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1-[(Imidazolidin-2-yl)imino]-1*H*-indoles as New Hypotensive Agents: Synthesis, In Vitro and In Vivo Biological Studies

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Abstract:

A series of 1-[(imidazolidin-2-yl)imino]-1*H*-indole analogues of hypotensive α_2-AR agonists 1-[(imidazolidin-2-yl)imino]-1*H*-indazoles, was synthesized and tested *in vitro* for their activities at α_1- and α_2-adrenoceptors as well as imidazoline I_1 and I_2 receptors. The most active 1-[(imidazolidin-2-yl)imino]-1*H*-indoles displayed high or moderate affinities for α_1-

and α_2-adrenoceptors and substantial selectivity for α_2-adrenoceptors over imidazoline-I_1 binding sites. The *in vivo* cardiovascular properties of indole derivatives **3** revealed that substitution at C-7 position of the indole ring may result in compounds with high cardiovascular activity. Among them, 7-fluoro congener **3g** showed the most pronounced hypotensive and bradycardic activities in this experiment at a dose as low as 10 μg/kg i.v. Metabolic stability of the selected compounds of type **3** were determined using both *in vitro* and *in silico* approaches. The results indicated that these compounds are not vulnerable to rapid first phase oxidative metabolism.

Key words: imidazolines, indoles, alpha-adrenoceptor ligands, circulatory activities, metabolic stability

Introduction

α-Adrenergic receptors (α-ARs) are involved in multiple physiological functions, particularly cardiovascular regulation. For example, activation of central α_2-ARs plays a prominent role in lowering blood pressure and, thus, it has been utilized clinically for many years in the treatment of hypertension (1,2). Other pharmacological effects of central α_2-ARs stimulation include behavioral functions, sedation, anxiolysis, analgesia and anaesthetic-sparing activity (3,4). α_2-ARs also mediate in insulin secretion, gastrointestinal motility, body temperature as well as seizure threshold (3,5-6). On the other hand, stimulation of α_1-ARs may protect from abnormal heart rate (7), whereas α_1-AR antagonists behave as vasodilators (8). The blockade of α_1-ARs may also be useful for the treatment of benign prostatic hyperplasia (8,9).

It is well known that imidazoline-containing compounds, such as clonidine or tolazoline, constitute the most vital set of drugs that interact with α-ARs (Figure 1, structure **A**). In addition, imidazoline I_1 binding sites (I_1-IBS or I_1-IRs for I_1-imidazoline receptors) have been postulated to participate in central antihypertensive effects of clonidine-like drugs (10,11). Thus, rational development of novel imidazoline-containing drugs has proven to be difficult owing to possible interaction with either α_1-ARs and α_2-ARs or I_1-imidazoline receptors. Moreover, it was found that even minor structural modification of the imidazoline-containing compounds could significantly affect their receptor affinity and selectivity (12,13).

Recently our SAR studies of imidazoline-containing α-adrenergic agents showed that the isosteric replacement of the indazole ring in 1-[(imidazolin-2-yl)methyl]indazoles **B** by the indole moiety led to compounds **C** (Figure 1), which exert different functional properties with respect to α-ARs. Indeed, imidazoline-containing indazoles **B** exhibited hypotensive properties due to α_1-AR antagonist activity (14), while their indole analogues **C** were described as partial α_{2A}-AR agonists showing clonidine-like cardiovascular pattern (15). Furthermore, we described 1-[(imidazolidin-2-yl)imino]-1*H*-indazoles **D** (Figure 1, marsanidine-like ligands), which proved to be hypotensive α_2-AR agonists (16-18). However, pharmacological effects of the displacement of the indazole ring in compounds **D** with the indole ring system have not been investigated previously. Therefore, the aim of this work was to determine the influence of the replacement of the indazole ring in 1-[(imidazolidin-2-yl)imino]-1*H*-indazoles **D** with the indole moiety on α-adrenergic affinity and selectivity as well as cardiovascular effects of the indole analogues **3**, thus obtained (Figure 1).

Methods and Materials

Chemistry

Melting points were measured on a Boetius apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer FTIR 1600 spectrometer. NMR spectra were recorded on a Varian Gemini 200 or a Varian Unity 500 apparatus. ^{1}H and ^{13}C chemical shifts were measured relative to the residual solvent signal at 7.26 ppm and 77.0 ppm ($CDCl_3$) or 2.50 ppm and 39.5 ppm (DMSO-d_6). Preparative thin layer chromatography was performed on silica gel 60 PF_{254} containing gypsum (Merck) with aid of Chromatotron® using the reported solvent systems. 1-Amino-1*H*-indoles **2a** (19), **2d** (20) and **2e** (21) were obtained according to published methods by reacting the commercially available 1*H*-indoles **1a, 1d** and **1e** with hydroxylamine-*O*-sulfonic acid (HOSA) as amination agent (20). 1-Amino-1*H*-indoles **2b** (22), **2c** (23) and **2j** (24) were synthesized as previously described from the commercially available 1*H*-indoles **1b-c** and **1j** using monochloramine as amination agent (23). The same procedure was applied to the synthesis of the previously not described 1-amino-1*H*-indoles **2f-i** (Appendix S1, Supporting Information). *N*-*tert*-butoxycarbonyl-2-methylthio-4,5-dihydro-1*H*-imidazole was obtained by reacting 2-methylthio-2-imidazoline with di-*tert*-butyl dicarbonate (25). *N*,*N'*-bis*(tert*-butoxycarbonyl)imidazolidine-2-thione was synthesized from imidazolidine-2-thione and di-*tert*-butyl dicarbonate (26).

Preparation of 1-[(Imidazolidin-2-yl)imino]-1*H*-indoles 3a-j

Synthesis of 1-[(imidazolidin-2-yl)imino]-1*H*-indoles 3a-i. **C**ompounds **3a-i** were obtained according to the method described by Sączewski *et al.* (27). A solution of the appropriate 1-amino-1*H*-indole (**2a-i**) (4.6 mmol) and *N*-*tert*-butoxycarbonyl-2-methylthio-4,5-dihydro-1*H*-imidazole (1.1 g, 5.1 mmol) in acetic acid (3 mL) was stirred at 62-65 °C (oil bath) for 16 h. Then the solvent was evaporated under reduced pressure, and the oily residue was treated with water (7 mL). The resulting suspension was made alkaline with 5% aqueous NaOH solution to pH 9.5 – 10, and extracted with dichloromethane (3 x 10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude product thus obtained was purified by preparative thin layer chromatography, followed by crystallization from suitable solvent if necessary.

The free bases **3a-i** were then converted into the corresponding water-soluble hydrochlorides with use of ca. 1.6 M methanolic solution of hydrochloride (in the case of **3a, 3d-e**), or ca. 2.5 M ethereal solution of hydrochloride (in the case of **3b-c**, **3f-h**) as well as hydrobromide salt with use of 48% hydrobromic acid solution (in the case of **3i**).

