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2-[(ARYLMETHOXY)IMINO]IMIDAZOLIDINES WITH POTENTIAL BIOLOGICAL ACTIVITIES

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Abstract: A series of 2-[(arylmethoxy)imino]imidazolidines was synthesized by reacting 2-chloro-4,5-dihydroimidazole with corresponding *O*-arylmethylhydroxylamines and evaluated for their α_1-, α_2-adrenergic and imidazoline I_1, I_2 receptor binding affinities. The most potent 2-[(naphthalen-1-ylmethoxy)imino]imidazolidine showed a high selectivity and good affinity for the [^{3}H]prazosin-labeled α_1-adrenoceptors (K_i = 107 nM). Representative compounds of this series were also tested *in vivo* for possible circulatory effects in rats after intravenous administration.

Keywords: 2-[(arylmethoxy)imino]imidazolidines, α-adrenergic receptors, imidazoline receptors, radioligand binding studies

Alpha-adrenergic receptors have proven to be attractive targets for drugs, especially in the treatment of cardiovascular diseases such as hypertension. Identification and characterization of alpha$_1$- and alpha$_2$-adrenergic receptor subtypes (1) had contributed considerably to the development of novel therapeutic strategies. The areas in which alpha-adrenergic agonists and antagonists may play role as therapeutic agents include effects mediated by receptors localized in central nervous system, arterial vascular smooth muscle cell membranes, vascular endothelial cells, myocardium, various compartments of kidney, extraocular muscles, platelets, pancreatic isles, liver cell membranes, urogenital system (bladder and prostate) as well as gastrointestinal tract. The recent interest into alpha-adrenergic receptors has been also stimulated by the introduction of new more selective compounds and easy accessibility of the test systems such as radioligand binding assays (2).

The discovery of clonidine, {[(2,6-dichlorophenyl)imino]imidazolidine}, has led to the synthesis and pharmacological evaluation of a large number of imidazoline-containing derivatives (3). However, the problem of the structure-biological activity relationships (SAR) became more complicated after the discovery made by Bousquet and his co-workers in 1984 of a new type of imidazoline receptors which participate in the circulatory effects of hypotensive drugs such as clonidine and moxonidine (4). Since then, rational design of imidazoline-containing drugs has proven to be difficult due to possible interactions with either alpha$_1$- and alpha$_2$-adrenoceptors or imidazoline I_1 receptors. It was found that even minor modifications of the structure could greatly change the alpha$_1$/alpha$_2$/I_1/I_2 selectivity profile.

As shown in Figure 1, studies aimed at elucidating the SAR of imidazoli(di)nes dealt mainly with three areas of structural modifications, i.e. modification of the aromatic portion (**A**), the bridge (**B**) and the imidazoline moiety (**C**).

Several topographical models of alpha$_1$-adrenergic (5-7), alpha$_2$-adrenergic (8, 9), imidazoline I_1 (10) and imidazoline I_2 receptor ligands (11, 12) have been developed in recent years as a result of retrospective approach rather than the rational design of highly selective compounds. For example, research carried out by our group led to the discovery of 4-Cl-indazim, [4-Cl-(2-(imidazolin-2-yl)indazole], with unprecedented for this class of compounds I_1/alpha$_2$ selectivity ratio of 3076 (13) and marsanidine, {1-[(imidazolidin-2-yl)imino]indazole}, showing a 3879-fold difference in affinity for alpha$_2$- adrenoceptors relative to imidazo-

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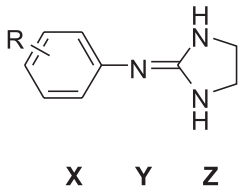


Figure 1. Three structural units: **X** (aromatic ring), **Y** (the bridge) and **Z** (imidazoline ring) accessible to chemical modifications

line I_1 receptors (14). However, three-dimensional structure of 4-Cl-indazim does not fit the features of the pharmacophoric model of selective I_2 ligands proposed by Baurin et al. (11) and stereochemical structure of marsanidine differs considerably from those of clonidine and other good alpha$_2$-adrenoceptor ligands described by Timmermans and van Zweiten (8).

Searching for new imidazolines which could cross easily the blood-brain barrier to interact with alpha$_2$-adrenergic and/or imidazoline receptors localized in central nervous system, we focused our attention at derivatives with decreased basicity, i.e. compounds which under physiological conditions (pH = 7.4) would exist mostly in the free base (unionized) form. In this paper we wish to describe a facile synthesis of a series of novel imidazoline-containing analogues of guanabenz (**A**) (15), aganodine (**B**) (16) and the potential antihypertensive drug (**C**) (17) which incorporate three-atom -CH_2-O-N= bridge connecting the aromatic ring and amidine moiety (Fig. 2). Initial comparison of the calculated pKa value (18) of the free base **5a**, which can be considered as a formal imidazolidin-2-one *O*-benzyl oxime (pKa = 7.16), with that designated for *clonidine* (pKa = 8.2, ref. 19) confirmed a possible decrease in basicity of this class of compounds.

It should be noted, that analogues of guanabenz incorporating -CH_2-O-N=C (MAOM) moiety as a bioisosteric replacement of the aryl group have been synthesized as alpha$_2$-adrenergic receptor agonists (20).

The newly prepared compounds of general formula **D** (Fig. 2) were tested *in vitro* for their affinities at alpha$_1$- and alpha$_2$-adrenoceptors as well as imidazoline I_1 and I_2 receptors. Possible circulatory activity of the most active compounds was also investigated *in vivo* after intravenous administration to normotensive Wistar rats.

