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Synthesis and biological activities of 2-[(heteroaryl)methyl]imidazolines

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ABSTRACT

A series of 2-[(heteroaryl)methyl]imidazolines was synthesized and tested for their activities at α_1- and α_2-adrenoceptors and imidazoline I_1 and I_2 receptors. The most active 2-[(indazol-1-yl)methyl]imidazolines showed high or moderate affinities for α_1- and α_2-adrenoceptors. However, their intrinsic activities at α_{2A}-adrenoceptors proved to be negligible. A selected 7-chloro derivative behaved as a potent α_1-adrenoceptor antagonist and exhibited peripherally mediated hypotensive effects in rats.

1. Introduction

Imidazoline-containing compounds constitute a valuable class of agents acting at α-adrenergic and/or imidazoline receptors.[1–4] For example, 2-(anilino)imidazolines such as clonidine and its analogues (structure **A**, Fig. 1) are non-selective α_2-adrenoceptor/I_1 receptor agonists widely used in clinical practice for the treatment of hypertension and in intensive care units as sedative, anxiolytic and analgesic agents, while other analogues of clonidine with a methylene bridge connecting the imidazoline ring with an aromatic moiety, represented by, for example, tolazoline (structure **B**), are α_1-adrenoceptor antagonists which behave as vasodilators. On the other hand, cirazoline with a methylenoxy spacer (structure **C**, X = O) is known to behave as an α_1-adrenoceptor agonist and an α_2 antagonist with vasoconstricting properties, and a series of 2-(anilinomethyl)imidazolines of type **C** (X = NH) has been described as potent α_1-adrenoreceptor agonists useful for the treatment of benign prostatic hyperplasia.[5–8]

Recently, we have disclosed a highly selective imidazoline-based partial α_2-adrenoceptor agonist *marsanidine* (1-[(imidazolidin-2-yl)imino]indazole, compound **D**, Fig. 1), from which emerged 7-methyl-marsanidine[9] and its positional analogues **E**,[10] compounds with potent hypotensive and diuretic activities.

In this report we describe the synthesis and biological activities of previously unexplored 2-(anilinomethyl)-imidazoline analogues of type **F** with a nitrogen atom incorporated into a heterocyclic ring system such as indazole, benzimidazole or benzotriazole. These newly prepared variants of 2-(anilinomethyl)imidazolines **C** (X = N) with partially restricted conformational freedom were tested for their in vitro binding affinities at α_1- and α_2-adrenoceptors and imidazoline I_1 and I_2 receptors, their intrinsic activity at α_{2A}-adrenoceptors as well as their in vivo effects on circulatory and CNS functions in the rat.

2. Results and discussion

2.1. Chemistry

As shown in Schemes 1 and 2, the reaction procedure leading to target compounds **3**, **5**, and **7** consisted in the reaction of corresponding azole sodium salts, generated by the treatment of indazoles **1a–i**, benzimidazoles **4a–h** or benzotriazoles **6a–c** with sodium hydride in anhydrous THF, with 2-chloromethylimidazoline **2** at ambient temperature. Thus, indazoles **1a–i** gave mixtures of 1- and 2-substituted alkylation products, from which indazol-1-yl compounds **3a–i** were separated in pure form in 26–34% yield

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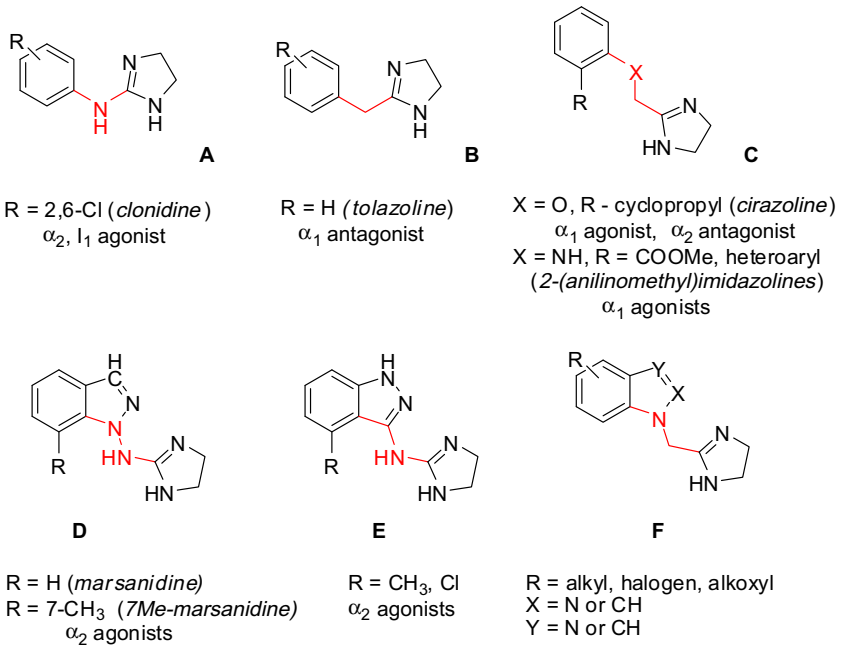
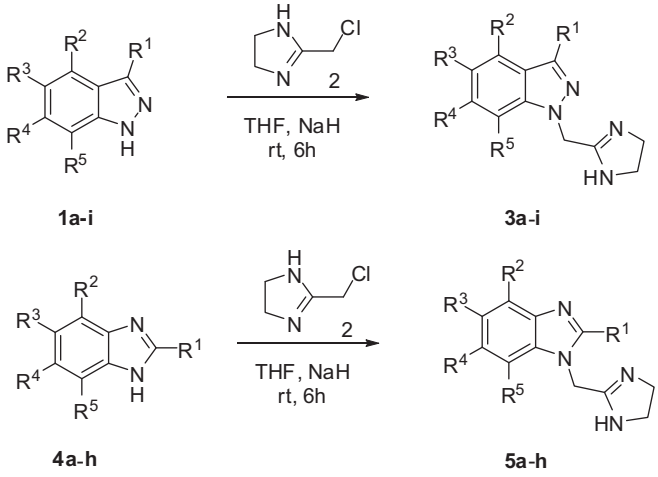


Figure 1. Structures of α-adrenergic ligands **A**–**C** and their analogues **D**–**F** with partially restricted conformational freedom.



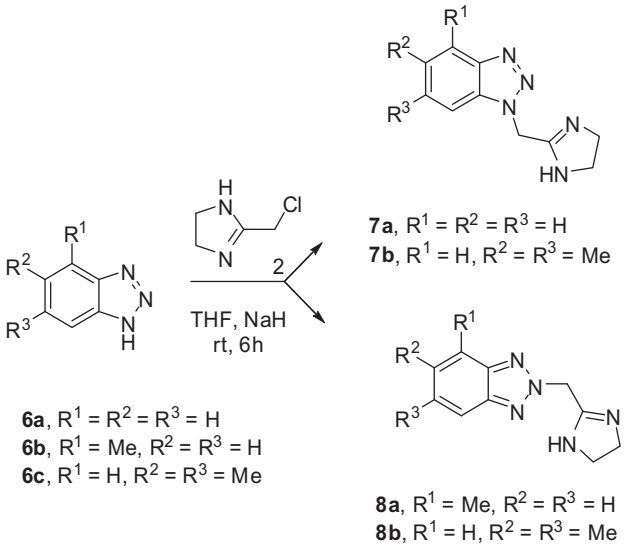
1a, **3a**, $R^1 = R^2 = R^3 = R^4 = R^5 = H$
1b, **3b**, $R^1 = Me$, $R^2 = R^3 = R^4 = R^5 = H$
1c, **3c**, $R^1 = H$, $R^2 = Me$, $R^3 = R^4 = R^5 = H$
1d, **3d**, $R^1 = H$, $R^2 = Cl$, $R^3 = R^4 = R^5 = H$
1e, **3e**, $R^1 = H$, $R^2 = OMe$, $R^3 = R^4 = R^5 = H$
1f, **3f**, $R^1 = R^2 = H$, $R^3 = Me$, $R^4 = R^5 = H$
1g, **3g**, $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = Me$
1h, **3h**, $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = Cl$
1i, **3i**, $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = F$