1-[(Imidazolidyn-2-yl)imino]-1*H*-indole (3a). Yield 0.50 g (55%); mp 140-142 °C (ethyl acetate/methanol, 9.5:0.5 v/v); IR (KBr, cm^{-1}) ν_{max} 3396, 3203, 2883, 1634, 1605, 1505, 1453, 1324, 1286, 1219, 1075, 1042, 745, 716; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.23-3.43 (m, 2H), 5.97 (s, 1H), 6.32 (d, J = 3.0 Hz, 1H), 6.57 (s, 1H), 6.89-7.07 (m, 2H), 7.13-7.15 (m, 2H), 7.50 (d, J = 7.7 Hz, 1H); ^{13}C NMR (50 MHz, DMSO-d_6,) δ 42.2, 42.8, 97.7, 109.9, 118.3, 120.3, 120.6, 126.0, 127.9, 134.6, 166.0. Anal. Calcd for $C_{11}H_{12}N_4$ (200.24): C, 65.98; H, 6.04; N, 27.98%. Found: C, 65.82; H, 5.97; N, 27.84%. **Hydrochloride of 3a**. Mp 211-213 °C (crystallization from ethanol/diethyl ether, 1:5 v/v); IR (KBr, cm^{-1}) ν_{max} 3256, 3154, 3090, 2893, 1667, 1630, 1457, 1309, 1225, 1081, 1048, 754. Anal. Calcd for $C_{11}H_{13}ClN_4$ (236.70): C, 55.82; H, 5.54; N, 23.67%. Found: C, 55.75; H, 5.39, N, 23.69%.

4-Fluoro-1-[(imidazolidin-2-yl)imino]-1*H*-indole (3b). Yield 0.67 g (67%); mp 156-157 °C (dichloromethane/ethyl acetate, 1:1 v/v → ethyl acetate); IR (KBr, cm^{-1}) ν_{max} 3402, 3172, 2955, 2877, 1643, 1620, 1501, 1227, 969, 739; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.40 (br s, 4H), 4.10-5.20 (br signal, 1H), 6.0-6.70 (br signal, 1H), 6.50 (d, J = 3.3 Hz, 1H), 6.69-6.78 (m, 1H), 7.04-7.09 (m, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 42.8, 94.8, 103.8 (d, J = 19.0 Hz),

105.7 (d, J = 3.4 Hz), 115.3 (d, J = 22.9 Hz), 122.2 (d, J = 8.0 Hz), 127.4, 137.5 (d, J = 11.4 Hz), 156.5 (d, J = 247 Hz), 166.5. Anal. Calcd for $C_{11}H_{11}FN_4$ (218.22): C, 60.55; H, 5.08; N, 25.67%. Found: C, 60.41; H, 5.19, N, 25.58%. **Hydrochloride of 3b**. Mp 181-182 °C; IR (KBr, cm^{-1}) ν_{max} 3086, 2922, 1656, 1622, 1492, 1236, 974, 743. Anal. Calcd for $C_{11}H_{12}ClFN_4$ (254.68): C, 51.88; H, 4.75; N, 21.99%. Found: C, 52.03; H, 4.87, N, 22.18%.

5-Fluoro-1-[(imidazolidin-2-yl)imino]-1*H*-indole (3c). Yield 0.46 g (46%); mp 58-60 °C (dichloromethane/ethyl acetate, 1:1 v/v → ethyl acetate); IR (KBr, cm^{-1}) ν_{max} 3397, 3171, 2954, 2878, 1644, 1615, 1427, 1283, 1211, 1117, 796, 715; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.42 (br s, 4H), 4.0-5.70 (br signal, 2H), 6.37 (d, J = 3.14 Hz, 1H), 6.91 (dt, J_1 = 2.5 Hz, J_2 = 9.1 Hz, 1H), 7.10 (d, J = 3.2 Hz, 1H), 7.16-7.25 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 42.8, 98.7 (d, J = 4.9 Hz), 105.4 (d, J = 23.6 Hz), 109.9 (d, J = 20 Hz), 110.3 (d, J = 2.9 Hz), 126.3 (d, J = 10.4 Hz), 128.7, 131.2, 158.0 (d, J = 233.4 Hz), 166.4. Anal. Calcd for $C_{11}H_{11}FN_4$ (218.22): C, 60.55; H, 5.08; N, 25.67%. Found: C, 60.63; H, 5.25, N, 25.69%. **Hydrochloride of 3c**. Mp 206-208 °C; IR (KBr, cm^{-1}) ν_{max} 3291, 3132, 3086, 2891, 1655, 1633, 1473, 1241, 1125, 946, 868, 809, 756 Anal. Calcd for $C_{11}H_{12}ClFN_4$ (254.68): C, 51.88; H, 4.75; N, 21.99%. Found: C, 51.73; H, 4.71, N, 22.08%.

5-Chloro-1-[(imidazolidin-2-yl)imino]-1*H*-indole (3d). Yield 0.54 g (50%); mp 108-110 °C (ethyl acetate/methanol, 9.5:0.5 v/v, crystallization from ethyl acetate); IR (KBr, cm^{-1}) ν_{max} 3405, 3391, 3133, 2965, 28757, 1643, 1604, 1498, 1447, 1283, 1213, 1076, 1042, 904, 792, 754, 711; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.35 (br s, 4H), 6.16 (br s, 1H), 6.34 (d, J = 2.9 Hz, 1H), 6.68 (br s, 1H), 7.04 (d, J = 8.5 Hz, 1H), 7.15 (d, J = 8.5 Hz, 1H), 7.24 (d, J = 2.9 Hz, 1H), 7.55 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 42.5, 97.7, 111.3, 119.5, 120.6, 123.1, 127.0, 129.7, 133.1, 166.0. Anal. Calcd for $C_{11}H_{11}ClN_4$ (234.69): C, 56.30; H, 4.72; N, 23.87%. Found: C, 56.24; H, 4.65; N, 23.80%. **Hydrochloride of 3d**. Mp 204-207 °C (crystallization from ethanol/diethyl ether, 1:5 v/v); IR (KBr, cm^{-1}) ν_{max} 3120, 3014, 2916, 1644, 1623, 1456, 1294, 1226, 1080, 1047, 792, 753, 720. Anal. Calcd for $C_{11}H_{12}Cl_2N_4$ (271.15): C, 48.73; H, 4.46; N, 20.66%. Found: C, 48.64; H, 4.31; N, 20.35%.

