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# *N*-(Imidazolidin-2-ylidene)-1-arylmethanamine Oxides: Synthesis, Structure and Pharmacological Evaluation

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A high yielding three-step procedure was applied for the synthesis of *N*-(imidazolidin-2-ylidene)-1-arylmethanamine oxides **3** (α-aminonitrones) starting from the easily accessible imidazolidin-2-one *O*-benzyl oxime **1**. The α-aminonitrone–α-iminohydroxyloamine tautomerism of these products was studied theoretically and the structures of the synthesised compounds were confirmed by single crystal X-ray crystallographic analysis. The compounds were evaluated *in vitro* for their binding affinities to $\alpha_1$ and $\alpha_2$ adrenoceptors as well as imidazoline $I_1$ and $I_2$ receptors. The highest potencies at the $\alpha_2$ adrenergic receptors were observed for compounds bearing biphenyl (**4h**, $K_i = 9$ nM) and naphthyl (**4i**, $K_i = 92$ nM) moieties. Compounds **4h** and **4i** were further tested *in vivo* for their cardiovascular and sedative-hypnotic effects in rats.

**Keywords:** Alpha-aminonitrones / Alpha-adrenergic receptors / Alpha-iminohydroxylamines / Imidazolines / Imidazoline receptors

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## Introduction

It is well known that imidazoline-containing compounds such as clonidine, phentolamine and naphazoline constitute the most vital set of α-adrenergic receptor ligands. The medical applications of these drugs mediated through activation of central $\alpha_2$ adrenergic autoreceptors include treatment of hypertension, suppression of opiate withdrawal, anaesthesia, attention-deficit hyperactivity disorder (ADHD), short statue, glaucoma and diarrhea [1–3]. In addition, $\alpha_2$ adrenergic agonists have shown organoprotective effects in various experimental models, and therefore attracted attention for the treatment of mechanically ventilated patients with sepsis [4]. Furthermore, $\alpha_2$ antagonists have found clinical uses for the treatment of Raynaud's phenomenon, impotence, obesity and noninsulin-dependent diabetes [5].

Specific imidazoline receptors have been proposed to be responsible for various pharmacological effects of imidazoline-containing drugs. These receptors constitute a heterogenous group of proteins which are divided into $I_1$, $I_2$ and $I_3$ binding sites [6]. Much work has been focused on the synthesis of selective agents acting at $I_2$ binding sites which recently have been identified within the brain creatine kinase enzyme (B-CK). Selective $I_2$ ligands may affect dopamine, 5-HT and noradrenaline release, and therefore can be used in the treatment of various psychiatric disorders including depression and Alzheimer's disease [7].

The major drawback of these drugs is lack of selectivity at either $\alpha_1$ and $\alpha_2$ adrenoceptors or imidazoline $I_1$ and $I_2$ receptors [8] which often results in undesired side effects. For this reason, the search for novel ligands with greater selectivity is of immense clinical interest [9].

A typical $\alpha_2$ adrenoceptor ligand consists of an imidazoline ring attached to an aryl group *via* a one- or two-atom spacer (structure **A**, Figure 1). Previous reports from our laboratories

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have dealt with the discovery of novel imidazoline-containing agents with a two-atom spacer incorporated into the 1*H*- or 2*H*-indazole ring system represented by *marsanidine* and its analogues which proved to be highly selective at $\alpha_2$ adrenoceptors [10, 11] and *4-Cl-indazim* with selectivity to imidazoline $I_2$ receptors [12, 13] (Figure 1). Other modifications that have been investigated include the placement of the hydroxyl group at the exocyclic nitrogen atom of 2-arylaminoimidazolines leading to structures of type **B** (Figure 1) that exhibited *in vitro* affinity to $\alpha_2$ adrenoceptors and inhibited human blood platelet aggregation [14], while *in vivo* they exhibited moderate hypotensive and tachycardic effects [15, 16].

Our present goal was to develop a facile synthetic route to *N*-(4,5-dihydro-1*H*-imidazol-2-yl)-*N*-(arylmethyl)hydroxylamines **C** (Figure 1) to probe the structural requirements for $\alpha_1$ and $\alpha_2$ adrenoceptors as well as imidazoline $I_1$ and $I_2$ receptors. Chemical modifications included aliphatic and aromatic substitution at the benzylic carbon atom. The elongation of the spacer between the aryl and the *N*-iminoimidazolidine moieties would result in less rigid structures, while the placement of the hydroxyl group would diminish both the lipophilic character and basicity of the guanidine compounds compared to classic 2-aryliminoimidazolidines [17, 18].

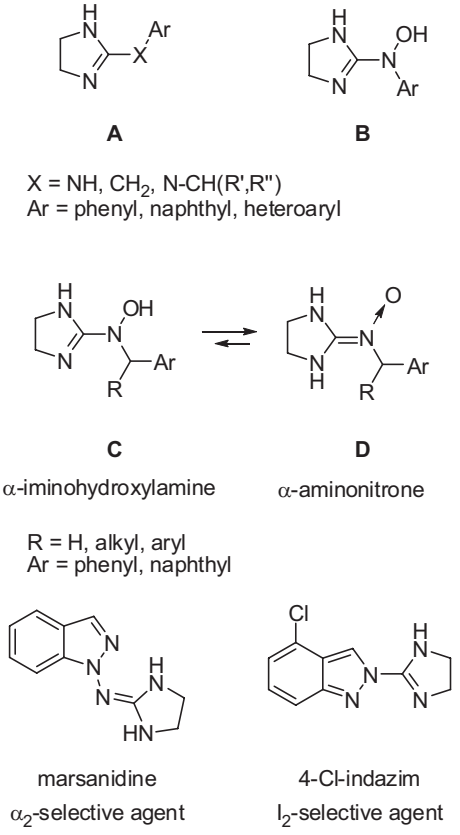


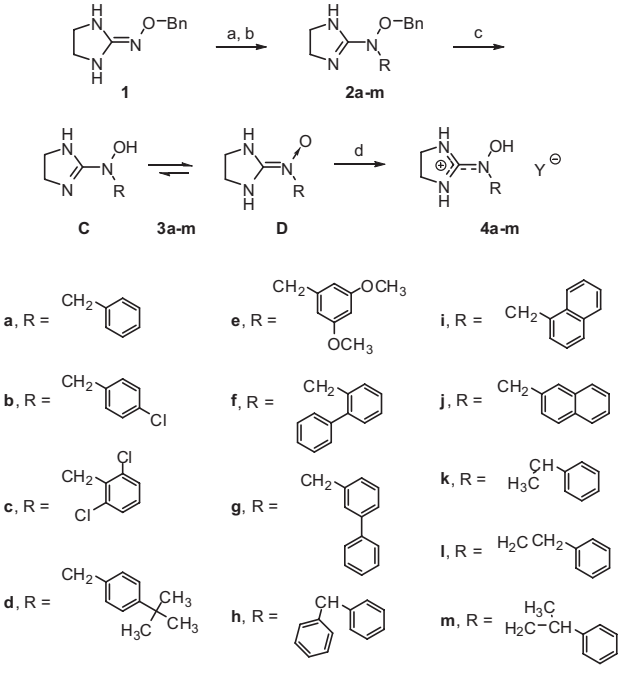

**Figure 1.** Imidazoline derivatives with activity at α-adrenergic and/or imidazoline receptors.

## Results and discussion

### Chemistry

Previously, the compounds of type **B** were obtained by reacting 2-chloroimidazoline with the corresponding *N*-arylhydroxylamines [14]; however, analogous reactions of *N*-benzylhydroxylamines were unsuccessful due to *O*-heteroalkylation and subsequent retro-ene reactions giving rise to the formation of aldimine and imidazolidin-2-one [19].