EXPERIMENTAL

Melting points determined are not corrected. FT-IR spectra were recorded using KBr tablet method. ^{1}H NMR and ^{13}C NMR spectra were acquired with Varian Gemini 200 or Varian Unity 500 spectrometers. Chemical shifts were measured relative to the residual solvent signal at 2.50 or 7.26 ppm and 39.5 or 77 ppm, respectively. Chromatographic purification of the compounds obtained was carried out by means of preparative thin-layer chromatography using Chromatotron apparatus (Harrison Research Inc. USA). The results of elemental C, H, N analyses for all newly prepared compounds were in agreement with calculated values within ± 0.4 %.

The following compounds were obtained according to the previously described procedures: 2-chloro-4,5-dihydroimidazole hydrogen sulfate by chlorination of imidazolidin-2-thione (21) and 2-(benzyloxyimino)imidazolidine by reacting 2-chloro-4,5-dihydro-imidazole with commercially available *O*-benzylhydroxylamine (22).

Chemistry

General procedure for preparation of 2-(arylmethoxy)isoindoline-1,3-diones **2** (23-25)

N-hydroxyphthalamide (**1**, 1.4 g, 8.58 mmol) was dissolved in dimethylformamide (DMF, 10 mL) and treated with triethylamine (1.2 mL, 8.58 mmol). To the red-colored solution thus obtained the corresponding arylmethyl halide (8.58 mmol) was added portion-wise and the reaction mixture was stirred at ambient temperature until the red color had disappeared. After 2 h, the reaction mixture was quenched with water (10 mL) and the resulting precipitate of the product **2** was filtered off, washed with water and dried under reduced pressure. Yield 90-95%. The isoindoline-1,3-diones **2b-q** (Scheme 1) were subjected to further reactions without purification.

General procedure for preparation of *O*-benzylhydroxylamines **3** (23-25)

The corresponding 2-(arylmethoxy)isoindoline-1,3-dione (**2**) (8.2 mmol) was dissolved in ethanol (30 mL) and treated with equimolar amount of 98% hydrazine hydrate followed by stirring of the reaction mixture at room temperature for 2 h. The resulting precipitate was filtered off and the filtrate was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure. The oily residue was dissolved in methylene chloride (5 mL) and purified on silica gel using a mixture of CH_2Cl_2 : hexanes (1:1, v/v) as the mobile phase to give pure hydroxylamine **3** in 90-95% yield. According to the above procedure, intermediate hydroxylamines **3b-q**

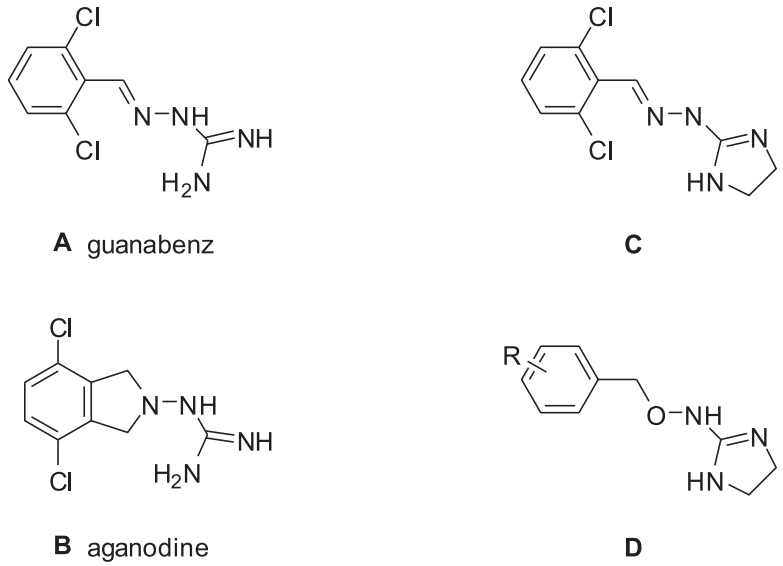


Figure 2. Known alpha$_2$-adrenoceptor ligands **A**, **B**, **C** and the newly designed 2-[(arylmethoxy)amino]imidazolines **D** with decreased basicity

(Scheme 1) were prepared and were used for the reaction with 2-chloro-4,5-dihydroimidazole.

General procedure for preparation of 2-[(arylmethoxy)imino]imidazolidine **6**

2-Chloro-4,5-dihydroimidazole hydrogen sulfate (1.65 g, 8.12 mmol) was dissolved in ice-cooled 10% aqueous NaOH (25 mL). The solution was extracted with methylene chloride (4 × 15 mL) and the combined organic layers were dried over anhydrous sodium sulfate and filtered. To the resulting solution of 2-chloro-4,5-dihydroimidazole free base (**4**) the corresponding *O*-benzylhydroxylamine **3** (8.12 mmol) was added and the reaction mixture was concentrated slowly under reduced pressure to volume of 5-7 mL maintaining the temperature below 25°C. After ca. 10 min, a rapid and exothermic reaction took place. The oily residue that deposited was made alkaline with 5% NaOH (pH = 10) and extracted with methylene chloride (3 × 15 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The oily residue thus obtained was dissolved in methylene chloride (5 mL) and purified on silica using ethyl acetate : methanol (99:1, v/v) as the mobile phase.

The free bases **5** were transformed into corresponding hydrochlorides or hydrobromides **6** required for biological tests by passing gaseous hydrogen chloride or hydrogen bromide through their methylene chloride or methanolic solution.