1-[(Imidazolidin-2-yl)imino]-5-methyl-1*H*-indole (3e). Yield 0.37 g (38%); mp 63-65 °C (ethyl acetate/methanol, 9.5:0.5 v/v); IR (KBr, cm^{-1}) ν_{max} 3387, 3173, 2871, 1644, 1499, 1473, 1328, 1283, 1211, 1077, 794, 756, 711; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.36

(s, 3H), 3.26-3.29 (m, 2H), 3.37-3.40 (m, 2H), 5.92 (s, 1H), 6.22 (d, *J* = 2.6 Hz, 1H), 6.54 (s, 1H), 6.87 (d, *J* = 7.8 Hz, 1H), 7.04 (d, *J* = 8.3 Hz, 1H), 7.08 (d, *J* = 2.6 Hz, 1H), 7.27 (s, 1H); ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ 21.9, 42.6, 43.3, 97.6, 110.1, 120.4, 122.7, 126.7, 127.1, 128.3, 133.5, 167.0. Anal. Calcd for $C_{12}H_{14}N_4$ (214.27): C, 67.27; H, 6.59; N, 26.15%. Found: C, 67.18; H, 6.47; N, 25.97%. **Hydrochloride of 3e**. Mp 224-226 °C (crystallization from acetonitrile); IR (KBr, cm^{-1}) ν_{max} 3132, 3079, 3025, 2926, 1651, 1630, 1471, 1327, 1299, 1224, 1085, 800, 762, 727. Anal. Calcd for $C_{12}H_{15}ClN_4$ (250.73): C, 57.48; H, 6.03; N, 22.35%. Found: C, 57.39; H, 5.97; N, 22.34%.

6-Fluoro-1-[(imidazolidin-2-yl)imino]-1*H*-indole (3f). Yield 0.46 g (46%); mp 118-120 °C (dichloromethane/ethyl acetate, 1:1 v/v → ethyl acetate); IR (KBr, cm^{-1}) ν_{max} 3395, 3169, 2955, 2877, 1643, 1614, 1483, 1329, 1284, 1218, 1166, 943, 800, 710; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.44 (br s, 4H), 4.10-6.20 (br signal, 2H), 6.40 (d, *J* = 3.3 Hz, 1H), 6.83 (dt, J_1 = 2.4 Hz, J_2 = 8.8 Hz, 1H), 6.97 (dd, J_1 = 2.1 Hz, J_2 = 9.9 Hz, 1H), 7.05 (d, *J* = 3.1 Hz, 1H), 7.48 (dd, J_1 = 5.2 Hz, J_2 = 8.7 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 42.8, 95.7 (d, *J* = 26.5 Hz), 99.2, 108.1 (d, *J* = 24.8 Hz), 121.6 (d, *J* = 10.1 Hz), 122.7, 127.8 (d, *J* = 3.7 Hz), 134.6 (d, *J* = 12.4 Hz), 160.0 (d, *J* = 237.6 Hz), 166.3. Anal. Calcd for $C_{11}H_{11}FN_4$ (218.22): C, 60.55; H, 5.08; N, 25.67%. Found: C, 60.71; H, 5.14, N, 25.74%. **Hydrochloride of 3f**. Mp 214-216 °C; IR (KBr, cm^{-1}) ν_{max} 3128, 2921, 1661, 1621, 1481, 1210, 943, 842, 801. Anal. Calcd for $C_{11}H_{12}ClFN_4$ (254.68): C, 51.88; H, 4.75; N, 21.99%. Found: C, 51.99; H, 4.92, N, 21.79%.

7-Fluoro-1-[(imidazolidin-2-yl)imino]-1*H*-indole (3g). Yield 0.39 g (39 %); 135-137 °C (dichloromethane/ethyl acetate, 1:1 v/v → ethyl acetate); IR (KBr, cm^{-1}) ν_{max} 3403, 3172, 2955, 2876, 1643, 1619, 1571, 1500, 1283, 1237, 1084, 782, 715; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.47 (br s, 4H), 4.10-5.0 (br signal, 1H), 5.50-6.50 (br signal, 1H), 6.41 (t, *J* = 3.0 Hz, 1H), 6.75-6.97 (m, 2H), 7.04 (d, *J* = 3.0 Hz, 1H), 7.32 (d, *J* = 7.7 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 42.9, 99.5, 107.1 (d, *J* = 17.3 Hz), 116.7 (d, *J* = 3.6 Hz), 119.2 (d, *J* = 6.4 Hz), 122.6 (d, *J* = 9.2 Hz), 128.9, 130.6 (d, *J* = 5.0 Hz), 149.9 (d, *J* = 245.8 Hz), 166.9. Anal. Calcd for $C_{11}H_{11}FN_4$ (218.22): C, 60.55; H, 5.08; N, 25.67%. Found: C, 60.61; H, 5.28, N, 25.51%. **Hydrochloride of 3g**. Mp 201-203 °C; IR (KBr, cm^{-1}) ν_{max} 3235, 3076, 2724, 1657, 1616, 1581, 1485, 1288, 1244, 784, 717, 670. Anal. Calcd for $C_{11}H_{12}ClFN_4$ (254.68): C, 51.88; H, 4.75; N, 21.99%. Found: C, 52.14; H, 4.41, N, 21.92%.

7-Chloro-1-[(imidazolidin-2-yl)imino]-1*H*-indole (3h). Yield 0.49 g (45%); colourless oil (dichlorometane → dichloromethane/ethyl acetate, 1:1 v/v → ethyl acetate); IR (liquid film, cm^{-1}) ν_{max} 3404, 3167, 2873, 1642, 1498, 1278, 1192, 935, 782, 717; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.33-3.62 (m, 4H), 4.32 (br s, 1H), 6.42-6.43 (m, 1H), 6.50 (br s, 1H), 6.94 (t, J = 7.7 Hz, 1H), 7.07-7.09 (m, 2H), 7.47 (dd, J_1 = 7.7 Hz, J_2 = 0.8 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 42.8, 98.9, 116.4, 119.5, 122.8, 129.2, 129.4, 167.2. Anal. Calcd for $C_{11}H_{11}ClN_4$ (234.69): C, 56.30; H, 4.72; N, 23.87%. Found: C, 56.41; H, 4.68; N, 23.80%. **Hydrochloride of 3h**. Mp 231-233 °C; IR (KBr, cm^{-1}) ν_{max} 3094, 3005, 2903, 2804, 1655, 1478, 1272, 1194, 1147, 793. Anal. Calcd for $C_{11}H_{12}Cl_2N_4$ (271.15): C, 48.73; H, 4.46; N, 20.66%. Found: C, 48.85; H, 4.32; N, 20.53%.