The synthetic approach to hydroxylamine derivatives **3** is based on our previous observation that *O*-sulfonyl imidazolidin-2-one oxime undergoes regioselective benzylation at the exocyclic N2 nitrogen atom [20]. As shown in Scheme 1, similar reaction of the previously obtained *O*-benzyl imidazolidin-2-one oxime **1** [21] with variously substituted benzyl and benzhydryl halides produces *O*-benzyl-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-*N*-[alkyl(aryl)]-hydroxylamines **2a–m** in high yields (85–90%). Later compounds were hydrogenated with use of gaseous hydrogen in the presence of 10% Pd/C to give the desired debenzylated products **3a–m** in excellent yields (95–99%). Finally, upon treatment of the free bases **3** with a methanolic solution of hydrochloride or hydrobromide, the




**Scheme 1.** Reagents and conditions. (a) RX, DMF, 12 h; (b) $K_2CO_3$; (c) $H_2$, 10% Pd/C; (d) HCl or HBr. X = Br for **a**–**h** and **j**–**m**. X = Cl for **i**. Y = Cl for **a**–**h**, **j**, **l** and **m**. Y = Br for **i** and **k**.

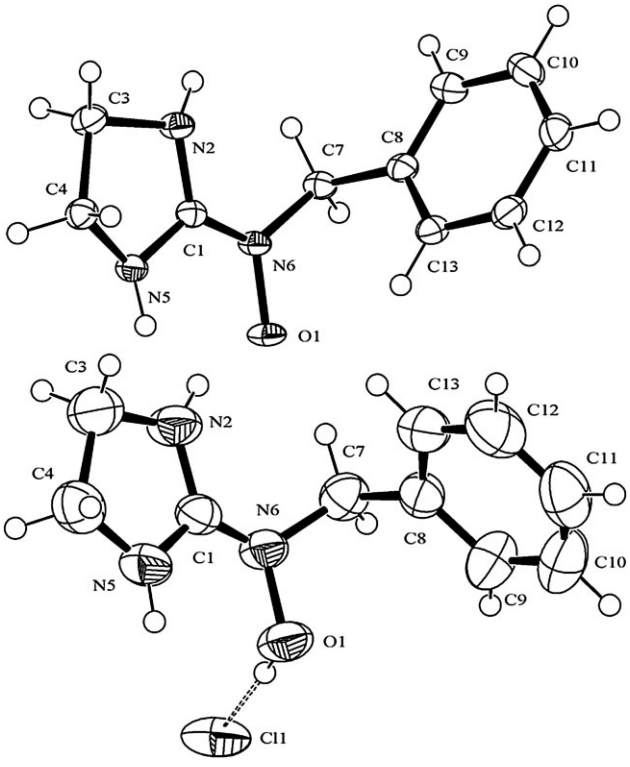



**Figure 2.** View of the molecular structures of **3a** *(top)* and **4a** *(bottom)* with displacement ellipsoids drawn at the 50% probability level.

water soluble addition salts **4a–m** suitable for biological evaluation were obtained.

By analogy to the previously investigated *N*-arylhydroxylamines of type **B** [22], compounds **3** may exist as a mixture of two tautomeric forms: α-iminohydroxylamine (**C**) and α-aminonitrone (**D**). We therefore felt it necessary to investigate in detail the structure of both the free base **3a** and corresponding salt **4a** by means of experimental methods and quantum-chemical calculations. Thus, in the $^{1}$H-NMR spectrum of **3a** run in $CD_3OD$ a singlet at 3.67 ppm integrated to four $CH_2CH_2$ protons of imidazolidine moiety indicates the presence of the α-aminonitrone tautomer **D**. This observation is further confirmed by the presence of single resonance attributable to $C_4$ and $C_5$ imidazolidine carbon atoms in the $^{13}$C-NMR spectrum at 45.1 ppm. Moreover, single crystal X-ray analysis of the free base **3a** obtained by crystallization from methanol confirmed that in the crystalline phase the α-aminonitrone form **D** exists (Figure 2).

To elucidate the possible tautomeric equilibrium we estimated the relative Gibbs free-energies $\Delta G$ (298.15 K) and dipole moments for **3a** tautomers: **C** (α-iminohydroxylamine) and **D** (α-aminonitrone). Calculations were performed using *ab initio* Hartree-Fock and DFT methods [23] both in the gas phase and using SM8 solvation models [24]. From the data presented in Table 1 one can infer that in polar solvents the formation of the α-aminonitrone **D** is energetically favorable. Nitrone **D** with higher dipole moment than those found for hydroxylamine **C** should also be better stabilized in polar solvents.

Basicity ($pK_a$ value) of the free base **3a** was determined by potentiometric titration. It appeared that the placement of the hydroxyl group at the nitrogen atom of the guanidine moiety resulted in the compound with diminished basicity ($pK_a = 7.7$) compared to classic 2-aryliminoimidazolidines such as clonidine ($pK_a = 8.2$). Thus, under physiological conditions ($pK_a = 7.4$) compound **3a** exists at about 66% in the free base form **D** leaving about 34% for the protonated form of type **4a**. The structure of the hydroxylamine hydrochloride **4a** resulting from protonation of the oxygen atom of the nitrone **D** was confirmed by single crystal X-ray analysis (Figure 2).

## Biology

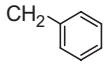
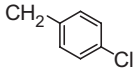
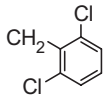
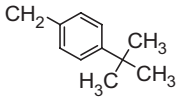
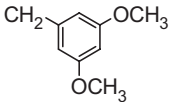
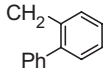
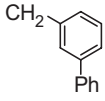
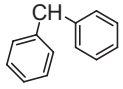
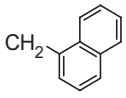
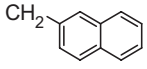
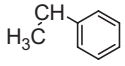
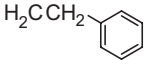
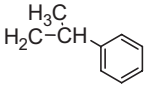
All the obtained compounds of type **4** have been evaluated for their affinities to $\alpha_1$ and $\alpha_2$ adrenoceptors as well as to imidazoline $I_1$ and $I_2$ receptors (Table 2). Radioligand binding assays were performed using $P_2$ membranes prepared from brains of male Sprague-Dawley rats (for $\alpha_1$, $\alpha_2$ and $I_2$ binding sites) or crude $P_2$ membranes obtained from kidneys of male Sprague-Dawley rats (for $I_2$ binding sites). The benzyl derivatives **4a–e** exhibited very weak micromolar affinities to α-adrenergic receptors and imidazoline $I_1$ receptors while the 4-chloro congener **4b** showed a moderate affinity to

**Table 1.** The relative Gibbs free-energies $\Delta G$ (298.15 K) and dipole moments for **3a** tautomers **C** (α-iminohydroxylamine) and **D** (α-aminonitrone) calculated in vacuum and using methanol or water SM8 [24] solvation models.

| Method | | Vacuum | | Methanol | | Water | |
|---|---|---|---|---|---|---|---|
| | | C | D | C | D | C | D |
| HF/6-31+G* | $\Delta G$ [kcal/mol] | 0 | 5.4 | 2.5 | 0 | 1.5 | 0 |
| | dipole moment [Debye] | 3.54 | 6.69 | 4.73 | 9.00 | 4.69 | 9.05 |
| B3LYP/6-31+G* | $\Delta G$ [kcal/mol] | 0 | 0.9 | 3.8 | 0 | 4.1 | 0 |
| | dipole moment [Debye] | 3.73 | 5.80 | 5.14 | 8.48 | 5.22 | 8.55 |