According to the above procedure the following compounds were obtained:

2-[(Benzyloxy)imino]imidazolidine hydrochloride (**5a**)

M.p. 141-146°C. ^{1}H-NMR (200 MHz, DMSO-d_6, δ ppm): 3.63 (s, 4H, CH_2), 4.88 (s, 2H, CH_2), 7.37–7.41 (m, 3H, CH), 7.46 (m, 2H, CH), 8.76 (s, 2H, NH), 12.12 (s, 1H, NH). IR (KBr, cm^{-1}): 3095, 2978, 1653, 1372, 1291, 1111, 966, 747. Analysis for $C_{10}H_{14}ClN_3O$.

2-[(Benzyloxy)imino]imidazolidine (**6a**)

M.p. 127-130°C, yield 89%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 3.31 (s, 4H, CH_2), 4.38 (bs, 1H, NH), 4.76 (s, 2H, CH_2), 4.84 (bs, 1H, NH), 7.22-7.35 (m, 5H, CH). ^{13}C-NMR ($CDCl_3$, δ ppm): 43.2, 76.0, 128.0, 128.6, 128.7, 139.1, 161.1. IR (KBr, cm^{-1}): 3228, 2282, 1643, 1496, 1452, 1283, 1063, 732.

2-[(4-Methylbenzyloxy)imino]imidazolidine hydrochloride (**5b**)

M.p. 180-184°C. IR (KBr, cm^{-1}): 3119, 2986, 2878, 1646, 1579, 1370, 1289, 1108, 999, 812. Analysis for $C_{11}H_{16}ClN_3O$.

2-[(4-Methylbenzyloxy)imino]imidazolidine (**6b**)

M.p. 58-61şC, yield 38.7%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 2.16 (s, 3H, CH_3), 3.39 (s, 4H, CH_2), 4.77 (s, 2H, CH_2), 4.85 (bs, 2H, NH),

7.12-7.16 (m, 2H, CH), 7.25-7.29 (m, 2H, CH). IR (KBr, cm^{-1}): 3359, 3197, 2856, 1658, 1494, 1453, 1282, 1097, 1068, 1018, 868, 793.

2-[(3-Methylbenzyloxy)imino]imidazolidine hydrobromide (**5c**)

M.p. 155-157şC. IR (KBr, cm^{-1}): 3150, 2982, 2868, 1641, 1588, 1111, 1009, 786, 669, 606. Analysis for $C_{11}H_{16}BrN_3O$.

2-[(3-Methylbenzyloxy)imino]imidazolidine (**6c**)

Oil, yield 11.3%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 2.35 (s, 3H, CH_3), 3.41 (s, 4H, CH_2), 4.20 (bs, 1H, NH), 4.79 (s, 2H, CH_2), 4.85 (bs, 1H, NH), 7.08-7.35 (m, 4H, CH). ^{13}C-NMR (50 MHz, $CDCl_3$, δ ppm): 21.9, 43.3, 76.2, 125.8, 128.7, 128.9, 129.5, 138.4, 138.7, 161.0.

2-[(4-Chlorobenzyloxy)imino]imidazolidine hydrochloride (**5d**)

M.p. 157–161°C. IR (KBr, cm^{-1}) 3309, 3151, 2967, 2853, 2764, 1628, 1595, 1571, 1491, 1285, 1086, 1013, 853, 812. Analysis for $C_{10}H_{13}Cl_2N_3O$.

2-[(4-Chlorobenzyloxy)imino]imidazolidine (**6d**)

M.p. 87–90 °C, yield 20.4%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 3.41 (s, 4H, CH_2), 4.77 (s, 2H, CH_2), 4.86 (bs, 2H, NH), 7.26-7.32 (m, 4H, CH). ^{13}C-NMR (50 MHz, $CDCl_3$, δ ppm): 43.3, 75.3, 128.9, 129.9, 133.8, 137.5, 161.1. IR (KBr, cm^{-1}): 3374, 3239, 2867, 1657, 1491, 1450, 1282, 1094, 1064, 1094, 1064, 1039, 813, 798.

2-[(4-Bromobenzyloxy)imino]imidazolidine hydrochloride (**5e**)

M.p. 88-89°C. IR (KBr, cm^{-1}): 3291, 2987, 2872, 1652, 1599, 1486, 1407, 1356, 1286, 1108, 1064, 1013, 1001, 902, 852, 798. Analysis for $C_{10}H_{13}BrClN_3O$.

2-[(4-Bromobenzyloxy)imino]imidazolidine (**6e**)

M.p. 109-110°C, yield 31.1%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 3.39 (s, 4H, CH_2), 4.75 (s, 2H, CH_2), 4.84 (bs, 2H, NH), 7.21-7.27 (m, 2H, CH), 7.41-7.48 (m, 2H, CH). ^{13}C-NMR (50 MHz, $CDCl_3$, δ ppm): 43.3, 75.3, 122.0, 130.3, 131.9, 138.0, 161.1. IR (KBr, cm^{-1}): 3377, 3228, 2864, 1658, 1488, 1447, 1282, 1093, 1060, 789.

2-[(4-Nitrobenzyloxy)imino]imidazolidine hydrochloride (**5f**)

M.p. 156-160şC. IR (KBr, cm^{-1}): 3305, 3052, 2967, 2858, 1631, 1595, 1531, 1349, 1295, 1105, 1008, 976, 851. Analysis for $C_{10}H_{13}ClN_4O_3$.