1-[(Imidazolidin-2-yl)imino]-7-methoxy-1*H*-indole (3i). Yield 0.65 g (61%); mp 179-180 °C (ethyl acetate/methanol, 9.5:0.5 v/v); IR (KBr, cm^{-1}) ν_{max} 3392, 3169, 2873, 1645, 1608, 1572, 1501, 1479, 1284, 1257, 1194, 1093, 1082, 1031, 965, 783, 711, 690; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.45 (s, 4H), 3.88 (s, 3H), 4.75 (br s, 2H), 6.37 (d, J = 3.0 Hz, 1H), 6.59 (d, J = 7.5 Hz, 1H), 6.95 (t, J = 7.5 Hz, 1H), 7.00 (d, J = 3.0 Hz, 1H), 7.24 (d, J = 7.5 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 43.4, 56.7, 99.2, 103.4, 114.4, 119.9, 124.2, 128.4, 129.4, 147.6, 167.2. Anal. Calcd for $C_{12}H_{14}N_4O$ (230.27): C, 62.58; H, 6.13; N, 24.33%. Found: C, 62.47; H, 5.98, N, 24.29%. **Hydrobromide of 3i**. Mp 150-152 °C; IR (KBr, cm^{-1}) ν_{max} 3135, 2906, 1656, 1631, 1582, 1486, 1463, 1443, 1385, 1338, 1286, 1257, 1097, 1084, 1050, 1007, 966, 784, 721, 690. Anal. Calcd for $C_{12}H_{15}BrN_4O$: C, 46.32; H, 4.86; N, 18.00%. Found: C, 46.02; H, 5.12; N, 17.73%.

Synthesis of 1-[(imidazolidin-2-yl)imino]-7-methyl-1*H*-indole (3j). **C**ompound **3j** was obtained according to the method described by F. Sączewski *et al.* (16). *Step 1*. To a stirred solution of 1-amino-7-methyl-1*H*-indole (**2j**) (0.40 g, 2.7 mmol), *N*,*N'*-bis*(tert*-butoxycarbonyl)imidazolidine-2-thione (1.23 g, 4.06 mmol), and triethylamine (0.97 g, 1.33 mL, 9.6 mmol) in DMF (4 mL) was added $HgCl_2$ (1.1 g, 4.06 mmol) at 0 °C. The reaction mixture was stirred for an additional 20 min at 0 °C, and then for 5 days at room temperature. The resulting dark-grey reaction mixture was diluted with ethyl acetate (40 mL) and filtered through a celite pad. The filtrates were washed with brine (3x) and water (1x), dried over $MgSO_4$, and finally concentrated under vacuum. The viscous residue was subjected to preparative thin layer chromatography eluting with hexane/dichloromethane (1:5 v/v)

followed by dichloromethane to afford 0.30 g 1-{[1,3-di(*tert*-butoxycarbonyl)imidazolidin-2-yl)]imino}-7-methyl-1*H*-indole (**4**). Yield 26%; mp 128-130 °C; IR (KBr, cm^{-1}) ν_{max} 2980, 2957, 2923, 1740, 1716, 1652, 1475, 1455, 1390, 1309, 1252, 1153, 1139, 988, 845, 787, 765, 717; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.83 (s, 9H), 1.50 (s, 9H), 2.70 (s, 3H), 3.79-3.87 (m, 4H), 6.38 (d, *J* = 3.3 Hz, 1H), 6.83-6.89 (m, 2H), 7.29-7.32 (m, 1H), 7.41 (d, *J* = 3.3 Hz, 1H). Anal. Calcd for $C_{22}H_{30}N_4O_4$ (414.50): C, 63.75; H, 7.30; N, 13.52%. Found: C, 63.89; H, 7.46; N, 13.49%. The obtained compound **4** was used for the next step without further purification. *Step 2*. A solution of the Boc-protected imidazolidine **4** (0.22 g, 0.72 mmol) in trifluoroacetic acid in dichloromethane (4 mL, 1:1, v/v) was stirred for 2 h at room temperature, and then the solvent and excess of trifluoroacetic acid were evaporated under reduced pressure. The oily residue was treated with water, made alkaline with 5% aqueous NaOH solution (pH 10-10.5), and the mixture was extracted with dichloromethane (3 x 15 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated to dryness. The crude product **3j** thus obtained was purified by preparative thin layer chromatography eluting with hexane/dichloromethane (9.5:0.5 v/v). The free base **3j** was then converted into hydrobromide salt by adding 48% hydrobromic acid solution to the solution of 1-[(imidazolidin-2-yl)imino]-7-methyl-1*H*-indole (**3j**) in methanol.

1-[(Imidazolidin-2-yl)imino]-7-methyl-1*H*-indole (3j). Yield 0.07 g (44%); mp 61-64 °C; IR (KBr, cm^{-1}) ν_{max} 3392, 3185, 2953, 2877, 1644, 1499, 1457, 1283, 1076, 1042, 781, 718; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.64 (s, 3H), 3.37 (br s, 2H), 3.50 (br s, 2H), 4.58 (br signal, 2H), 6.42-6.43 (m, 1H), 6.90 (d, *J* = 6.8 Hz, 1H), 6.95-6.98 (m, 1H), 7.00 (d, *J* = 2.9 Hz, 1H), 7.45 (d, *J* = 7.8 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) 18.7, 43.2, 99.3, 119.1, 119.7, 122.1, 124.1, 127.3, 128.2, 133.4, 167.1. Anal. Calcd for $C_{12}H_{14}N_4$ (214.27): C, 67.27; H, 6.59; N, 26.15. Found: C, 67.38; H, 6.31; N, 25.87%. **Hydrobromide of 3j**. Mp: 189-192 °C (crystallization from ethanol/diethyl ether, 1:5 v/v); IR (KBr, cm^{-1}) ν_{max} 3190, 3095, 2925, 1654, 1606, 1458, 1384, 1278, 1214, 1078, 782, 721. Anal. Calcd for $C_{12}H_{15}BrN_4$ (295.18): C, 48.83; H, 5.12; N, 18.98%. Found: C, 48.67; H, 4.93; N, 18.85%.

Pharmacological methods

Radioligand binding assays

I_1-imidazoline site binding assay. Kidneys were obtained *post mortem* from male Sprague-Dawley rats (250-280 g) and crude P_2 membranes prepared according to the methods of Lione *et al.* (28). Binding of [^{3}H]clonidine (3 nM, Perkin-Elmer) was investigated in the presence of 10 µM rauwolscine to preclude radioligand binding to α_2-ARs. The specific component was defined by 10 µM rilmenidine; under these conditions, the site labeled represents a model of the central I_1 binding site (29). 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 4.03 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-ARs, α_2-ARs and I_2-binding site assays. Brains were obtained *post mortem* from male Sprague-Dawley rats (250-280 g) and crude P_2 membranes were prepared (28). 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) (30) (1 nM), the α_1-AR antagonist radioligand [^{3}H]prazosin (1 nM) or the α_2-AR antagonist radioligand [^{3}H]RX821002 (2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1*H*-imidazole) (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) (31) for I_2 binding, 10 µM phenylephrine for α_1-ARs and 10 µM rauwolscine to define α_2-AR 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 (32). The resulting values are given as the means ±SEM (standard error of measurement) 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 commercial rodent chow (Labofeed-B, Poland). Tap water was available ad libitum. Rats were anesthetized by *i.p.* injection of thiopental (Sandoz, Austria) at 70 mg/kg body weight and anesthesia was maintained 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. Tracheotomy was performed. Catheters were inserted into the carotid artery for monitoring of MAP and HR, 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 a stable baseline. 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 compound was administered as a 100 μl bolus through the venous catheter at doses of either 0.01 or 0.1 mg/kg depending on cardiovascular potency of a tested compound. The time of administration of a compound was assumed as "time 0". MAP and HR were monitored directly and sampled continuously at 100 Hz as described previously (33), using Biopac Systems, Inc., Model MP 100 (Goleta, CA, USA). The recorded results were elaborated with the help of the ACQKnowledge (Goleta, CA, USA) analysis system and were selected, scaled and filtered to remove accidental signal disturbances. The recorded time domain transient data are presented as graphs with the help of Excel (Microsoft, USA).