4a, **5a**, $R^1 = R^2 = R^3 = R^4 = R^5 = H$
4b, **5b**, $R^1 = Me$, $R^2 = R^3 = R^4 = R^5 = H$
4c, **5c**, $R^1 = Et$, $R^2 = R^3 = R^4 = R^5 = H$
4d, **5d**, $R^1 = Ph$, $R^2 = R^3 = R^4 = R^5 = H$
4e, **5e**, $R^1 = Bz$, $R^2 = R^3 = R^4 = R^5 = H$
4f, **5f**, $R^1 = H$, $R^2 = Me$, $R^3 = R^4 = R^5 = H$
4g, **5g**, $R^1 = R^2 = H$, $R^3 = R^4 = Me$, $R^5 = H$
4h, **5h**, $R^1 = Et$, $R^2 = H$, $R^3 = R^4 = Me$, $R^5 = H$

Scheme 1. Preparation of indazol-1-yl derivatives **3a**–**i** and benzimidazol-1-yl derivatives **5a**–**h**.

by preparative thin layer chromatography on silica gel using chromatotron. In a similar manner, benzimidazol-1-yl derivatives **5a**–**h** were obtained in 42–83% yield. On the other hand, from the reaction of sodium salts of benzotriazoles **6a**–**c** with 2-chloromethylimidazoline **2**, both the 1-substituted products **7a**–**b** and 2-substituted products **8a**–**b** could be separated in 34–35% and 10–12% yield, respectively. For biological testing, the products **3**, **5**, **7** and **8** were further converted into the corresponding water-soluble hydrochlorides with use of methanolic hydrochloric acid solution or by passing gaseous hydrogen chloride through dichloromethane solutions of the free base.

Structures of all newly prepared compounds were confirmed by C, H, N elemental analyses as well as IR and NMR spectroscopic data (see Section 4).



Scheme 2. Preparation of benzotriazol-1-yl derivatives **7a**,**b** and benzotriazol-2-yl derivatives **8a**,**b**.

2.2. Radioligand binding assays

The in vitro assay involved the investigation of the affinities of 2-[(heteroaryl)methyl]-imidazolines **3a–i**, **5a–h**, **7a–b** and **8a–b** for α_1- and α_2-adrenoceptors as well as imidazoline I_1 and I_2 receptors. All ligands were used in form of water soluble hydrochloride salts and the results of the receptor binding assays are presented in Table 1.

In general, all of the tested compounds exhibited very low affinity for imidazoline I_1 receptors (K_i values in the range of 646–96400 nM). Similar results were obtained for imidazoline I_2 receptors with the only the exception of the 5-methylindazole derivative **3f**, which exhibited high affinity (K_i = 22 nM).

Table 1
Binding affinity data for compounds **3a–i**, **5a–h**, **7a–b**, and **8a–b**

Compound	α_1 K_i (nM)[a]	α_2 K_i (nM)[a]	I_1 IC_{50} (nM)[b]	I_2 K_i (nM)[a]
3a	272 ± 42	18.5 ± 5.9	19400 (*n* = 1)	42200 (*n* = 1)
3b	95 ± 27	435 ± 99	21300 (*n* = 1)	359 ± 88
3c	409 ± 39	197 ± 28	12900 (*n* = 1)	116 ± 44
3d	187 ± 77	130 ± 27	5150 ± 688	2850 ± 492
3e	1670 ± 393	305 ± 59	32900 (*n* = 1)	382 ± 72
3f	363 ± 89	268 ± 59	45400 (*n* = 1)	22 ± 7.4
3g	101 ± 28	607 ± 97	96400 (*n* = 1)	6840 ± 879
3h	14.1 ± 3.9	6.4 ± 2.3	856 ± 239	554 ± 288
3i	107 (*n* = 1)	12.6 (*n* = 1)	16400 (*n* = 1)	156 (*n* = 1)
5a	929 ± 48	3250 ± 479	43900 (*n* = 1)	6100 ± 410
5b	1290 ± 599	1020 ± 163	9390 ± 1059	ND[c]
5c	5500 ± 610	5000 ± 900	44200 (*n* = 1)	34100 (*n* = 1)
5d	1110 ± 440	7440 ± 680	28100 (*n* = 1)	ND[c]
5e	30900 (*n* = 1)	11200 (*n* = 1)	21000 (*n* = 1)	ND[c]
5f	1500 (*n* = 1)	273 (*n* = 1)	77700 (*n* = 1)	24400 (*n* = 1)
5g	1500 ± 740	2260 ± 490	74000 (*n* = 1)	28000 (*n* = 1)
5h	12900 ± 5300	7410 ± 620	93300 (*n* = 1)	81 ± 12
7a	1340 ± 547	5110 ± 1189	56800 (*n* = 1)	810 ± 269
7b	8040 (*n* = 1)	1560 (*n* = 1)	16800 (*n* = 1)	26300 (*n* = 1)
8a	1540 (*n* = 1)	499 (*n* = 1)	48800 (*n* = 1)	78 ± 55
8b	6070 (*n* = 1)	1320 (*n* = 1)	646 (*n* = 1)	233 (*n* = 1)

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

[b] Molar concentration of the test compounds that displaces 50% of specifically bound [^{3}H]clonidine in rat kidney membranes in the presence of rauwolscine.

[c] ND—no displacement up to 1,00,000 nM.

The most interesting results, however, were found within a series of indazole derivatives **3a–i** which were characterised by moderate or high affinity for α-adrenoceptors, depending on the substitution pattern. The unsubstituted compound **3a** showed high affinity for α_2-adrenoceptors (K_i = 18.5 nM) and moderate affinity for α_1-adrenoceptors (K_i = 272 nM), while the 3-, 4- and 5-substituted analogues **3b–g** showed affinities about 7–33 times lower at α_2-adrenoceptors (K_i values in the range of 130–607 nM) and comparable or lower affinities at α_1-adrenoceptors (K_i in the range of 95–1670 nM). It should be pointed out that the highest affinity at both the α_1- and α_2-adrenoceptors was exhibited by compound **3h** with a chlorine atom placed at position 7 of the indazole ring (K_i = 14 and 6.4 nM, respectively). Compound **3h** also displayed submicromolar affinities to imidazoline I_1 and I_2 receptors (K_i values = 856 and 554 nM, respectively).

Summing up, from an analysis of the results of in vitro binding assays presented in Table 1 it can be concluded that indazole-containing compounds **3** constitute an interesting class of nonselective $\alpha_1\alpha_2$-adrenoceptor ligands of which the most active was the 7-Cl derivative **3h** that bears resemblance to the previously described agents of type **C**, **D** and **E** (Fig. 1).

2.3. Functional [^{35}S]GTPγS binding assays

Compounds **3a** (R = H) and **3h** (R = 7-Cl) with the highest binding affinity at α_2-adrenoceptors and the compound **3g** (R = 7-Me) which displayed higher α_2-adrenoceptor binding affinity compared to its affinity at α_1-adrenoceptors were subjected to [^{35}S]GTPγS binding experiments to determine their intrinsic activity and agonist potency at recombinant human α_{2A}-adrenoceptors.[11,12] For SAR purposes, noradrenaline, clonidine as well as marsanidine and its 7-Me and 7-Cl derivatives representing nitrogen-bridged analogues of type **E** were also included as controls.

As shown in Figure 2 and Table 2, in contradistinction to the natural full agonist noradrenaline as well as the partial agonists clonidine and the marsanidine derivatives, the newly prepared compounds of type **3** only weakly stimulated the binding of [^{35}S]GTPγS to CHO cell membranes expressing recombinant human α_{2A}-adrenoceptors. Their intrinsic activities estimated relative to noradrenaline were found to be very low (6.3, 6.3 and 7.4% for compounds **3a**, **3g** and **3h**, respectively). Moreover, their agonist potencies expressed as EC_{50} values were in the micromolar range.