2-[(4-Nitrobenzyloxy)imino]imidazolidine (**6f**)

M.p. 134-138°C, yield 27.7%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 3.48 (s, 4H, CH_2), 4.50 (bs, 2H, NH), 4.93 (s, 2H, CH_2), 7.52 (d, 2H, CH, J = 8.7 Hz), 8.18 (d, 2H, CH, J = 8.7 Hz). ^{13}C-NMR (50 MHz, $CDCl_3$, δ ppm): 43.3, 74.8, 124.0, 128.7, 146.8, 147.8, 161.0. IR (KBr, cm^{-1}): 3433, 3224, 2872, 1666, 1511, 1345, 1272, 1085, 1075, 872, 736.

2-[(2,6-Dichlorobenzyloxy)imino]imidazolidine hydrochloride (**5g**)

M.p. 215-225°C. IR (KBr, cm^{-1}): 3262, 3120, 2991, 2873, 1644, 1604, 1580, 1436, 1289, 1201, 909, 780, 766. Analysis for $C_{10}H_{12}Cl_3N_3O$.

2-[(2,6-Dichlorobenzyloxy)imino]imidazolidine (**6g**)

M.p. 121-125°C, yield 48.1%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 3.42 (s, 4H, CH_2), 4.85 (bs, 2H, NH), 5.06 (s, 2H, CH_2), 7.11-7.31 (m, 3H, CH). ^{13}C-NMR (50 MHz, $CDCl_3$, δ ppm): 43.3, 70.4, 128.8, 130.4, 133.5, 137.6, 162.1. IR (KBr, cm^{-1}): 3437, 3189, 2952, 2875, 2858, 1659, 1433, 1283, 1087, 1031, 989, 769.

2-[(3,5-Dimethoxybenzyloxy)imino]imidazolidine hydrochloride (**5h**)

M.p. 117–125°C. IR (KBr, cm^{-1}): 3174, 2958, 2845, 2779, 1631, 1594, 1435, 1363, 1300, 1283, 1211, 1163, 1000, 933, 842. Analysis for $C_{12}H_{18}ClN_3O_3$.

2-[(3,5-Dimethoxybenzyloxy)imino]imidazolidine (**6h**)

M.p. 99-102°C, yield 38.2%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 3.41 (s, 4H, CH_2), 3.78 (s, 6H, OCH_3), 4.76 (s, 2H, CH_2), 4.80 (bs, 2H, NH), 6.37 (t, 1H, CH, J = 2.3 Hz), 6.53 (d, 2H, CH, J = 2.3 Hz). ^{13}C-NMR (50 MHz, $CDCl_3$, δ ppm): 43.3, 55.8, 76.2, 100.3, 106.3, 141.2, 161.1, 161.2. IR (KBr, cm^{-1}): 3424, 3199, 2876, 1663, 1609, 1595, 1460, 1431, 1349, 1292, 1207, 1158, 1088, 1064, 1051, 1038.

2-[(4-Isopropylbenzyloxy)imino]imidazolidine hydrochloride (**5i**)

M.p. 175-180°C. IR (KBr, cm^{-1}): 3124, 2961, 2855, 1634, 1594, 1290, 1056, 830. Analysis for $C_{13}H_{20}ClN_3O$.

2-[(4-Isopropylbenzyloxy)imino]imidazolidine (**6i**)

M.p. 144–148°C, yield 56.7%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 1.24 (d, 6H, CH_3, J = 7 Hz),

2.80-3.00 (m, 1H, CH, J = 7 Hz), 3.40 (bs, 4H, CH_2), 4.80 (s, 2H, CH_2), 7.19-7.27 (m, 2H, CH), 7.30-7.34 (m, 2H, CH). ^{13}C-NMR (50 MHz, $CDCl_3$, δ ppm): 24.5, 34.4, 43.2, 76.0, 126.8, 128.8, 136.2, 148.8, 160.9. IR (KBr, cm^{-1}): 3397, 3226, 2958, 1659, 1283, 1103, 813.

2-[(4-*tert*-Butylbenzyloxy)imino]imidazolidine hydrochloride (**5j**)

M.p. 151-160°C. IR (KBr, cm^{-1}): 3151, 2961, 2861, 2788, 1638, 1602, 1371, 1292, 1009, 856, 832, 818, 666. Analysis for $C_{14}H_{22}ClN_3O$.

2-[(4-*tert*-Butylbenzyloxy)imino]imidazolidine (**6j**)

M.p. 64-66°C, yield 40%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 1.31 (s, 9H, CH_3), 3.37 (bs, 4H, CH_2), 4.79 (s, 2H, CH_2), 4.92 (bs, 2H, NH), 7.20-7.40 (m, 4H, CH). ^{13}C-NMR (50 MHz, $CDCl_3$, δ ppm): 31.3, 34.4, 42.7, 75.5, 125.1, 128.0, 135.1, 136.2, 150.5, 160.6. IR (KBr, cm^{-1}): 3426, 3274, 3180, 2962, 2900, 2869, 1673, 1631, 1485, 1361, 1280, 1093, 1034, 823.

2-[(Biphenyl-2-ylmethoxy)imino]imidazolidine hydrochloride (**5k**)

M.p. 170-174şC. IR (KBr cm^{-1}): 3418, 3148, 2962, 2855, 2784, 1633, 1590, 1450, 752, 710. Analysis for $C_{16}H_{18}ClN_3O$.