ANOVA was performed for ΔMAP and ΔHR, calculated as the difference in MAP and in HR from baseline measurements ("time 0") for each group, as described previously (33). This allowed for direct comparison of responses to treatment between groups. Data were analyzed by ANOVA for repeated measurements, using Statistica StatSoft software (StatSoft, Inc., Tulsa, USA), after test compound or vehicle administration. 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.

Metabolic stability

Stock solutions of studied compounds were prepared at concentration of 1 mM in DMSO. Incubations were performed in the presence of 1 mM of NADPH (Sigma-Aldrich, St. Louis, MO, USA) and 1 mg/mL of human liver microsomes (Sigma-Aldrich, St. Louis, MO, USA) in potassium phosphate buffer (0.1 M, pH 7.4). Incubation was carried out in incubator at 37 °C with agitation and started by addition of compound of interest at final concentration of 1 μM (34,35). Directly after start of incubation and after 5, 15, 30, 45 and 60 min reaction was ended by adding the equal volume of cold acetonitrile containing 1 μM of IS (alprazolam). All samples were immediately centrifuged (10 min 10 000 rpm) and resulted supernatant was directly analyzed or kept in −80 °C until LC-MS analysis. $\ln(A/A_{IS})$ was plotted versus incubation time (A – peak area of compound of interest, A_{IS} – peak area of internal standard). *In vitro* metabolic half-life ($t_{1/2}$) was calculated from the slope of the linear regression, according to equation reported in (34,35).

LC-MS analysis was performed on an Agilent 1260 system coupled to SingleQuad 6120 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Ascentis Express ES-CN column (2.1 x 100 mm, 2.7 μm, Sigma-Aldrich, St. Louis, MO, USA) was used in reversed-phase mode with gradient elution starting with 5% of phase A (10 mM ammonium formate buffer in water, pH 3) and 95% of phase B (10 mM ammonium formate in acetonitrile-water mixture, 50:5 v/v, pH 3). The amount of phase B was linearly increased to 100% in 5 minutes. Total analysis time was 10 min at 25 °C, flow rate was 0.4 mL/min and the injection volume was 10 μL. The mass spectrometer was equipped with electrospray ionization source and ionization mode was positive. Mass analyzer was set individually to each compound to detect pseudomolecular ions $[M+H^+]$. MSD parameters of the ESI source were as follows: nebulizer pressure 50 psig (N_2), drying gas 13 mL/min (N_2), drying gas temperature 300 °C, capillary voltage 3.5 kV, fragmentor voltage 100 V.

Metabolic stability was additionally assessed by *in silico* technique proposed by Zaretzki *et al.* based on a model build on molecular descriptors and trained using neural networks (36).

X-ray structure analysis

Diffraction data for single crystal of compound **3d** were collected with an Oxford Diffraction, XcaliburE diffractometer at 293(2) K. Using Olex-2 (37), the structure was solved with SIR2004 (38) using direct methods and refined with ShelXL-2014 (39). H atoms bonded to C were placed geometrically and refined as riding on their carriers. H atoms bonded to N were freely refined with isotropic temperature factors.

Crystal data for $C_{11}H_{11}ClN_4$ (**3d**) (M = 234.69 g/mol): monoclinic, space group $P2_1/c$ (no. 14), a = 11.9693(6) Å, b = 9.1986(4) Å, c = 21.5853(11) Å, β = 105.513(5)°, V = 2290.0(2) Å^3, Z = 8, T = 293(2) K, μ(MoKα) = 0.311 mm^{-1}, *Dcalc* = 1.361 g/cm^3, 14010 reflections measured (8.342° ≤ 2Θ ≤ 50.05°), 4016 unique (R_{int} = 0.0375, R_{sigma} = 0.0479) which were used in all calculations. The final R_1 was 0.0473 (I > 2σ(I)) and wR_2 was 0.1038 (all data). CCDC deposit number: CCDC 1479707.

Results and Discussion

Chemistry

The known 1-amino-1*H*-indoles **2a** (19), **2b** (22), **2c** (23), **2d** (20), **2e** (21) and **2j** (24) as well as the novel derivatives **2f-i** were prepared using previously described procedures (20,23) (Scheme S1, Supporting Information). As shown in Scheme 1, 1-amino-1*H*-indoles **2a-i** were then reacted with *N*-Boc-2-methylthio-4,5-dihydro-1*H*-imidazole (25) in acetic acid to obtain, after deprotection of the formed intermediates **A**, the target free bases **3a-i** (27). The synthesis of the analogue **3j** was accomplished by reacting 1-amino-1*H*-indole **2j** with *N,N'*-bis-Boc-imidazolidine-2-thione (26) in the presence of $HgCl_2$, followed by cleavage of the protecting group upon treatment of the Boc-protected compound **4** with TFA/CH_2Cl_2 (Scheme 1) (16).

For biological studies, compounds **3a-j** were converted into water-soluble hydrochlorides or hydrobromides with the use of methanolic or ethereal solution of hydrochloride or hydrobromic acid solution, respectively.

Structures of the newly prepared compounds **3a-j** were confirmed by C, H, N elemental analyses, IR and NMR spectroscopic data as well as X-ray structure analysis of the compound **3d** (Figure 2).

Single X-ray analysis of compound **3d** confirmed that as expected, the imidazolidin-2-imine tautomer exists in the crystalline phase. Moreover, comparison of the crystal structures of indole **3d** and 4-Cl-marsanidine of type **D** (16) revealed that both compounds in solid phase adopt similar conformation, where benzazole and imidazolidin-2-imine fragments are nearly perpendicular (dihedral angle of 87.04° and 80.75°, respectively).

As revealed by the data in Table 1, the tested compounds **3a-j** were in general nearly inactive at the I_1 binding sites (K_i values in the range of 607–159000 nM), with the exception of the 4-F-indole derivative **3b** having moderate affinity for I_1-receptors (K_i = 241 nM). Similar results were obtained for imidazoline I_2 receptors (K_i values ranging from 354 to 2708 nM).

