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Fluorinated analogues of marsanidine, a highly α_2-AR / imidazoline I_1 binding site - selective hypotensive agent. Synthesis and biological activities

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Abstract

The aim of these studies was to establish the influence of fluorination of the indazole ring on the pharmacological properties of two selective α_2-adrenoceptor (α_2-AR) agonists: 1-[(imidazolidin-2-yl)imino]-1*H*-indazole (marsanidine, **A**) and its methylene analogue 1-[(4,5-dihydro-1*H*-imidazol-2-yl)methyl]-1*H*-indazole (**B**). Introduction of fluorine into the indazole ring of **A** and **B** reduced both binding affinity and α_2-AR/I_1 imidazoline binding site selectivity. The most α_2-AR-selective ligands were 6-fluoro-1-[(imidazolidin-2-yl)imino]-1*H*-indazole (**6c**) and 7-fluoro-1-[(imidazolidin-2-yl)imino]-1*H*-indazole (**6d**). The in vivo cardiovascular properties of fluorinated derivatives of **A** and **B** revealed that in both cases the C-7 fluorination leads to compounds with the highest hypotensive and bradycardic activities. The α_2-AR partial agonist **6c** was prepared as a potential lead compound for development of a radiotracer for PET imaging of brain α_2-ARs.

Keywords: marsanidine, indazole, α_2-adrenoceptor, Selectfluor

1. Introduction

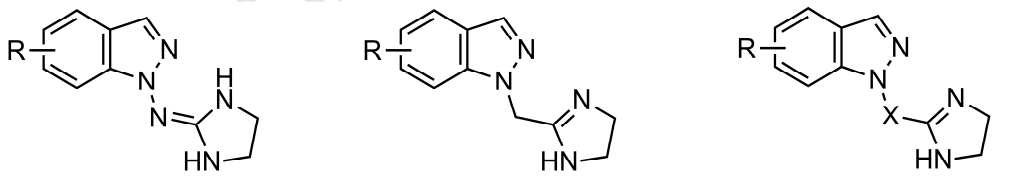
α_2-Adrenoceptors (α_2-ARs) represent a subfamily of G-protein coupled receptors mediating many of the central and peripheral actions of the endogenous catecholamines norepinephrine and epinephrine. In humans and other mammals, they are present as three subtypes, α_{2A}-, α_{2B}- and α_{2C}-ARs [1], with α_{2A} responsible for most of the hitherto recognized physiological functions of α_2-ARs, which include regulation of sympathetic nervous system activity, arterial blood pressure, insulin secretion, gastrointestinal motility, body temperature and seizure threshold [2–6]. α_2-ARs are also involved in different aspects of central nervous system functioning. They regulate noradrenergic neurotransmission via two mechanisms: somato-dendritic autoreceptors inhibit the firing of noradrenergic neurons in the locus coeruleus and in other noradrenergic brain nuclei, and activation of presynaptic α_2-autoreceptors inhibits norepinephrine release from nerve endings as part of a negative feedback loop [2,7]. The pharmacological effects of activation of central α_2-heteroreceptors

include sedation, anxiolysis, analgesia and anaesthetic-sparing activity [8]. α_2-ARs also contribute to the modulation of a number of mental functions, among others mood, cognition and behaviour [2,5,9–14]. α_2-AR functions may be disturbed in many neurological and psychiatric disorders such as Alzheimer's disease [15–19], depression [20,21], long-term stress responses [22–24] and anxiety disorders [25,26], where they also represent potential therapeutic drug targets. It is thus considered important to try to develop approaches to investigate brain α_2-ARs in vivo, in order to enable investigation and diagnostics of and drug development for these conditions.

There are numerous reports on the alteration of pharmacological properties of biologically active compounds on fluorination that could be ascribed to changes in pKa [27–29], lipophilicity [30], conformation [31,32], chemical/metabolic stability [33–39] or propensity to participate in intermolecular interactions [40–43]. According to the results obtained by Kirk et al. [44], fluorine substitution of the aromatic ring of adrenergic agonists may have marked and site-of-fluorination-dependent influences on their receptor affinity and selectivity. Therefore, it seemed reasonable to expand our previous SAR studies of 1-[(imidazolidin-2-yl)imino]-1*H*-indazoles **A** (**Fig. 1**) [45–47] and 1-[(4,5-dihydro-1*H*-imidazol-2-yl)methyl]-1*H*-indazoles **B** (**Fig. 1**) [48], α_2-AR ligands, by including fluorine-substituted derivatives **6a–e** and **8a–c** (**Fig. 1**).

