1-[(Imidazolidin-2-yl)imino]indazole. Highly α_2/I_1 Selective Agonist: Synthesis, X-ray Structure, and Biological Activity

Franciszek Sączewski,*[,†] Anita Kornicka,*[,†] Apolonia Rybczyńska,[‡] Alan L. Hudson,[§] Shu Sean Miao,[§] Maria Gdaniec,[#] Konrad Boblewski,[‡] and Artur Lehmann[‡]

Department of Chemical Technology of Drugs and Department of Pathophysiology, Medical University of Gdańsk, 80-416 Gdańsk, Poland, Department of Pharmacology, University of Alberta, Edmonton, T6G 2R3, Canada, and Faculty of Chemistry, A. Mickiewicz University, 60-780 Poznań, Poland

Received February 3, 2008

Novel benzazole derivatives bearing a (imidazolidin-2-yl)imino moiety at position 1 or 2 were synthesized by reacting 1-amino- or 2-aminobenzazoles with *N*,*N*′-bis(*tert*-butoxycarbonyl)imidazolidine-2-thione in the presence of $HgCl_2$. Structures of 1-[(imidazolidin-2-yl)imino]indazole (marsanidine, **13a**) and free base of the 4-Cl derivative **12e** were confirmed by X-ray single crystal structure analysis. Compound **13a** was found to be the selective α_2-adrenoceptor ligand with α_2-adrenoceptor/imidazoline I_1 receptor selectivity ratio of 3879, while 1-[(imidazolidin-2-yl)imino]-7-methylindazole (**13k**) proved to be a mixed α_2-adrenoceptor/imidazoline I_1 receptor agonist with α_2/I_1 selectivity ratio of 7.2. Compound **13k** when administered intravenously to male Wistar rats induced a dose-dependent decrease in mean arterial blood pressure (ED_{50} = 0.6 μg/kg) and heart rate, which was attenuated following pretreatment with α_{2A}-adrenoceptor antagonist RX821002. Compound **13a** may find a variety of medical uses ascribed to α_2-adrenoceptor agonists, and its 7-methyl derivative **13k** is a good candidate for development as a centrally acting antihypertensive drug.

Introduction

α_2-Adrenoceptors are widely expressed in many tissue types and mediate a multitude of functions in both peripheral organs and within the central nervous system, and the α_2-adrenoceptor agonists represent a unique class of compounds because of a wide range of medicinal uses reported in the literature.[1–5] For example, activation of central α_2-adrenoceptors in the brain stem has been utilized clinically for many years in the treatment of hypertension.[2,3] However, this original explanation has later been challenged by a concurrent "imidazoline hypothesis" that assumes the existence of the imidazoline receptors and attributes the sympathoinhibition to activation of I_1 imidazoline receptors in the medulla oblongata.[6–10]

Nevertheless, because of some degree of similarity between the two receptor classes and the fact that most α_2-adrenoceptor agonists, including prototypical clonidine, also have comparable affinity for I_1 imidazoline receptors, it is still difficult to dissociate imidazoline receptors from α_2-adrenoceptors both pharmacologically and functionally. Moreover, α_2-adrenoceptors and imidazoline receptors usually colocalize in the central nervous system, which raises a question as to whether a link between these two types might exist. In this context, a comprehensive review by Szabo should be mentioned,[11] in which a critical analysis of the above hypotheses led the author to a conclusion that the sympathoinhibitory effects of clonidine-like drugs are best explained by activation of α_2-adrenoceptors. On the other hand, Bousquet pointed out that it is possible to produce a hypotensive effect by activating either α_2-adrenoceptors or imidazoline receptors within the central nervous system, and a potentiating interaction exists between these two receptor classes.[12] Whether the mechanism of this interaction involves molecular receptor−receptor interference on the ventral medullary cardiovascular neurons or an interaction between receptors located on different neuronal structures but with effects converging to inhibit the vasomotor tone remains to be demonstrated. To achieve this goal, however, new compounds with imidazoline structure and a very high selectivity for α_2-adrenoceptors vis-à-vis imidazoline I_1 receptors will be required.

Currently, a growing interest in the field of developing highly selective α_2-adrenoceptor agonists has been stimulated by their potential applications as analgesic, sedative, anxiolytic, hemodynamic-stabilizing, and organ-protective agents.[13,14] Prominent examples of such compounds include imidazole-containing dexmedetomidine (**1**)[15,16] and spirooxazoline derivative S18616 (**2**).[17] Mivazerol (**3**) is an example of the imidazole derivative designed for the prevention of myocardial infarction in perioperative patients,[18,19] while spiroimidazoline S19014 (**4**) has been assessed as antimigraine agent.[20–22] Other effects of clonidine-like agents on central noradrenergic transmitter and modulator functions that are not related to hypertension include the stimulation of human growth hormone and the decrease of the output of nearly all secretory glands, such as lacrimal, salivary, gastric secretory, and sweat glands.[23]

Recently, in connection with our previous studies on novel imidazoline compounds[24–26] we have turned our attention to the [(imidazolidin-2-yl)imino]indazoles. As shown in Figure 1, the previously described 4-substituted indazole derivative **5** (indanidine) exhibited selectivity for α_1-adrenoceptors[27] while its 6-substituted analogues of type **6** were claimed in patent literature to be agonists of α_2-adrenoceptors,[28] although no data regarding α_2/I_1 selectivity ratio were given.

A priori, it was not obvious what effect translocation of the iminoimidazolidine moiety might have with regard to α-adrenoceptor agonism. As such, we were interested in analogues of type **7** (Figure 1) wherein the iminoimidazolidine moiety had

* To whom correspondence should be addressed. For F.S.: phone, (48-58)-3493250; fax, (48-58)-3493257; e-mail, saczew@amg.gda.pl. For A.K.: phone, (48-58)-3493250; fax, (48-58)-3493257; e-mail, a.kornicka@amg.gda.pl.

† Department of Chemical Technology of Drugs, Medical University of Gdańsk.

‡ Department of Pathophysiology, Medical University of Gdańsk.

§ University of Alberta.

A. Mickiewicz University.

10.1021/jm800112s CCC: $40.75
Published on Web 06/03/2008

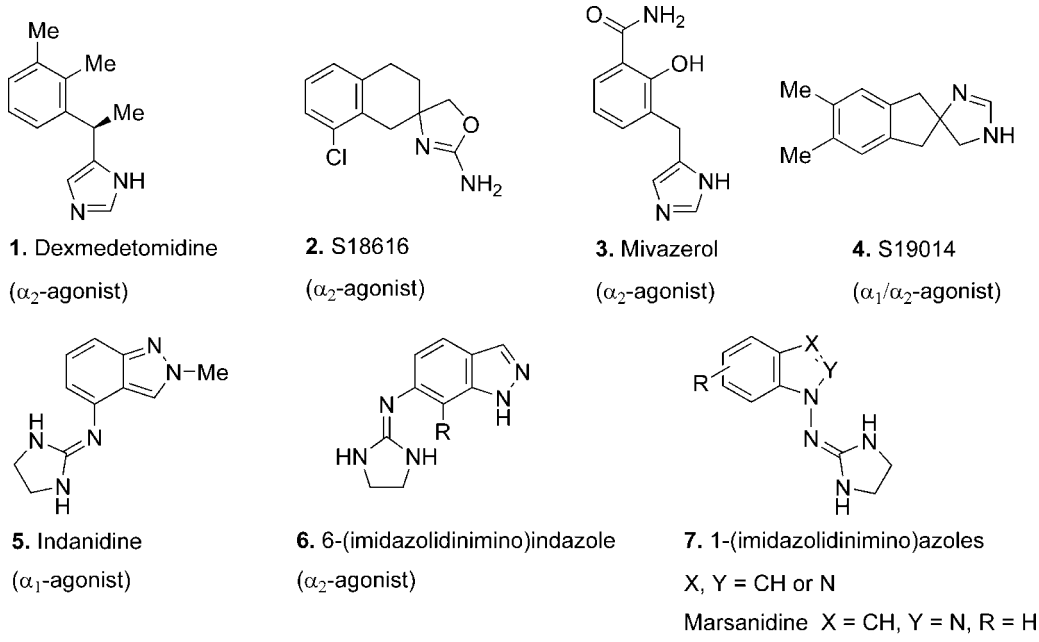
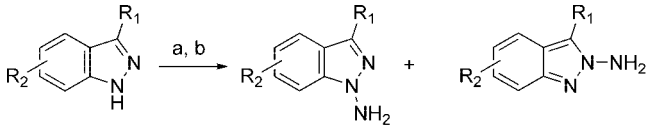


Figure 1. Ligands of medicinal interest selective for α_1- and α_2-adrenoceptors.