2.4. In vivo cardiovascular effects

Based on the results of the in vitro experiments described above, the most interesting compound **3h** (R = 7-Cl) was selected for

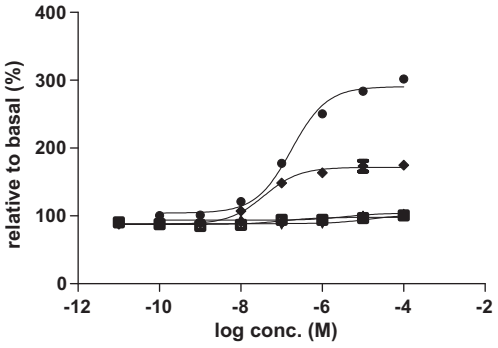


Figure 2. Graphical presentation of [^{35}S]GTPγS binding results with CHO cell membranes expressing recombinant human α_{2A}-adrenoceptors: the full agonist noradrenaline (●) and the partial agonist clonidine (♦) dose-dependently stimulated [^{35}S]GTPγS binding but compounds **3a** (□), **3g** (▲) and **3h** (▽) were almost inactive in this assay. Values shown are means ± s.e.m. from 3 independent experiments.

Table 2
Characterization of [^{35}S]GTPγS binding to CHO cell membranes expressing recombinant human α_{2A}-adrenoceptors: estimates of agonist potency (EC_{50}) and intrinsic activity relative to the natural full agonist noradrenaline. Values shown are means ± s.e.m. from 3 independent experiments for compounds **3a**, **3g** and **3h** and 3–19 independent experiments for the other compounds

Ligand	EC_{50} (nM)	Intrinsic activity (% of Noradrenaline)
Noradrenaline	118 ± 14	100
Clonidine	30 ± 4	51 ± 2
Marsanidine[a]	163 ± 66	18 ± 2
7-Me-marsanidine[a]	37 ± 9	32 ± 5
7-Cl-marsanidine[a]	15 ± 2	48 ± 3
3a	4500 ± 4300	6.3 ± 1.6
3g	17,400 ± 15,500	6.3 ± 0.6
3h	16,600 ± 5800	7.4 ± 1.0

[a] These results have been published previously in Sączewski F. et al. **2011**.[10]

evaluation of possible cardiovascular effects in anaesthetized rats. We reasoned that this compound, characterized by very weak intrinsic activity at α_{2A}-adrenoceptors, could still prove to be vasoactive by being a mixed α_1-adrenoceptor/α_2-adrenoceptor antagonist. Its in vivo hemodynamic effects were evaluated in anaesthetized rats by monitoring changes in blood pressure and heart rate after intravenous infusion of **3h** using procedures described previously.[13] The post-infusion data were compared to baseline values and are presented as ΔMAP and ΔHR in Figures 3 and 4, respectively. We found that administration of **3h** at a dose of 0.1 mg/kg induced highly significant decreases in blood pressure (ΔMAP = −47/7 ± 4.3 mmHg) and heart rate (ΔHR = −131.8 ± 11/5 bpm).

Clonidine-like centrally acting antihypertensive agents, with their main pharmacological actions mediated by α_{2A}-adrenoceptor activation, exert biphasic hemodynamic effects after intravenous administration: an initial short-lasting increase in arterial blood pressure resulting from a peripheral vasoconstrictor response is followed by a prolonged centrally mediated hypotensive effect. Upon administration of **3h**, however, an immediate fall of blood pressure was observed with no initial pressor response. In view of the in vitro finding that the imidazoline derivative **3h** has negligible intrinsic activity at α_{2A}-adrenoceptors we put forward a hypothesis that the possible mechanism of the observed hypotensive effect might involve blockade of peripheral α_1-adrenoceptors and/or α_2-adrenoceptors that are widely expressed in vascular beds. Testing of this hypothesis was performed by examining the interaction of **3h** with the selective α_1-adrenoceptor agonist methoxamine that has vasoconstrictor and positive inotropic properties. As shown in Figure 3, the intravenous infusion of methoxamine at 10 μg/kg/min, 10 min prior to **3h** administration, markedly attenuated the hypotensive action of the compound. A further increase of the methoxamine infusion rate to 20 μg/kg/min almost completely abolished the hypotensive effect of **3h**.

On the other hand, as shown in Figure 4, co-administration of methoxamine with **3h** only slightly attenuated the decrease in heart rate (this difference did not reach statistical significance in comparison to the **3h** group). Since activation of α_1-adrenoceptors in the myocardium by methoxamine causes a positive chronotropic response[14,15] and this effect was not fully inhibited by **3h**, the newly prepared imidazoline compound may produce a negative chronotropic effect on the heart independent of α-adrenoceptors. A possible mechanism may, for example, involve β-adrenoceptors[16] or sodium channel blockade.[17]

2.5. Loss of righting reflex (LORR) test

α_2-Adrenoceptor agonists such as clonidine and dexmedetomidine have been used in anaesthesiology to induce a sedative-hypnotic state somewhat similar to physiological non-rapid eye movement (non-REM) sleep.[18] Therefore, the compound **3h** with high affinity and poor efficacy at α_2-adrenoceptors was investigated to determine whether or not it shows capacity to activate the non-REM sleep pathway in rats. This anesthetic state was evaluated by induction of loss of the righting reflex (LORR) over a wide range of doses. The results presented in Table 3 indicate that at low doses of 10–500 μg/kg, the righting reflex was present, and at the highest administered doses of 1000 and 2000 μg/kg compound **3h** produced marked CNS actions with rapid onset. Within 5–8 min post-injection rats displayed loss of spontaneous

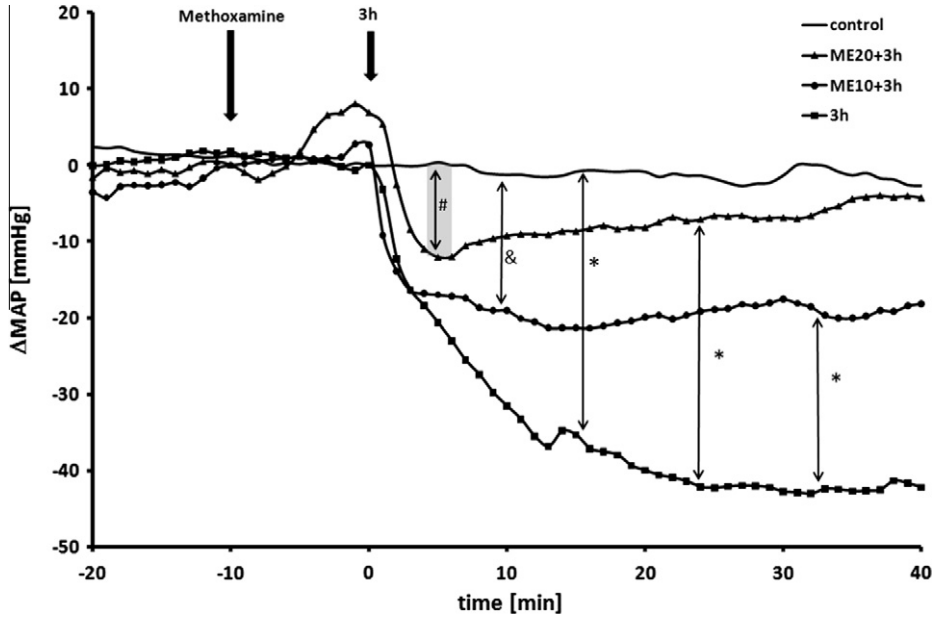


Figure 3. Effect of 0.1 mg/kg of **3h** in the absence and presence of 10 (ME10) and 20 (ME20) μg/kg/min of methoxamine (ME) on ΔMAP (mean arterial blood pressure) calculated as the difference of MAP between sequential measurements and time 0 of the experiment. Each point represents a mean value of ΔMAP from five to seven experiments. Comparisons were made using ANOVA for repeated measurements and Fisher's test. Significant differences (*) p <0.001 were found for comparisons **3h** versus control, **3h** versus ME20+**3h** and **3h** versus ME10+**3h**; (&) p <0.01 was found for comparison of ME10+**3h** versus the control group. Significance (#) p <0.05 was found for ME20+**3h** versus the control group between 3 and 5 min of the experiment (shaded part).