Positron emission tomography (PET) is a molecular imaging technique that offers the potential to reveal distribution and concentration of protein targets (receptors, transporters, enzymes) in living organisms by the use of specific ligands [49,50] labelled with short-lived positron emitting radionuclides, most commonly ^{11}C or ^{18}F [51,52]. Imaging of brain receptors with PET is dependent on the availability of selective high-affinity ligands that are amenable for labelling with a positron emitter and demonstrate good permeability of the blood-brain barrier. For example, the possibility of imaging of α_2-ARs with PET has been extensively investigated in recent years and several α_2-AR ligands have been labelled with positron emitters and evaluated in animals and humans [53–62]. Promising results were obtained for [^{11}C]yohimbine [61] and [^{11}C]MPTQ ([N-methyl-^{11}C] 5-methyl-3-[4-(3-phenylallyl)-piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroisoxazolo[4,3-c]quinolone) [62].

In view of the above information our next goal was to develop a fluorination method suitable for ^{18}F labelling of compounds **A** or **B**.



A
R= H (marsanidine), alkyl, alkoxyl, Cl

B
R= H, alkyl, alkoxyl, Cl

6a-e X= NH, R= F
8a-d X= CH, R= F

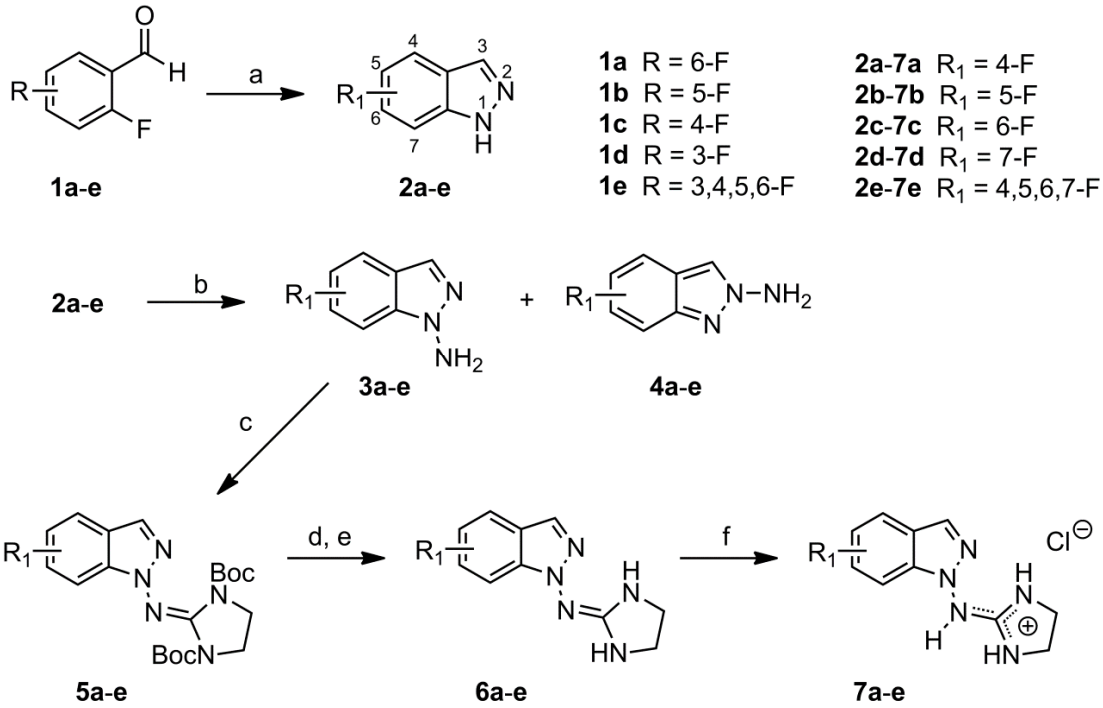
Fig. 1. Structures of marsanidine and its analogues.

2. Results and Discussion

2.1 Chemistry

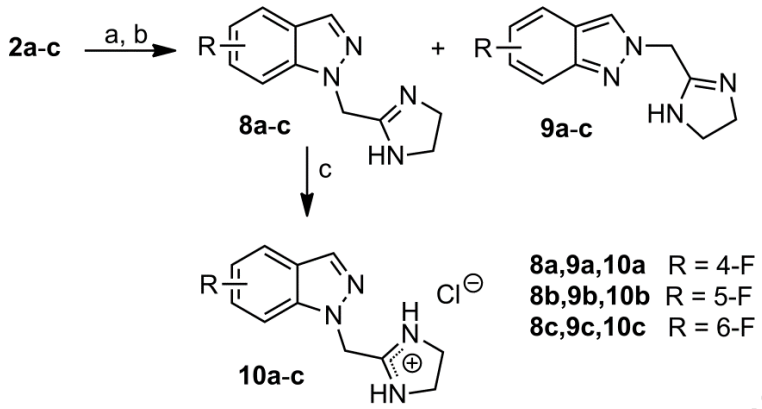
The fluorinated derivatives of marsanidine (**6a–e**) were obtained according to the procedure previously described [45] and depicted in **Scheme 1**. Condensation of 2-fluorobenzaldehydes **1a–e**