Scheme 1. Synthesis of the Aminoindazoles[a]



9a, 10a, $R^1 = R^2 = H$
9b, 10b, R^1 = 3-Me, $R^2 = H$
9c, 10c, R^1 = 3-Ph, $R^2 = H$
9d, 10d, $R^1 = H$, R^2 = 4-Me
9e, 10e, $R^1 = H$, R^2 = 4-Cl
9f, 10f, $R^1 = H$, R^2 = 4-OMe
9g, 10g, $R^1 = H$, R^2 = 5-Me
9h, 10h, $R^1 = H$, R^2 = 5-Cl
9i, 10i, $R^1 = H$, R^2 = 6-Me
9j, 10j, $R^1 = H$, R^2 = 6-OMe
9k, 10k, $R^1 = H$, R^2 = 7-Me

[a] Reagents and conditions: (a) hydroxylamine-*O*-sulfonic acid (HOSA) (2.6 molar equiv), 6% NaOH/H_2O, EtOH, 20 min, 55 °C, 1 h, room temp, then 1 h, 10 °C; (b) CH_2Cl_2, chromatography on silica gel, 21−60% for **9a**−**k**, 8−30% for **10a**−**k**.

been shifted to the 1- or 2-position in order to obtain a new series of clonidine-like compounds with decreased basicity of the guanidine grouping and, hopefully, with a new pharmacological profile. In this work, the importance of the properly substituted indazole ring for α_2-adrenoceptor affinity and selectivity has been explored. The in vivo biological effects of these newly prepared positional analogues of **5** and **6** have also been investigated.

Results and Discussion

Synthesis. Target compounds of type **7** may be considered as cyclic *N*,*N*′,*N*″-trisubstituted guanidines. Most methods for preparation of guanidine derivatives are based on the reaction of the corresponding amine with electrophilic precursors of the guanidine moiety.[29] Therefore, as depicted in Scheme 1, the synthesis of our target molecules commenced with the N-amination of corresponding indazole **8a**−**k** to afford mixtures of 1-amino- and 2-aminoindazoles **9a**−**k** and **10a**−**k**, which could be separated by column chromatography.

Then an effort was made to introduce the imidazoline group under variety of conditions and with a number of guanylating reagents. However, treatment of **9** or **10** with 2-chloroimidazoline[30,31] at mild as well as at elevated temperature and prolonged reaction time led to the extensive decomposition of starting material. Because of a poor nucleophilic character of *N*-aminoazoles,[32] similar difficulty in guanylation was observed with 2-methylthioimidazoline. Therefore, the alternative route for preparation of compounds **12a**−**k** and **15a**−**f** was considered by employing Kim's guanidine synthesis.[33] As shown in Scheme 2, aminoindazoles **9** and **10**[34] were treated with the preformed *N*,*N*′-bis-Boc-imidazolidine-2-thione[35] in the presence of $HgCl_2$ to give bis-Boc-protected guanidines **11a**−**k** and **14a**−**f** in 30−59% and 45−61% yields, respectively. This method followed by deprotection with TFA resulted in the desired 1-[(imidazolidin-2-yl)imino]indazoles **12a**−**k** and 2-[(imidazolidin-2-yl)imino]indazoles **15a**−**f**, which upon treatment with methanolic solution of HCl were converted into the corresponding hydrochlorides **13a**−**k** and **16a**−**f**.

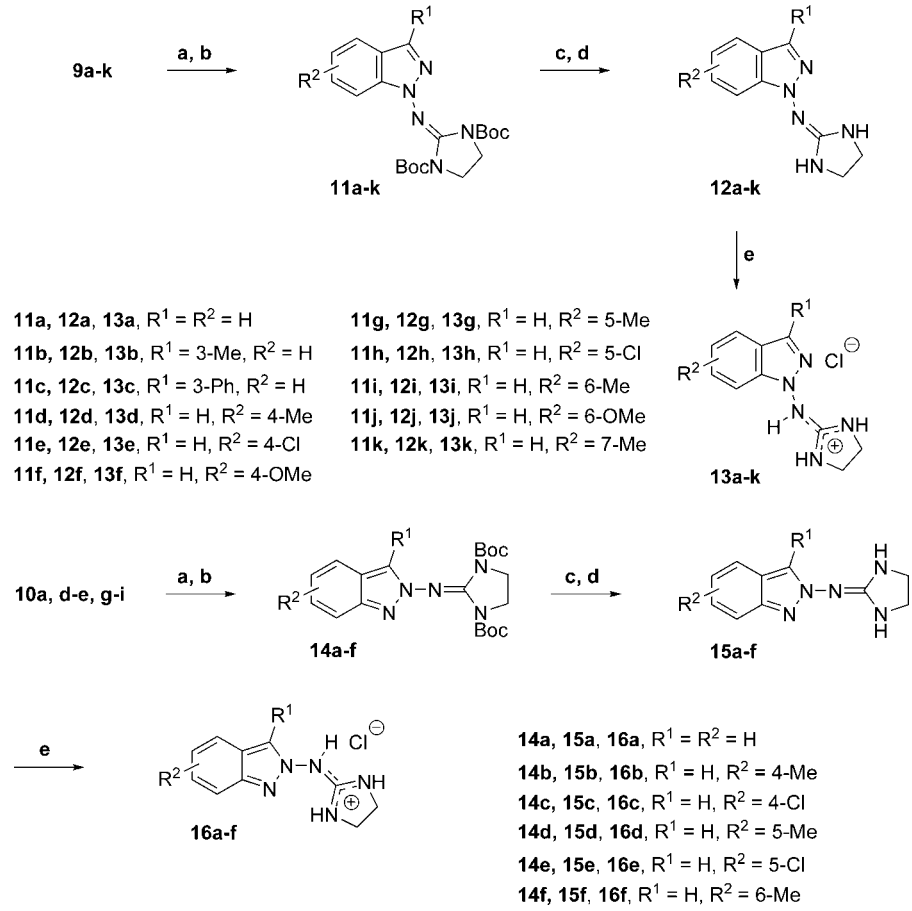
For SAR studies involving variation of the azolyl moiety, the previously described 1-aminobenzimidazoles **17a**,**b**[36,37] (X = CH) and 1-aminobenzotriazole **17c**[38] (X = N) were prepared by amination of corresponding azoles and, as shown in Scheme 3, subjected to the reaction with *N*,*N*′-bis(*tert*-butoxycarbonyl)-imidazolidine-2-thione to give Boc-protected 1-(iminoimidazolidine)azoles **18a**−**c**. The free bases **19a**−**c** were obtained upon cleavage of the protecting group and were in turn used to prepare the desired hydrochloride salts **20a**−**c**.

Structures of the final free bases and corresponding hydrochloride salts were confirmed by elemental analyses as well as IR and NMR spectroscopic data (see Experimental Section).

Crystal Structure Analysis and Molecular Modeling. The molecular structures of the free bases **12** and hydrochlorides **13** were further confirmed by X-ray crystal structure analysis of **12e** and **13a**, which are shown in Figures 2 and 3, respectively.

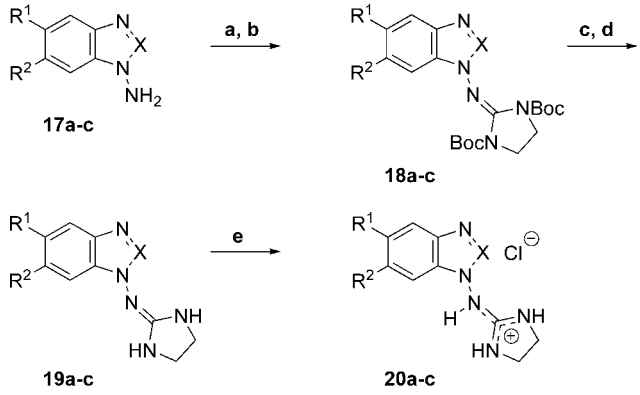
The molecule of **12e** (Figure 2) consists of two fragments, a planar indazole ring and a roughly planar imidazolidin-2-imine group, which form dihedral angle of 80.75(5)°. The torsion angles along the C−N and N−N bonds connecting these two fragments are 0.0(3)° and 107.3(2)°. The last value indicates that there is practically no conjugation between the p orbitals

Scheme 2. Synthesis of the 1- and 2-[(Imidazolidin-2-yl)imino]indazoles[a]



[a] Reagents and conditions: (a) *N*,*N*′-bis-Boc-imidazolidine-2-thione (1.5 molar equiv), $HgCl_2$ (1.5 molar equiv), Et_3N (3.5 molar equiv), anhydrous DMF, 0 °C, 20 min, then room temp, 5 days or room temp, 3 days then 6 h, 85 °C for **11k**; (b) EtOAc, chromatography on silica gel, then crystallization from suitable solvent, 30−59% for **11a**−**k**, 45−61% for **14a**−**f**; (c) 50% TFA/CH_2Cl_2, 2 h, room temp; (d) 10% NaOH/H_2O, 5 °C, 50−76% for **12a**−**k**, 51−72% for **15a**−**f**; (e) HCl/MeOH, 5 °C, then room temp, 30 min, 56−75% for **13a**−**k**, 59−77% for **16a**−**f**.

Scheme 3. Synthesis of the 1-[(Imidazolidin-2-yl)imino]azoles[a]



[a] Reagents and conditions: (a) *N*,*N*′-bis-Boc-imidazolidine-2-thione (1.5 molar equiv), $HgCl_2$ (1.5 molar equiv), Et_3N (3.5 molar equiv), anhydrous DMF, 0 °C, 20 min, then room temp, 5 days; (b) EtOAc, chromatography on silica gel, then crystallization from suitable solvent, 40−62%; (c) 50% TFA/CH_2Cl_2, 2 h, room temp; (d) 10% NaOH/H_20, 5 °C, 65−80%; (e) HCl/MeOH, 5 °C, then room temp, 30 min, 59−77%.

