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DRUG SYNTHESIS

1-[(IMIDAZOLIN-2-YL)AMINO]INDOLINE AND 1-[(IMIDAZOLIN-2-YL)AMINO]1,2,3,4-TETRAHYDROQUINOLINE DERIVATIVES: NEW INSIGHTS INTO THEIR CIRCULATORY ACTIVITIES

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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 α_1- and α_2-adrenoceptors as well as imidazoline I_1 and I_2 receptors. The compounds most potent at either α_1- or α_2-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, α-adrenoceptors, imidazoline receptors, hypertensive effect, hypotensive effect

Imidazoline-containing agents acting at α_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 salivation (1-11). On the other hand, the therapeutic potential of agents which selectively interact with α_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 = CH_2) such as xylometazoline, oxymetazoline and naphazoline induce an increase in blood pressure due to peripheral α_1-adrenergic receptor stimulation, while

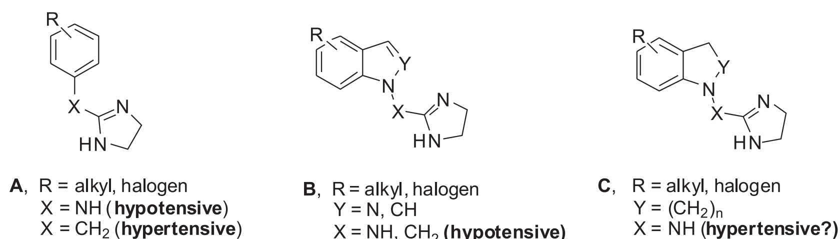

Figure 1. Imidazoline derivatives with circulatory activity

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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 α_2-adrenoceptor activation (17). Our previous investigations on α-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 α_2-adrenoceptor agonist or α_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 μg/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 α-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*H*-imidazole (29), *N*-*tert*-butoxycarbonyl-2-methylthio-4,5-dihydro-1*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

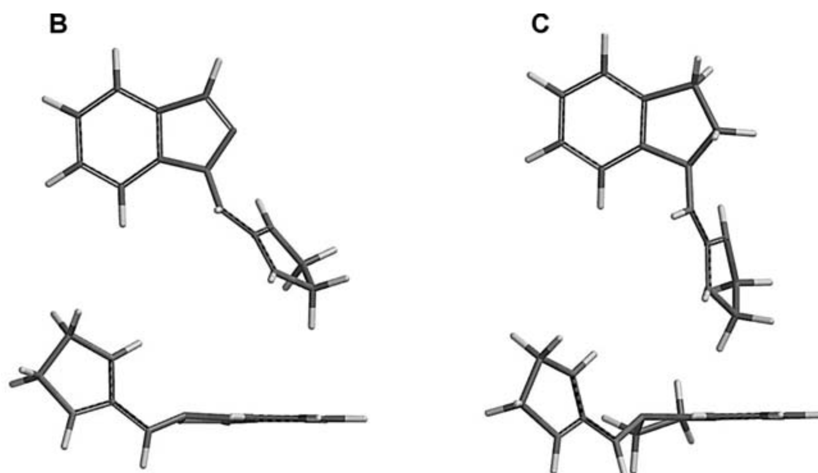


Figure 2. Minimum energy conformations of indazole derivative **B** (*left*) and indoline derivative **C** (*right*) optimized at density functional level of theory (B3LYP) with the 6-31G* basis set.

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-tertahydroquinolines **8a**,**b** without purification.

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

To a suspension of $LiAlH_4$ (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 $LiAlH_4$. The reaction mixture was filtered under reduced pressure and the filter cake was washed with dichloromethane. The combined filtrates were dried over $MgSO_4$ and concentrated to dryness under reduced pressure. *N*-aminoindoline **3d** and *N*-amino-1,2,3,4-tetrahydroquinoline **8b** thus obtained were converted to 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}): 3044, 2900, 2680, 2513, 1605, 1575, 1540, 1454, 1075, 879, 824, 767, 705, 598; ^1H NMR (200 MHz, DMSO-d_6, δ, ppm): 10.69 (br. s, 3H, NH_3^+), 7.30–721 (m, 2H), 7.09–7.02 (m, 1H), 3.64 (t, 2H, *J* = 7.8 Hz), 3.02 (t, 2H, *J* = 7.8 Hz). ^{13}C NMR (50 MHz, DMSO-d_6, δ, ppm): 151.1, 129.9, 129.7, 127.6, 122.8, 110.9, 55.4, 27.1.