with hydrazine furnished the respective fluoro-1*H*-indazoles **2a–e** [63]. The concurrent 1- and 2-amination of the latter was accomplished by the use of hydroxylamine-*O*-sulfonic acid (HOSA). The isolated 1-amino-1*H*-indazoles (**3a–e**) were then reacted with *N*,*N*′-bis-Boc-imidazolidine-2-thione [64] in the presence of $HgCl_2$ to obtain bis-Boc-protected compounds **5a–e**. Deprotection of the latter with TFA/CH_2Cl_2 afforded the target fluoro-substituted marsanidine analogues **6a–e.**



Scheme 1. Synthesis of fluorinated derivatives of marsanidine. Reagents and conditions: a) $NH_2NH_2{\cdot}H_2O$, Δ, 12–48h, 20–40 % yield for **2a–d** or $NH_2NH_2{\cdot}H_2O$/THF, 0 °C then 70 °C, 48 h, 13 % yield for **2e**; b) HOSA (2.6 molar equiv.), 6 % NaOH/H_2O, EtOH, 55 °C, 20 min., then room temperature 1.5 h, 20–40 % yield for **3a–e**; c) *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., room temperature 5–7 days, 20–70 % yield for **5a–e**; d) 50 % TFA/CH_2Cl_2, room temperature, 2 h; e) 10 % NaOH/H_2O, 60–88 % yield for **6a–e**; f) 2.6 M HCl/Et_2O.

The synthesis of monofluoro-substituted derivatives of **B** was accomplished according to **Scheme 2** [48]. The indazole sodium salts, generated upon treatment of the corresponding indazoles with sodium hydride in THF, were reacted with the preformed 2-chloromethylimidazoline [65]. From the resulting mixture of 1- and 2-alkylated indazoles compounds **8a–c** were isolated in 25 to 40 % yield.



Scheme 2. Synthesis of fluorinated derivatives **8a–c** of 1-((4,5-dihydro-1*H*-imidazol-2-yl)methyl)-1*H*-indazole. Reagents and conditions: a) NaH, THF; b) 2-chloromethylimidazoline, 25–40 % yield for **8a–c**; c) 2.5 M HCl/Et_2O.

For biological studies, compounds **6a–e** and **8a–c** were converted into hydrochloride salts **7a–e** and **10a–c**, respectively, with the use of ethereal solution of hydrochloride. Structures of all newly prepared compounds were confirmed by C, H, N elemental analyses as well as IR and NMR spectroscopic data.

2.2 Pharmacological evaluation

2.2.1. Radioligand binding assays

In vitro radioligand binding assays involved the investigation of the affinity and selectivity of the newly prepared compounds **6a–e** and **8a–c** for α_1- and α_2-ARs as well as imidazoline I_1 and I_2 binding sites. A summary of all these properties together with the data obtained for previously reported marsanidine [45,46], **B** and **8d** [48] are displayed in **Table 1**. All of the monofluoro-substituted marsanidine derivatives **6a–d** and analogues **8a–d** were bound with high to moderate affinity to α_2-ARs. In both series of compounds, fluorination at C-4 proved to be detrimental for α_2-AR binding, as **8a** (K_i = 187.3 nM) and **6a** (K_i = 416 nM) displayed 10- to 30-fold higher K_i values than **B** (K_i = 18.5 nM) and marsanidine (K_i = 14.0 nM). On the contrary, substitution at C-6 or C-7 yielded compounds with the highest activity at α_2-AR in both series. Given the above results, the inactivity of the perfluorinated **6e** at α_2-AR was rather surprising.

Apart from being high to moderate affinity α_2-AR ligands, compounds **8a–c** displayed high affinity for I_2 imidazoline binding sites with K_i values ranging from 8.193 to 21.87 nM. This property may be ascribed to fluorine substitution of the indazole moiety since **B** is inactive at I_2 binding sites (K_i = 42 200 nM).

In comparison to the parent marsanidine, compounds **6a–d** displayed definitely lower I_1/α_2 selectivity, with K_i ratios ranging from 1.4 to 789; **6b** had nearly similar affinity for I_1 binding sites and α_2-ARs. Worth noting is also their moderate (**6b–d**) or low (**6a**) selectivity for α_2- versus α_1-ARs. The highest affinity for α_2-ARs, close to that of marsanidine, was observed for compounds **6c** and **6d** (K_i = 26.2 and 31.0 nM). These two compounds also displayed the highest overall selectivity within the **6a–d** and **8a–d** series. Their α_1/α_2, I_1/α_2 and I_2/α_2 selectivity ratios all exceeded 35 (α_1/α_2 for **6c**) and ranged up to 11 263 (I_2/α_2 for **6d**).