**Table 2.** Experimental binding affinities of salts **4** to α-adrenergic and imidazoline receptors.

| Compd 4 | R | $\alpha_1$ receptor $K_i$ (nM)[a] | $\alpha_2$ receptor $K_i$ (nM)[a] | $I_1$ receptor $IC_{50}$ (nM)[b] | $I_2$ receptor $K_i$ (nM)[a] |
|---|---|---|---|---|---|
| **a** |  | 18 100 | 5150 | 2210 | 303 |
| **b** |  | 5440 | 8260 | 42 600 | 72.7 |
| **c** |  | 6340 | 1700 | 955 | 2340 |
| **d** |  | 2010 | 5800 | 15 800 | 5160 |
| **e** |  | 7130 | 19 700 | 105 000 | 12 000 |
| **f** |  | 970 | 3850 | 7090 | 25 400 |
| **g** |  | 373 | 910 | 77 800 | 794 |
| **h** |  | 31 | 9 | 1020 | 46 |
| **i** |  | 137 | 92 | 1640 | 41.5 |
| **j** |  | 1040 | 4880 | 87 300 | 2570 |
| **k** |  | 8680 | 3220 | 144 000 | 38 200 |
| **l** |  | 2090 | 5990 | 80 600 | 49.4 |
| **m** |  | 2660 | 8500 | ND[c] | 517 |

[a] $K_i$ affinity values for $\alpha_1$-adrenoreceptors, $\alpha_2$-adrenoreceptors and $I_2$ imidazoline binding sites were assessed by measuring the ability of the test compounds to displace [$^3$H]prazosin (rat brain membranes), [$^3$H]RX821002 (rat brain membranes) or [$^3$H]2BFI (rat brain membranes), respectively.
[b] Molar concentration of the test compounds that displaces 50% of specifically bound [$^3$H]clonidine (rat kidney membranes).
[c] ND – no displacement

the imidazoline $I_2$ receptor ($K_i$ = 72.7 nM). Poor activity was also observed for compounds **4f–g** incorporating the diphenyl moiety. The most interesting results, however, were obtained for lipophilic derivatives **4h–j**. Thus, the benzhydryl congener **4h** proved to be a non-selective ligand of $\alpha_2$, $\alpha_1$ and $I_2$ receptors ($K_i$ values = 9 nM, 31 nM and 46 nM, respectively). When the benzhydryl scaffold was replaced by a 1-naphthyl structure (**4i**), the affinity at $\alpha_1$ and $\alpha_2$ adrenoceptors was reduced while the activity at the $I_2$ receptor was retained. In turn, a change from 1-naphthyl (**4i**) to 2-naphthyl (**4j**) structure led to a considerable decrease of activity.

Interestingly enough, elongation of the aliphatic bridge, i.e. going from *N*-benzyl derivative **4a** to *N*-phenylethyl derivative **4l**, resulted in an increase of the binding affinity to imidazoline $I_2$ receptors ($K_i$ = 49 nM vs. 303 nM) which have been associated with various CNS disorders, such as Alzheimer's disease, Huntington's disease, depression, and malignancy of human gliomas [25, 26].

The compounds most active at $\alpha_1$ and $\alpha_2$ adrenoceptors, i.e. **4h** and **4i**, were then evaluated in anaesthetized male Wistar rats for possible cardiovascular effects. The phentolamine congener **4h** administered intravenously did not affect the mean arterial blood pressure (MAP) at doses up to 0.1 mg/kg, however, at that dose it lowered the heart rate (HR) by 30 bpm. As phentolamine blocks $\alpha_1$ adrenergic receptors, lowers blood pressure and in consequence increases the heart rate [27], the mechanism underlying the bradycardic action of **4h** should be different and may involve $\beta_1$ adrenergic receptor blockage. The naphazoline analogue **4i** administered i.v. at dose 1 mg/kg showed biphasic effect on blood pressure: initial hypertension (ΔMAP = 14 mmHg) followed by a prolonged hypotension (ΔMAP = 15 mmHg). Apparently, the transient vasoconstriction is probably mediated by vascular $\alpha_{2B}$ adrenoceptors or results from peripheral $\alpha_1$ agonist activity. Upon crossing the blood-brain barrier, compound **4i** elicits a hypotensive effect, which is characteristic of the centrally acting $\alpha_2$ adrenoceptor agonists.

Compounds **4h** and **4i** were further tested for the ability to activate the non-REM sleep pathway in rats which was determined by the loss of righting reflex (LORR) test (see Experimental for details). Both compounds were well tolerated at the doses tested (5–500 μg/kg), however, no LORR was observed for either compound. Thus, we found no evidence for induction of a sedative-hypnotic state following intraperitoneal injection of **4h** and **4i**.

## Conclusion

All the synthesized congeners of the series **4a–m** exhibited very weak binding affinities to imidazoline $I_1$ receptors. Compounds **4b**, **4h**, **4i** and **4l** demonstrated good affinity to imidazoline $I_2$ receptors. Of special interest are the selective $I_2$ receptor ligands, i.e. compounds **4b** and **4l** which, due to low lipophilicity, are not expected to cross the blood-brain barrier, and therefore could find applications for the treatment of vascular hyperplasia [28] or as enhancers of supraspinal morphine analgesia [29].

The most active compound at $\alpha_1$ and $\alpha_2$ adrenoceptors was **4h** which did not show the haemodynamic and sedative effects. Such pharmacological profile may prove beneficial for therapeutic applications other than the treatment of hypertension.

Finally, it is worth noting that the free bases of type **3** bearing nitrone moiety may be regarded as a potential neuroprotective agent in preventing the death of cells exposed to enhanced oxidative stress and damage [30]. Further studies are planned to confirm the above assumptions.

## Experimental

Melting points were measured on a Boetius 524 apparatus and are uncorrected. $^1$H- and $^{13}$C-NMR spectra were recorded using a Varian Unity 200 apparatus at 200 MHz and 50 MHz, respectively, using TMS as an internal standard. Results of C,H,N elemental analyses were within ±0.4% of theoretical values.

### Chemistry

#### *O-Benzyl-N-(arylalkyl)-N-(4,5-dihydro-1H-imidazol-2-yl)-hydroxylamines **2***

General procedure: Imidazolidin-2-one *O*-benzyl oxime [21] (0.5 g, 2.61 mmol) and the corresponding arylalkyl bromide or chloride (2.61 mmol) were dissolved in DMF (2 mL) and stirred overnight. The resulting suspension was evaporated under reduced pressure, alkalized with 5% NaOH solution (5 mL) and extracted with methylene chloride (3 × 10 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered and evaporated. The oily residue was purified on silica with use of chromatotron (ethyl acetate/methanol 95:5). Yields 85–90%.

#### *N,O-Dibenzyl-N-(4,5-dihydro-1H-imidazol-2-yl)-hydroxylamine **2a***

M.p.: 108–110°C; $^1$H-NMR (500 MHz, $CDCl_3$) δ 3.63 (4H, bs, $CH_2$), 4.51 (2H, s, $CH_2$), 4.65 (2H, s, $CH_2$), 4.70 (1H, bs, NH), 7.16–7.20 (2H, m, CH), 7.31–7.36 (4H, m, CH), 7.39 (2H, t, $J$ = 7.2 Hz, CH), 7.49 (2H, d, $J$ = 7.2 Hz, CH); $^{13}$C-NMR (125 MHz, $CDCl_3$) δ 46.0, 57.4, 78.4, 128.0, 128.6, 128.8, 128.9, 129.5, 130.1, 135.6, 136.8, 167.4; IR (KBr, $cm^{-1}$) 3139, 3064, 2948, 2848, 1592, 1582, 1497, 1454, 1277, 1089, 1002, 990, 840, 749, 698.