_N_-amino-8-methyl-1,2,3,4-tetrahydroquinoline hydrochloride (8b)

Yield: 3.2 g (77%); m.p. 178–181°C. IR (KBr, cm^{-1}): 2926, 2830, 2744, 2660, 1596, 1578, 1522, 1458, 769; ^1H NMR (200 MHz, DMSO-d_6, δ, ppm): 10.0 (br. s, 3H NH_3^+), 7.13–7.02 (m, 3H), 3.43–3.38 (m, 2H), 2.83 (t, 2H, *J* = 6.7 Hz), 2.39 (s, 3H), 2.03–1.98 (m, 2H). ^{13}C NMR (50 MHz, DMSO-d_6, δ, ppm): 141.4, 134.7, 131.7, 128.8, 127.9, 126.9, 50.7, 25.8, 17.6, 15.8.

_N_-[(imidazolin-2-yl)amino]indolines (4a–d) and _N_-[(imidazolin-2-yl)amino-1,2,3,4-tetra-hydroquinolines (9a,b) and corresponding hydrochlorides 5a–d and 10a,b.

Method A: To a stirred solution of 2-chloro-4,5-dihydro-1*H*-imidazole (25 mmol) in dichloromethane (30 mL) the corresponding *N*-aminoindoline (17 mmol) was added and the mixture was stirred for 24 h at room temperature. The precipitated solid was collected by filtration under reduced pressure. The crude product in the form of hydrochloride was dissolved in water and washed with dichloromethane to remove impurities. The aqueous phase was made alkaline (pH~10) with 5% aqueous NaOH and extracted with dichloromethane. The combined organic extracts were dried over $MgSO_4$ and evaporated to dryness to obtain the product in the form of free base as a white solid. The free base was dissolved in methylene chloride (5 mL) and the 3.2 M solution of hydrochloride in diethyl ether (3 mL) was added. The precipitated hydrochloride salt was collected by filtration.

According to the above procedure the following compounds were obtained:

_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-d_6, 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, CH_2), 3.46 (s, 4H, CH_2), 3.06 (t, 2H, CH_2). ^{13}C-NMR (50 MHz, DMSO-d_6, 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-d_6, δ, ppm): 8.79 (s, 2H, NH), 7.24–7.42 (m, 3H), 7.12 (t, 1H), 4.09 (t, 2H, CH_2), 3.75 (s, 4H, CH_2), 3.22 (t, 2H, CH_2),

_N_-[(imidazolidin-2-yl)imino]-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, $CDCl_3$, δ, ppm): 7.03–7.10 (m, 2H), 6.69–6.77 (m, 1H), 6.52–6.56 (m, 1H), 5.28 (br. s, 2H, 2 ´ NH), 3.42 (s, 4H), 3.04 (dd,1H, J_1 = 14.9 Hz, J_2 = 7.0 Hz), 2.52 (dd, 1H, J_1 = 14.9 Hz, J_2 = 11.4 Hz), 1.29 (d, 3H, *J* = 7.0 Hz). ^{13}C NMR (50 MHz $CDCl_3$, δ, ppm): 166.65 (C=N), 153.76, 128.57, 126.93, 123.70, 119.31, 109.90, 65.75, 42.42, 36.15, 18.96.

_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,

2966, 2902, 1679, 1600, 1477, 1459, 1286, 1069, 750, 670. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 10.39 (br. s, 1H, NH), 8.85 (br. s 1H, NH), 8.47 (br. s 1H, NH), 7.14–7.18 (m, 2H), 6.91 (t, 1H, J = 7.8 Hz), 6.69 (d, 1H, J = 7.8 Hz), 3.63–3.71 (m, 5H), 3.15 (dd, 1H, J_1 = 15.6 Hz, J_2 = 7.8 Hz), 2.56 (m, 2H), 1.35 (d, 3H, J = 7.8 Hz).