2-[(Biphenyl-2-ylmethoxy)imino]imidazolidine (**6k**)

M.p. 112-115°C, yield 51.7%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 3.37 (bs, 4H, CH_2), 4.05 (bs, 1H, NH), 4.51 (bs, 1H, NH), 4.79 (s, 2H, CH_2), 7.27-7.41 (m, 8H, CH), 7.55-7.59 (m, 1H, CH). ^{13}C-NMR (50 MHz, $CDCl_3$, δ ppm): 43.2, 74.1, 127.5, 127.8, 128.2, 128.6, 129.7, 130.2, 130.4, 135.7, 141.5, 142.3, 161.1. IR (KBr, cm^{-1}): 3403, 3202, 2866, 1654, 1482, 1278, 1033, 741.

2-[(Biphenyl-3-ylmethoxy)imino]imidazolidine hydrochloride (**5l**)

M.p. 129-131°C. IR (KBr, cm^{-1}): 3113, 3060, 2978, 2876, 1642, 1598, 1481, 1370, 1291, 1108, 812, 765. Analysis for $C_{16}H_{18}ClN_3O$.

2-[(Biphenyl-3-ylmethoxy)imino]imidazolidine (**6l**)

M.p. 95-97°C, yield 35.5%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 3.41 (bs, 4H, CH_2), 4.08 (bs, 1H, NH), 4.84 (bs, 1H, NH), 4.91 (s, 2H, CH_2), 7.30-7.631 (m, 9H, CH). ^{13}C-NMR (50 MHz, $CDCl_3$, δ ppm): 43.3, 76.1, 126.9, 127.5, 127.6, 127.7 (two overlapping signals), 129.2 (two overlapping signals), 139.5, 141.6, 141.7, 160.9. IR (KBr, cm^{-1}): 3454, 3203, 3053, 2870, 1655, 1479, 1286, 1091, 1058, 1036, 939, 768, 703.

2-[(Naphthalen-1-ylmethoxy)imino]imidazolidine hydrochloride (**5m**)

M.p. 132-136°C. IR (KBr, cm^{-1}): 3114, 2961, 2854, 2777, 1634, 1588, 1364, 1293, 791, 773. Analysis for $C_{14}H_{16}ClN_3O$.

2-[(Naphthalen-1-ylmethoxy)imino]imidazolidine (**6m**)

M.p. 109-113°C, yield 16%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 3.38 (bs, 4H, CH_2), 4.10 (bs, 1H, NH), 4.75 (bs, 1H, NH), 5.29 (s, 2H, CH_2), 7.27-7.56 (m, 4H, CH), 7.80-7.89 (m, 2H, CH), 8.17-8.20 (m, 1H, CH). ^{13}C-NMR (50 MHz, $CDCl_3$, δ ppm): 43.3, 74.7, 124.7, 125.8, 126.2, 126.7, 127.7, 129.0, 129.2, 132.6, 134.1, 134.2, 161.0. IR (KBr, cm^{-1}): 3236, 2916, 2886, 1648, 1498, 1281, 1100, 798.

2-[(Naphthalen-2-ylmethoxy)imino]imidazolidine hydrochloride (**5n**)

M.p. 128-135°C. IR (KBr, cm^{-1}): 3189, 2856, 2776, 1638, 1582, 1386, 1292, 1116, 1103, 814. Analysis for $C_{14}H_{16}ClN_3O$.

2-[(Naphthalen-2-ylmethoxy)imino]imidazolidine (**6n**)

M.p. 79-81°C, yield 46.7%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 3.38 (bs, 4H, CH_2), 4.10 (bs, 1H, NH), 4.85 (bs, 1H, NH), 5.00 (s, 2H, CH_2), 7.27-7.56 (m, 3H, CH), 7.78-7.88 (m, 4H, CH). ^{13}C-NMR (50 MHz, $CDCl_3$, δ ppm): 43.3, 76.2, 126.2, 126.4, 126.7, 127.4, 128.1, 128.4 (two overlapping signals), 133.5, 133.8, 136.6, 161.0. IR (KBr, cm^{-1}): 3444, 3221, 2856, 1653, 1488, 1477, 1299, 1279, 1088, 920, 764.

2-[(Benzhydryloxy)imino]imidazolidine hydrobromide (**5o**)

M.p. 162-164°C. IR (KBr, cm^{-1}): 3151, 2958, 2839, 2772, 1634, 1596, 1341, 1277, 997, 688. Analysis for $C_{16}H_{18}BrN_3O$.

2-[(Benzhydryloxy)imino]imidazolidine (**6o**)

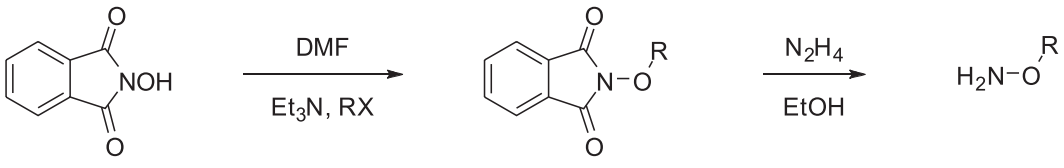
M.p 171-174° °C, yield 29.6%. ^{1}H-NMR (200 MHz, $CDCl_3$, d ppm): 3.41 (bs, 4H, CH_2), 4.50 (bs, 2H, NH), 5.84 (s, 1H, CH), 7.20-7.39 (m, 10H, CH). ^{13}C-NMR (50 MHz, $CDCl_3$, δ ppm): 43.3, 86.9, 127.8, 128.0, 128.8, 142.2, 160.8. IR (KBr, cm^{-1}): 3449, 3166, 3026, 1638, 1494, 1451, 1092, 751, 707.