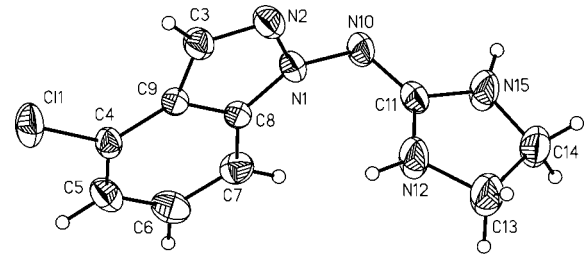


Figure 2. X-Ray structure of compound **12e** showing atomic labels and displacement ellipsoids at the 50% probability level. Bond lengths (Å): C3−N2 1.325(2), N2−N1 1.3594(18), N1−N10 1.4019(17), N10−C11 1.307(2), C11−N12 1.333(2), C11−N15 1.3480(19). Torsion angles (deg): N12−C11−N10−N1 0.0(3), C11−N10−N1−N2 107.3(2).

of the sp^2 hybridized indazole N1 atom and the exocyclic N atom of the imidazoline ring. Indeed, the exocyclic N−N bond length of 1.402(2) Å agrees well with the standard single bond length between the sp^2-hybridized N atoms (1.401 Å).[39] The indazole N1 atom is slightly pyramidal, being displaced 0.093(2) Å from the plane defined by its three bonding atoms. The twist around the exocyclic N−N bond, which renders the molecule of **12e** chiral in the solid state, determines also distances of the imidazoline N atoms to the centroid of the indazole benzene ring (4.41 and 5.64 Å). In compound **13a** (Figure 3), which is a close analogue of **12e**, the exocyclic N atom is protonated and the two N−N bonds become of equal length. Nonetheless, the degree of pyramidalization of the indazole N1 atom remains practically unaltered, with N1 displaced 0.115(2) Å from the

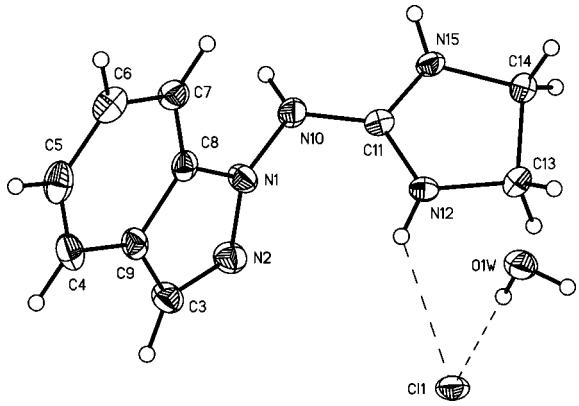


Figure 3. X-Ray structure of compound **13a** showing atomic labels and displacement ellipsoids at the 50% probability level. Bond lengths (Å): C3−N2 1.3159(19), N2−N1 1.3739(16), N1−N10 1.3758(16), N10−C11 1.3365(18), C11−N12 1.3184(17), C11−N15 1.3241(18). Torsion angles (deg): N12−C11−N10−N1 −3.5(2), C11−N10−N1−N2 −72.51(17).

plane of the three bonding atoms. A strong twist of the imidazolidin-2-iminium fragment relative to the indazole ring [dihedral angle of 65.18(4)°] also makes this molecule chiral; however, an opposite direction of the twist compared with **12e** increases the distances of the imidazoline N atoms from the centroid of the indazole benzene ring (5.07 and 5.95 Å).

The series of 1-[(imidazolidin-2-yl)imino]indazoles **12** share structural similarities with the antihypertensive clonidine-like drugs,[23] which in solution exists in the imino form. We have examined tautomers of **12a** by quantum chemical calculations using density functional (B3LYP/6.31G**) calculations.[40] As shown in Figure 4, the imino-imidazolidine tautomer **A** was calculated to be substantially lower in energy than the amino-imidazoline tautomer **B** (ΔE = 11.05 kcal/mol). Moreover, on the basis of their calculated dipole moments, tautomer **A** (3.34 D) would be predicted to predominate over **B** (1.99 D) in polar solvents.

As shown by conformational analysis and crystallographic data, **12** can adopt a variety of conformations that involve a relatively small energy expense. Thus, puckered conformation required for interaction with α_2-adrenoceptors[23] can be achieved at an energy expense of about 7 kcal/mol by rotation around N1−N10 single bond. In order to compare the global energy minimum planar geometry of **12a** with those of clonidine, the electrostatic potential was generated. Comparison between 3D electrostatic potential maps[40] of **12a** and clonidine (Figure 5) shows that superimposable negative wells appear around the exocyclic nitrogen atoms. However, separated electrostatic regions are positioned around the phenyl moiety of indazole ring of **12a**.

Previously established model for central antihypertensive agents also demonstrated the importance of decreased basicity of the guanidine moiety by introduction of electronegative substituents into the aromatic ring.[23] We expected that unlike other arylimino-imidazolidines, which are ionized at physiological pH (pK_a of clonidine of 8.2; 14% of un-ionized form at pH 7.4), the corresponding 1-[(imidazolidin)imino]indazoles **13** would have much lower basicity (pK_a < 8) because of the electron withdrawing effect of the azaaromatic ring. Indeed, at physiological pH it is very likely that agonists **13a** and **13k** with pK_a values of 6.32 and 6.53, respectively (Table 1), exist primarily as the neutral free bases (92% and 88%, respectively). This would likely facilitate their ability to cross the blood−brain barrier.

Biological Activity. In Vitro Radioligand Binding Study. The in vitro assays involved the investigation of the affinity and selectivity of the newly prepared imidazoline ligands for the α_2-adrenoceptor, imidazoline I_1, and imidazoline I_2 binding sites, closely related receptor types. All compounds were used in form of hydrochloride salts. A summary of all these biological properties is displayed in Table 1.

In general, all of the tested compounds showed very low or no affinity for imidazoline I_2 receptors. Initial evaluation of the two isomeric indazole compounds **13a** and **16a** established that only 1-substituted isomer **13a** possessed significant activity at α_2-adrenoceptors (K_i = 14.0 nM) and a very high α_2/I_1 selectivity ratio of 3879 (Table 1). Compound **16c**, which can be regarded as a partially constrained analogue of the well-known antihypertensive drug guanabenz, showed very weak affinity for α_2-adrenoceptors (K_i = 3136 nM) and extremely low activity at imidazoline I_1 receptors (K_i = 47 840 nM). The poor activity of the 1-substituted benzimidazoles **20a**,**b** and 1-substituted benzotriazole analogue **20c** was also surprising because the active indazole **13a** possessed the same substitution pattern.

Further SAR studies suggested that the α_2-adrenoceptor affinity of the 1-substituted indazole derivatives **13** was strongly influenced by the nature and position of substituent, and therefore, our work focused on the modification of the substitution pattern. First, placement of either methyl or phenyl group at position 3 was not tolerated, as evidenced by considerable decrease in affinity for α_2-adrenoceptors ranging from 1838- to 5058-fold for compounds **13c** and **13b**, respectively. Translocation of the methyl group from position 3 to 4, 5, or 6 led to active compounds **13d**, **13g**, and **13i** (K_i of 37.1, 44.2, and 49.1 nM, respectively). However, corresponding 4-Cl and 5-Cl-substituted analogues **13e** and **13h** were 2- to 3-fold less active (K_i of 61.6 and 114.2 nM, respectively) and compound **13f** bearing electron-donating OMe group at position 4 proved to be inactive at α_2-adrenoceptors (K_i = 69.63 μM).

Interestingly, within a homologous series of Me-substituted derivatives, the 7-Me analogue **13k** retained activity at α_2-adrenoceptors (K_i = 53.5 nM), whereas its affinity for imidazoline I_1 receptor increased by about 2 orders of magnitude (K_i = 387 nM, α_2/I_1 selectivity ratio = 7.2). The graphical representations (Figures 6 and 7) illustrate the differences in selective profiles of these compounds.

Summing up, the above results suggest that binding of the indazole derivatives **13** may occur at multiple classes of receptor sites, depending on the substitution pattern. Compound **13a** shows highest affinity to rat brain α_2-adrenoceptors and a very high α_2/I_1 selectivity ratio. On the other hand, 7-Me-analogue **13k** is characterized by good affinity to α_2-adrenoceptors and moderate affinity to imidazoline I_1 receptors, and therefore, it bears resemblance to clonidine-like "hybrid drugs".

In Vivo Cardiovascular Activities. The cardiovascular properties of **13a** and its selected derivatives were evaluated after intravenous infusion in anaesthetized rats using procedure previously described.[41] Changes in mean arterial blood pressure and heart rate were measured directly. These measurements were compared to the baseline values and are presented as ΔMAP and ΔHR in Table 2.

In general, most of the tested compounds showed a biphasic effect on blood pressure: transient hypertension followed by prolonged hypotension effect. However, no clear correlation was found between in vivo potencies and affinities of the compounds studied to α_2-adrenoceptors. For example, cardiovascular evaluation of **13a** showed that this compound exerts significant effect

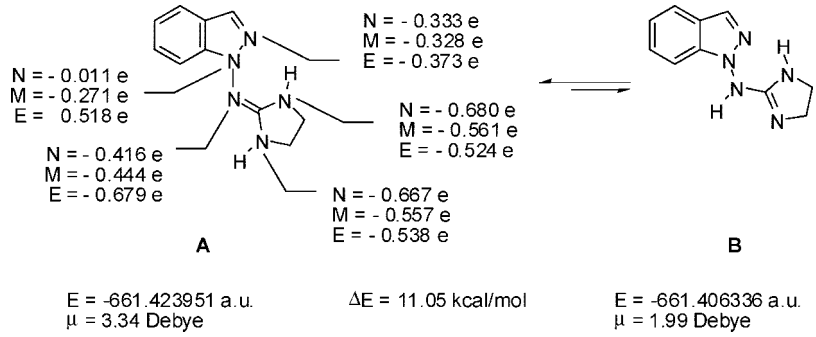


Figure 4. Molecular diagram of 1-[(imidazolidin-2-yl)imino]indazole (**12a**): *N* = natural charges; M = charges derived from Mulliken population analysis; *E* = charges derived from electrostatic potential.

