 3.10–2.85 (m, 2H).

The free base 9b was obtained as a viscous oil which was transformed into hydrochloride 10b without characterization.

N-[(imidazolin-2-yl)amino]-1,2,3,4-tetrahydro-8-methylquinoline hydrochloride (10b).

Yield 20%; m.p. 248–251°C. IR (KBr, cm–1): 3265, 3133, 3069, 3033, 3012, 2955, 2929, 2854, 1657, 1482, 1460, 1441, 1374, 1302, 1284, 1261, 1129, 884, 771; ‘H NMR (200 MHz, DMSO-d6, δ ppm): 6.99 δ, 6.88 δ, 3.87–3.83 (m, 1H), 3.68 (br s, 2H), 3.27–3.21 (m, 1H), 3.15–3.10 (m, 2H), 2.82–2.75 (m, 1H), 2.17 (s, 3H). 13C NMR (50 MHz, DMSO-d6, δ ppm): 160.8 (C=N), 147.6, 130.1, 129.2, 122.89, 122.85, 57.0, 43.3, 42.2, 27.4, 16.7.

Radioligand binding assays

1-Binding site assay

Kidneys were obtained post mortem from male Sprague–Dawley rats (250–280 g) and crude P2

N-[(imidazolin-2-yl)amino]-7-methylindoline hydrochloride (5c).

Yield 7%; m.p. 112–115°C (lit. (24) m.p. 194–196°C). IR (KBr, cm–1): 3145, 2966, 2908, 2865; 1665; 1604. ‘H NMR (200 MHz, DMSO-d6, δ ppm): 10.54 (s, 1H, NH), 8.98 (s, 1H, NH), 8.37 (s, 1H, NH), 7.06 (d, 1H, J = 7.3), 6.95 (d, 1H, J = 7.8), 6.88 (dd, 1H, J1 = 7.3, J2 = 7.8 Hz), 3.87–3.83 (m, 1H), 3.68 (br s, 2H), 3.27–3.21 (m, 1H), 3.15–3.10 (m, 1H), 2.82–2.75 (m, 1H), 2.17 (s, 3H). 13C NMR (50 MHz, DMSO-d6, δ ppm): 160.8 (C=N), 147.6, 130.1, 129.2, 122.89, 122.85, 57.0, 43.3, 42.2, 27.4, 16.7.

N-[(imidazolin-2-yl)amino]-4-chloroindoline (4d).

Yield 20%; m.p. 174–176°C. IR (KBr, cm–1): 3388, 3132, 2975, 1609, 1599, 1451, 1279, 1262, 1109, 768. ‘H NMR (200 MHz, CDCl3, δ ppm): 6.99 (t, 1H, J = 7.7 Hz), 6.71 (d, 1H, J = 8.1 Hz), 6.49 (d, 1H, J = 7.7 Hz), 2.56 (br s, 2H, NH), 3.43 (s, 4H), 3.38 (t, 2H, J = 7.7 Hz), 2.93 (t, 2H, J = 7.7 Hz). 13C NMR (50 MHz, CDCl3, δ ppm): 166.2, 155.5, 130.0, 128.7, 127.2, 119.5, 108.6, 56.8, 42.7, 27.4.

N-[(imidazolin-2-yl)amino]-1,2,3,4-tetrahydro-8-methylquinoline hydrochloride (10a).

Yield: 49%; m.p. 269–272°C. IR (KBr, cm–1): 3305, 3161, 2947, 2856, 1654, 1489, 1451, 1302, 1275, 741. ‘H NMR (200 MHz, DMSO-d6, δ ppm): 6.79–6.91 (m, 2H), 6.61–6.66 (m, 1H), 6.42–6.50 (m, 1H), 6.14 (s, 2H, NH), 3.27 (s, 4H, CH2), 3.05 (t, 2H, CH2), 2.67 (t, 2H, CH2), 1.94–2.06 (m, 2H, CH2).

N-[(imidazolin-2-yl)amino]-1,2,3,4-tetrahydroquinoline (9a).