2-[(Trityloxy)imino]imidazolidine hydrochloride (**5p**)

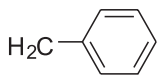
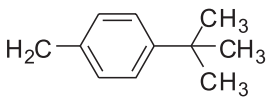
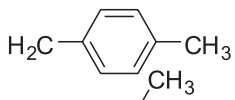
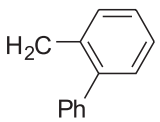
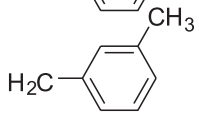
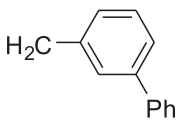
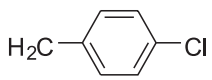
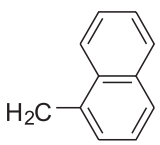
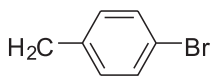
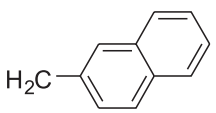
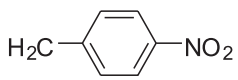
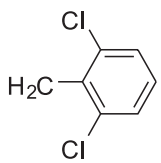
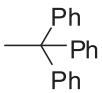
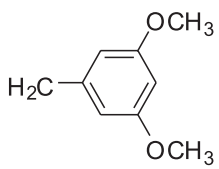
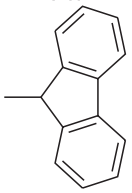
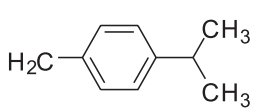
M.p. 184-192°C. IR (KBr, cm^{-1}): 3420, 3186, 3062, 2887, 1652, 1620, 1599, 1490, 1446, 1222, 759, 703, 636. Analysis for $C_{22}H_{22}ClN_3O$.

2-[(Trityloxy)imino]imidazolidine (**6p**)

M.p. 210-214°C, yield 26%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 3.18-3.20 (m, 4H, CH_2), 4.11 (bs, 1H, NH), 4.64 (bs, 1H, NH), 7.18-7.45 (m, 15H, CH). ^{13}C-NMR (50 MHz, $CDCl_3$, δ ppm): 43.2, 90.0,



1 **2a-q** **3a-q**

	R	X		R	X
a		Br	j		Br
b		Br	k		Br
c		Br	l		Br
d		Br	m		Cl
e		Br	n		Br
f		Br	o		Br
g		Br	p		Br
h		Br	q		Br
i		Br			

Scheme 1. Synthesis of *O*-benzylhydroxylamines **3**

127.2, 127.9, 129.5, 145.4, 161.4. IR (KBr, cm^{-1}): 3443, 3173, 3057, 3026, 2866, 1643, 1490, 1447, 1282, 1223, 1091, 972, 919, 749, 703.

2-[(9*H*-Fluoren-9-yloxy)imino]imidazolidine hydrochloride (**5q**)

M.p. 154-158°C. IR (KBr, cm^{-1}): 3366, 3175, 2908, 1641, 1609, 1449, 1355, 1313, 1286, 1106, 1009, 963, 893, 746. Analysis for $C_{16}H_{16}ClN_3O$.

2-[(9*H*-Fluoren-9-yloxy)imino]imidazolidine (**6q**)

M.p. 89-90°C, yield 39%. ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): 3.38 (s, 4H, CH_2), 4.36 (bs, 1H, NH), 4.74 (bs, 1H, NH), 5.82 (s, 1H, CH), 7.25-7.42 (m, 4H, CH), 7.64-7.78 (m, 4H, CH). ^{13}C-NMR (50 MHz, $CDCl_3$, δ ppm): 43.2, 83.9, 120.2, 126.4, 128.0, 129.3, 141.1, 144.3, 161.6. IR (KBr, cm^{-1}) 3396, 3231, 3041, 2955, 2884, 1657, 1495, 1450, 1280, 1101, 1034, 945, 922, 734.

Pharmacology

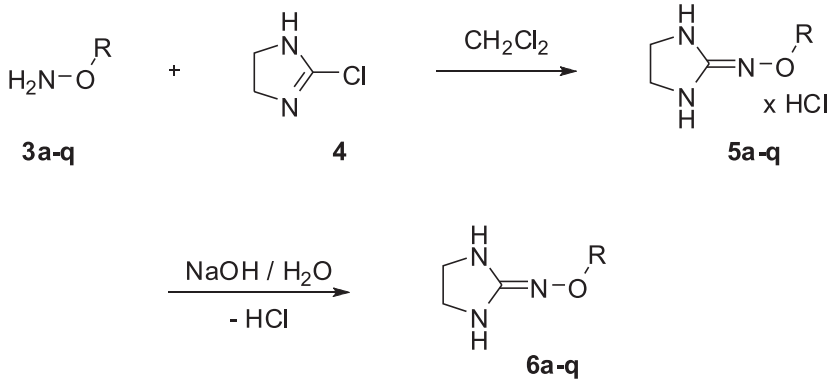
Radioligand Binding Assays

Membrane preparation.