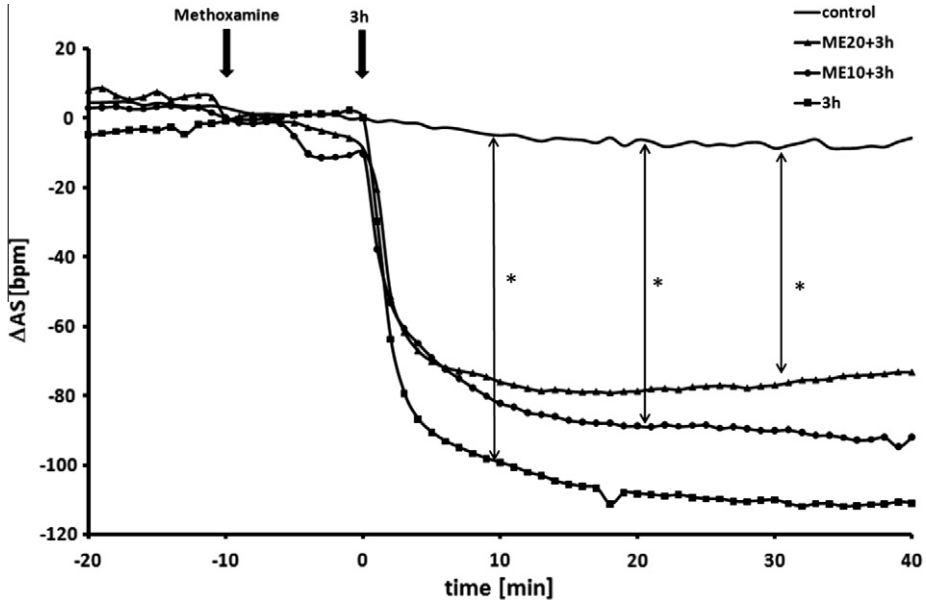


Figure 4. Effect of 0.1 mg/kg of **3h** in the absence and presence of 10 (ME10) and 20 (ME20) μg/kg/min of methoxamine on ΔHR (heart rate) calculated as the difference of HR between sequential measurements and time 0 of the experiment. Each point represents a mean value of ΔHR from five to seven experiments. Comparisons were made using ANOVA for repeated measurements and Fisher's test. Significant differences (*) p <0.001 were found for comparisons **3h**, ME20+**3h** and ME10+**3h** versus control.

Table 3
LORR dose–response results for compound **3h** at 30 min after i.p. injection

Animal	Weight (g)	Dose (μg/kg)	LORR
1	260	10	No
2	253	100	No
3	258	500	No
4	260	1000	Yes
5	251	2000	Yes

locomotor activity and reduced motor co-ordination, suggestive of locomotor impairment without a true hypnotic action as the righting reflex was slowed but not completely lost. After 30 min, the rats remained in a calm, subdued state, remaining still with no exploratory behaviour and showing little resistance to handling. However, reflex responses such as paw grasping and the righting reflex had returned to normal.

The above results regarding reduced locomotor activity after systemic administration of **3h** require further investigation but are briefly commented here. They agree with previously described findings that also α_1-adrenoceptors participate in regulation of locomotor behaviour of rats and mice.[19–21] Moreover, it was found that both pharmacologic and genetic inhibition of α_1-adrenoceptor activation inhibited dopamine-mediated locomotor hyperactivity.[22,23] Hence, α_1-antagonists were considered as a new approach to treat dopamine-induced dyskinesia. However, since decreased locomotor activity was observed only in animals treated with relatively high doses of **3h** (1–2 mg/kg), we cannot exclude that this depressant effect may result from a profound decrease in blood pressure. Such observations have been made previously upon treatment of experimental animals with various hypotensive agents.[24]

Interestingly, the onset of CNS effects was accompanied by changes in body temperature, which suggests activity in the hypothalamus and possibly in the same brain nuclei that are involved in regulation of non-REM sleep. In contrast to clonidine and dexmedetomidine, the effect on temperature regulation appeared to be hyper- rather than hypothermic. Although sedation is usually accompanied by a decrease in body temperature, hypnotic agents have previously been reported to produce increases, decreases or even biphasic effects on body temperature.[25] Therefore, given the fairly rapid onset and short-lasting action (<30 min) of the effects of **3h** noted in the present study, further testing using a higher initial dose (>500 mg/kg) and temperature monitoring will be necessary for a more comprehensive assessment. Changes in the release of dopamine, 5-HT and other neurotransmitters leading to the hyperthermic effect of **3h** cannot be excluded.[25]

3. Conclusion

In this paper we have described the synthesis and binding affinities of four series of novel analogues of 2-(anilinomethyl) imidazolines with partially restricted conformational freedom. Indazol-1-yl derivatives of type **3** exhibited high or moderate affinity to both α_1- and α_2-adrenoceptors. However, these compounds showed negligible intrinsic activity at α_{2A}-adrenoceptors. Hypotensive responses to the most active derivative 7-chloro-1-((4,5-dihydro-1*H*-imidazol-2-yl)methyl)-1*H*-indazole (**3h**) were progressively reduced by increasing doses of the α_1-adrenoceptor agonist methoxamine, which suggests that this effect may result from peripheral vascular effects rather than central sympathetic depression, and the newly prepared imidazoline compound **3h** may be regarded as a prototype of a new class of α_1-adrenoceptor antagonists.

4. Experimental

4.1. Chemistry

Melting points were measured on a Buchi 535 apparatus and are uncorrected. IR spectra were taken in KBr pellets on a Perkin–Elmer FTIR 1600 spectrometer. NMR spectra were recorded on a Varian Gemini 200 or a Varian Unity 500 apparatus. ^{1}H and ^{13}C chemical shifts were measured relative to the residual solvent signal at 7.26 ppm ($CDCl_3$) or 2.50 ppm and 39.5 ppm (DMSO-d_6). Chromatography was performed on silica gel 60 PF_{254} containing gypsum (Merck) using the reported solvent systems. Indazoles **3c**, **3f**, **3g** and **3d**, **3e**, **3h**, **3i** were prepared according to the

Rüchardt and Hassmann procedure[26] or using *o*-fluorobenzaldehydes,[27] respectively. The benzimidazoles **4a**–**h** and benzotriazoles **6a**–**c** were obtained from the corresponding *o*-phenylenediamines. 2-(Chloromethyl)-4,5-dihydro-1*H*-imidazole hydrochloride was synthesized from 2-chloroacetonitrile and ethylenediamine.[28]

4.2. General procedure for the preparation of compounds 3, 5, 7, and 8

Sodium hydride (0.53 g, 6.6 mmol, 60% oil dispresion) was added to the stirred solution of the corresponding azole (3.3 mmol) in anhydrous THF (2 mL) at room temperature. After 15 min, freshly prepared 2-(chloromethyl)-4,5-dihydro-1*H*-imidazole **2** (0.47 g, 4.0 mmol) was added and the reaction mixture was stirred at ambient temperature for 6 h. The *N*-alkylation products **3**, **5**, **7** and **8** were isolated upon quenching the reaction mixture with water (5 mL) followed by extraction with dichloromethane (3 × 5 mL). The combined organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure. The oily residue thus obtained was purified on silica with use of chromatotron (Et_3N/MeOH/AcOEt 1:5:100). All products were very polar with R_f values close to 0.05.

The *N*-alkylation reaction of indazoles (**1**) provided the desired N1 substituted products (**3**) along with the N2 substituted side product. The latter compounds demonstrated considerably lower R_f than **3** and were not isolated in pure form. The alkylation reactions of benzotriazoles **6a** and **6c** allowed the isolation of N1 substituted products **7a**–**b** (higher R_f) and N2 isomer **8b** (lower R_f). However, in the case of 4-methyl-benzotriazole **6b** only the N2 substituted isomer **8a** was isolated as a pure product.