#### *O-Benzyl-N-(4-chlorobenzyl)-N-(4,5-dihydro-1H-imidazol-2-yl)hydroxylamine **2b***

M.p.: 76–78°C; $^1$H-NMR (200 MHz, $CDCl_3$) δ 3.65 (4H, bs, $CH_2$), 4.56 (2H, s, $CH_2$), 4.59 (2H, s, $CH_2$), 4.70 (1H, bs, NH), 7.15–7.45 (9H, m, CH); $^{13}$C-NMR (50 MHz, $CDCl_3$) δ 46.0, 56.8, 78.6, 128.9, 129.1, 129.2, 129.7, 131.6, 134.0, 135.6, 135.8, 167.3; IR (KBr, $cm^{-1}$) 3184, 3031, 2933, 2870, 1603, 1498, 1283, 1088, 800, 752, 734, 698.

### *O-Benzyl-N-(2,6-dichlorobenzyl)-N-(4,5-dihydro-1H-imidazol-2-yl)hydroxylamine* ***2c***

M.p.: 140–144°C; $^1$H-NMR (500 MHz, $CDCl_3$) δ 3.40–3.49 (2H, m, $CH_2$), 3.85–3.94 (2H, m, $CH_2$), 4.33 (2H, s, $CH_2$), 4.59 (1H, bs, NH), 5.01 (2H, s, $CH_2$), 7.13–7.17 (2H, m, CH), 7.26 (1H, t, $J$ = 7.8 Hz, CH), 7.30–7.34 (3H, m, CH), 7.40 (2H, d, $J$ = 7.8 Hz, CH); $^{13}$C-NMR (125 MHz, $CDCl_3$) δ 45.0, 52.6, 79.1, 128.7, 128.8, 128.9, 129.5, 130.0, 132.3, 135.2, 137.8, 167.3; IR (KBr, $cm^{-1}$) 3155, 2981, 2922, 2856, 1604, 1562, 1491, 1459, 1437, 1279, 1091, 1003, 991, 780, 754, 692.

### *O-Benzyl-N-(4-tert-butylbenzyl)-N-(4,5-dihydro-1H-imidazol-2-yl)hydroxylamine* ***2d***

M.p.: 96–98°C; $^1$H-NMR (500 MHz, $CDCl_3$) δ 1.36 (9H, s, $CH_3$), 3.64 (4H, bs, $CH_2$), 4.54 (2H, s, $CH_2$), 4.60 (1H, bs, NH), 4.63 (2H, s, $CH_2$), 7.15–7.17 (2H, m, CH), 7.33–7.35 (4H, m, CH), 7.41–7.43 (3H, m, CH); $^{13}$C-NMR (50 MHz, $CDCl_3$) δ 31.9, 35.0, 45.0, 57.2, 78.7, 125.7, 129.0, 129.2, 129.8, 130.1, 133.8, 135.8, 151.1, 167.4; IR (KBr, $cm^{-1}$) 3172, 2962, 2865, 1595, 1492, 1457, 1280, 1007, 986, 830, 757, 749, 698.

### *O-Benzyl-N-(4,5-dihydro-1H-imidazol-2-yl)-N-(3,5-dimethoxybenzyl)hydroxylamine* ***2e***

M.p.: 66–68°C; $^1$H-NMR (200 MHz, $CDCl_3$) δ 3.63 (4H, bs, $CH_2$), 3.79 (6H, s, $OCH_3$), 4.15 (1H, bs, NH), 4.58 (2H, s, $CH_2$), 4.59 (2H, s, $CH_2$), 6.43 (1H, t, $J$ = 2.3 Hz, CH), 6.64 (2H, d, $J$ = 2.3 Hz, CH), 7.19–7.35 (5H, m, CH); $^{13}$C-NMR (50 MHz, $CDCl_3$) δ 44.5, 55.8, 57.9, 78.7, 100.5, 108.0, 129.0, 129.1, 129.7, 135.9, 139.4, 161.2, 167.5; IR (KBr, $cm^{-1}$) 3157, 2946, 2846, 1597, 1463, 1345, 1283, 1203, 1146, 1052, 824, 752, 700.

### *O-Benzyl-N-(biphenyl-2-ylmethyl)-N-(4,5-dihydro-1H-imidazol-2-yl)hydroxylamine* ***2f***

M.p.: 165–166°C; $^1$H-NMR (200 MHz, DMSO-$d_6$) δ 3.41 (4H, bs, $CH_2$), 4.48 (2H, s, $CH_2$), 4.50 (2H, s, $CH_2$), 6.00 (1H, bs, NH), 7.03–7.07 (2H, m, CH), 7.25–7.40 (11H, m, CH), 7.59–7.61 (1H, m, CH); $^{13}$C-NMR (50 MHz, DMSO-$d_6$) δ 45.2, 53.8, 77.1, 127.3, 127.4, 127.5, 128.3 (two signals), 128.4, 129.3, 129.5, 130.0, 130.1, 134.4, 135.7, 140.8, 142.1, 166.4; IR (KBr, $cm^{-1}$) 3167, 2956, 2854, 1590, 1492, 1456, 1278, 1004, 991, 758, 699.

### *O-Benzyl-N-(biphenyl-3-ylmethyl)-N-(4,5-dihydro-1H-imidazol-2-yl)hydroxylamine* ***2g***

Obtained as an oil; $^1$H-NMR (200 MHz, $CDCl_3$) δ 3.64 (4H, bs, $CH_2$), 4.45 (1H, bs, NH), 4.56 (2H, s, $CH_2$), 4.71 (2H, s, $CH_2$), 7.14–7.72 (14H, m, CH); $^{13}$C-NMR (50 MHz, $CDCl_3$) δ 45.0, 57.7, 78.7, 127.0, 127.7, 127.8, 129.1, 129.2 (two signals), 129.3 (two signals), 129.8 (two signals), 135.8, 137.5, 141.4, 141.7, 167.5.

### *N-Benzhydryl-O-benzyl-N-(4,5-dihydro-1H-imidazol-2-yl)hydroxylamine* ***2h***

M.p.: 118–120°C; $^1$H-NMR (500 MHz, $CDCl_3$) δ 3.28–3.37 (2H, m, $CH_2$), 3.72–3.81 (2H, m, $CH_2$), 4.30 (2H, bs, $CH_2$), 4.68 (1H, bs, NH), 6.49 (1H, s, $CH_2$), 7.10–7.12 (2H, m, CH), 7.29–7.36 (9H, m, CH), 7.44–7.46 (4H, m, CH); $^{13}$C-NMR (125 MHz, $CDCl_3$) δ 45.3, 54.4, 69.2, 79.1, 127.5, 128.4, 128.7, 128.8, 129.4, 129.8, 135.5, 139.7, 166.3; IR (KBr, $cm^{-1}$) 3159, 3062, 3027, 2940, 2870, 1605, 1494, 1454, 1285, 1001, 750, 723, 699.

### *O-Benzyl-N-(4,5-dihydro-1H-imidazol-2-yl)-N-(naphthalen-1-ylmethyl)hydroxylamine* ***2i***

Obtained as an oil, $^1$H-NMR (500 MHz, $CDCl_3$) δ 3.71 (4H, bs, $CH_2$), 4.13 (2H, s, $CH_2$), 5.16 (2H, s, $CH_2$), 6.94–6.98 (2H, m, CH), 7.22–7.30 (3H, m, CH), 7.49–7.64 (4H, m, CH), 7.88–7.93 (2H, m, CH), 8.32–8.35 (1H, m, CH).