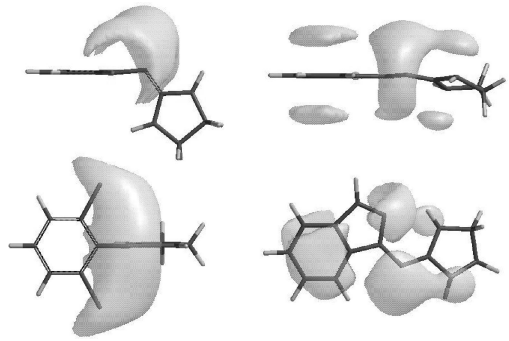


Figure 5. Comparison of the electrostatic potential maps of marsanidine free base (**12a**) (right) and clonidine free base (left) isocontoured at −20 kcal/mol.

on blood pressure at the dose of 0.1 mg/kg (ΔMAP = −30.9 mmHg) and heart rate (ΔHR = −49.0 bpm). Similar or slightly lower hypotensive activity was observed for 4- and 6-methylated derivatives **13d** and **13i** (ΔMAP = −29.0 and −23.4 mmHg, respectively). On the other hand, 5-Me congener **13g**, which was equally active at α_2-adrenoceptors, exhibited a very weak cardiovascular activity (ΔMAP = −4.9 mmHg). The most striking discrepancy was observed for the 7-Me derivative **13k**, which exhibited similar affinity for α_2-adrenoceptors and much higher hypotensive effect when administered intravenously at the same dose (ΔMAP = −43.5 mmHg, ED_{50} = 0.6 μg/kg, iv).

As shown in Figure 8, changes of ΔMAP induced by **13a**, **13k**, and standard drug moxonidine were highly significant in comparison to control group and reached $p < 0.01$, $p < 0.001$, and $p < 0.001$, respectively. Similarly, **13k** and moxonidine injections induced immediate decrease in ΔHR, reaching high significance: $p < 0.001$ and $p < 0.01$ in comparison to control and **13a** groups, respectively (Figure 9). Nevertheless, **13a** administration resulted in weaker but also significant ($p < 0.05$) decrease of ΔHR in comparison to control animals.

It was previously found[42,43] that the selective α_2-adrenoceptor antagonists could reverse the central hypotensive effects of highly selective α_2-adrenoceptor agonists or prevent hypotension elicited by clonidine-like drugs binding to α_2-adrenoceptors and to imidazoline I_1 receptors. Therefore, we have examined the interaction of **13k** with RX821002,[a] a selective α_{2A}-adrenoceptor antagonist.[44,45] This compound was chosen because of its imidazoline structure and previous finding that hypotensive effects of clonidine-like drugs may be mediated through stimulation of α_{2A}-adrenoceptors.[46] As shown in Figure 10, imidazoline-containing α_{2A}-antagonist RX821002 at 5 μg/kg, administered iv 5 min prior to **13k** (administered at dose 10 μg/kg iv), attenuated hypotensive action of **13k**, and at dose 10 μg/kg it was even more effective at reversing **13k**-induced hypotensive effect.

The data presented above support the hypothesis according to which an interaction between imidazoline I_1 receptors and α_2-adrenoceptors might explain the mechanism of the hypotensive effects of clonidine-like "hybrid drugs" binding to both α_2-adrenoceptors and imidazoline I_1 receptors.[47,48]

In summary, we have shown that methyl substituent at position 7 has a significant role in determining overall cardiovascular profile of the investigated indazole derivatives **13**. Compound **13k** with affinities for both α_2-adrenoceptors and imidazoline I_1 receptors confers the most potent hypotensive and negative chronotropic properties. However, whether the long-lasting hypotension of these compounds is likely to be attributable to their central sympathetic depression rather than peripheral vascular effects[11] remains to be established.

Conclusions

From the above-discussed results one can draw the following conclusions: (i) placement of iminoimidazolidine moiety at the N1 nitrogen atom of an azaaromatic ring, such as indazole, benzimidazole, and benzotriazole, decreases basicity of formal guanidine grouping, which is a prerequisite for hypotensive properties of clonidine-like drugs; (ii) 1-[(imidazolidin-2-yl)imino]indazoles (**13**) exhibit structure-dependent affinities for α_2-adrenoceptors and α_2/I_1 selectivity ratio. The most active at α_2-adrenoceptors unsubstituted indazole derivative **13a** (K_i = 14 nM) shows a very high α_2/I_1 selectivity ratio of 3879 vs dexmedetomidine's ratio of 32 (i.e., over 100-fold difference) and therefore may serve as a pharmacological tool and find a variety of medical uses, especially as a more cardiostable α_2 agonist for organoprotection and anesthesia; (iii) 7-Me analogue **13k**, the most potent hypotensive agent in this series (ED_{50} = 0.6 μg/kg), is characterized by good affinity for α_2-adrenoceptors (K_i = 53 nM) and moderate affinity for imidazoline I_1 receptors (K_i = 387 nM). Compound **13k** may therefore serve as a lead structure for further development of novel antihypertensive drugs.

To explain the differences observed between in vitro binding affinities and in vivo circulatory activities of **13**, further studies on their pharmacokinetics are needed. Binding affinities of **13a** for human α_1-adrenoceptor as well as α_2-adrenoceptor subtypes

[a] Abbreviations: Tris, 2-amino-2-(hydroxymethyl)propane-1,3-diol; RX821002, 2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline; 2BFI, 2-(2-benzofuranyl)-2-imidazoline; BU224, 2-(4,5-dihydroimidazol-2-yl)quinoline.

Table 1. Binding Affinities, α_2/I_1 Selectivity, and pK_a Data for Compounds **13a**−**k**, **16a**−**f**, and **20a**−**c**

compd	α_2 K_i (nM)[a]	I_1 IC_{50} (nM)[b]	I_2 K_i (nM)[a]	α_2/I_1 selectivity ratio	pK_a[c]
13a	14.05 ± 2.7	54500.0 ± 16730	16900.0 ± 5900	3879.0	6.32
13b	71070.0 ± 5080	Nd	32610.0 ± 8130		
13c	25830.0 ± 4508	76510.0 ± 29880	684.0 ± 74.3	2.96	
13d	37.1 ± 9.8	38530.0 ± 7930	10370.0 ± 2860	1038.54	6.20
13e	61.6 ± 22.6	31460.0 ± 2348	11080.0 ± 3930	510.71	5.77
13f	69630.0 ± 15570	13500.0 ± 2880	5984.0 ± (n = 1)	0.19	6.03
13g	44.2 ± 8.3	20310.0 ± 7840	7159.0 ± 1277	459.50	6.30
13h	114.2 ± 39.4	16980.0 ± 3670	20620.0 ± 1393	148.69	5.88
13i	49.1 ± 7.8	Nd	19170.0 ± 1730		
13j	96.2 ± 28.4	Nd	33640.0 ± 6330		6.21
13k	53.5 ± 18.4	387.0 ± 97.3	2520.0 ± 593	7.23	6.53
16a	78343.3 ± 21851	Nd	77826.7 (n = 2)		
16b	11850.0 ± 19320	Nd	35110.0 ± 14190		
16c	3136.0 ± 793	47840.0 ± 8210	13140.0 ± 2990	15.26	
16d	45620.0 ± 18670	13420.0 ± 5567	11440.0 ± 2830	0.29	
16e	74090.0 ± 25069	24670.0 (n = 1)	33200.0 (n = 1)	0.33	
16f	50680.0 ± 21188	19730.0 (n = 1)	42880.0 ± 11830	0.39	
20a	6016.3 ± 895	>100000	>10000	>16.62	
20b	10072.7 ± 13930	Nd	>10000		
20c	>10000	84633.4	>10000	<8.46	

[a] K_i affinity values for α_2-adrenoreceptors and I_2 imidazoline binding sites were assessed by measuring the ability of test compounds to displace [^{3}H]RX821002 and rauwolscine (rat brain membranes) or [^{3}H]2BFI and BU224 (rat brain membranes), respectively. [b] Molar concentration of test compounds that displaces 50% of specifically bound [^{3}H]clonidine. Nd: not determined. [c] pK_a values were determined at 25 °C by potentiometric titration with TiNet 2.5 software.

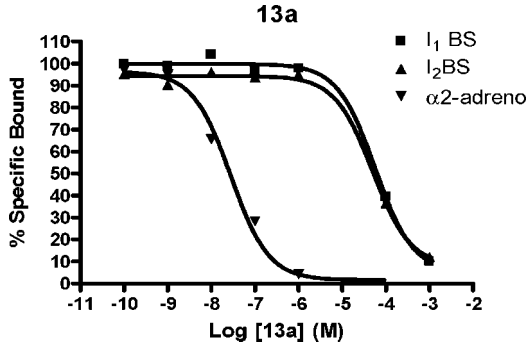


Figure 6. Competition binding curves of marsanidine (**13a**) on α_2-adrenoceptors, imidazoline I_1, and imidazoline I_2 receptors.