***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. ^{1}H-NMR (200 MHz, DMSO-d_6, 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, CH_2), 3.05 (t, 2H, CH_2), 2.67 (t, 2H, CH_2), 1.94–2.06 (m, 2H, CH_2). ^{13}C-NMR (50 MHz, DMSO-d_6, δ, ppm): 165.88, 148.99, 128.08, 126.23, 122.39, 116.58, 113.88, 50.93, 42.38 (2 C), 27.02, 22.35.

***N*-[(imidazolin-2-yl)amino]-1,2,3,4-tetrahydroquinoline hydrochloride (10a)**

Yield: 49%; m.p. 269–272°C. IR (KBr, cm^{-1}): 3113, 2930, 2890, 1661, 1606, 1452, 1284, 752. ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 8.76 (s, 2H, NH), 7.02–7.13 (m, 2H), 6.70–6.84 (m, 2H), 3.67 (s, 4H, CH_2), 3.34 (s, 2H, CH_2), 2.71 (s, 2H, CH_2), 2.01 (s, 2H, CH_2).

Method B: A suspension of the appropriate *N*-aminoindoline or N-amino-1,2,3,4-tetrahydroquinoline (12.8 mmol) and *N-tert*-butoxycarbonyl-2-methylthio-4,5-dihydro-1*H*-imidazole (3.32 g, 15.36 mmol) in acetic acid (8 mL) was stirred at 60°C (oil bath) for 16 h and then the solvent was evaporated under reduced pressure. The viscous residue was treated with water (7 mL) and to the resulting mixture was added dropwise 5% aqueous NaOH to pH 9.5–10. The resulting mixture was extracted with dichoromethane (3 × 20 mL). The combined organic phases were dried over $MgSO_4$ and evaporated to dryness. The oily residue was purified by chromatography on silica gel eluting with ethyl acetate and then ethyl acetate/methanol/triethylamine (8 : 1 : 1, v/v/v). The resulting *N*-[(imidazolin-2-yl)amino]amine free bases were transformed into the corresponding hydrochloride salts by treating with 3.2 M solution of hydrochloride in diethyl ether.

According to the above procedure the following compounds were obtained:

The free base **4c** was obtained as a viscous oil, and therefore, it was transformed into hydrochloride **5c** without characterization.

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. ^{1}H NMR (200 MHz, DMSO-d_6, 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, J_1 = 7.3, J_2 = 7.8 Hz), 3.87–3.83 (m, 1H), 3.68 (br. s, 4H), 3.27–3.21 (m, 1H), 3.15–3.10 (m, 1H), 2.82–2.75 (m, 1H), 2.17 (s, 3H). ^{13}C NMR (50 MHz, DMSO-d_6, 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, 3162; 2857, 1650; 1599, 1451, 1279, 1262, 1109, 768. ^{1}H NMR (200 MHz, $CDCl_3$, 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), 5.26 (br. s, 2H, NH), 3.43 (s, 4H), 3.38 (t, 2H, J = 7.7 Hz), 2.93 (t, 2H, J = 7.7 Hz). ^{13}C NMR (50 MHz, $CDCl_3$, 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]-4-chloroindoline hydrochloride (5d)**

Yield 100%; m.p. 235–238°C. IR (KBr, cm^{-1}): 3082, 3030, 2871, 2798, 2719, 1664, 1604, 1589, 1449, 1261, 1129, 884, 771; ^{1}H NMR (200 MHz, DMSO-d_6, δ, ppm): 10.75 (s, 1H, NH), 8.79 (br. s, 2H, 2×NH), 7.18 (t, 1H, J_1 = 8.0 Hz), 6.95 (d, 1H, J = 8.0 Hz), 6.66 (d, 1H, J = 8.0 Hz), 3.80–3.50 (m, 6H), 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, 1274, 1102, 764. ^{1}H NMR (200 MHz DMSO-d_6, δ, ppm): 10.53 (s, 1H, NH), 9.03 (s, 1H, NH), 7.92 (s, 1H, NH), 7.27–6.96 (m, 3H), 3.66 (s, 4H), 3.27–3.24 (m, 1H), 3.15–3.13 (m, 1H), 2.79–2.74 (m, 2H), 2.12 (s, 3H), 2.00–1.92 (m, 1H), 1.76–1.70 (m, 1H). ^{13}C NMR (50 MHz, DMSO-d_6, δ, ppm): 159.4 (C=N), 142.3, 132.6, 129.3, 128.8, 127.8, 124.3, 53.4, 43.1, 42.4, 26.7, 17.2, 17.8;