### *O-Benzyl-N-(4,5-dihydro-1H-imidazol-2-yl)-N-(naphthalen-2-ylmethyl)hydroxylamine* ***2j***

Obtained as an oil; $^1$H-NMR (200 MHz, $CDCl_3$) δ 3.65 (4H, s, $CH_2$), 4.51 (2H, s, $CH_2$), 4.60 (1H, bs, NH), 4.82 (2H, s, $CH_2$), 7.09–7.18 (2H, m, CH), 7.22–7.32 (3H, m, CH), 7.43–7.55 (2H, m, CH), 7.61–7.68 (1H, m, CH), 7.80–7.91 (4H, m, CH); $^{13}$C-NMR (50 MHz, $CDCl_3$) δ 45.0, 57.9, 78.7, 126.4, 126.6, 128.1, 128.2, 128.4, 128.5, 129.0, 129.1, 129.2, 129.7, 133.4, 133.8, 134.6, 135.8, 167.5.

### *O-Benzyl-N-(4,5-dihydro-1H-imidazol-2-yl)-N-(1-phenylethyl)hydroxylamine* ***2k***

M.p.: 72–73°C; $^1$H-NMR (200 MHz, $CDCl_3$) δ 1.62 (3H, d, $J$ = 6.8 Hz, $CH_3$), 3.61 (4H, s, $CH_2$), 4.35 (1H, bs, NH), 4.50–4.60 (2H, m, $CH_2$), 5.34 (1H, q, $J$ = 6.8 Hz, CH), 7.15–7.22 (2H, m, CH), 7.29–7.43 (6H, m, CH), 7.52–7.60 (2H, m, CH); $^{13}$C-NMR (50 MHz, $CDCl_3$) δ 15.2, 45.0, 61.3, 79.5, 128.1, 128.6, 129.0, 129.1 (two signals), 129.6, 135.9, 140.8, 167.0; IR (KBr, $cm^{-1}$) 3156, 3030, 2934, 2880, 1591, 1497, 1453, 1371, 1279, 989, 752, 699.

### *O-Benzyl-N-(4,5-dihydro-1H-imidazol-2-yl)-N-phenethylhydroxylamine* ***2l***

M.p.: 76–79°C; $^1$H-NMR (500 MHz, $CDCl_3$) δ 3.02 (2H, t, $J$ = 7.5 Hz, $CH_2$), 3.59 (4H, bs, $CH_2$), 3.78 (2H, t, $J$ = 7.5 Hz), 4.84 (2H, s, $OCH_2$), 4.86 (1H, bs, NH), 7.15–7.45 (10H, m, CH); $^{13}$C-NMR (50 MHz, $CDCl_3$) δ 32.4, 42.8, 48.0, 77.5, 126.2, 128.4, 128.7, 128.8, 129.0, 129.1, 135.6, 139.2, 166.1; IR (KBr, $cm^{-1}$) 3234, 3024, 2930, 2867, 1645, 1607, 1494, 1458, 1374, 1275, 1215, 1063, 1005, 740, 697.

### *O-Benzyl-N-(4,5-dihydro-1H-imidazol-2-yl)-N-(2-phenylpropyl)hydroxylamine* ***2m***

Obtained as an oil; $^1$H-NMR (200 MHz, $CDCl_3$) δ 1.34 (3H, d, $J$ = 7.3 Hz, $CH_3$), 3.29 (1H, q, $J$ = 7.3 Hz, CH), 3.52 (4H, s, $CH_2$), 3.75 (2H, d, $J$ = 7.3 Hz, $CH_2$), 4.78 (1H, bs, NH), 4.62–4.78 (2H, m, $OCH_2$), 7.18–7.40 (10H, m, CH); $^{13}$C-NMR (50 MHz, $CDCl_3$) δ 20.5, 38.3, 49.5, 60.6, 77.8, 126.8, 128.0, 128.9, 129.1 (two signals), 129.5, 136.1, 145.4, 167.5.

### *N-(Imidazolidin-2-ylidene)-1-arylalkylamine oxides* ***3***

General procedure: The corresponding hydroxylamine **2** (0.5 g) was dissolved in methanol (5 mL). The substrate was hydrogenated in the presence of 10% Pd/C (0.05 g) by passing gaseous hydrogen through the solution (1 h) or with use of pressurized reactor (0.5 h). The progress of the reaction was monitored by TLC. The filtration and evaporation of the reaction mixture furnished a solid residue which upon rinsing with diethyl ether gave pure product **3**. Yields 95–99%. The free bases **3** thus obtained were converted into corresponding addition salts **4** upon treatment with methanolic solutions of gaseous hydrochloride or hydrobromide.

*N-(Imidazolidin-2-ylidene)-1-phenylmethanamine oxide* ***3a***
M.p.: 192–197°C; $^1$H-NMR (200 MHz, $CD_3OD$) δ 3.67 (4H, s, $CH_2$), 4.63 (2H, s, $CH_2$), 4.94 (2H, s, NH), 7.33–7.37 (5H, m, CH); $^{13}$C-NMR (50 MHz, $CD_3OD$) δ 45.1, 57.6, 129.1, 129.3, 129.9, 137.1, 158.8; IR (KBr, $cm^{-1}$) 3028, 2858, 2656, 1694, 1519, 1494, 1480, 1290, 1264, 1111, 741, 702.

*Hydrochloride of N-(imidazolidin-2-ylidene)-1-phenylmethanamine oxide* ***4a***
M.p.: 129–131°C; $^1$H-NMR (500 MHz, DMSO-$d_6$) δ 3.69 (4H, s, $CH_2$), 4.87 (2H, s, $CH_2$), 4.94 (2H, s, NH), 7.33–7.39 (5H, m, CH), 8.98 (2H, bs, NH), 11.17 (1H, s, OH); $^{13}$C-NMR (125 MHz, DMSO-$d_6$) δ 43.9, 56.3, 128.7, 129.1, 129.2, 135.5, 161.1. Anal. calcd. for $C_{10}H_{14}ClN_3O$: C, 52.75; H, 6.20; N, 18.45; found: C, 52.44; H, 6.51; N, 18.31.

*1-(4-Chlorophenyl)-N-(imidazolidin-2-ylidene)-methanamine oxide* ***3b***
M.p.: 159–163°C; $^1$H-NMR (500 MHz, $CD_3OD$) δ 3.70 (4H, s, $CH_2$), 4.62 (2H, s, $CH_2$), 4.97 (2H, s, NH), 7.34–7.40 (4H, m, CH); $^{13}$C-NMR (50 MHz, $CD_3OD$) δ 45.1, 56.9, 129.9, 131.1, 134.9, 135.9, 159.3; IR (KBr, $cm^{-1}$) 3058, 2886, 1686, 1522, 1489, 1292, 1113, 1017, 801.

*Hydrochloride of 1-(4-chlorophenyl)-N-(imidazolidin-2-ylidene)methanamine oxide* ***4b***
M.p.: 155–160°C; $^1$H-NMR (200 MHz, DMSO-$d_6$) δ 3.67 (4H, s, $CH_2$), 4.87 (2H, s, $CH_2$), 7.36–7.48 (4H, m, CH), 9.01 (2H, bs, NH), 11.18 (1H, bs, OH); $^{13}$C-NMR (50 MHz, DMSO-$d_6$) δ 43.5, 55.2, 128.7, 130.7, 132.9, 134.0, 160.7; IR (KBr, $cm^{-1}$) 3198, 3123, 3023, 2833, 1661, 1626, 1570, 1492, 1290, 1115, 1093, 1014, 802. Anal. calcd. for $C_{10}H_{13}Cl_2N_3O$: C, 45.82; H, 5.00; N, 16.03; found: C, 45.76; H, 5.12; N, 15.94.