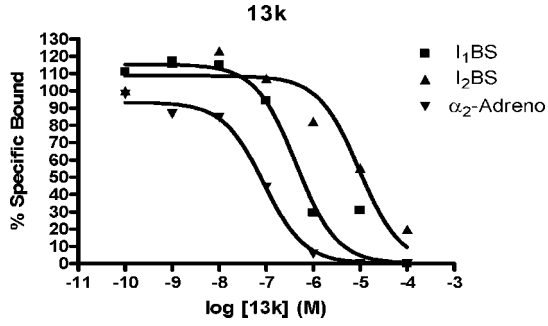


Figure 7. Competition binding curves of 7-Me-marsanidine (**13k**) on α_2-adrenoceptors, imidazoline I_1, and imidazoline I_2 receptors.

α_{2A}, α_{2B}, and α_{2C} are also planned, and the results of these studies will be described elsewhere.

Experimental Section (See Also Supporting Information)

Chemistry. Melting points were measured on a Büchi 535 apparatus and are not corrected. IR spectra were taken in KBr pellets on a Perkin-Elmer FTIR 1600 spectrometer. NMR spectra were recorded on a Varian Gemini 200 or Varian Unity 500 apparatus. ^{1}H and ^{13}C chemical shifts were measured relative to the residual solvent signal at 7.26 ppm ($CDCl_3$) or 2.50 and 39.5 ppm (DMSO-d_6). Chromatography was performed on silica gel 60 (230−400 mesh ASTM, Fluka) using the reported solvent systems. Analysis results of C, H, N were within ±0.4% of the theoretical values.

Indazole (**8a**),[49] 3-methylindazole (**8b**),[34] 3-phenylindazole (**8c**),[34] 4-methylindazole (**8d**),[49] 4-chloroindazole (**8e**),[49] 4-methoxyindazole (**8f**),[50] 5-methylindazole (**8g**),[49] 5-chloroindazole (**8h**),[49] 6-methylindazole (**8i**),[49] 6-methoxyindazole (**8j**),[50] 7-methylindazole (**8k**),[49] 1-aminoindazole (**9a**),[34] 1-amino-3-methylindazole (**9b**),[34] 1-amino-3-phenylindazole (**9c**),[34] 2-aminoindazole (**10a**),[34] 2-amino-3-methylindazole (**10b**),[34] 2-amino-3-phenylindazole (**10c**),[34] *N*,*N*′-bis(*tert*-butoxycarbonyl)imidazolidine-2-thione,[35] 1-aminobenzimidazole (**17a**),[36] 1-amino-5,6-dimethylbenzimidazole (**17b**),[37] and 1-aminobenzotriazole (**17c**)[38] were obtained as previously reported.

General Procedure for the Preparation of 1-[(Imidazolidin-2-yl)imino]indazoles 12a−k and Their Hydrochlorides 13a−k. Step 1. To a stirred solution of the appropriate 1-aminoindazole **9a**−**k** (2.5 mmol), *N*,*N*′-bis(*tert*-butoxycarbonyl)imidazolidine-2-thione[35] (1.12 g, 3.7 mmol), and Et_3N (0.88 g, 1.21 mL, 8.7 mmol) in anhydrous DMF (4 mL) was added $HgCl_2$ (1.0 g, 3.7 mmol) at 0 °C. The reaction mixture was stirred for an additional 20 min at 0 °C and then at room temperature for 5 days or for 3 days followed by heating at 85 °C for 6 h (in the case of **9k**). The resulting dark-gray reaction mixture was diluted with EtOAc (40 mL), filtered off, and washed with EtOAc (3 × 10 mL). The filtrates were washed successively with brine (3 × 20 mL) and water (3 × 20 mL), dried over $MgSO_4$, and finally concentrated under vacuum. The viscous residue (a mixture of unreacted *N*,*N*′-bis-Boc-imidazolidine-2-thione and Boc-protected 2-iminoimidazolidines **11a**−**k**) thus obtained was separated by flash column chromatography on silica gel. *N*,*N*′-Bis-Boc-imidazolidine-2-thione was eluted first and isolated in 17−25% yields.

In this manner the following compounds were obtained.

1-{[1,3-Di(*tert*-butoxycarbonyl)imidazolidin-2-yl]imino}indazole (11a). 11a was obtained from 1-aminoindazole (**9a**,[34] 0.33 g) and EtOAc/$CHCl_3$ (0.1:10) as eluent: yield 0.58 g (59%); mp 128−130 °C (*n*-heptane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.03 (s, 9H, CH_3), 1.6 (s, 9H. CH_3) 3.9 (t, 2H, CH_2), 4.03 (t, 2H, CH_2), 7.16 (m, 1H), 7,38 (m, 1H) 7.66 (m, 2H) 7.9 (s, 1H). Anal. ($C_{20}H_{27}N_5O_4$ (401.46)) C, H, N.

1-{[1,3-Di(*tert*-butoxycarbonyl)imidazolidin-2-yl]imino}-7-methylindazole (11k). 11k was obtained from 1-aminoindazole **9k** (0.37 g) and EtOAc/$CHCl_3$ (0.1:3) as eluent: yield 0.31 g (30%); mp 160−162 °C (*n*-heptane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.02 (s, 9H, CH_3), 1.57 (s, 9H. CH_3), 2.84 (s, 3H, CH_3), 3.9 (t, 2H, CH_2), 4.06 (t, 2H, CH_2), 7.04 (m, 1H), 7,14 (m, 1H), 7.49 (m, 1H), 7.89 (s, 1H). Anal. ($C_{21}H_{29}N_5O_4$ (415.49)) C, H, N.

Step 2. A solution of the appropriate Boc-protected 2-iminoimidazolidine **11a**−**k** (2.0 mmol) from step 1 in 50% trifluoroacetic

Table 2. Effects of Compounds **13a**,**c**−**k** at 0.1 mg/kg iv on Mean Arterial Blood Pressure (MAP) and Heart Rate (HR) in Anesthetized Rats

	time after application of tested compounds (min), ΔMAP (mmHg)[a] and ΔHR (bpm)[a,b]				
compd	10	30	60	90	n^c
13a	−6.9 ± 4.4 (−48.5 ± 23.5)	−30.9 ± 4.7 (−49.0 ± 26.5)	−29.0 ± 9.3 (−31.4 ± 11.1)	−22.1 ± 6.7 (−9.7 ± 8.6)	4
13c	−4.1 ± 0.9 (−17.7 ± 3.5)	−7.6 ± 1.9 (−10.1 ± 4.9)	−10.0 ± 2.4 (−11.1 ± 8.1)	−11.4 ± 3.0 (−12.7 ± 10.2)	5
13d	−17.7 ± 4.2 (−104.2 ± 10.5)	−29.0 ± 4.3 (−88.3 ± 11.4)	−14.1 ± 3.2 (−22.7 ± 17.2)	−10.1 ± 1.6 (−2.1 ± 12.1)	4
13e	−10.9 ± 7.4 (−60.8 ± 18.0)	−14.0 ± 4.1 (−32.2 ± 15.5)	−12.3 ± 2.7 (−24.3 ± 11.4)	−10.9 ± 3.8 (−19.0 ± 7.9)	5
13f	−3.7 ± 1.8 (−18.6 ± 5.4)	−7.6 ± 1.7 (−9.7 ± 3.9)	−11.0 ± 2.5 (−12.5 ± 6.4)	−12.9 ± 2.0 (−11.6 ± 8.5)	5
13g	−4.1 ± 1.7 (−104.2 ± 10.5)	−3.6 ± 2.3 (−3.2 ± 4.5)	−4.6 ± 3.0 (−1.0 ± 9.5)	−4.9 ± 3.6 (−3.0 ± 10.1)	5
13h	−0.2 ± 0.7 (0.6 ± 1.2)	−1.4 ± 1.4 (7.2 ± 3.4)	−2.8 ± 1.4 (12.8 ± 7.8)	−1.1 ± 1.8 (28.6 ± 4.9)	4
13i	−13.2 ± 7.2 (−71.8 ± 14.9)	−23.4 ± 0.9 (−43.5 ± 14.8)	−20.8 ± 2.6 (−26.4 ± 11.0)	−11.0 ± 3.6 (−22.2 ± 6.0)	4
13j	−16.5 ± 5.7 (−33.0 ± 13.9)	−17.1 ± 5.4 (−23.6 ± 12.1)	−11.6 ± 4.6 (−4.1 ± 16.0)	−5.3 ± 5.7 (14.3 ± 15.3)	4
13k	−14.8 ± 6.9 (−110.0 ± 16.7)	−40.6 ± 4.2 (−115.0 ± 12.6)	**−43.5 ± 4.6** (−122.9 ± 12.6)	−43.2 ± 4.1 (−119.9 ± 13.5)	7
control	−1.2 ± 0.8 (−4.9 ± 1.7)	−1.3 ± 2.0 (−8.6 ± 2.7)	−3.5 ± 1.8 (−6.2 ± 6.3)	−9.1 ± 2.9 (−19.8 ± 11.9)	5

[a] Values are the mean ± SE. [b] Values in parentheses. [c] Number of experiments.