Yield: 58%; m.p. 153–154°C. IR (KBr, cm–1): 3405, 3161, 2947, 2856, 1654, 1489, 1451, 1302, 1275, 741. ‘H NMR (200 MHz, DMSO-d6, δ ppm): 8.76 (s, 2H, NH), 7.02–7.13 (m, 2H), 6.70–6.84 (m, 2H), 3.67 (s, 4H, CH2), 3.34 (s, 2H, CH2), 2.71 (s, 2H, CH2), 2.01 (s, 2H, CH2).
membranes were prepared according to the methods of Lione et al. (31). Binding of [3H]clonidine (3 nM, PerkinElmer) was investigated in the presence of 10 nM rauwolscine to preclude radioligand binding to α2-adrenoceptors. The specific component was defined by 10 nM rilmenidine; under these conditions, the site labelled represents a model of the central I1 binding site (32). Membrane aliquots (400 µL, 0.2–0.5 mg protein) were incubated with 11 concentrations of the test compounds over the range 0.1 nM – 10 µM. Incubations were carried out in 50 mM Tris-HCl buffer (pH 7.4) at room temperature for 45 min. Bound radioligand and free radioactivity were separated by rapid filtration through pre-soaked (0.5% polyethyleneimine) glass-fibre filters (Whatman GF/B). Trapped radioligand was determined by liquid scintillation counting and the data were analyzed with GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego, CA, USA) to yield IC50 values (the concentration of test compound that displaces 50% of specifically bound [3H]clonidine).

**α1- and α2-adrenoceptor and I1-receptor binding assays**

Brains were obtained post mortem from male Sprague–Dawley rats (250–280 g) and crude P2 membranes were prepared (31). Membrane aliquots (400 µL, 0.2–0.3 mg protein) were incubated with 11 concentrations of the test compounds over the range 0.1 nM – 100 µM in the presence of the selective I1 binding site radioligand [3H]2BFI (2-(2-benzofuranyl)-2-imidazoline) (32) (1 nM), the α1-adrenoceptor antagonist radioligand [3H]prazosin (1 nM) or the α2-adrenoceptor antagonist radioligand [3H]RX821002 (2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1H-imidazole) (33) (1 nM) in a final volume of 500 µL. Non-specific binding was determined using 10 µM BU224 (2-(4,5-dihydroimidazol-2-yl)quinoline) (34) for I1 binding, 10 µM phenylephrine for α1-adrenoceptors and 10 µM rauwolscine to define α2-adrenoceptor binding. Incubations were performed in triplicate at room temperature and were 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. The data were analyzed by iterative non-linear regression curve fitting procedures with GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, CA, USA). Each experiment was analyzed individually and equilibrium dissociation constants (Ki) were determined by the method of Cheng and Prusoff (35). The resulting values are given as the means ± SEM of three or four separate experiments.

**In vivo studies: mean arterial blood pressure (MAP) and heart rate (HR) in rats**

Male Wistar rats, weighing 200–290 g, were purchased from the Animal House of the Medical University of Gdańsk, Poland. All in vivo experiments were approved by the Local Ethical Committee on Animal Experiments. The animals were fed a commercial rodent chow (Labofeed–B, Poland). Tap water was available ad libitum. Rats were anesthetized by i.p. injection of thiopental (Sandoz, Austria) at a dose of 70 mg/kg body weight and maintained under anesthesia by thiopental supplementation (30 µg/kg/min) during the experiment. The animals were placed on a heated table, and body temperature was maintained between 36 and 37°C. Tracheostomy was performed. Catheters were inserted into the carotid artery for blood pressure and heart rate monitoring, into a jugular vein for infusions, and into the bladder for free diuresis. After all surgical procedures, a 40 min recovery period was allowed to establish steady state. The rats were infused with isotonic saline (Fresenius Kabi, Poland) supplemented with thiopental at a rate of 1.2 mL/h. After 40 min of saline infusion, the tested compounds were administered as a 100 µL bolus through the venous catheter at a dose of 0.1 mg/kg. The time of administration of the compound was assumed as “time 0”. Mean arterial blood pressure (MAP) and heart rate (HR) were monitored directly and sampled continuously at 100 Hz, as described previously (36) using Biopac Systems, Inc., Model MP 100 (Goleta, CA, USA). The results of recordings were elaborated with the help of the ACQKnowledge (Goleta, CA, USA) analysis system and were selected, scaled and filtered to remove signal disturbances. The recorded time domain transient data are presented as graphs with the help of Excel (Microsoft, USA).