*1-(2,6-Dichlorophenyl)-N-(imidazolidin-2-ylidene)-methanamine oxide* ***3c***
M.p.: 176–182°C; $^1$H-NMR (200 MHz, $CD_3OD$) δ 3.79 (4H, s, $CH_2$), 4.92 (2H, s, $CH_2$), 4.95 (2H, s, NH), 7.27–7.45 (3H, m, CH); $^{13}$C-NMR (50 MHz, $CD_3OD$) δ 44.9, 51.5, 129.7, 131.4, 131.7, 138.2, 159.1; IR (KBr, $cm^{-1}$) 3420, 2945, 2866, 1685, 1582, 1563, 1495, 1436, 1289, 1208, 1006, 835, 782, 769.

*Hydrochloride of 1-(2,6-dichlorophenyl)-N-(imidazolidin-2-ylidene)methanamine oxide* ***4c***
M.p.: 248–252°C; $^1$H-NMR (200 MHz, DMSO-$d_6$) δ 3.72 (4H, s, $CH_2$), 5.06 (2H, s, $CH_2$), 7.40–7.56 (5H, m, CH), 8.99 (2H, bs, NH), 10.86 (1H, s, OH); $^{13}$C-NMR (50 MHz, DMSO-$d_6$) δ 43.5, 51.2, 129.1, 129.3, 131.5, 136.6, 160.8; IR (KBr, $cm^{-1}$) 3115, 3031, 2852, 1660, 1564, 1435, 1295, 1114, 1088, 935, 777, 770. Anal. calcd. for $C_{10}H_{12}Cl_3N_3O$: C, 40.50; H, 4.08; N, 14.17; found: C, 40.45; H, 4.14; N, 14.02.

*1-(4-tert-Butylphenyl)-N-(imidazolidin-2-ylidene)-methanamine oxide* ***3d***
M.p.: 188–193°C; $^1$H-NMR (500 MHz, DMSO-$d_6$) δ 1.28 (9H, s, $CH_3$), 3.42 (4H, s, $CH_2$), 4.44 (2H, s, $CH_2$), 7.25 (2H, d, $J$ = 8.3 Hz, CH), 7.33 (2H, d, $J$ = 8.3 Hz, CH); $^{13}$C-NMR (50 MHz, DMSO-$d_6$) δ 31.2, 34.1, 48.4, 56.5, 124.7, 128.3, 134.8, 149.2, 166.7; IR (KBr, $cm^{-1}$) 3054, 2962, 2866, 1694, 1516, 1477, 1289, 1267, 1008, 831, 680, 548.

*Hydrochloride of 1-(4-tert-butylphenyl)-N-(imidazolidin-2-ylidene)methanamine oxide* ***4d***
M.p.: 178–180°C; $^1$H-NMR (200 MHz, DMSO-$d_6$) δ 1.27 (9H, s, $CH_3$), 3.67 (4H, s, $CH_2$), 4.82 (2H, s, $CH_2$), 7.29 (2H, d, $J$ = 8.3 Hz, CH), 7.39 (2H, d, $J$ = 8.3 Hz, CH); 8.87 (bs, 2H, NH), 11.14 (s, 1H, OH); $^{13}$C-NMR (50 MHz, DMSO-$d_6$) δ 31.4, 34.6, 43.4, 55.6, 125.5, 128.5, 132.1, 150.7, 160.6. Anal. calcd. for $C_{14}H_{22}ClN_3O$: C, 59.25; H, 7.81; N, 14.81; found: C, 59.01; H, 8.06; N, 14.69.

*1-(3,5-Dimethoxyphenyl)-N-(imidazolidin-2-ylidene)-methanamine oxide* ***3e***
M.p.: 172–175°C.

*Hydrochloride of 1-(3,5-dimethoxyphenyl)-N-(imidazolidin-2-ylidene)methanamine oxide* ***4e***
M.p.: 179–181°C; $^1$H-NMR (200 MHz, DMSO-$d_6$) δ 3.68 (4H, s, $CH_2$), 3.74 (6H, s, $OCH_3$), 4.78 (2H, s, $CH_2$), 6.45–6.47 (1H, m, CH), 6.54–6.55 (2H, m, CH), 8.96 (2H, bs, NH), 11.18 (1H, s, OH); $^{13}$C-NMR (50 MHz, DMSO-$d_6$) δ 43.4, 55.5, 56.0, 99.7, 106.6, 137.2, 160.8 (two signals); IR (KBr, $cm^{-1}$) 3122, 2844, 1647, 1629, 1609, 1597, 1474, 1429, 1351, 1208, 1164, 1069, 833. Anal. calcd. for $C_{12}H_{18}ClN_3O_3$: C, 50.09; H, 6.31; N, 14.60; found: C, 49.98; H, 6.68; N, 14.39.

*1-(Biphenyl-2-yl)-N-(imidazolidin-2-ylidene)methanamine oxide* ***3f***
Obtained as an oil.

*Hydrochloride of 1-(biphenyl-2-yl)-N-(imidazolidin-2-ylidene)methanamine oxide* ***4f***
M.p.: 165–170°C; $^1$H-NMR (500 MHz, DMSO-$d_6$) δ 3.65 (4H, b, $CH_2$), 4.84 (2H, s, $CH_2$), 7.27–7.49 (9H, m, CH), 8.81 (2H, bs, NH), 11.01 (1H, s, OH); $^{13}$C-NMR (50 MHz, DMSO-$d_6$) δ 43.4, 54.1, 127.6, 127.8, 128.0, 128.6, 129.3, 130.4, 131.9, 140.0, 141.6, 160.7; IR (KBr, $cm^{-1}$) 3120, 2857, 1638, 1573, 1480, 1294, 1106, 1010, 744, 702. Anal. calcd. for $C_{16}H_{18}ClN_3O$: C, 63.26; H, 5.97; N, 13.83; found: C, 63.41; H, 6.05; N, 13.58.

*1-(Biphenyl-3-yl)-N-(imidazolidin-2-ylidene)methanamine oxide* ***3g***
Obtained as an oil.

*Hydrochloride of 1-(biphenyl-3-yl)-N-(imidazolidin-2-ylidene)methanamine oxide* ***4g***
M.p.: 161–163°C; $^1$H-NMR (200 MHz, DMSO-$d_6$) δ 3.69 (4H, s, $CH_2$), 4.93 (2H, s, $CH_2$), 7.36–7.54 (5H, m, CH), 7.60–7.72 (4H, m, CH), 9.01 (2H, bs, NH), 11.21 (1H, s, OH); $^{13}$C-NMR (50 MHz, DMSO-$d_6$) δ 43.2, 55.7, 126.3, 126.7, 126.8, 127.4, 127.6, 129.0, 129.1, 135.4, 139.9, 140.3, 160.5; IR (KBr, $cm^{-1}$) 3113, 3029, 2857, 1636, 1569, 1480, 1454, 1292, 1109, 759, 725, 704. Anal. calcd. for $C_{16}H_{18}ClN_3O$: C, 63.26; H, 5.97; N, 13.83; found: C, 63.46; H, 6.13; N, 13.86.

*N-(Imidazolidin-2-ylidene)-1,1-diphenylmethanamine oxide* ***3h***
M.p.: 162–164°C; IR (KBr, $cm^{-1}$) 3413, 3041, 2931, 2884, 1663, 1514, 1445, 1289, 1143, 1095, 866, 741, 728, 703.