For the I_1-binding site assay kidneys were obtained post-mortem from male Sprague Dawley rats (250-280 g) and for the I_2-binding site assay, α_1-adrenoceptor and α_2-adrenoceptor assay, brains were removed post-mortem from the same animals. Crude P_2 membranes were prepared according to methods of Lione et al. (26). The tissues were prepared as follows. All procedures were carried out at 4°C unless otherwise stated. The tissues were homogenized in 10 volumes of ice-cold buffer (50 mM Tris-HCl, 1 mM $MgCl_2$ and 320 mM sucrose, pH 7.4) with the aid of a polytron. The homogenates were centrifuged (1000 × *g* for 10 min) and the precipitates discarded. The kidney and brain supernatants were centrifuged a second time (32000 × *g* for 20 min) and the supernatants discarded, with the remaining precipitates making up the crude P_2 membrane preparations. These were washed twice in excess buffer (50 mM Tris-HCl, 1mM $MgCl_2$) at room temperature, 30 mL were added, the precipitates re-suspended and centrifuged (32000 × *g* for 20 min). The washed membrane preparations were stored at – 70°C until use. Prior to use they were thawed and washed (as above) a further two times. Membrane aliquots (400 µL, 0.2-0.3 mg protein) were incubated with 11 concentrations of the test compounds (0.1 nM – 100 µM) in a final buffer volume of 500 mL (50 mM Tris-HCl, 1amM $MgCl_2$).

Binding assays

For the I_1-binding site assay [^{3}H]clonidine (3 nM, Perkin Elmer) was bound to kidney membranes in the presence of 10 mM rauwolscine to preclude binding to α_2-adrenoceptors, the specific component was defined by 10 mM rilmenidine, under these conditions the site labelled is a model of the central I_1 binding site (27). For the I_2 binding site assay [^{3}H]2BFI (1 nM, GE Healthcare) was bound to brain membranes and the specific component of binding defined with 10 µM BU224. For the α_1-adrenoceptor binding assay [^{3}H]prazosin (0.5 nM, Perkin Elmer) was bound to brain membranes and the specific component of binding defined with 10 µM phenylephrine. For the α_2-adrenoceptor binding assay [^{3}H]RX821002 (1 nM, GE Healthcare) was bound to brain membranes and the specific component of binding defined with 10 µM rauwolscine. Each incubation was performed in triplicate, at room



Scheme 2. Synthesis of 2-[(arylmethoxy)imino]imidazolidines **6**

Table 1. Experimental binding affinities of 2-[(arylmethoxy)imino]imidazolidine salts (**5**) to a-adrenergic and imidazoline receptors.

5	a_1-receptor K_i (nM)	a_2-receptor K_i (nM)	I_1-receptor IC_{50} (nM)	I_2-receptor K_i (nM)
a	NT	6652	6422	927
c	1280	ND	8150	2290
d	35900	ND	55200	11100
e	25500	ND	ND	39100
g	7110	57100	10600	1650
h	ND	ND	ND	1570
i	36400	ND	ND	9000
j	34000	ND	42200	4680
l	2020	ND	ND	1850
m	107	ND	ND	6170
n	4950	91310	26400	8250
o	553	ND	ND	ND
p	2420	2281	ND	ND
q	755	16500	38500	4170

ND – no displacement; NT – not tested

temperature and allowed to reach equilibrium (45 min). Bound and free radioactivities were separated by rapid filtration through pre-soaked (0.5% polyethylenimine) 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. Data were analyzed by iterative non-linear regression curve fitting procedures in GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego, California, USA). Each experiment was analyzed individually and the equilibrium dissociation constant (K_i), determined by the method of Cheng and Prusoff (28) and the resulting K_i values are given as the means ± SD for 3-4 separate experiments. As previously reported, the K_D of [^{3}H]clonidine binding to I_1-binding sites can vary (26), therefore the results for I_1-binding are presented as IC_{50} values.

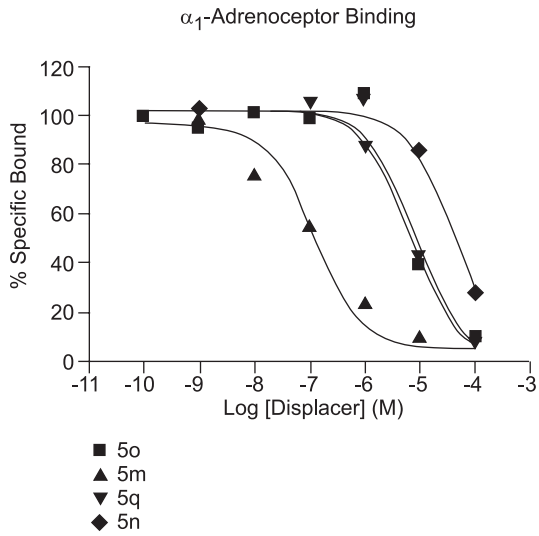


Figure 3. [^{3}H]Prazosin binding (0.5 nM) to rat whole brain membranes

In vivo studies

Mean Arterial Blood Pressure (MAP) and Heart Rate (HR) in Rats

Male Wistar rats, weighing 200-250 g, were purchased from the Animal House of the Polish Academy of Sciences, Warsaw, Poland. All experiments were approved by the Local Ethical Committee on Animal Experiments. The animals were fed a commercial rodent chow (Labofeed-B, Poland) and tap water, available *ad libitum*. Rats were anesthetized by *ip* injection of thiopental (Sandoz, Austria) at a dose 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 in all experimental groups. Catheters were inserted into the carotid artery for pressure and heart rate monitoring, into a jugular vein for infusions, and into the bladder for free diuresis. Blood pressure and heart