ANOVA was performed for DMAP and DHR, calculated as the difference in MAP and in HR from baseline measurements (“time 0”) for each group, as described previously (36). This allowed direct comparisons of responses to treatments between the groups. Data were analyzed with ANOVA with repeated measurements, using Statistica StatSoft.
Scheme 1. Synthesis of 1-[(imidazolin-2-yl)amino]indolines

Method A: 2-chloro-4,5-dihydro-1H-imidazole, DCM, 20°C, 12-24 h; for R = H, 2-CH₃
Method B: N-tert-butoxycarbonyl-2-methylthio-4,5-dihydro-1H-imidazole, acetic acid, 60°C, 16 h; for R = 7-CH₃, 4-Cl

Scheme 2. Synthesis of 1-[(imidazolin-2-yl)amino]-1,2,3,4-tetrahydroquinolines

Method A: 2-chloro-4,5-dihydro-1H-imidazole, DCM, 20°C, 12-24 h; for R = H
Method B: N-tert-butoxycarbonyl-2-methylthio-4,5-dihydro-1H-imidazole, acetic acid, 60°C, 16 h; for R = 8-CH₃
When a treatment effect was significant, post hoc comparisons were performed using Fisher’s test. A value of p < 0.05 was considered statistically significant.

Molecular modeling studies were performed using B3LYP/6-31G* density functional model as implemented into Spartan 08 version 1.2, Wavefunction Inc. Irvine, CA, USA.

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Comp.</th>
<th>α₁, Kᵢ (nM)</th>
<th>α₂, Kᵢ (nM)</th>
<th>I₁, IC₅₀ (nM)</th>
<th>I₂, Kᵢ (nM)</th>
<th>Selectivity αᵡ αᵢ</th>
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<tr>
<td>5a</td>
<td>1340 ± 7.6</td>
<td>3640 ± 456</td>
<td>123.8 ± 45.4</td>
<td>52.1 ± 25.7</td>
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<tr>
<td>5b</td>
<td>30.2 ± 2.4</td>
<td>6.09 ± 2.49</td>
<td>39.71 ± 6.05</td>
<td>431.7 ± 276.1</td>
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<tr>
<td>5c</td>
<td>27.1 ± 4.61</td>
<td>0.75 ± 0.11</td>
<td>1543 ± 1233</td>
<td>48.3 ± 5.93</td>
<td>36.1</td>
<td></td>
</tr>
<tr>
<td>5d</td>
<td>106.0 ± 14.07</td>
<td>3.1 ± 0.37</td>
<td>5777 ± 5652</td>
<td>49.8 ± 4.65</td>
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<tr>
<td>10a</td>
<td>69.8 ± 7.6</td>
<td>4.94 ± 0.66</td>
<td>1423 ± 112.4</td>
<td>14.4 ± 1.36</td>
<td>14.1</td>
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<tr>
<td>10b</td>
<td>528 ± 239</td>
<td>5244 ± 9.19</td>
<td>3076 ± 2647</td>
<td>62.7 ± 5.05</td>
<td>10.1</td>
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Table 1. Binding affinities of compounds 5a-d and 10a,b.

<table>
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<tr>
<th>Time after application of tested compound (min)</th>
<th>Time after application of tested compound (min)</th>
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<td>∆MAP (mmHg)</td>
<td>∆HR (bpm)</td>
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<td>5</td>
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<td>15</td>
<td>30</td>
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Table 2. Effect of compounds 5a, 5c, 10a and 10b at 0.1 mg/kg i.v. on mean arterial blood pressure (MAP) in anesthetized rats.

Table 3. Effect of compounds 5b, 5c, 10a and 10b at 0.1 mg/kg i.v. on heart rate (HR) in anesthetized rats.
RESULTS AND DISCUSSION

Chemistry

The title indoline-containing (5a-d) and 1,2,3,4-tetrahydroquinoline-containing (10a,b) compounds have been synthesized according to the procedures depicted in Scheme 1 and Scheme 2, respectively. First, the indolines 1 and 1,2,3,4-tetrahydroquinolines 6 were converted into corresponding N-nitroso derivatives 2 and 7 by the treatment with amyl nitrite, followed by the reduction with LiAlH₄ to give N-amino compounds 3 and 8. Then, upon treatment of 3a, 3b and 8a with 2-chloro-4,5-dihydro-1H-imidazole and 3c, 3d and 8b with $N$-tert-butoxycarbonyl-2-methylthio-4,5-dihydro-1H-imidazole the desired imidazoline derivatives 4a-d and 9a,b were obtained. For the purposes of biological tests free bases thus obtained were converted into the corresponding hydrochlorides 5a-d and 10a,b. Structures of the products thus obtained were confirmed by elemental analysis as well as by IR and NMR spectroscopic data.