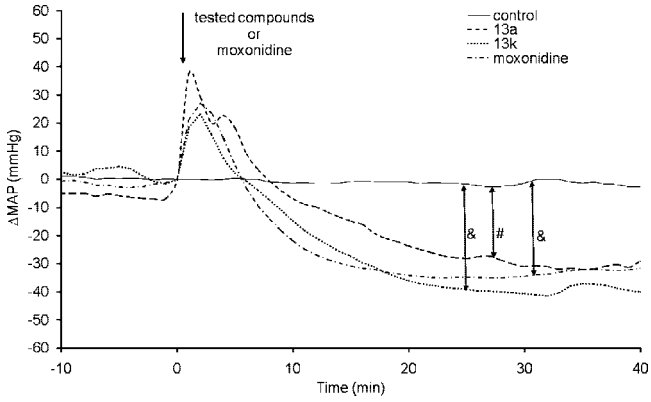


Figure 8. Effect of 0.1 mg/kg of **13a**, **13k**, and moxonidine on ΔMAP (calculated as the difference of MAP between sequential measurement and time 0 of experiment) in rats. Each point represents the mean value of ΔMAP for four to seven experiments. Comparisons were made using ANOVA with repeated measures and Fisher and Duncan tests. Significances (&) $p < 0.001$, (&) $p < 0.001$, and (#) $p < 0.01$ were found for comparisons of **13k**, moxonidine, and **13a** versus control group, respectively.

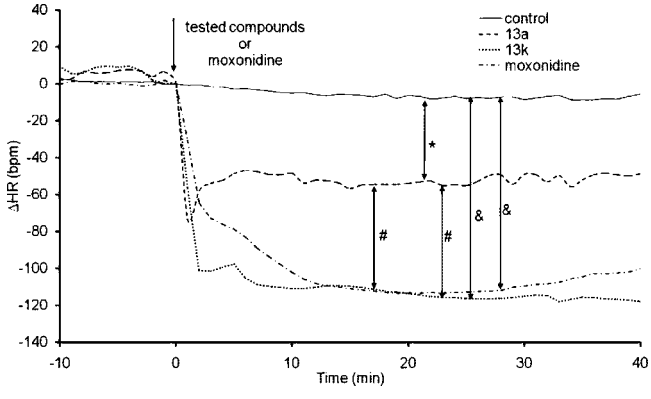


Figure 9. Effect of 0.1 mg/kg of **13a**, **13k**, and moxonidine on ΔHR (calculated as the difference of HR between sequential measurement and time 0 of experiment) in rats. Each point represents mean value of ΔHR for four to seven experiments. Comparisons were made using ANOVA with repeated measures and Fisher and Duncan tests. Significances (&) $p < 0.001$, (&) $p < 0.001$, and (∗) $p < 0.05$ were found for comparisons of **13k**, moxonidine, and **13a** versus control group, respectively. Significance (#) $p < 0.01$ was found for comparisons of **13a** versus **13k** and moxonidine groups.

acid in CH_2Cl_2 (8 mL) was stirred at room temperature for 2 h, and then the solvent and excess of trifluoroacetic acid were evaporated under reduced pressure. The viscous residue was treated with water (7 mL), and the resulting mixture or solution was made alkaline (pH 10−10.5) with 10% aqueous NaOH solution at 5 °C.

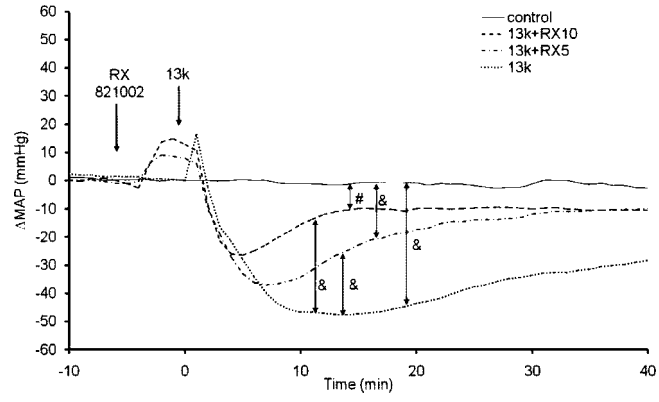


Figure 10. Effect of 10 μg/kg of **13k** in the absence and presence of 5 (RX5) and 10 (RX10) μg/kg of RX 821002 on ΔMAP (calculated as the difference of MAP between sequential measurement and time 0 of experiment) in rats. Each point represents mean value of ΔMAP for four to seven experiments. Comparisons were made using ANOVA with repeated measures and Fisher and Duncan tests. Significance (&) $p < 0.001$ was found for comparisons of the following: **13k** versus control, **13k** + RX5 (5 μg/kg of RX 821002), and **13k** + RX10 (10 μg/kg of RX 821002); control versus **13k** + RX5 (5 μg/kg of RX 821002). Significance (#) $p < 0.01$ was found for comparison of **13k** + RX10 (10 μg/kg of RX 821002) and control.

The precipitate thus obtained was filtered off and purified by crystallization from suitable solvent.

In this manner the following compounds were obtained.

1-[(Imidazolidin-2-yl)imino]indazole (12a). 12a was obtained from **11a** (0.80 g): yield 0.24 g (60%); mp 172−174 °C (2-propanol); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.88 (s, 1H), 7.69 (d, J = 7.8 Hz, 1H), 7.34 (d, J = 8.3 Hz, 1H), 7.27 (t, 1H), 7.05 (t, 1H), 6.65 (s, 1H), 6.47 (s, 1H), 3.61−3.41 (m, 4H). Anal. ($C_{10}H_{11}N_5$ (201.23)) C, H, N.

1-[(Imidazolidin-2-yl)imino]-7-methylindazole (12k). 12k was obtained from **11k** (0.83 g): yield 0.30 g (70%); mp 201−204 °C (MeCN); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.80 (s, 1H), 7.47 (d, J = 7.8 Hz, 1H), 6.96−6.88 (m, 2H), 6.62 (s, 1H), 6.20 (s, 1H), 3.40−3.36 (m, 4H), 2.61 (s, 3H). Anal. ($C_{11}H_{13}N_5$ (215.25)) C, H, N.

Step 3. To an ice-cold suspension of the appropriate 2-iminoimidazolidine **12a**−**k** (1.0 mmol) from step 2 in anhydrous methanol (7 mL) was added dropwise HCl/MeOH solution (d = 5.67 g/100 mL, 0.78 mL, 1.2 mmol). The cooling bath was removed, and the resulting solution was stirred at room temperature for 30 min. Then the solvent was evaporated under reduced pressure to dryness. The crude product thus obtained was purified by crystallization from suitable solvent.

In this manner the following compounds were obtained.

1-[(Imidazolidin-2-yl)imino]indazole Hydrochloride (13a). 13a was obtained from 2-iminoimidazolidine **12a** (0.20 g): yield 0.18 g (75%); mp 195−196 °C (EtOH/Et_2O); ^{1}H NMR (200 MHz, DMSO-

d_6) δ 12.59 (bs, 1H), 8.97 (s, 2H), 8.25 (s, 1H), 7.86 (d, $J = 8.0$ Hz, 1H), 7.54 (d, $J = 4.0$ Hz, 2H), 7.33−7.25 (m, 1H), 3.72 (s, 4H). Anal. ($C_{10}H_{12}ClN_5$ (237.69)) C, H, N.

1-[(Imidazolidin-2-yl)imino]-7-methylindazole Hydrochloride (13k). 13k was obtained from 2-iminoimidazolidine **12k** (0.21 g): yield 0.15 g (63%); mp 189−190 °C (EtOH/Et_2O); ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.78 (bs, 1H), 8.98 (s, 2H), 8.21 (s, 1H), 7.67 (d, $J = 7.7$ Hz, 1H), 7.29−7.12 (m, 2H), 3.74 (s, 4H), 2.55 (s, 3H). Anal. ($C_{11}H_{14}ClN_5$ (251.71)) C, H, N.

X-ray Crystallography of 12e and 13a. The intensity data of the crystals were collected using a KumaCCD diffractometer. The crystals structures were solved with SHELXS-97[51] and refined with SHELXL-97.[52] Crystallographic data for the structures **12e** and **13a** are available in Supporting Information.

Pharmacology. 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 prepared according to methods of Lione et al.[53] [^{3}H]Clonidine (3 nM, Perkin-Elmer) was bound in the presence of 10 μM rauwolscine to preclude binding to α_2-adrenoceptors. The specific component was defined by 10 μM rilmenidine. Under these conditions the site labeled is a model of the central I_1 binding site.[54] Membrane aliquots (400 μL, 0.2−0.5 mg of protein) were incubated with 11 concentrations of the test compound 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 ligand and free radioactivity were separated by rapid filtration through presoaked (0.5% polyethylamine) glass-fiber filters (Whatman GFB). Trapped ligand was determined by liquid scintillation counting, and data were analyzed by GraphPad Prism, version 3.02, for Windows (GraphPad Software, San Diego, CA) to yield IC_{50} values (the concentration of drug that displaces 50% of specifically bound [^{3}H]clonidine).