### *Hydrochloride of N-(imidazolidin-2-ylidene)-1,1-diphenylmethanamine oxide* ***4h***

M.p.: 198–205°C; $^1$H-NMR (200 MHz, DMSO-$d_6$) δ 3.69 (4H, s, $CH_2$), 6.82 (1H, s, CH), 7.33–7.39 (10H, m, CH), 9.30 (2H, bs, NH), 10.80 (1H, s, OH); $^{13}$C-NMR (50 MHz, DMSO-$d_6$) δ 43.4, 67.1, 128.2, 128.7, 128.9, 137.9, 161.8; IR (KBr, $cm^{-1}$) 3062, 1624, 1582, 1568, 1496, 1454, 1292, 1106, 1030, 748, 729, 700. Anal. calcd. for $C_{16}H_{18}ClN_3O$: C, 63.26; H, 5.97; N, 13.83; found: C, 63.03; H, 5.64; N, 13.66.

### *N-(Imidazolidin-2-ylidene)-1-(naphthalen-1-yl)-methanamine oxide* ***3i***

Obtained as an oil.

### *Hydrobromide of N-(imidazolidin-2-ylidene)-1-(naphthalen-1-yl)methanamine oxide* ***4i***

M.p.: 222–227°C; $^1$H-NMR (500 MHz, DMSO-$d_6$) δ 3.65 (4H, s, $CH_2$), 3.84 (2H, bs, NH), 4.90 (2H, s, $CH_2$), 7.49–7.56 (2H, m, CH), 7.58–7.66 (2H, m, CH), 7.92–7.96 (1H, m, CH), 7.98–8.06 (2H, m, CH), 8.77 (1H, bs, OH); $^{13}$C-NMR (50 MHz, DMSO-$d_6$) δ 42.9, 44.0, 123.6, 125.2, 125.7, 126.4, 126.8, 128.6, 128.9, 130.8, 132.5, 133.6, 159.8; IR (KBr, $cm^{-1}$) 3169, 3068, 1682, 1602, 1380, 1066, 807, 780. Anal. calcd. for $C_{14}H_{16}BrN_3O$: C, 52.19; H, 5.01; N, 13.04; found: C, 52.02; H, 5.24; N, 13.09.

### *N-(Imidazolidin-2-ylidene)-1-(naphthalen-2-yl)-methanamine oxide* ***3j***

M.p.: 170–174°C; $^1$H-NMR (200 MHz, DMSO-$d_6$) δ 3.43 (4H, s, $CH_2$), 4.64 (2H, s, $CH_2$), 7.42–7.56 (3H, m, CH), 7.80–7.93 (4H, m, CH), 8.00 (2H, bs, NH); IR (KBr, $cm^{-1}$) 3042, 2943, 2896, 1682, 1525, 1296, 1113, 819, 752.

### *Hydrochloride of N-(imidazolidin-2-ylidene)-1-(naphthalen-2-yl)methanamine oxide* ***4j***

M.p.: 206°C dec.; $^1$H-NMR (200 MHz, DMSO-$d_6$) δ 3.70 (4H, s, $CH_2$), 5.04 (2H, s, $CH_2$), 7.50–7.55 (3H, m, CH), 7.91–7.95 (4H, m, CH), 9.03 (2H, bs, NH), 11.22 (1H, s, OH); $^{13}$C-NMR (50 MHz, DMSO-$d_6$) δ 43.5, 56.1, 126.5 (two signals), 126.7, 127.6, 127.9, 128.0, 128.4, 132.6, 132.8, 133.0, 160.7; IR (KBr, $cm^{-1}$) 3146, 3035, 1680, 1561, 1300, 1117, 811, 756, 716, 602. Anal. calcd. for $C_{14}H_{16}ClN_3O$: C, 60.54; H, 5.81; N, 15.13; found: C, 60.48; H, 5.90; N, 15.02.

### *N-(Imidazolidin-2-ylidene)-1-phenylethanamine oxide* ***3k***

Obtained as an oil; $^1$H-NMR (200 MHz, $CDCl_3$) δ 1.55 (3H, d, $J$ = 6.8 Hz, $CH_3$), 3.38 (4H, s, $CH_2$), 4.92 (2H, bs, NH), 5.01 (1H, q, $J$ = 6.8 Hz, CH), 7.20–7.46 (5H, m, CH); $^{13}$C-NMR (50 MHz, $CDCl_3$) δ 17.7, 45.7, 60.2, 127.4, 127.9, 128.8, 141.3, 160.0.

### *Hydrobromide of N-(imidazolidin-2-ylidene)-1-phenylethanamine oxide* ***4k***

M.p.: 147–154°C; IR (KBr, $cm^{-1}$) 3114, 2882, 2853, 1634, 1559, 1452, 1297, 1130, 1069, 987, 703; $^1$H-NMR (500 MHz, DMSO-$d_6$) δ 1.55 (3H, d, $J$ = 6.8 Hz, $CH_3$), 3.69 (4H, s, $CH_2$), 5.24 (1H, q, $J$ = 6.8 Hz, CH), 7.32–7.43 (5H, m, CH); $^{13}$C-NMR (125 MHz, DMSO-$d_6$) δ 17.9, 43.8, 59.9, 127.9, 128.7, 129.2, 139.6, 161.3. Anal. calcd. for $C_{11}H_{16}BrN_3O$: C, 46.17; H, 5.64; N, 14.68; found: C, 45.87; H, 5.99; N, 14.41.

### *N-(Imidazolidin-2-ylidene)-2-phenylethanamine oxide* ***3l***

M.p.: 148–155°C; $^1$H NMR (200 MHz, DMSO-$d_6$) δ 2.84 (2H, t, $J$ = 7.5 Hz, $CH_2$), 3.37 (4H, s, $CH_2$), 3.48 (2H, t, $J$ = 7.5 Hz, $CH_2$), 7.15–7.32 (5H, m, CH), 7.60 (2H, bs, NH); IR (KBr, $cm^{-1}$) 3022, 2930, 2870, 1682, 1519, 1498, 1284, 1201, 1108, 755, 707, 670.

### *Hydrochloride of N-(imidazolidin-2-ylidene)-2-phenylethanamine oxide* ***4l***

M.p.: 152–157°C; $^1$H-NMR (200 MHz, DMSO-$d_6$) δ 2.93 (2H, t, $J$ = 7.5 Hz, $CH_2$), 3.56 (4H, s, $CH_2$), 3.83 (2H, t, $J$ = 7.5 Hz, $CH_2$), 7.21–7.32 (5H, m, CH), 8.73 (2H, bs, NH), 11.19 (1H, s, OH); $^{13}$C-NMR (50 MHz, DMSO-$d_6$) δ 32.1, 43.3, 53.6, 126.7, 128.6, 129.3, 138.0, 160.5; IR (KBr, $cm^{-1}$) 3088, 3036, 2899, 2844, 1673, 1561, 1453, 1296, 1206, 1112, 1078, 943, 756, 706, 646. Anal. calcd. for $C_{11}H_{16}ClN_3O$: C, 54.66; H, 6.67; N, 17.38; found: C, 54.50; H, 6.98; N, 17.13.