It is pertinent to note, that the already patented compounds 5a (23) and 10a (24) were prepared by different method, i.e., by reacting N-amino-indoline 3a and N-amino-1,2,3,4-tetrahydroquinoline 8a, respectively, with 2-bromoethyl isocyanate followed by imidazoline ring closure upon treatment of corresponding $N$-chloroethylurea with aqueous

![Figure 3](image-url)
NaOH. No spectral data for both the free bases or corresponding hydrochloride salts have previously been described.

**Binding affinities at α<sub>1</sub>-, α<sub>2</sub>-adrenoceptors and imidazoline I<sub>1</sub> and I<sub>2</sub> receptors**

Radioligand binding experiments of α<sub>1</sub>-adrenoceptors and imidazoline I<sub>2</sub> receptors were conducted using crude P<sub>2</sub> rat brain membranes, and crude P<sub>2</sub> rat kidney membranes were used for I<sub>1</sub> receptors. Equilibrium dissociation constants (K<sub>i</sub>) were determined by the method of Cheng & Prusoff (35) and the resulting values are presented in Table 1 as the mean ± SEM for 3 or 4 separate experiments.

As shown in Table 1, the unsubstituted compound 5a showed a poor affinity for both the α<sub>1</sub>- and α<sub>2</sub>-adrenoceptors with K<sub>i</sub> = 1340 nM and 3640 nM, respectively. Compound 5b with CH<sub>3</sub> substituent at position 2 displayed enhanced activity at α<sub>1</sub>- (K<sub>i</sub> = 30.2 nM) and α<sub>2</sub>- (K<sub>i</sub> = 6.09 nM) receptors, but still a negligible α<sub>1</sub>/α<sub>2</sub> selectivity ratio of 4.96. The highest difference in potencies at α<sub>1</sub>- and α<sub>2</sub>-adrenoceptors was showed by 7-CH<sub>3</sub> and 4-Cl -substituted α<sub>1</sub>-adrenoceptors receptors (Table 2, Fig. 3A, ∆MAP = -28 and -26 mmHg, respectively). Thus, in the circulatory system of Wistar rats the vasorelaxant response after which significant reduction of arterial blood pressure was observed (Table 2, Fig. 3A, ∆HR = -154 and -133 bpm, respectively).

The negative chronotropic effect observed in the present study deserves a special attention. It is well known that the human heart expresses α<sub>1</sub>-adrenoceptors albeit at much lower levels than β<sub>1</sub>-adrenoceptors (37). However, the role of α<sub>2</sub>-receptors in cardiac physiology is still a matter of debate, contrary to their well established effects in regulation of blood flow by inducing constriction of major arteries smooth muscles (38). Very recent study on papillary muscles obtained from rat heart ventricles indicated that stimulation of α<sub>2</sub>-adrenoceptors inhibits cardiac excitation-contraction coupling through tyrosine phosphorylation of β<sub>1</sub>-adrenoceptors (39). Moreover, experiments performed on human cardiac myocytes indicated expression of α<sub>1A</sub>- and α<sub>1B</sub>-adrenoceptor subtypes (40) that are considered as cardioprotective proteins (41). In view of the above information, the immediate sharp fall in the heart rate observed after intravenous administration of tested compounds (Table 3, Fig. 3B, ∆HR = -154 and -133 bpm, respectively) might possibly be mediated by cardiac α<sub>1</sub>-adrenoceptors activation.

In conclusion, the present studies extend the results previously described in patent literature, showing that imidazoline-containing indolines 5 and 1,2,3,4-tetrahydroquinolines 10 administered at dose of 0.1 mg/kg i.v. elicit long-lasting hypotensive and bradycardic effects attributable to their ability to stimulate central α<sub>2</sub>-adrenoceptors, and therefore, should
not be classified as hypertensive agents. This study has widened the scope of developing imidazoline derivatives as promising antihypertensive agents.

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