Radioligand binding assays

I_1-Binding site assay

Kidneys were obtained *post mortem* from male Sprague–Dawley rats (250–280 g) and crude P_2

membranes were prepared according to the methods of Lione et al. (31). Binding of [^{3}H]clonidine (3 nM, PerkinElmer) was investigated in the presence of 10 mM rauwolscine to preclude radioligand binding to α_2-adrenoceptors. The specific component was defined by 10 mM rilmenidine; under these conditions, the site labelled represents a model of the central I_1 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 – 100 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 GFB). 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 IC_{50} values (the concentration of tested ligand that displaces 50% of specifically bound [^{3}H]clonidine).

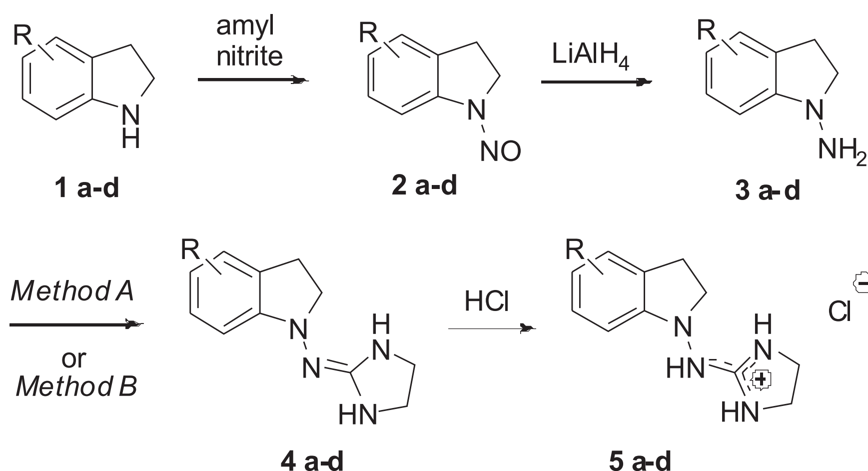
α_1- and α_2-adrenoceptor and I_2-receptor binding assays

Brains were obtained *post mortem* from male Sprague–Dawley rats (250–280 g) and crude P_2 membranes were prepared (31). Membrane aliquots (400 µL, 0.2–0.3 mg protein) were incubated with 11 concentrations of the tested compounds over the range 0.1 nM – 100 µM in the presence of the selective I_2 binding site radioligand [^{3}H]2BFI (2-(2-benzofuranyl)-2-imidazoline) (32) (1 nM), the α_1-adrenoceptor antagonist radioligand [^{3}H]prazosin (1 nM) or the α_2-adrenoceptor antagonist radioligand [^{3}H]RX821002 (2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1*H*-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 I_2 binding, 10 µM phenylephrine for α_1-adrenoceptors and 10 µM rauwolscine to define α_2-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 (K_i) 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).

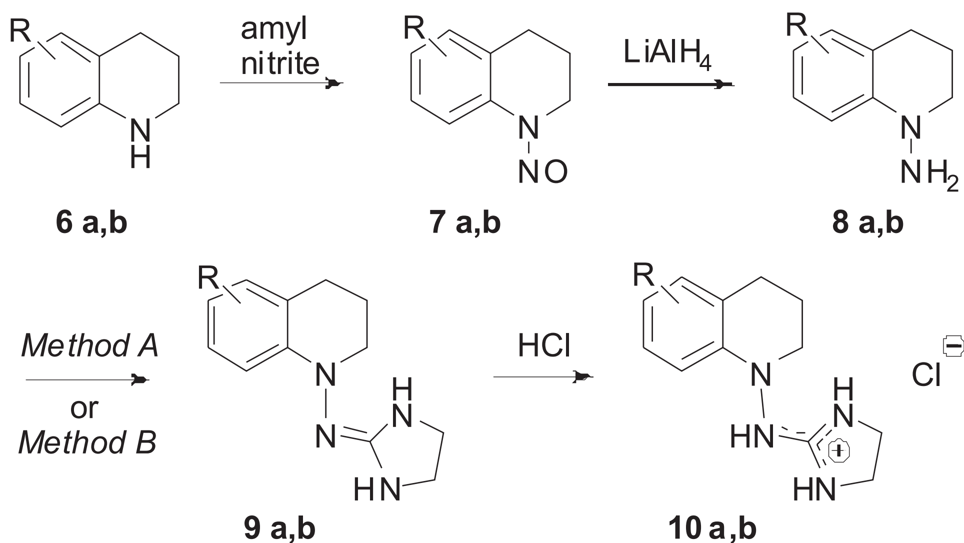
ANOVA was performed for DMAP and DHR, calculated as the difference in MAP and in HR from baseline measurements (“time 0”) for each group, as described previously (36). This allowed direct comparisons of responses to treatments between the groups. Data were analyzed with ANOVA with repeated measurements,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