On the other hand, the indole derivatives **3a-j** were bound with moderate or high affinity to α-ARs. Accordingly, the unsubstituted compound **3a** showed good affinity for α_1-ARs (K_i = 70.2 nM) and high affinity for α_2-ARs (K_i = 5.33 nM). However, placement of substituents at positions C-4 or C-5 of the indole ring resulted in 3- to 12-fold decreases of the binding properties (**3b**: α_1K_i = 348 nM, α_2K_i = 63.6 nM; **3c**: α_1K_i = 165 nM, α_2K_i = 23.4 nM; **3d**: α_1K_i = 214 nM, α_2K_i = 57.9 nM; **3e**: α_1K_i = 118 nM, α_2Ki = 16.5 nM), while the 6-F- substituted derivative **3f** stayed equipotent to its unsubstituted counterpart **3a** (α_1K_i = 70.77 nM and α_2K_i = 7.073 nM). Interestingly, within a series of 7-substituted derivatives, the 7-F, 7-Cl and 7-CH_3 analogues **3g**, **3h** and **3j** retained comparably high affinity for α_2-ARs, and displayed the following K_i values: 12 nM for **3g** (7-F), 9.95 nM for **3h** (7-Cl) and 12.09 nM for **3j** (7-CH_3), whereas their potencies for α_1-ARs were much more different. It appeared that the 7-Cl congener **3h** was 4-times more active at α_1-ARs than 7-F- and 7-CH_3-substituted indoles **3g** and **3j** (K_i of 38.85, 167 and 166 nM, respectively). On the other hand, placement of OCH_3 group at position 7 (**3i**) reduced both the α_1- and α_2-AR interactions (K_i = 807 and 151 nM, respectively). The data presented in Table 2 indicate that the presence of a small substituent at position 7 characterized by positive lipophilic contribution is suitable for high affinity at α_2-ARs (**3g**: 7-F π = 0.14; **3h**: 7-Cl π = 0.71; **3j:** 7-CH_3 π = 0.56) (40). Moreover, 7-Cl substituent, which exhibits high lipophilicity as well as relatively high electronegativity (41), is responsible for binding affinity to α_1-ARs.

In comparison to the previously investigated indazole analogues **D** (Table 1, values given in parentheses) (16,17,27), the indoles **3a-j** displayed 2- to 7-fold increases in receptor binding affinity for α_2-ARs with different degrees of selectivity for α_2-ARs versus I_1 binding sites (I_1/α_2 selectivity ratios ranging from 4 to 9636). The highest difference in potencies at α_2-ARs and I_1-imidazoline receptors was showed by unsubstituted indole **3a** as well as 5-CH_3- and 7-F-substituted derivatives **3e** and **3g** (I_1/α_2 selectivity ratio of 1300, 9636 and 1269, respectively). It is worth noting that the investigated indole derivatives **3** displayed relatively low selectivity for α_2-ARs versus α_1-ARs (α_1/α_2 selectivity ratios in the range of 3.7–14, Table 1).

In vivo *cardiovascular effects*

To determine the effects of the displacement of the indazole ring in 1-[(imidazolidin-2-yl)imino]-1*H*-indazoles **D** with the indole ring on the cardiovascular properties, the newly prepared compounds **3** were evaluated in anesthetized rats by directly measuring changes in blood pressure and heart rate after intravenous administration at doses of either 0.01 or 0.1 mg/kg depending on cardiovascular potency of a tested compound and using previously described procedure (33). The post-infusion data were compared to baseline values and are presented as ΔMAP and ΔHR in Table 3.

First, most of the tested compounds **3** elicited a biphasic effect on blood pressure after intravenous administration that is transient hypertension followed by a prolonged hypotension effect. The observed changes were similar to those obtained in the case of marsanidine-like ligands **D** (16,17) and might be due to the stimulation of peripheral α_1-ARs or vascular α_2-ARs (a short-term increase in blood pressure) followed by activation of central α_2-ARs (a long-lasting hypotensive effect) (3,42,43). The pressure phase was accompanied by a marked bradycardia, which persisted through the longer hypotensive phase. The above cardiovascular effects elicited by **3a**, **3f** and **3g** are also presented in Figures S1 and S2 (Supporting Information).

In our previous SAR analysis on marsanidine-like ligands **D** (16,17,44) it has been revealed that substitution at the C-7 atom of the indazole ring with chlorine, fluorine or methyl group led to compounds with high cardiovascular properties, wherein the greatest hypotensive activity was noted for the congener bearing a fluorine that is 7-F-marsanidine of

type **D** (17). A similar trend was found for indoles **3** as 7-F-substituted derivative **3g** displayed the largest hemodynamic effects in the respective **3a-j** series (Table 3). It should be mentioned that hypotensive and negative chronotropic effects of **3g** administered at a dose of 0.01 mg/kg (ΔMAP = −65.0 mmHg, ΔHR = −148 bpm) were larger than those of 7-F-marsanidine at a higher dose of 0.1 mg/kg (ΔMAP = −59.4 mmHg, ΔHR = −128 bpm) (17). In comparison to the indazole ring system the indole moiety is more lipophilic ($logP_{indole}$ 2.14 versus $logP_{indazole}$ 1.77) (40). Therefore it appears that the more lipophilic nature and the higher α_2-binding affinity of 7-F-indole **3g** with respect to that of 7-F-indazole **D** may facilitate its ability to penetrate the brain-blood-barrier and account for its more efficacious activity than 7-F-indazole **D**, which is a partial α_2-AR agonist (17,18). In addition, under identical experimental conditions, changes of ΔMAP induced by **3g** were much greater as compared to those of **3a** and **3f** (Table 3, Figure S1) even though they showed higher activity at α_2-ARs (Table 1). Similarly, **3g** administration resulted in a greater decrease of ΔHR in comparison to that of **3a** and **3f** (Table 3, Figure S2).

As expected, 7-Cl and 7-CH_3-substitued derivatives **3h** and **3j** with high affinity to α_2-ARs also displayed efficacious hypotensive effects at a dose of 0.1 mg/kg (ΔMAP = –42.3 and –40.2 mmHg, respectively), however slightly lower than those of their 7-Cl (ΔMAP = –49.6 mmHg) (17) and 7-CH_3 (ΔMAP = –43.5 mmHg) (16) indazole analogues **D** at the same dose. Moreover, intravenous administration of these compounds elicited a significant bradycardic effects (ΔHR = −124.6 and −158.2 bpm, respectively). On the other hand, 5-CH_3-substituted indole **3e** with similar receptor affinity profile as compared to 7-CH_3 congener **3j** and the highest α_2- versus I_1-selectivity of 9636 (Table 1) exhibited much less pronounced cardiovascular activity (ΔMAP = –21.8 mmHg, ΔHR = −94.8 bpm). It should be pointed out that 4-F congener **3b**, characterized by good affinity for α_2-ARs and moderate affinity for I_1 imidazoline binding sites, showed greater hypotensive activity (ΔMAP = –51.7 mmHg) than that of **3h** and **3j**. Therefore, both I_1-imidazoline and α_2-adrenergic receptors might be possible mediators of the observed hypotensive effect of the tested compound **3b** (10-11,16). Finally, much lower cardiovascular activity was observed for 5-Cl and 7-OMe derivatives **3d** and **3i** (**3d**: ΔMAP = –11.4 mmHg, ΔHR = −39.9 bpm; **3i**: ΔMAP = –16.6 mmHg, ΔHR = −66.0 bpm), although they displayed relatively moderate affinity for α_2-ARs (K_i = 57.9 and 151 nM, respectively).