### *N-(Imidazolidin-2-ylidene)-2-phenylpropan-1-amine oxide* ***3m***

M.p.: 132–137°C; $^1$H-NMR (200 MHz, $CDCl_3$) δ 1.34 (3H, d, $J$ = 7.5 Hz, $CH_3$), 2.00 (2H, bs, NH), 3.30–3.55 (5H, m, $CH_2$, CH), 3.96 (2H, d, $J$ = 7.5 Hz, $CH_2$), 7.15–7.45 (5H, m, CH); $^{13}$C-NMR (50 MHz, $CDCl_3$) δ 18.2, 36.8, 43.3, 59.3, 126.7, 127.8, 128.3, 142.6, 160.2; IR (KBr, $cm^{-1}$) 3158, 3062, 2964, 2899, 1680, 1553, 1452, 1378, 1292, 1086, 1015, 773, 707.

### *Hydrochloride of N-(imidazolidin-2-ylidene)-2-phenylpropan-1-amine oxide* ***4m***

M.p.: 62–65°C; $^1$H-NMR (500 MHz, DMSO-$d_6$) δ 1.20 (3H, d, $J$ = 6.8 Hz, $CH_3$), 3.21–3.25 (1H, m, CH), 3.54 (4H, m, $CH_2$), 3.62–3.66 (1H, m, CH), 3.80–3.84 (1H, m, CH), 7.20–7.34 (5H, m, CH) 8.65 (2H, bs, NH), 11.04 (1H, s, OH); $^{13}$C-NMR (50 MHz, DMSO-$d_6$) δ 18.8, 36.6, 43.3, 58.3, 126.9, 127.8, 128.6, 143.4, 160.5. Anal. calcd. for $C_{12}H_{18}ClN_3O$: C, 56.36; H, 7.09; N, 16.43; found: C, 56.12; H, 7.27; N, 16.30.

## 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 methods of Lione *et al.* [31]. [$^3$H]Clonidine (3 nM, Perkin Elmer) was bound in the presence of 10 μM rauwolscine to preclude binding to $\alpha_2$ adrenoceptors. The specific component was defined by 10 μM 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 0.01 μM to 100 mM. 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% polyethylamine) glass-fibre filters (Whatman GFB). Trapped radioligand was determined by liquid scintillation counting and the data were analysed 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).

### $\alpha_1$, $\alpha_2$ 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 according to methods of Lione *et al*. [31]. Membrane aliquots (400 μL, 0.2–0.3 mg protein) were incubated with 11 concentrations of the tested compounds over the range 0.01 nM to 100 μM in the presence of the selective $I_2$ binding site ligand [$^3$H]2BFI [33] (1 nM), the $\alpha_1$ adrenoceptor antagonist [$^3$H]prazosin (1nM) or the $\alpha_2$ adrenoceptor antagonist [$^3$H]RX821002 [34] (1 nM) in a final volume of 500 μL. Non-specific binding was determined using 10 μM BU224 [35] for defining $I_2$ binding, 10 μM phenylephrine for $\alpha_1$-adrenoceptors and 10 μM rauwolscine to define $\alpha_2$ adrenoceptor binding. Each incubation was performed in triplicate at room temperature and was 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. Data were analysed by iterative non-linear regression curve fitting procedures with GraphPad Prism. Each experiment was analysed individually and equilibrium dissociation constants ($K_i$) were determined by the method of Cheng and Prusoff [36]. The resulting values are given as means of 3 or 4 separate experiments.

### *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 Medical University of Gdansk, Poland. All 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 anaesthetized by i.p. injection of thiopental (Sandoz, Austria) at the dose 70 mg/kg body weight and maintained under anaesthesia 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 the 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 a dose of 0.1 mg/kg. Arterial blood pressure and heart rate were monitored directly and sampled continuously at 100 Hz, as described previously [37], using Biopac Systems, Inc., Model MP 100 (Goleta, CA, USA). The results of measurements were elaborated with the help of the ACQKnowledge (Goleta, CA, USA) measurement 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 [37]. This allowed for direct comparison of responses to treatment between groups. Data were analysed by ANOVA with repeated measurements, using Statistica StatSoft software (StatSoft, Inc., Tulsa, USA), after test compound or vehicle administration. When the effect was significant, *post hoc* comparisons were performed using Fisher's test. A value of $p < 0.05$ was considered statistically significant. This allowed direct comparison of responses to treatment between groups during the entire experiment or for selected time-points.

### Loss of righting reflex (LORR) test

The ability of compounds to activate the non-REM sleep pathway in animals is determined by induction of the loss of righting reflex (LORR). If an animal is placed on its back, it will normally turn over immediately, returning to an upright position (the righting reflex). However, with increasing loss of consciousness (such as during sedation) this ability is lost and the animal will remain in a supine position. The novel compounds **4h** and **4i** were solubilised in sterile saline for intra-peritoneal (i.p.) injection at the following doses, (i) 5 μg/kg; (ii) 50 μg/kg and (iii) 500 μg/kg. The dose range was based upon previous studies of $\alpha_2$ agonist sedative-hypnotic efficacy in rats [38]. Adult male Sprague-Dawley rats weighing 250–280 g received a single i.p. injection and were observed for LORR ($n = 3$ per dose). If, after 5 min, no LORR was observed, the dose was repeated, up to a maximum dosage of 1000 μg/kg. A selection of brains were retained following perfusion-fixation with 4% paraformaldehyde.

### X-ray crystal structure analysis

The diffraction data for single crystals of compounds **3a** and **4a** were collected with an Oxford Diffraction SuperNova diffractometer using Cu Kα radiation. The intensity data were collected and processed using Oxford Diffraction CrysAlis Software [39]. The structures were solved by direct methods with the program SHELXS-97 [40] and refined by full-matrix least-squares method on $F^2$ with SHELXL-97 [40].

Crystal data for **3a** $C_{10}H_{13}N_3O$, orthorhombic, space group Pbca, $a = 9.4805(1)$, $b = 10.1116(1)$, $c = 20.1214(1)$ Å, $V = 1928.90(3)$ Å$^3$, $Z = 8$, $T = 130$ K, $d_x = 1.317$ g cm$^{-3}$, $\mu$(Cu Kα) $= 0.718$ mm$^{-1}$, 25743 data were collected up to $\theta_{max} = 75.55°$ for a crystal with dimensions $0.69 \cdot 0.08 \cdot 0.08$ mm$^3$ ($R_{int} = 0.0253$, $R_\sigma = 0.0083$). Final R indices for 1887 reflections with $I > 2\sigma(I)$ and 136 refined parameters are: $R_1 = 0.0317$, $wR_2 = 0.0823$ ($R_1 = 0.0330$, $wR_2 = 0.0833$ for all 1987 data).

Crystal data for **4a** $C_{10}H_{14}ClN_3O$, triclinic, space group P-1, $a = 8.3310(4)$, $b = 8.7350(5)$, $c = 9.6385(5)$ Å, $\alpha = 63.059(6)$, $\beta = 83.530(4)$, $\gamma = 67.902(5)°$, $V = 577.93(7)$ Å$^3$, $Z = 2$, $T = 293$ K, $d_x = 1.308$ g cm$^{-3}$, $\mu$(Cu Kα) $= 2.759$ mm$^{-1}$, 8341 data were collected up to $\theta_{max} = 75.16°$ for a crystal with dimensions $0.49 \cdot 0.27 \cdot 0.22$ mm$^3$ ($R_{int} = 0.0151$, $R_\sigma = 0.0084$). Final R indices for 2217 reflections with $I > 2\sigma(I)$ and 148 refined parameters are: $R_1 = 0.0416$, $wR_2 = 0.01017$ ($R_1 = 0.0422$, $wR_2 = 0.1022$ for all 2267 data).

Crystallographic data for compounds **3a** and **4a** have been deposited with the Cambridge Crystallographic Data Centre, with the deposition numbers CCDC 785379 and 785378, respectively [41].

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