The products **3**, **5**, **7** and **8** were then converted into water-soluble hydrochloride salts suitable for biological tests with use of methanolic hydrochloric acid solution or by passing gaseous hydrogen chloride through dichloromethane solution of the corresponding free base.

4.2.1. 1-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-1*H*-indazole (3a)

A white solid (0.20 g, 30%), mp = 132–135 °C; ^{1}H NMR ($CDCl_3$): δ 3.59 (s, 4H, CH_2), 4.57 (br s, 1H, NH), 5.21 (s, 2H, CH_2), 7.15–7.23 (m, 1H, CH), 7.38–7.54 (m, 2H, CH), 7.72–7.77 (m, 1H, CH), 8.05 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 47.9, 50.4, 109.7, 121.6, 121.7, 124.6, 127.5, 134.8, 140.4, 163.6; ν_{max} 3082, 2926, 2856, 2797, 1617, 1508, 1413, 1239, 1173, 985, 907, 831, 752, 741 cm^{-1}; MS (ESI) *m*/*z*: 201 [M+H]$^+$. Hydrochloride of **3a**; mp = 196 °C dec.; Anal. Calcd ($C_{11}H_{13}ClN_4$): C, 55.82; H, 5.54; N, 23.67. Found: C, 55.65; H, 5.63; N, 23.39.

4.2.2. 1-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-3-methyl-1*H*-indazole (3b)

A white solid (0.23 g, 33%), mp = 141–144 °C; ^{1}H NMR ($CDCl_3$): δ 2.58 (s, 3H, CH_3), 3.62 (s, 4H, CH_2), 4.75 (br s, 1H, NH), 5.20 (s, 2H, CH_2), 7.14–7.21 (m, 1H, CH), 7.37–7.49 (m, 2H, CH), 7.64–7.69 (m, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 12.4, 47.5, 50.3, 109.6, 120.9, 124.1, 127.5, 141.3, 143.5, 164.0; ν_{max} 3146, 2926, 2859, 1620, 1499, 1274, 1176, 919, 750 cm^{-1}; MS (ESI) *m*/*z*: 215 [M+H]$^+$. Dihydrochloride of **3b**; mp = 210 °C dec.; Anal. Calcd ($C_{12}H_{16}Cl_2N_4$): C, 50.19; H, 5.62; N, 19.51. Found: C, 50.33; H, 5.81; N, 19.76.

4.2.3. 1-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-4-methyl-1*H*-indazole (3c)

A white solid (0.22 g, 31%), mp = 133–135 °C; ^{1}H NMR ($CDCl_3$): δ 2.60 (s, 3H, CH_3), 3.57 (s, 4H, CH_2), 4.94 (br s, 1H, NH), 5.20 (s, 2H, CH_2), 6.93–6.97 (m, 1H, CH), 7.30–7.32 (m, 2H, CH), 8.05 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 19.0, 47.9, 50.2, 107.1, 121.7, 125.1, 127.7, 132.1, 133.7, 140.4, 163.8; ν_{max} 3180, 2952, 2881, 2794, 1633, 1506, 1439, 1295, 1237, 1154, 978, 847, 784, 771 cm^{-1}; MS (ESI) *m*/*z*: 215 [M+H]$^+$. Hydrochloride of **3c**; mp = 234–238 °C; Anal. Calcd ($C_{12}H_{15}ClN_4$): C, 57.48; H, 6.03; N, 22.35. Found: C, 57.17; H, 6.22; N, 22.29.

4.2.4. 4-Chloro-1-((4,5-dihydro-1*H*-imidazol-2-yl)methyl)-1*H*-indazole (3d)

A white solid (0.24 g, 31%), mp = 103–109 °C; ^{1}H NMR ($CDCl_3$): δ 3.61 (s, 4H, CH_2), 4.92 (br s, 1H, NH), 5.24 (s, 2H, CH_2), 7.18 (d, *J* = 8.0 Hz, 1H, CH), 7.35 (dd, *J* = 8.0 Hz, *J* = 8.0 Hz, 1H, CH), 7.45 (d, *J* = 8.0 Hz, 1H, CH), 8.14 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 47.8, 49.9, 108.2, 121.2, 123.7, 127.0, 128.0, 133.4, 141.1, 163.1; ν_{max} 3212, 2960, 2940, 2866, 1613, 1496, 1355, 1281, 1163, 982, 776 cm^{-1}; MS (ESI) *m*/*z*: 235 [M+H]$^+$. Hydrochloride of **3d**; mp = 225–231 °C; Anal. Calcd ($C_{11}H_{12}Cl_2N_4$): C, 48.74; H, 4.46; N, 20.66. Found: C, 48.58; H, 4.71; N, 20.61.

4.2.5. 1-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-4-methoxy-1*H*-indazole (3e)

A white solid (0.20 g, 26%), mp = 94–97 °C; ^{1}H NMR ($CDCl_3$): δ 3.50 (br s, 1H, NH), 3.57 (s, 4H, CH_2), 3.96 (s, 3H, OCH_3), 5.24 (s, 2H, CH_2), 6.49 (d, *J* = 7.7 Hz, 1H, CH), 7.07 (d, *J* = 7.7 Hz, 1H, CH), 7.32 (t, *J* = 7.7 Hz, 1H, CH), 8.10 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 48.0, 50.36, 55.9, 100.5, 102.4, 116.6, 128.9, 132.8, 142.2, 154.3, 163.6; ν_{max} 3475, 3113, 2940, 2871, 1625, 1601, 1513, 1392, 1268, 1055, 986, 772 cm^{-1}; MS (ESI) *m*/*z*: 231 [M+H]$^+$. Dihydrochloride of **3e**; mp = 96–99 °C; Anal. Calcd ($C_{12}H_{16}Cl_2N_4O$): C, 47.54; H, 5.32; N, 18.48. Found: C, 47.80; H, 5.41; N, 18.74.

4.2.6. 1-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-5-methyl-1*H*-indazole (3f)

A white solid (0.19 g, 27%), mp = 135–140 °C; ^{1}H NMR ($CDCl_3$): δ 2.45 (s, 3H, CH_3), 3.57 (s, 4H, CH_2), 5.02 (br s, 1H, NH), 5.18 (s, 2H, CH_2), 7.24 (d, *J* = 8.4 Hz, 1H, CH), 7.40 (d, *J* = 8.4 Hz, 1H, CH), 7.49 (s, 1H, CH), 7.94 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 21.7, 47.8, 50.2, 109.4, 120.6, 125.0, 129.6, 131.2, 134.2, 139.1, 163.9; ν_{max} 3106, 3073, 2928, 2868, 2795, 1615, 1509, 1293, 1240, 1175, 1142, 985, 825, 790, 578 cm^{-1}; MS (ESI) *m*/*z*: 215 [M+H]$^+$. Dihydrochloride of **3f**; mp = 100–103 °C; Anal. Calcd ($C_{12}H_{16}Cl_2N_4$): C, 50.19; H, 5.62; N, 19.51. Found: C, 50.26; H, 5.91; N, 19.62.

4.2.7. 1-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-7-methyl-1*H*-indazole (3g)

A white solid (0.22 g, 31%), mp = 143–147 °C; ^{1}H NMR ($CDCl_3$): δ 2.77 (s, 3H, CH_3), 3.57 (s, 4H, CH_2), 4.47 (br s, 1H, NH), 5.40 (s, 2H, CH_2), 7.02–7.15 (m, 2H, CH), 7.57 (d, 1H, CH), 8.01 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 19.9, 50.2, 50.4, 119.5, 121.1, 122.0, 125.4, 129.4, 135.2, 139.9, 164.7; ν_{max} 3094, 2933, 2870, 2786, 1623, 1502, 1426, 1408, 1292, 1238, 986, 833, 778, 749 cm^{-1}; MS (ESI) *m*/*z*: 215 [M+H]$^+$. Hydrochloride of **3g**; mp = 248–255 °C; Anal. Calcd ($C_{12}H_{15}ClN_4$): C, 57.48; H, 6.03; N, 22.35. Found: C, 57.39; H, 6.24; N, 22.33.