a R = H; **b** R = 2-CH_3; **c** R = 7-CH_3; **d** R = 4-Cl

Method A: 2-chloro-4,5-dihydro-1*H*-imidazole, DCM, 20°C, 12-24 h; for R = H, 2-CH_3
Method B: *N*-*tert*-butoxycarbonyl-2-methylthio-4,5-dihydro-1*H*-imidazole, acetic acid, 60°C, 16 h; for R = 7-CH_3, 4-Cl

Scheme 1. Synthesis of 1-[(imidazolin-2-yl)amino]indolines



a R = H; **b** R = 8-CH_3;

Method A: 2-chloro-4,5-dihydro-1*H*-imidazole, DCM, 20°C, 12-24 h; for R = H
Method B: *N*-*tert*-butoxycarbonyl-2-methylthio-4,5-dihydro-1*H*-imidazole, acetic acid, 60°C, 16 h; for R = 8-CH_3

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

Table 1. Binding affinities of compounds **5a-d** and **10a**,**b**.

Comp. No.	α_1, K_i (nM)	α_2, K_i (nM)	I_1, IC_{50} (nM)	I_2, K_i (nM)	Selectivity α_1/α_2
5a	1340 ± 7.6	3640 ± 456	123.8 ± 45.4	52.1 ± 25.7	0.368
5b	30.2 ± 2.4	6.09 ± 2.49	39.71 ± 6.05	431.7 ± 276.1	4.96
5c	27.1 ± 4.61	0.75 ± 0.11	1543 ± 1233	48.3 ± 5.93	36.1
5d	106.0 ± 14.07	3.1 ± 0.37	5777 ± 5652	49.8 ± 4.65	34.2
10a	69.8 ± 7.6	4.94 ± 0.66	1423 ± 112.4	14.4 ± 1.36	14,1
10b	528 ± 239	5244 ± 9.19	3076 ± 2647	62.7 ± 5.05	10.1

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.

	Time after application of tested compound (min) ΔMAP (mmHg)				
	2	5	15	30	60
5b n = 4	19.9 ± 3.9*	27.1 ± 3.3*	1.9 ± 5.9	-16.0 ± 1.9*	-23.6 ± 1.2*
5c n = 5	25.5 ± 1.7*	29.1 ± 1.8*	-6.3 ± 2.4	-19.0 ± 1.4*	-28.0 ± 1.8*
10a n = 4	23.7 ± 6.8*	37.7 ± 2.8*§	3.4 ± 2.7	-14.5 ± 3.6&	-26.2 ± 2.9*
10b n = 5	21.4 ± 3.8*	14.2 ± 6.7*	-14.4 ± 4.4#$	-14.5 ± 1.9 #	-13.7 ± 2.1 &
Control n = 5	0.0 ± 0.2	0.4 ± 0.7	-0.8 ± 0.7	-1.3 ± 2.0	-3.5 ± 1.8

Values are the mean ± SE. n – number of experiments. Comparisons were made using ANOVA with repeated measures and Fisher test. Significance: (*) $p < 0.001$, (#) $p < 0.002$, (&) $p < 0.05$ *vs*. control group; (§) $p < 0.001$ *vs*. **10b** group; ($) $p < 0.002$ *vs*. **5b** group.