α_2- and I_2-Binding Site Assays. Crude P_2 brain membranes were prepared as follows. All procedures were carried out at 4 °C unless otherwise stated. Rat brains (male Sprague−Dawley rats, 250−280 g) were taken and homogenized in 10 volumes of ice cold buffer (50 mM Tris-HCl, 1 mM $MgCl_2$, and 320 mM sucrose, pH 7.4). The homogenate was centrifuged (1000*g* for 10 min) and the precipitate discarded. The supernatant was centrifuged a second time (32000*g* for 20 min) and the supernatant discarded, with the remaining precipitate making up the crude P_2 membrane preparation. This was washed twice in excess buffer (50 mM Tris-HCl, 1 mM $MgCl_2$) at room temperature. An amount of 30 mL was added, and the precipitate was resuspended 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) an additional two times. Membrane aliquots (400 μL, 0.2−0.3 mg of protein) were incubated with 11 concentrations of the test compound over the range 0.01 nM to 100 μM in the presence of the selective I_2 binding site ligand [^{3}H]2BFI[55] (1 nM) or the α_2-adrenoceptor antagonist [^{3}H]RX821002[44] (1 nM), to final volume of 500 μL. Nonspecific binding was determined using 10 μM BU224,[56] I_2 binding and 10 μM rauwolscine, α_2-adrenoceptor binding. Each incubation was performed in triplicate at room temperature and allowed to reach equilibrium (45 min). Bound radioactivity and free radioactivity were separated by rapid filtration through presoaked (0.5% polyethyleimine) glass-fiber 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 nonlinear regression curve fitting procedures in GraphPad Prism, version 3.02, for Windows (GraphPad Software, San Diego, CA). Each experiment was analyzed individually and the equilibrium dissociation constant (K_i) determined by the method of Cheng and Prusoff,[57] and the resulting values are given as the mean ± SEM for three to four 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 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 α_2-adrenoceptors (RX821002) was given iv at a dose of 5 or 10 μg/kg 5 min before the tested compound **13k**.

Arterial blood pressure and heart rate were monitored directly and sampled continuously at 100 Hz, as we described previously,[41] 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 a 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.[41] 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.

Acknowledgment. This research was supported in part by the Polish Ministry of Science and Higher Education, Poland (Grant N40500532/0458).

Supporting Information Available: General procedures for the preparation of compounds **9d−k**, **10d−k**, **12a−k**, **13a−k**, **15a−f**, **16a−f**, **19a−c**, and **20a−c**; elemental analysis results of reported compounds; and crystallographic data of compounds **12e** and **13a**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Koblinger, W. Drugs as Tools in Research on Adrenoceptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1986**, *332*, 113–117.
(2) Armach, B. T. Unique Presynaptic α_2-Receptors and Specificity of the Antihypertensive Agent Moxonidine. *Arzneim.-Forsch./Drug Res.* **1988**, *38*, 1435–1441.
(3) Van Zweiten, P. A. Pharmacology of Alpha$_2$-Adrenoceptor Agonist Rilmenidine. *Am. J. Cardiol.* **1988**, *61*, D6−D14.
(4) Koblinger, W.; Pichler, L. Centrally Acting Drugs (Clonidine, Methyldopa, Guanfacine). In *Handbook of Experimental Pharmacology. Pharmacology of Antihypertensive Therapeutics*; Ganten, D., Murlow, P. J. Eds.; Springer-Verlag: Berlin, Germany, **1990**; Vol. 93, p 227.
(5) Ruffolo, R. R., Jr.; Nichols, A. J.; Stadel, J. M.; Hieble, J. P. Pharmacologic and Therapeutic Applications of α_2-Adrenoceptor Subtypes. *Annu. Rev. Pharmacol. Toxicol.* **1993**, *32*, 243–279.
(6) Verbeuren, T. J.; Yuan, A. T. D.; Koenig-Bererd, E.; Viton, P. Rilmenidine. *Cardiovasc. Drug. Rev.* **1990**, *8*, 56–66.
(7) Bousquet, P.; Bricca, G.; Dontenwill, M.; Feldman, J.; Greney, H.; Belcourt, A.; Stutzmann, J.; Tibrica, E. From theα_2-Adrenoceptors to the Imidazoline Preferring Receptors. *Fundam. Clin. Pharmacol.* **1992**, *6* (Suppl. 1), 15a.
(8) Chrisp, P.; Faulds, D. Moxonidine. *Drugs* **1992**, *44*, 993–995.