4.2.8. 7-Chloro-1-((4,5-dihydro-1*H*-imidazol-2-yl)methyl)-1*H*-indazole (3h)

A white solid (0.26 g, 34%), mp = 159–163 °C; ^{1}H NMR ($CDCl_3$): δ 3.59 (s, 4H, CH_2), 4.90 (br s, 1H, NH), 5.60 (s, 2H, CH_2), 7.09 (t, *J* = 7.6 Hz, 1H, CH), 7.38 (d, 7.6 Hz, 1H, CH), 7.64 (d, 7.6 Hz, 1H, CH), 8.07 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 44.1, 44.7, 111.1, 115.1, 117.1, 121.9, 123.2, 129.9, 131.2, 159.0; ν_{max} 3156, 3104, 2932, 2872, 2799, 1620, 1503, 1236, 1133, 963, 839, 773, 733 cm^{-1}; MS (ESI) *m*/*z*: 235 [M+H]$^+$. Hydrochloride of **3h**; mp = 242–250 °C; Anal. Calcd ($C_{11}H_{11}ClN_4$): C, 56.30; H, 4.72; N, 23.87. Found: C, 56.02; H, 4.99; N, 23.61.

4.2.9. 7-Fluoro-1-((4,5-dihydro-1*H*-imidazol-2-yl)methyl)-1*H*-indazole (3i)

A white solid (0.39 g, 54%), mp = 171–173 °C; ^{1}H NMR ($CDCl_3$): δ 3.57 (s, 4H, CH_2), 5.01 (br s, 1H, NH), 5.33 (s, 2H, CH_2), 7.00–7.09 (m, 2H, CH), 7.43–7.52 (m, 1H, CH), 8.03 (d, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 49.7 (d, J = 4.8 Hz), 50.1, 112.3 (d, J = 16.9 Hz), 117.4 (d, J = 4.5 Hz), 122.2 (d, J = 5.5 Hz), 128.5 (d, J = 3.9 Hz), 129.9 (d, J = 12.9 Hz), 135.5, 140.9 (d, J = 247.2 Hz), 164.0; ν_{max} 3143, 3102, 2937, 2866, 2794, 1621, 1583, 1501, 1434, 1412, 1316, 1248, 1236, 1174, 866, 836, 783, 733 cm^{-1}; MS (ESI) m/z: 219 [M+H]$^+$. Hydrochloride of **3i**; mp = 244–248 °C; Anal. Calcd ($C_{11}H_{11}FN_4$): C, 60.54; H, 5.08; N, 25.67. Found: C, 60.46; H, 5.21; N, 25.51.

4.2.10. 1-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-1*H*-benzimidazole (5a)

A white solid (0.50 g, 76%), mp = 197–201 °C; ^{1}H NMR ($CDCl_3$): δ 3.60 (br s, 1H, NH), 3.63 (s, 4H, CH_2), 4.99 (s, 2H, CH_2), 7.31–7.37 (m, 2H, CH), 7.47–7.49 (m, 1H, CH), 7.80–7.81 (m, 1H, CH), 7.88–7.89 (m, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 44.0, 50.4, 109.9, 120.8, 123.0, 123.9, 134.0, 143.3, 144.0, 162.3; ν_{max} 3066, 2930, 2870, 2803, 1616, 1516, 1498, 1459, 1423, 1367, 1247, 1206, 1193, 1175, 988, 766, 753, 740 cm^{-1}; MS (ESI) m/z: 201 [M+H]$^+$. Dihydrochloride of **5a**; mp = 285–288 °C; Anal. Calcd ($C_{11}H_{14}Cl_2N_4$): C, 48.37; H, 5.17; N, 20.51. Found: C, 48.21; H, 5.34; N, 20.37.

4.2.11. 1-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-2-methyl-1*H*-benzimidazole (5b)

A white solid (0.57 g, 81%), mp = 151–156 °C; ^{1}H NMR ($CDCl_3$): δ 2.59 (s, 3H, CH_3), 3.60 (br s, 1H, NH), 3.63 (s, 4H, CH_2), 4.92 (s, 2H, CH_2), 7.23–7.38 (m, 3H, CH), 7.66–7.70 (m, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 14.3, 43.0, 50.5, 109.3, 119.9, 123.0, 123.2, 135.5, 143.1, 152.3, 162.8; ν_{max} 3091, 2928, 2870, 1613, 1515, 1467, 1450, 1407, 1289, 1244, 1160, 1037, 768, 752, 741 cm^{-1}; MS (ESI) m/z: 215 [M+H]$^+$. Dihydrochloride of **5b**; mp = 173–178 °C; Anal. Calcd ($C_{12}H_{16}Cl_2N_4$): C, 50.19; H, 5.62; N, 19.51. Found: C, 50.03; H, 5.88; N, 19.45.

4.2.12. 1-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-2-ethyl-1*H*-benzimidazole (5c)

A white solid (0.59 g, 78%), mp = 162–168 °C; ^{1}H NMR ($CDCl_3$): δ 1.33 (t, J = 7.5 Hz, 3H, CH_3), 2.78 (q, J = 7.5 Hz, 2H, CH_2), 3.59 (s, 4H, CH_2), 4.46 (br s, 1H, NH), 4.89 (s, 2H, CH_2), 7.21–7.36 (m, 3H, CH), 7.66–7.70 (m, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 11.9, 21.2, 42.6, 50.4, 109.4, 119.9, 122.9, 123.2, 135.6, 143.0, 156.7, 163.0; ν_{max} 3084, 2975, 2935, 2868, 1613, 1518, 1465, 1415, 1243, 1206, 985, 743 cm^{-1}; MS (ESI) m/z: 229 [M+H]$^+$. Dihydrochloride of **5c**; mp = 254–259 °C; Anal. Calcd ($C_{13}H_{18}Cl_2N_4$): C, 51.84; H, 6.02; N, 18.60. Found: C, 51.70; H, 6.32; N, 18.51.

4.2.13. 1-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-2-phenyl-1*H*-benzimidazole (5d)

A white solid (0.63 g, 69%), mp = 151–155 °C; ^{1}H NMR (DMSO-d_6): δ 3.45 (s, 4H, CH_2), 4.84 (s, 2H, CH_2), 6.69 (s, 1H, CH), 7.22–7.33 (m, 2H, CH), 7.45–7.60 (m, 4H, CH), 7.64–7.75 (m, 1H, CH), 7.85–7.91 (s, 2H, CH); ^{13}C NMR (DMSO-d_6): δ 40.1, 43.0, 111.1, 119.3, 122.3, 122.7, 128.9, 129.5, 130.0, 130.4, 136.6, 142.8, 153.5, 163.4; ν_{max} 3067, 2929, 2859, 1607, 1463, 1443, 1392, 1237, 974, 745, 692 cm^{-1}; MS (ESI) m/z: 277 [M+H]$^+$. Dihydrochloride of **5d**; mp = 171–175 °C; Anal. Calcd ($C_{17}H_{18}Cl_2N_4$): C, 58.46; H, 5.19; N, 16.04. Found: C, 58.52; H, 5.33; N, 15.82.

4.2.14. 2-Benzyl-1-((4,5-dihydro-1*H*-imidazol-2-yl)methyl)-1*H*-benzimidazole (5e)

A white solid (0.70 g, 73%), mp = 147–152 °C; ^{1}H NMR ($CDCl_3$): δ 3.42 (s, 4H, CH_2), 3.61 (br s, 1H, NH), 4.33 (s, 2H, CH_2), 4.85 (s, 2H, CH_2), 7.20–7.36 (m, 7H, CH), 7.38–7.40 (m, 1H, CH), 7.79–7.81 (m, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 34.8, 43.1, 50.3, 109.9, 120.2, 123.1, 123.6, 127.7, 129.2, 129.4, 135.9, 136.4, 143.0, 153.7, 162.5; ν_{max} 3219, 3031, 2939, 2869, 1618, 1506, 1466, 1411, 1234, 1165, 765, 746, 711 cm^{-1}; MS (ESI) m/z: 291 [M+H]$^+$. Dihydrochloride of **5e**; mp = 158–163 °C; Anal. Calcd ($C_{18}H_{20}Cl_2N_4$): C, 59.51; H, 5.55; N, 15.42. Found: C, 59.71; H, 5.68; N, 15.79.