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

	Time after application of tested compound (min) ΔHR (bpm)				
	2	5	15	30	60
5b n = 4	-170.4 ± 20.1*&	-131.7 ± 13.9*#	-91.9 ± 22.9*	-88.7 ± 20.0*	-100.6 ± 14.2*#
5c n = 5	-107.3 ± 26.9*#	-94.4 ± 12.8*	-142.3 ± 12.0*#	-154.4 ± 9.2*&	-145.1 ± 13.1*&
10a n = 4	-132.7 ± 24.8*§	-96.0 ± 19.1*	-92.0 ± 16.5*	-96.1 ± 19.7*	-91.7 ± 27.1*
10b n = 5	-61.7 ± 1.4	-79.1 ± 6.9*	-83.0 ± 9.8*	-76.8 ± 5.3*	-58.1 ± 9.3*
Control n = 5	-0.6 ± 1.1	-2.9 ± 0.9	-6.0 ± 1.5	-8.6 ± 2.7	-6.2 ± 6.3

Values are the mean ± SE. n – number of experiments. Comparisons were made using ANOVA with repeated measures and Fisher test. Significance: (*) $p < 0.001$ *vs*. control group; (#) $p < 0.05$, (&) $p < 0.001$, (§) $p < 0.01$ *vs*. **10b** group.

software (StatSoft, Inc., Tulsa, USA). 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.

RRSULTS 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 $LiAlH_4$ to give *N*-amino compounds **3** and **8**. Then, upon treatment of **3a**, **3b** and **8a** with 2-chloro-4,5-dihydro-1*H*-imidazole and **3c**, **3d** and **8b** with *N-tert*-butoxycarbonyl-2-methylthio-4,5-dihydro-1*H*-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

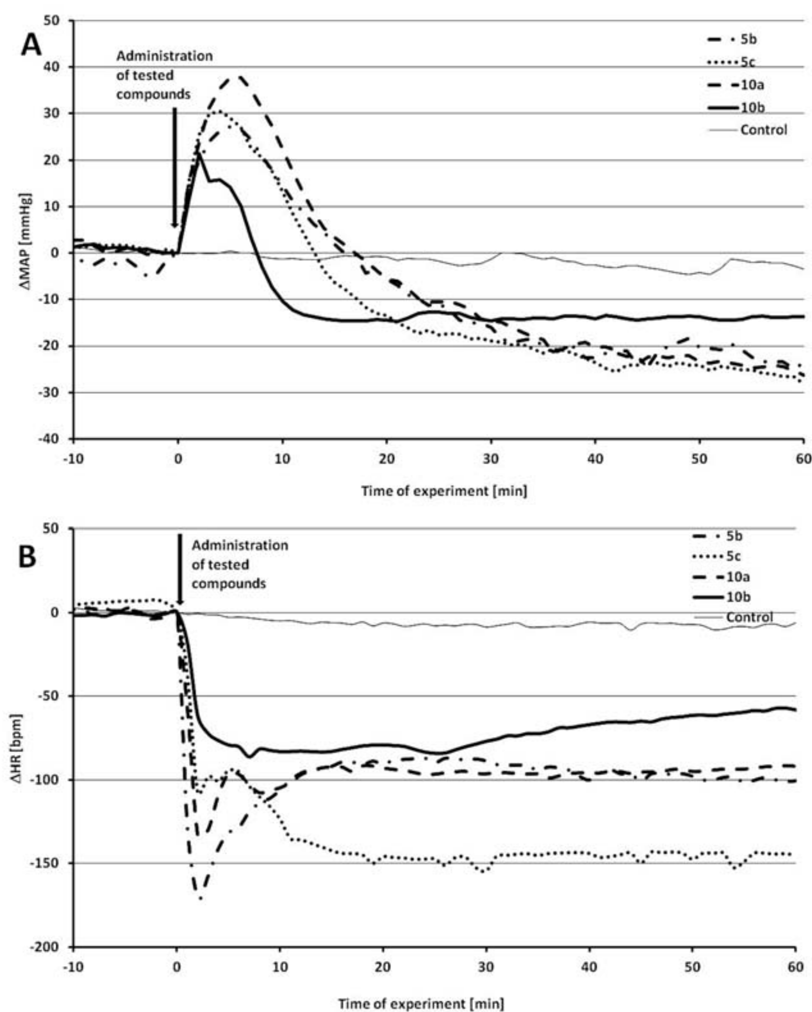


Figure 3. Effect of compounds: **5b**, **5c**, **10a**, **10b**, 100 ěg/kg b.w., and control group on (**A**) ΔMAP and (**B**) ΔHR (calculated as the difference in the MAP or HR between the sequential measurements and time 0 of the experiment) in rats. Each point represents the mean value of ΔMAP or ΔHR for four to five experiments

NaOH. No spectral data for both the free bases or corresponding hydrochloride salts have previously been described.