(9) Molderings, G. J.; Göthert, M. Imidazoline Binding Sites and Receptors in Cardiovascular Tissue. *Gen. Pharmacol.* **1999**, *32*, 17–22.
(10) Ernsberger, P.; Damon, T. H.; Graff, L. M.; Schafer, S. G.; Christen, M. O. Moxonidine a Centrally Acting Antihypertensive Agent Is a Selective Ligand for I_1-Imidazoline Sites. *J. Pharmacol. Exp. Ther.* **1993**, *264*, 172–176.
(11) Szabo, B. Imidazoline Antihypertensive Drugs: A Critical Review on Their Mechanism of Action. *Pharmacol. Ther.* **2002**, *93*, 1–35.
(12) Bousquet, P. Identification and Characterization of I_1 Imidazoline Receptors. Their Role in Blood Pressure Regulation. *Am. J. Hypertens.* **2000**, *13*, 845–848.
(13) Sanders, R. D.; Maze, M. α-Adrenoceptor Agonists. *Curr. Opin. Invest. Drugs* **2007**, *8*, 25–33.
(14) Mantz, J. $Alpha_2$-Adrenoceptor Agonists: Analgesia, Sedation, Anxiolysis, Haemodynamics, Respiratory Function and Weaning. *Bailliere's Clin. Anaesthesiol.* **2000**, *14*, 433–448.
(15) Gerlah, A. T. Dexmedetomidine: An Updated Review. *Ann. Pharmacother.* **2007**, *41*, 245–252.
(16) Pandharipande, P. P.; Pun, B. T.; Herr, D. L.; Maze, M.; Girard, T. D.; Miller, R. R.; Shintani, A. K.; Thompson, J. L.; Jackson, J. C.; Deppen, S. A.; Stiles, R. A.; Dittus, R. S.; Bernard, G. R.; Ely, E. W. Effect of Sedation with Dexmedetomidine vs Lorazepam on Acute Brain Dysfunction in Mechanically Ventilated Patients: The Mends Randomized Controlled Trial. *JAMA, J. Am. Med. Assoc.* **2007**, *298*, 2644–2653.
(17) Millan, M. J.; Dekeyne, A.; Newman-Tancredi, A.; Cussac, D.; Audinot, V.; Milligan, G.; Duqueyroix, D.; Girardon, S.; Mullot, J.; Boutin, J. A.; Nicolas, J.-P.; Renouard-Try, A.; Lacoste, J.-M.; Cordi, A. A. S18613 a Highly Potent, Spiroimidazoline Agonist at α_2-Adrenoceptors: I. Receptor Profile, Antinociceptive and Hypothermic Actions in Comparison with Dexmedetomidine and Clonidine. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 1192–1205.
(18) Richer, C.; Gober, J.; Noyer, M.; Wulfert, E.; Giudicelli, J. F. Peripheral $Alpha_2$-Adrenoceptor-Mediated Sympathoinhibitory Effects of Mivazerol. *Fundam. Clin. Pharmacol.* **1996**, *10*, 529–537.
(19) Priebe, H.-J. Perioperative Myocardial Infraction. Aetiology and Prevention. *Br. J. Anaesth.* **2005**, *95*, 3–19.
(20) Cordi, A. A.; Lacoste, J. M.; Descombes, J.-J.; Courchay, Ch.; Vanhautte, P. M.; Laubie, M.; Verbeuren, T. J. Design, Synthesis and Structure−Activity Relationships of a New Series of α_2-Adrenergic Agonists: Spiro[(1,3-Diazacyclopent-1-ene)-5,2′-(1′,2′,3′,4′-tetrahydronaphthalene)]. *J. Med. Chem.* **1995**, *38*, 4056–4069.
(21) Cordi, A. A.; Lacoste, J. M.; Le Borgne, F.; Herve, Y.; Vaysse-Ludot, L.; Descombes, J.-J.; Courchay, Ch.; Laubie, M.; Verbeuren, T. J. (*S*)-Spiro[(1,3-diazacyclopent-1-ene)-5,2′-(7′-methyl-1′,2′,3′,4′-tetrahydronaphthalene)]: Resolution, Stereospecific Synthesis and Preliminary Pharmacological Characterization as a Potential α-Adrenergic Agonist. *J. Med. Chem.* **1997**, *40*, 2931–2935.
(22) Kapoor, K.; Willems, E. W.; Maassen VanDenBrink, A.; Hillgers, J. P. C.; Cordi, A. A.; Vaysettes-Courchay, C. Assessment of Anti-Migraine Potential of a Novel α-Adrenoceptor Agonist S19014: Effects on Porcine Carotid and Regional Haemodynamics and Human Coronary Artery. *Cephalalgia* **2004**, *24*, 425–438.
(23) Stähle, H. A Historical Perspective: Development of Clonidine. *Bailliere's Clin. Anaesthesiol.* **2007**, *14*, 237–246.
(24) Sączewski, F.; Kobierska, E.; Dębowski, T.; Charakchieva-Minol, S.; Mokrosz, M.; Gdaniec, M.; Nowak, E. Synthesis, Structure and Binding of Some 2-Imidazolines to Rat Brain $Alpha_1$- and $Alpha_2$-Adrenergic Receptors. *Arch. Pharm. (Weinheim, Ger.)* **2000**, *333*, 425–430.
(25) Sączewski, F.; Hudson, A. L.; Tyacke, R. J.; Nutt, D. J.; Man, J.; Tabin, P.; Sączewski, J. 2-(4,5-Dihydro-1*H*-imidazol-2-yl)indazole (Indazim) Derivatives as Selective I_2 Imidazoline Receptor Ligands. *Eur. J. Pharm. Sci.* **2003**, *20*, 201–208.
(26) Sączewski, F.; Tabin, P.; Tyacke, R. J.; Maconie, A.; Sączewski, J.; Kornicka, A.; Nutt, D. J.; Hudson, A. L. 2-(4,5-Dihydroimidazol-2-yl)benzimidazoles as Highly Selective Imidazoline I_2/Adrenergic α_2 Receptor Ligands. *Bioorg. Med. Chem.* **2006**, *14*, 6679–6685.
(27) Mathy, M. J.; Thoolen, M. J. M. C.; Timmermans, P. B. M. W. M.; van Zweiten, P. A. Sgd 101/75: Cardiovascular Effects in Various Animal Preparations; Interactions with Vascular Postjunctional α_1- and α_2-Adrenoceptors. *Br. J. Pharmacol.* **1984**, *81*, 255–262.
(28) Patent application: Cupps, T. L.; Bogdan, S. E.; Henry, R. T.; Sheldon, R. J.; Seibel, W. L.; Ares, J. J. Preparation and Formulation of 6-(Imidazolinylamino)indazoles asα_2-Adrenoceptor Agonists. WO1998023609, 1998.
(29) Sączewski, F. Functions Containing an Iminocarbonyl Group and Any Elements Other Than a Halogen or Chalcogen. In *Comprehensive Organic Functional Group Transformations*; Katritzky, A. R., Taylor, R. J. K. Eds.; Elsevier: Amsterdam, The Netherlands, 2005; Vol. 6, pp 605−659.
(30) Trani, A.; Belasio, E. Synthesis of 2-Chloro-2-imidazoline and Its Reactivity with Aromatic Amines, Phenols and Thiophenols. *J. Heterocycl. Chem.* **1974**, *11*, 257–264.
(31) Sączewski, F.; Sączewski, J. Application of 2-Chloro-4,5-dihydroimidazole in Heterocyclic and Medicinal Chemistry. *Trends Heterocycl. Chem.* **2003**, *9*, 19–31.
(32) Claramunt, R. M.; Sanz, D.; Catalan, J.; Fabero, F.; Garcia, N. A.; Foces-Foces, C.; Limas-Saiz, A. L.; Elguero, J. Aminoazoles. Part 2. Basicity and Protonation Site of *N*-Aminoazoles: an Experimental (pK_a, ^{13}C and ^{15}N NMR Spectroscopy and Crystallography) and Theoretical Study. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1687–1699.
(33) Kim, K. S.; Quian, L. Improved Method for the Preparation of Guanidines. *Tetrahedron Lett.* **1993**, *34*, 7677–7680.
(34) Adger, B. M.; Bradbury, S.; Keating, M.; Rees, Ch. W.; Storr, R. C.; Williams, M. T. 1,2,3-Benzotriazines. *J. Chem. Soc. Perkin Trans. 1* **1975**, 31–40.
(35) Dardonville, Ch.; Goya, P.; Rozas, I.; Alsasua, A.; Martin, M. I.; Borrego, M. J. New Aromatic Iminoimidazolidine Derivatives as α_1-Adrenoceptor Antagonists: A Novel Synthetic Approach and Pharmacological Activity. *Bioorg. Med. Chem.* **2000**, *8*, 1567–1577.
(36) Somei, M.; Matsubara, M.; Kanda, Y.; Natsume, M. A Novel *N*-Amination Method and Its Application to the Preparation of *N*-Aminoheterocycles. *Chem. Pharm. Bull.* **1978**, *26*, 2522–2534.
(37) Kuz'menko, V. V.; Komissarov, V. N.; Simonov, A. M. Unusual Fisher Reaction in 1-Aminobenzimidazole. Synthesis of Pyrido[1,2-*a*]benzimidazole. *Khim. Geterotsikl. Soedin.* **1981**, *11*, 1497–1502.
(38) Knight, D. W.; Little, P. B. 1-Aminobenzotriazole Functionalisation Using Directed Metallation: New Routes to Chromanes and Chromenes Using Intramolecular Benzyne Trapping by Alcohols. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2343–2355.
(39) Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen, A. G.; Taylor, R. Tables of Bond Lengths Determined by X-Ray and Neutron Diffraction. Part 1. Bond Lengths in Organic Compounds. *J. Chem. Soc., Perkin Trans. 2* **1987**, S1–S19.
(40) The geometry of tautomers **13aA** and **13aB** were fully optimized by the DFT method at the B3LYP/6-31G** level using the Spartan program. Total energies, atomic charges, and 3D electrostatic potential maps were calculated at the same level.
(41) Rybczyńska, A.; Boblewski, K.; Lehmann, A.; Orlewska, C.; Foks, H.; Drewnowska, K.; Hoppe, A. Calcimimetic NPS R-568 Induces Hypotensive Effect in Spontaneously Hypertensive Rats. *Am. J. Hypertens.* **2005**, *18*, 364–371.
(42) Urban, R.; Szabo, B.; Starke, K. Involvement of $Alpha_2$-Adrenoceptors in the Cardiovascular Effects of Moxonidine. *Eur. J. Pharmacol.* **1995**, *282*, 19–28.
(43) Head, G. A.; Chan, C. K. S.; Burke, S. L. Relationship between Imidazoline and α_2-Adrenoceptors Involved in the Sympatho-Inhibitory Actions of Centrally Acting Antihypertensive Agents. *J. Auton. Nerv. Syst.* **1998**, *72*, 163–169.
(44) Langin, D.; Lafontan, M.; Stillings, M. R.; Paris, H. [^{3}H]RX821002: A New Tool for the Identification of α_{2A}-Adrenoceptors. *Eur. J. Pharmacol.* **1989**, *167*, 95–104.
(45) Hudson, A. L.; Nutt, D. J. In Vitro Autoradiography of [^{3}H]RX821002 in Rat CNS, a New Ligand for Identifying α_2-Adrenoceptors. *Br. J. Pharmacol.* **1990**, *100*, 345P
(46) Vayssettes-Courchay, Ch.; Bouysset, F.; Cordi, A. A.; Laubie, M.; Verbeuren, T. J. A. Comparative Study of the Reversal by Different α_2-Adrenoceptor Antagonists of the Central Sympatho-Inhibitory Effect of Clonidine. *Br. J. Pharmacol.* **1996**, *117*, 587–593.
(47) Head, G. A.; Burke, S. L.; Chan, C. K. Central Imidazoline Receptors and Centrally Acting Anti-Hypertensive Agents. *Clin. Exp. Hypertens.* **1997**, *19*, 591–605.
(48) Bousquet, P. I1 Receptors, Cardiovascular Function and Metabolism. *Am. J. Hypertens.* **2001**, *14*, 317S–321S.
(49) Rűchardt, Ch.; Hassmann, V. Durchfűhrung der Jacobsonschen Indazolsynthese im Eintopfverfahren. *Liebigs Ann. Chem.* **1980**, 908–927.
(50) Lukin, K.; Hsu, M. C.; Fernando, D.; Leanna, M. R. New Practical Synthesis of Indazoles via Condensation of *o*-Fluorobenzaldehydes and Their *O*-Methyloximes with Hydrazine. *J. Org. Chem.* **2006**, *71*, 8166–8172.
(51) Sheldrick, G. M. *SHELXS-97. Program for the Solution of Crystal Structures*; University of Göttingen: Göttingen, Germany, 1997.
(52) Sheldrick, G. M. *SHELXL-97. Program for the Refinement of Crystal Structures*; University of Göttingen: Göttingen, Germany 1997.
(53) Lione, L. A.; Nutt, D. J.; Hudson, A. L. Characterisation and Localisation of [^{3}H]2-(2-Benzofuranyl)-2-imidazoline Binding in Rat Brain: A Selective Ligand for Imidazoline I_2 Receptors. *Eur. J. Pharmacol.* **1998**, *353*, 123–135.
(54) Ernsberger, P.; Graves, M. E.; Graff, L. M.; Zakieh, N.; Nguyen, P.; Collins, L. A.; Westbrooks, K. L.; Johnson, G. G. I_1-Imidazoline Receptors. Definition, Characterisation, Distribution and Transmembrane Signaling. *Ann. N.Y. Acad. Sci.* **1995**, *763*, 22–43.

(55) Hudson, A. L.; Chapleo, C. B.; Lewis, J. W.; Husbands, S.; Grivas, K.; Mallard, N. J.; Nutt, D. J. Identification of Ligands Selective for Central I_2 Imidazoline Binding Sites. *Neurochem. Int.* **1997**, *30*, 47–54.

(56) Nutt, D. J.; French, N.; Handley, S.; Hudson, A. L.; Husbands, S.; Jackson, H.; Jordan, S.; Lalies, M.; Lewis, J.; Lione, L.; Mallard, N.; Pratt, J. Functional Studies of Specific Imidazoline-2 Receptor Ligands. *Ann. N.Y. Acad. Sci.* **1995**, *763*, 125–139.

(57) Cheng, Y. C.; Prusoff, W. H. Relationship between the Inhibition Constant (K_i) and the Concentration of Inhibitor Which Causes 50% Inhibition (IC_{50}) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

JM800112S