It is generally assumed that a short-time hypertensive phase followed by a long lasting decrease in blood pressure with accompanying bradycardia is a common effect of the centrally acting α_2-AR agonists, such as clonidine (45,46). The bradycardia induced by α_2-AR agonists relates to their central sympatholytic action with unopposed vagal tone, presynaptic inhibition of noradrenaline release, or direct vagotonic effect (6,47). Recently, our studies concerning cardiovascular effects of marsanidine and 7-CH_3-marsanidine in vagotomised rats have revealed that cardiovascular activities of these compounds are not due to vagal nerves, nevertheless, vagotomy enhanced sensitivity of the sympathetic pathways for the tested compounds (48). Hence, we propose that the long-lasting heart rate decrease and hypotensive effect of the tested imidazoline-containing indoles **3** result from their central sympathetic depression. However, taking into account their relatively high or moderate affinities to α_1-ARs, a contribution of α_1-ARs to cardiac function can not be excluded (49-51).

Metabolic stability

Metabolic stability assessed in the presence of human liver microsomes and NADPH as a cofactor showed that the studied compounds **3a**, **3f** and **3h** were stable under these conditions. After 60 min of incubation *ca* 90% of studied compounds remained unchanged. Additionally, XenoSite, *in silico* model proposed by Zaretzki *et al*. (36) showed that the studied derivatives **3a**, **3f** and **3h** are characterized by low vulnerability to undergo metabolic reactions mediated by cytochrome P-450 as depicted in Figure S3 (Supporting Information). Our results indicate high stability of the tested compounds, especially for first phase metabolism.

Conclusion

The displacement of the indazole ring in marsanidine-like ligands **D** (16,17) with the indole moiety results in compounds **3** with higher affinity at α_2-ARs and high or moderate affinity at α_1-ARs. The pronounced hypotensive and bradycardic effects of novel α-adrenergic ligands **3g**, **3h** and **3j** are in agreement with our previous observation that C-7 substitution of the azaaromatic ring, such as indazole (16,17,44), plays a crucial role in cardiovascular activities of this class of imidazoline-containing compounds. Similar hemodynamic activity profile of marsanidine-like compounds **D** and indole derivatives **3** suggests that the hypotensive effect of indoles **3** is mediated by central α_2-ARs. In

contradistinction to our earlier observation that the bioisosteric 1-[(imidazolin-2-yl)methyl]indazoles (**B**) and 1-[(imidazolin-2-yl)methyl]indoles (**C**) exert different hemodynamic effects (15), the present studies indicate that 1-[(imidazolidin-2-yl)imino]indazoles (**D**) and their indole analogues **3** produce similar cardiovascular effects. Moreover, the results of *in vitro* metabolic stability studies as well as *in silico* model revealed that indoles **3** are not vulnerable to rapid first phase oxidative metabolism.

Conflict of interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Preparation and analytical data of 1-amino-1*H*-indoles **2f-i.**

Scheme S1. Synthesis of 1-amino-1*H*-indoles **2a-j**.

Figure S1. Effect of 0.01 mg/kg of **3a**, **3f** and **3g** on ΔMAP (calculated as the difference of MAP between sequential measurement and time 0 of the experiment) in rats. The lines represent the mean values of ΔMAP for four or five experiments. Comparison were made using ANOVA with repeated measures and Fisher's test. Significance (*) $p < 0.001$ was found for comparisons of the following: **3f** and **3g** versus control group; **3a** and **3f** versus **3g**. Significance (#) $p < 0.01$ was found for comparison of **3a** versus control group. Significance (&) $p < 0.05$ was found for comparison of **3a** and **3f**.

Figure S2. Effect of 0.01 mg/kg of **3a**, **3f** and **3g** on ΔHR (calculated as the difference of HR between sequential measurement and time 0 of the experiment) in rats. The lines represent the mean values of ΔHR for four or five experiments. Comparison were made using ANOVA with repeated measures and Fisher's test. Significance (*) $p < 0.001$ was found for comparisons of the following: **3a** and **3f** versus **3g** and **3g** versus control group. Significance (#) $p < 0.01$ was found for comparison of **3a** and **3f** versus control group.

Figure S3. Output from XenoSite *in silico* tool for prediction of sites of metabolism. Color scale bar shows probability to undergo metabolic reactions mediated by cytochrome P-450 isoforms present in human liver microsomes.

Figures Legends

1. **Figure 1**. Known imidazoline α-adrenoceptor ligands **A-D** and the newly designed 1-[(imidazolidin-2-yl)imino]-1*H*-indoles **3**.

2. **Figure 2.** Molecule structure of **3d** with displacement ellipsoids drawn at the 50% probability level.

Scheme Legend

1.

2. **Scheme 1.** Synthesis of 1-[(imidazolidin-2-yl)imino]-1*H*-indole derivatives **3a-j.**

Table Legends

1**. Table 1.** Binding affinity data for the prepared indoles **3a-j** and selected indazoles of type **D** (values given in parentheses)

2. **Table 2.** Hydrophobic constants (π) and Pauling electronegativities (χ) for substituents (R) of indole derivatives and binding affinities of **3g-j** to α-adrenergic receptors

3**. Table 3.** Effects of the prepared indoles **3a-j** at 0.01 or 0.1 mg/kg i.v. on mean arterial blood pressure (MAP) and heart rate (HR) in anesthetized rats

Table 1. Binding affinity data for the prepared indoles **3a-j** and selected indazoles of type **D** (values given in parentheses)