Binding affinities at α_1-, α_2-adrenoceptors and imidazoline I_1 and I_2 receptors

Radioligand binding experiments of α_2-adrenoceptors and imidazoline I_2 receptors were conducted using crude P_2 rat brain membranes, and crude P_2 rat kidney membranes were used for I_1 receptors. Equilibrium dissociation constants (K_i) 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_1- and α_2-adrenoceptors with K_i = 1340 nM and 3640 nM, respectively. Compound **5b** with CH_3 substituent at position 2 displayed enhanced activity at α_1- (K_i = 30.2 nM) and α_2- (K_i = 6.09 nM) receptors, but still a negligible α_1/α_2 selectivity ratio of 4.96. The highest difference in potencies at α_1- and α_2-adrenoceptors was showed by 7-CH_3 and 4-Cl -substituted indolines **5c** and **5d** (α_2 K_i = 0.75 and 3.1 nM, respectively; α_1/α_2 selectivity ratio = 36.19 and 34.24, respectively). It should be pointed out that unsubstituted 1,2,3,4-tetrahydroisoquinoline compound **10a** also displayed good affinity for α_2-adrenoceptors (K_i = 4.94 nM) and a moderate affinity for α_1-adrenoceptor (K_i = 69.8 nM), while the 8-CH_3 congener **10b** proved to be less potent.

It is worth mentioning here the noticeable affinities of indoline **5b** at imidazoline I_1 receptors (IC_{50} = 39.7 nM) and 1,2,3,4-tetrahydroquinoline **10a** at imidazoline I_2 receptors (K_i = 14.4 nM).

Effect on arterial blood pressure and heart rate

Intravenous administration of compounds **5b**,**c** and **10a**,**b** at dose 0.1 mg/kg in thiopental-anesthetized male Wistar rats caused a short-lasting pressor response after which significant reduction of arterial blood pressure was observed (Table 2, Fig. 3A). The most pronounced changes in blood pressure were observed for indoline **5c** and 1,2,3,4-tetrahydroquinoline derivative **10a**, i.e., the compounds with relatively high α_1- and α_2-adrenoceptor affinities (Table 1). The initially observed increase in blood pressure resulting from activation of vascular α_1-adrenoceptors (Table 2, Fig. 3A, ΔMAP = 29 and 37 mmHg, respectively) was followed by a long-lasting hypotensive effect (ΔMAP = -28 and -26 mmHg, respectively). Thus, in the circulatory system of Wistar rats the investigated compounds behaved as nonselective stimulators of both α_1- and α_2-adrenoceptors, which was further confirmed by a pronounced bradycardic effect elicited by these compounds (Table 3, Fig. 3B, ΔHR = -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_1-adrenoceptors albeit at much lower levels than β-adrenoceptors (37). However, the role of α_1-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_1-adrenoceptors inhibits cardiac excitation-contraction coupling through tyrosine phosphorylation of β_1-adrenoceptors (39). Moreover, experiments performed on human cardiac myocytes indicated expression of α_{1A}- and α_{1B}-adrenoceptors 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) might possibly be mediated by cardiac α_1-adrenoceptors activation.

On the other hand, cardiac function is under the control of the sympathetic and parasympathetic nervous systems. Whereas sympathetic stimulation leads to an increase of cardiac function, the effects of the parasympathetic system are the opposite and vagal stimulation exerts negative inotropic, negative chronotropic and negative chromotropic effects in the heart (42). These observations stay in agreement with our recent experiments performed on vagotomized rats, indicating that although cardiovascular effects of imidazoline compunds of type **B** (Fig. 1, X = NH, *marsanidine*, *7-Me-marsanidine*) are not mediated by the vagal nerves, vagotomy enhanced the sensitivity of sympathetic pathways for tested compounds (43). Therefore, the long-lasting heart rate decrease and hypotensive effect of tested compounds might be mediated through activation of the central α_2-adrenoceptors and the subsequent decrease of sympathetic activity [44, 45].

CONCLUSION

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_2-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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