rate were constantly monitored to the end of experiment. After all surgical procedures, a 40 min recovery period was allowed to establish steady state. During the whole experiment, 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 administrated as 100 μL bolus through venous catheter at a dose of 0.1 mg/kg. The antagonist of alpha$_2$-adrenoceptors (RX821002) was given *iv* at a dose of 5 or 10 μg/kg 5 min before the tested compound. Arterial blood pressure and heart rate were monitored directly and sampled continuously at 100 Hz using Biopac Systems, Inc., model MP 100 (Goleta, CA). The results of measurements were elaborated with the help of the ACQKnowledge (Goleta, CA) measurement system that is selected, scaled, and filtered to remove accidental signal disturbances. The recorded time domain transient data have been presented as graphs with the help of Excel (Microsoft). Statistical ANOVAs of mean arterial blood pressure (MAP) and heart rate (HR) were performed for ΔMAP and ΔHR, calculated as the difference in MAP and in HR between sequential measurements and the time of compound application ("time 0") for each group, as we described previously (29) This allowed for direct comparison of responses to treatment between groups when baselines differed. Data were analyzed by ANOVA with repeated measures, using Statistica StatSoft software (StatSoft Inc., Tulsa, OK), after test compounds or vehicle treatment. When the effect was significant, *post hoc* comparisons were performed using Duncan and Fisher tests. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Chemistry

The planned syntheses required use of a large number of commercially unavailable hydroxylamines (**3**) which were obtained in excellent yields by *O*-alkylation of *N*-hydroxyphthalimide (**1**) with corresponding benzyl (arylmethyl) halide followed by cleavage of the intermediary 2-(benzyloxy)isoindoline-1,3-dione (**2**) with hydrazine (Scheme 1). The hydroxylamines (**3b-q**) were subjected to the reaction with 2-chloro-4,5-dihydroimidazole (**4**) to give hydrochlorides **5b-q** which were further converted into the free bases **6b-q** as shown in Scheme 2. According to the above procedure compound **5a** was obtained from **4** and the commercially available *O*-benzylhydroxylamine **3a** (22). Water soluble salts **5a**-**q** suitable for biological investigations were obtained upon treatment of **6a**-**q** with gaseous HCl or HBr in either the methylene chloride or methanolic solution (Scheme 2).

The potentiometric titration experiments (30) revealed the p*K*a constants ranging from 6.28 for the 4-nitro (**5f**) analogue to 6.75 for the 4-*tert*-butyl (**5j**) congener. Therefore, as expected, at physiological pH = 7.4 these compounds should predominantly exist in form of unionized free bases.

Structures of the final products **5** and **6** were confirmed by elemental analyses as well as IR and NMR spectroscopic data presented in Experimental Section.

Pharmacology

The results of radioligand binding studies of 2-[(arylmethoxy)imino]imidazolidine salts (**5a**-**q**) using α_1- and α_2-adrenergic as well as imidazoline I_1 and I_2 receptors are presented in Table 1. In general, this class of imidazoline-containing compounds exhibited rather poor (micromolar range) or no affinity (not detected) for all but α_1 receptors being investigated.

For example, several tested imidazolidines **5** showed weak affinities to imidazoline I_2 receptors with K_i values ranging between 0.927 mM for *O*-benzyl derivative **5a** to 39.1 mM for *p*-Br-benzyl analogue **5e**. Weak affinities were observed for congeners bearing 3,5-dimethoxy (**5h**) and 2,6-dichloro (**5g**) substituents at the phenyl ring (K_i values = 1.57 and 1.65 mM, respectively). It is worth noting that compounds with bulky aromatic substituents such as *O*-benzhydryl (**5o**) and *O*-trityl (**5p**) showed virtually no affinity at this type of imidazoline receptors. These results are consistent with the previous findings that good I_2 receptor ligands require planar arrangement of both the aromatic and imidazoline rings (9, 17).

Compound **5a** also proved to be most active at imidazoline I_1 receptors and α_2-adrenoceptors showing K_i values of 6.42 and 6.65 μM, respectively.

Much more encouraging results were obtained from studies using α_1-adrenergic receptors, since 3,5-dimethoxybenzyl derivative (**5h**) was the only compound that did not show affinity to these receptors. Other *O*-benzyl analogues substituted at the position 4 of the phenyl ring (**5d**, **5e**, **5i**, **5j**) exhibited weak binding affinities with the K_i values ranging from 25.5 to 35.9 μM. However, the most active compounds in the series were analogues with bulky aryl substituents. As shown in Table 1 and Figure 3, the benzhydryl derivative (**5o**) and its constrained fluorene congener (**5q**) displayed affinities in nanomolar range (K_i = 553 and 755 nM, respective-

ly). The highest α_1-binding affinity was observed for the 1-naphthalene analogue **5m** with K_i value of 107 nM. Interestingly, its 2-naphthalene congener **5n** revealed 50-fold lower affinity (K_i = 4.95 μM). Also 3-biphenyl (**5l**) and trityl (**5p**) derivatives showed weak affinities to α_1-adrenoceptors with K_i = 2.02 and 2.42 μM, respectively). In terms of selectivity, the benzhydryl derivative **5o** proved to be the most selective showing no displacement for the α_2, I_1, and I_2 binding sites.

Finally, it should be pointed out that the *in vivo* tests of the selected compounds **5h**, **5m**, and **5o** showed no effect on mean arterial blood pressure and heart rate after intravenous administration to normotensive male Wistar rats at doses up to 10 mg/kg. It is known that 2-iminoimidazoline derivatives bearing naphthalene or tetrahydronaphthalene moiety such as naphazoline, tramazoline and tetrahydrozoline are mixed α_1- and α_2-adrenergic receptor agonists used as nasal decongestants (1). Lack of circulatory activities of compounds **5** tested may therefore result from either the proved poor affinity to α_2-adrenoceptors or a very low intrinsic activity at α_1-adrenoceptors.

Ackonwledgment

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30. The p*K*a values were determined at 25°C by potentiometric titration with TiNet 2.5 software.

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