Compound		Binding affinities[a]				Selectivity ratio	
No	**R**	**α_1 K_i (nM)[b]**	**α_2 K_i (nM)[b]**	**I_1 IC_{50} (nM)[c]**	**I_2 K_i (nM)[b]**	**α_1/α_2**	**I_1/α_2**
3a (D: 13a)[d]	H	70.2 (n = 1)[e] *(nd)*[g]	5.33 (n = 1)[e] *(14.05)*	6930 (n = 1)[e] *(54500)*	1840 (n = 1)[e] *(16900)*	13	1300 *(3879)*
3b (D: 6a)[g]	4-F	348 ± 146.5 *(3807)*	63.6 ± 8.561 *(416)*	241 ± 7.024 *(14776)*	2127 ± 351.8 *(6177)*	5.5	4 *(35)*
3c (D: 6b)[g]	5-F	165 ± 25.48 *(2320)*	23.4 ± 1.986 *(64.33)*	22557 ± 15338 *(91.65)*	335.7 ± 103.5 *(5703)*	7	964 *(1.4)*
3d (D: 13h)[d]	5-Cl	214 (n = 1)[e] *(nd)*[f]	57.9 (n = 1)[e] *(114.2)*	17300 (n = 1)[e] *(16980)*	1150 (n = 1)[e] *(20620)*	3.7	299 *(149)*
3e (D: 13g)[d]	5-CH_3	118 (n = 1)[e] *(nd)*[f]	16.5 (n = 1)[e] *(44.2)*	159000 (n = 1)[e] *(20310)*	354 (n = 1)[e] *(7159)*	7	9636 *(459)*
3f (D: 6c)[g]	6-F	70.77 ± 9.51 *(918.3)*	7.073 ± 0.3886 *(26.20)*	5270 ± 2659 *(20675)*	422.7 ± 43.21 *(21967)*	10	745 *(789)*
3g (D: 6d)[g]	7-F	167 ± 26.1 *(1625)*	12 ± 0.7371 *(30.97)*	15230 ± 6203 *(7740)*	1087 ± 432.9 *(348833)*	14	1269 *(250)*
3h (D)[h]	7-Cl	38.85 ± 5.88 *(1010)*	9.95 ± 2.52 *(30.3)*	949.7 ± 532.8 *(46800)*	2708 ± 1022 *(15000)*	4	95 *(1544)*
3i	7-OCH_3	807 (n = 1)[e]	151 (n = 1)[e]	607 (n = 1)[e]	748 (n = 1)[e]	5.3	4
3j (D: 13k)[d]	7-CH_3	166.0 ± 47.7 *(nd)*[f]	12.09 ± 2.37 *(53.5)*	7152 ± 6775 *(387)*	2536 ± 950.5 *(2520)*	13.7	591.6 *(7.2)*

[a] Values given are means ± SEM of three or four independent experiments.

[b] K_i affinity values for α_1-adrenoceptors, α_2-adrenoceptors, and I_2 imidazoline binding sites were assessed by measuring the ability of the tested compounds to compete with [^{3}H]prazosin, [^{3}H]RX821002 or [^{3}H]2BFI binding to rat brain membranes.

[c] Molar concentration of the tested compounds that displaces 50% of specifically bound [^{3}H]clonidine in rat kidney membranes in the presence of rauwolscine (I_1 imidazoline binding sites).

[d] The results have been published earlier in Sączewski *et al.* (16).

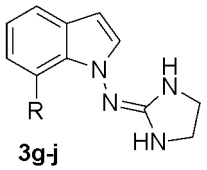
[e] n: number of experiments.

[f] nd: not determined.

[g] The results have been published earlier in Wasilewska *et al*. (17).

[h] The results have been published earlier in Sączewski *et al.* (27).

Table 2. Hydrophobic constants (π) and Pauling electronegativities (χ) for substituents (R) of indole derivatives and binding affinities of **3g-j** to α-adrenergic receptors



3g-j

Compound					
No	**R**	**π**[a]	**χ**[b]	**$\alpha_1 K_i$ (nM)**	**$\alpha_2 K_i$ (nM)**
3g	F	0.14	4.0	167	12
3h	**Cl**	**0.71**	**3.0**	**38.85**	9.95
3i	OCH_3	–0.02	2.7	807	151
3j	CH_3	0.56	2.3	166	12.09

[a] According to ref. 40.

[b] According to ref. 41.

Table 3. Effects of the prepared indoles **3a-j** at 0.01 or 0.1 mg/kg i.v. on mean arterial blood pressure (MAP) and heart rate (HR) in anesthetized rats

Compound		Dose (mg/kg)	$+\Delta MAP_{max}$[a,d] (mmHg)	*p*	$-\Delta MAP_{max}$[b,d] (mmHg)	*p*	$-\Delta HR_{max}$[c,d] (bpm)	*p*	*n*[e]
No	**R**								
3a	H	0.01	21.1±7.0	<0.03	–24.0 ± 3.8	<0.003	–80.4 ± 12.6	<0.001	5
3b	4-F	0.1	35.8 ± 5.5	<0.001	–51.7 ± 5.9	<0.001	–136.3 ± 17.4	<0.001	4
3c	5-F	0.1	25.3 ± 5.4	<0.003	–28.4 ± 4.3	<0.001	–110.8 ± 12.6	<0.001	4
3d	5-Cl	0.1	8.5 ± 4.1	–	–11.4 ± 1.9	–	–39.9 ± 1.5	<0.005	4
3e	5-CH_3	0.1	26.6 ± 4.7	<0.002	–21.8 ± 0.9	<0.001	–94.8 ± 5.6	<0.001	7
3f	6-F	0.01	14.6 ± 3.4	<0.009	–37.2 ± 2.1	<0.001	–87.4 ± 7.7	<0.001	4
3g	7-F	0.01	15.3 ± 1.6	<0.001	–65.0 ± 1.1	<0.001	–148 ± 4.2	<0.001	4
3h	7-Cl	0.1	26.6 ± 5.2	<0.002	–42.3 ± 2.9	<0.001	–124.6 ± 14.0	<0.001	5
3i	7-OCH_3	0.1	0.5 ± 0.3	–	–16.6 ± 1.3	<0.001	–66.0 ± 7.5	<0.001	5
3j	7-CH_3	0.1	18.4 ± 0.8	<0.001	–40.2 ± 3.9	<0.001	–158.2 ± 13.0	<0.001	5
control[f]			3.4 ± 0.9	–	–6.2 ± 1.7	–	–22.9 ± 3.6	–	5

[a] The maximal hypertensive effect [mmHg] of a compound observed during 60 min after injection.

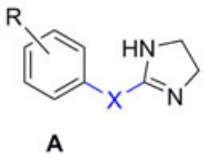
[b] The maximal hypotensive effect [mmHg] of a compound observed during 60 min after injection.

[c] The maximal negative chronotropic effect [bpm] of a compound observed during 60 min after injection.

[d] Values given are means ± SEM from *n* independent experiments.

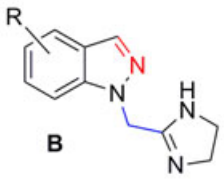
[e] Number of experiments.

[f] Saline vehile injection. Comparisons were performed using the Student's *t* test; *p* significance versus control.



A

R = 2,6-Cl, X = NH (*clonidine*)
α_2/I_1-agonist
R = H, X = CH_2 (*tolazoline*)
α_1/α_2-antagonist

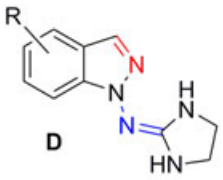


B

R = H, halogen, alkyl, alkoxyl
α_1-antagonists (hypotensives)

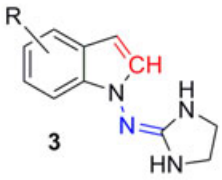
C

R = H, halogen, alkyl, alkoxyl
α_2-agonists (hypotensives)



D

R = H (*marsanidine*)
R = F, Cl, CH_3, OCH_3
(*marsanidine-like ligands*)
α_2-agonists (hypotensives)



3

R = H, halogen, alkyl, alkoxyl