4.2.15. 1-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-4-methyl-1*H*-benzimidazole (5f)

A white solid (0.30 g, 42%), mp = 152–159 °C; ^{1}H NMR ($CDCl_3$): δ 2.58 (s, 3H, CH_3), 3.62 (s, 4H, CH_2), 5.01 (s, 2H, CH_2), 5.10 (br s, 1H, NH), 7.06–7.10 (m, 1H, CH), 7.18–7.30 (m, 2H, CH), 7.91 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 16.8, 43.9, 50.1, 107.4, 123.3, 123.8, 130.6, 133.6, 142.5, 143.1, 162.7; ν_{max} 3075, 2937, 2867, 1620, 1498, 1360, 1243, 986, 762 cm^{-1}; MS (ESI) m/z: 215 [M+H]$^+$. Dihydrochloride of **5f**; mp = 235–240 °C; Anal. Calcd ($C_{12}H_{16}Cl_2N_4$): C, 50.19; H, 5.62; N, 19.51. Found: C, 50.11; H, 5.82; N, 19.66.

4.2.16. 1-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-5,6-dimethyl-1*H*-benzimidazole (5g)

A white solid (0.63 g, 83%), mp = 180–185 °C; ^{1}H NMR ($CDCl_3$): δ 2.36 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 3.62 (s, 4H, CH_2), 4.28 (br s, 1H, NH), 4.82 (s, 2H, CH_2), 7.21 (s, 1H, CH), 7.50 (s, 1H, CH), 7.72 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 20.7, 21.0, 44.2, 50.5, 110.2, 120.9, 132.2, 132.7, 133.4, 142.7, 163.0; ν_{max} 3082, 2930, 2864, 1617, 1498, 1239, 1222, 986, 981 cm^{-1}; MS (ESI) m/z: 229 [M+H]$^+$. Dihydrochloride of **5g**; mp = 219–223 °C; Anal. Calcd ($C_{13}H_{18}Cl_2N_4$): C, 51.84; H, 6.02; N, 18.60. Found: C, 51.73; H, 6.13; N, 18.56.

4.2.17. 1-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-2-ethyl-5,6-dimethyl-1*H*-benzimidazole (5h)

A white solid (0.68 g, 80%), mp = 185–188 °C; ^{1}H NMR ($CDCl_3$): δ 1.42 (t, J = 7.8 Hz, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.86 (q, J = 7.8 Hz, 2H, CH_2), 3.60 (br s, 4H, CH_2), 4.35 (br s, 1H, NH), 4.88 (s, 2H, CH_2), 7.12 (s, 1H, CH), 7.49 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 11.9, 20.5, 20.8, 21.0, 42.5, 109.3, 119.9, 131.4, 132.0, 133.8, 141.4, 155.6, 163.0; ν_{max} 3082, 2930, 2864, 1617, 1498, 1239, 1222, 986, 981 cm^{-1}; MS MS (ESI) m/z: 257 [M+H]$^+$. Dihydrochloride of **5h**; mp = 252–260 °C; Anal. Calcd ($C_{15}H_{22}Cl_2N_4$): C, 54.72; H, 6.73; N, 17.02. Found: C, 54.48; H, 6.95; N, 17.01.

4.2.18. 1-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-1*H*-benzotriazole (7a)

A white solid (0.23 g, 35%), mp = 180–183 °C; ^{1}H NMR ($CDCl_3$): δ 3.62 (s, 4H, CH_2), 4.86 (br s, 1H, NH), 5.48 (s, 2H, CH_2), 7.35–7.44 (m, 1H, CH), 7.49–7.57 (m, 1H, CH), 7.65–7.69 (m, 1H, CH), 8.02–8.07 (m, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 46.8, 50.24, 110.2, 120.5, 124.9, 128.6, 133.6, 146.5, 161.8; ν_{max} 3067, 2936, 2863, 2797, 1618, 1509, 1456, 1415, 1240, 1161, 1101, 983, 786, 745 cm^{-1}; MS (ESI) m/z: 202 [M+H]$^+$. Hydrochloride of **7a**; mp = 255–258 °C; Anal. Calcd ($C_{10}H_{12}ClN_5$): C, 50.53; H, 5.09; N, 29.46. Found: C, 50.47; H, 5.23; N, 29.13.

4.2.19. 1-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-5,6-dimethyl-1*H*-benzotriazole (7b)

A white solid (0.26 g, 34%), mp = 154–157 °C; ^{1}H NMR ($CDCl_3$): δ 2.37 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 3.59 (s, 4H, CH_2), 4.63 (br s, 1H, NH), 7.36 (s, 1H, CH), 7.72 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 20.4, 20.9, 46.4, 50.0, 108.9, 118.9, 132.1, 134.2, 138.5, 145.2, 161.4; ν_{max} 3260, 2952, 2871, 1613, 1499, 1454, 1284, 1226, 1104, 977, 850 cm^{-1}; MS (ESI) m/z: 230 [M+H]$^+$. Dihydrochloride of **7b**; mp = 249–251 °C; Anal. Calcd ($C_{12}H_{17}Cl_2N_5$): C, 47.69; H, 5.67; N, 23.17. Found: C, 47.55; H, 5.72; N, 23.16.

4.2.20. 2-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-4-methyl-2*H*-benzotriazole (8a)

A white solid (0.07 g, 10%), mp = 118–120 °C; ^{1}H NMR ($CDCl_3$): δ 2.67 (s, 3H, CH_3), 3.67 (s, 4H, CH_2), 4.75 (br s, 1H, NH), 5.58 (s, 2H, CH_2), 7.14–7.18 (m, 1H, CH), 7.28–7.36 (s, 1H, CH), 7.68–7.72 (m, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 17.8, 50.4, 54.2, 117.6, 128.3, 128.5, 130.2, 141.4, 144.1, 161.9; ν_{max} 3160, 2945, 2869, 1620, 1503, 1276, 1239, 981 cm^{-1}; MS (ESI) *m*/*z*: 216 [M+H]$^+$. Dihydrochloride of **8a**; mp = 205–210 °C; Anal. Calcd ($C_{11}H_{15}Cl_2N_5$): C, 45.85; H, 5.25; N, 24.30. Found: C, 45.94; H, 5.41; N, 24.51.

4.2.21. 2-((4,5-Dihydro-1*H*-imidazol-2-yl)methyl)-5,6-dimethyl-2*H*-benzotriazole (8b)

A white solid (0.17 g, 22%), mp = 167–170 °C; ^{1}H NMR ($CDCl_3$): δ 2.40 (s, 6H, CH_3), 3.66 (s, 4H, CH_2), 4.20 (br s, 1H, NH), 5.57 (s, 2H, CH_2), 7.59 (s, 2H, CH); ^{13}C NMR ($CDCl_3$): δ 21.4, 50.5, 54.8, 117.0, 137.9, 144.7, 161.7; ν_{max} 3201, 2949, 2866, 1631, 1553, 1504, 1365, 1273, 1236, 1170, 997, 852 cm^{-1}; MS (ESI) *m*/*z*: 230 [M+H]$^+$. Dihydrochloride of **8a**; mp = 231–235 °C; Anal. Calcd ($C_{12}H_{17}Cl_2N_5$): C, 47.69; H, 5.67; N, 23.17. Found: C, 47.76; H, 5.83; N, 23.14.

4.3. Radioligand binding assays

4.3.1. I_1-Binding site assay

Kidneys were obtained *post mortem* from male Sprague–Dawley rats (250–280 g) and crude P_2 membranes were prepared according to the methods of Lione et al.[29] Binding of [^{3}H]clonidine (3 nM, Perkin–Elmer) was investigated in the presence of 10 μM rauwolscine to preclude radioligand binding to α_2-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.[30] Membrane aliquots (400 μL, 0.2–0.5 mg protein) were incubated with 11 concentrations of the test compounds over the range 0.01–100 μM. Incubations were carried out in 50 mM Tris–HCl buffer (pH 7.4) at room temperature for 45 min. Bound radioligand and free radioactivity were separated by rapid filtration through pre-soaked (0.5% polyethyleneimine) glass-fibre filters (Whatman GFB). Trapped radioligand was determined by liquid scintillation counting and the data were 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).

4.3.2. α_1-, α_2- and I_2-Binding assays

Brains were obtained *post mortem* from male Sprague–Dawley rats (250–280 g) and crude P_2 membranes were prepared.[29] 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–100 μM in the presence of the selective I_2 binding site radioligand [^{3}H]2BFI (2-(2-benzofuranyl)-2-imidazoline)[30] (1 nM), the α_1-adrenoceptor antagonist radioligand [^{3}H]prazosin (1 nM) or the α_2-adrenoceptor antagonist radioligand [^{3}H]RX821002 (2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1*H*-imidazole)[31] (1 nM) in a final volume of 500 μL. Non-specific binding was determined using 10 μM BU224 (2-(4,5-dihydroimidazol-2-yl)quinoline)[32] for I_2 binding, 10 μM phenylephrine for α_1-adrenoceptors and 10 μM rauwolscine to define α_2-adrenoceptor binding. Incubations were performed in triplicate at room temperature and were allowed to reach equilibrium (45 min). Bound and free radioactivity were separated by rapid filtration through pre-soaked (0.5% polyethyleneimine) glass–fibre filters (Whatman GF/B). Filters were then washed twice with 5 mL of ice-cold buffer and membrane-bound radioactivity remaining on the filters was determined by liquid scintillation counting. The data were 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.[33] The resulting values are given as means of three or four separate experiments except where compounds demonstrated low affinity and were tested in a singular experiment as indicated.

4.4. Estimation of agonist potency and efficacy

4.4.1. Cell culture and transfections

Adherent Chinese hamster ovary (CHO) cells (K1 strain) (American Type Culture Collection, Manassas, VA, USA), stably expressing cDNAs encoding the human α_{2A}-adrenoceptor subtype were produced previously by Pohjanoksa et al. using the expression vector pMAMneo (Clontech, Palo Alto, CA, USA) that contains a neomycin analogue G418 (Geneticin®, Calbiochem, San Diego, CA, USA) resistance gene.[35] Cells were cultured in α-minimum essential medium (GIBCO™, Invitrogen, Carlsbad, CA, USA) supplemented with 26 mM $NaHCO_3$, 5% heat-inactivated foetal bovine serum, penicillin (50 units/mL), streptomycin (50 μg/mL) and 200 μg/mL G418. Before the functional [^{35}S]GTPγS binding assays, the cultured cells were tested for their capacity to bind the α_2-adrenoceptor antagonist radioligand [^{3}H]RX821002 (2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1*H*-imidazole). Thereafter, confluent cells were harvested into chilled phosphate-buffered saline, pelleted and frozen at −70 °C.

4.4.2. Membrane preparation

All procedures were performed on ice. CHO cell pellets were thawed and suspended in hypotonic lysis buffer (10 mM Tris–HCl, 0.1 mM EDTA, 0.32 mM sucrose, pH 7.4) and homogenised using an Ultra-Turrax homogeniser (3 × 10 s at 800 rpm). The homogenate was centrifuged at 23,000 g for 30 min, and the pellet was re-homogenised and again centrifuged at 23,000 g for 30 min. The membrane pellet was suspended in hypotonic lysis buffer and stored at −70 °C until used. Protein concentrations were determined with the method of Bradford[12] using bovine serum albumin as reference.

4.4.3. [^{35}S]GTPγS binding assay

Agonist-induced stimulation of [^{35}S]GTPγS binding was measured essentially as described previously.[11] Briefly, membranes were thawed and diluted with binding buffer (25 mM Tris, 1 mM EDTA, 5 mM $MgCl_2$, 20 mM NaCl, 1 μM GDP, 1 mM DTT, 30 μM ascorbic acid, pH 7.4). Incubations were performed on 96-well Millipore MultiScreen MSFBN glass-fibre filter plates (Millipore Corp., Bedford, MA, USA). Samples containing 5 μg of membrane protein were incubated with 8–7 serial dilutions of the test compounds and 0.1 nM [^{35}S]GTPγS. Reactions were terminated after 30 min incubation at RT by rapid vacuum filtration using a Millipore MultiScreen Vacuum Manifold. The filter plates were washed three times with ice-cold wash buffer (20 mM Tris, 1 mM EDTA, 5 mM $MgCl_2$, pH 7.4). Filters were dried and 50 μl SuperMix scintillation cocktail was added into each well. The incorporated radioactivity was measured using a Wallac 1450 Betaplate scintillation counter (PerkinElmer Wallac, Turku, Finland). All experiments were performed in duplicate and repeated at least three times. Analysis of the results with GraphPad Prism software yielded estimates of agonist potency (EC_{50}) and efficacy (intrinsic activity in comparison to the natural full agonist noradrenaline).

4.5. In vivo studies: mean arterial blood pressure (MAP) and heart rate (HR) in rats

Male Wistar rats, weighing 200–290 g, were purchased from the Animal House of the Medical University of Gdańsk, Poland. All in vivo experiments were approved by the Local Ethical Committee

on Animal Experiments. The animals were fed a commercial rodent chow (Labofeed–B, Poland). Tap water was available ad libitum. Rats were anaesthetized by i.p. injection of thiopental (Sandoz, Austria) at a 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 a rate of 1.2 mL/h. After 40 min of saline infusion, the tested compound (**3h**) was administered as a 100 μL bolus through the venous catheter at a dose of 0.1 mg/kg. The time of administration of the compound was assumed as ''time 0''. The *i.v*. infusion of methoxamine, a potent agonist of α_1-adrenoceptors, at a dose 10 or 20 μg/kg/min was started 10 min before administration of **3h**. Arterial blood pressure and heart rate were monitored directly and sampled continuously at 100 Hz, as described previously,[13] using Biopac Systems, Inc., Model MP 100 (Goleta, CA, USA). The results of recordings were elaborated with the help of the ACQKnowledge (Goleta, CA, USA) analysis system and were selected, scaled and filtered to remove signal disturbances. The recorded time domain transient data are presented as graphs with the help of Excel (Microsoft, USA).

ANOVA of 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.[13] This allowed direct comparisons of responses to treatments between the groups. Data were analysed with ANOVA for repeated measurements, using Statistica StatSoft software (StatSoft, Inc., Tulsa, USA). When a treatment effect was significant, *post hoc* comparisons were performed using Fisher's test. A value of $p < 0.05$ was considered statistically significant.

4.6. In vivo loss of righting reflex (LORR) test

The compound **3h** was solubilised in sterile saline for intra-peritoneal (i.p.) injection at the following initial doses, (i) 5 μg/kg; (ii) 50 μg/kg and (iii) 500 μg/kg. This dose range was based on previous studies on the sedative-hypnotic effects of α_2-adrenoceptor agonists in rats.[34]

One day prior to testing, the animals were familiarised with the experimenter in order to minimise handling stress during the experiment. During testing, adult male Sprague–Dawley rats weighing 250–270 g received a single i.p. injection and were observed for LORR. If, after 5 min, no LORR was observed, the dosing was repeated, up to a maximum cumulative dose of 2000 μg/kg. If LORR was not achieved, a standard neurological assessment (including motor coordination, reflexes and arousal) was conducted to probe for evidence of hypnosis or mild sedation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.11.025. These data include MOL files and InChiKeys of the most important compounds described in this article.

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