Table 1.
Binding affinity data for marsanidine, **B**, **6a–e** and **8a–d**

Compd	α_1 K_i (nM)[a,c]	α_2 K_i (nM)[a,c]	I_1 IC_{50} (nM)[b,c]	I_2 K_i (nM)[a,c]
marsanidine[d]	nd[f]	14.05 ± 2.7	54550 ± 16730	16900 ± 5900
6a	3807 ± 1342	416.0 ± 16.2	14776 ± 7893	6177 ± 4962
6b	2320 ± 293	64.33 ± 4.39	91.65 ± 16.54	5703 ± 2656
6c	918.3 ± 52.6	26.20 ± 6.30	20675 ± 20513	21967 ± 3735
6d	1625 ± 475.2	30.97 ± 1.93	7740 ± 5522	348833 ± 306639
6e	19793 ± 18003	3717 ± 220.2	5827 ± 3402	22733 ± 817.2
B[e]	272 ± 42	18.5 ± 5.9	19400 (n=1)[g]	42200 (n=1)[g]
8a	534.7 ± 118.4	187.3 ± 40.26	1765 ± 1200	21.87 ± 4.703
8b	476.3 ± 267.5	32.17 ± 9.60	2419 ± 2057	10.38 ± 5.44
8c	518.3 ± 159.9	29.73 ± 3.04	33456 ± 24023	8.193 ± 3.43
8d[e]	107 (n=1)[g]	12.6 (n=1)[g]	16400 (n=1)[g]	156 (n=1)[g]

[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 tested compounds to compete with [^{3}H]prazosin, [^{3}H]RX821002 or [^{3}H]2BFI binding to rat brain membranes. [b] Molar concentration of the tested compounds that displaces 50% of specifically bound [^{3}H]clonidine in rat kidney membranes in the presence of rauwolscine (I_1 imidazoline binding sites). [c] Values given are means ± s.e.m. from 3–4 independent experiments. [d] The results have been published earlier in Sączewski F. et al. [45]. [e] The results have been published earlier in Sączewski J. et al. [48]. [f] nd: not determined. [g] n: number of experiments.

The most α_2-AR-selective ligands **6c** and **6d** were further evaluated for their affinity for the different human α_2-AR subtypes (**Table 2**). In this analysis, **6c** and **6d** displayed selectivity towards α_{2A}- versus α_{2C}-ARs and were slightly more active at α_{2A}- than at α_{2B}-ARs. Since α_{2A}-ARs are the most abundant in the brain [3,66–71], while the density of α_{2B}-ARs in the brain is too small to be detected with PET [62], one can expect that compounds **6c** and **6d** could be suitable ligands for selective α_{2A}-AR imaging.

Table 2.

Binding affinities of **6c** and **6d** obtained with [^{3}H]RS-79948-197 to human α_2-AR subtypes expressed in CHO cell membranes. Results expressed as K_i (nM) and their 95 % confidence intervals of three independent experiments.

	Human		
Compd	α_{2A}-AR	α_{2B}-AR	α_{2C}-AR
marsanidine	52 (39–70)	79 (63–99)	640 (520–780)***
6c	33 (27–41)	72 (54–92)**	600 (510–710)***
6d	65 (54–77)	158 (130–190)**	1200 (960–1400)**

Statistical significance of differences from the α_{2A}-AR is shown by symbols: **P<0.01; ***P<0.001 tested with unpaired t-tests.

2.2.2. In vivo cardiovascular effects

The potential pharmacological effects of centrally acting α_2-AR agonists include hypotension and bradycardia [2–6]. To determine the influence of fluorination of the indazole ring on the cardiovascular properties of compounds **A** and **B** the high-affinity α_2-AR ligands **6b–d**, **8b** and **8c** were subjected to in vivo studies in anesthetized rats after intravenous infusion of the tested compounds using previously described procedures [72]. Changes in mean arterial blood pressure (MAP) and heart rate (HR) were measured and compared to baseline values. The results are expressed as ΔMAP and ΔHR and are presented in **Table 3**.

The previously reported [45,46,48] SAR analysis concerning marsanidine and its methylene analogue **B** indicated that substitution at the C-7 atom of the indazole nucleus with chlorine or methyl group resulted in compounds with high cardiovascular activity, exceeding that of the congeners. The same rule applied to C-7 fluorination as compounds **6d** and **8d** [48] displayed the largest hemodynamic effects in the respective **6b**–**6d** and **8b**–**8d** series. It is worth noting that hypotensive effects of **6d** administered at a dose of 0.1 mg/kg were larger than those of 7-chloro- (ΔMAP = –49.6 mmHg, unpublished data) and 7-methylmarsanidine (ΔMAP = –43.5 mmHg [45]). On the other hand, **8d** (ΔMAP = – 29.5 mmHg) reduced arterial blood pressure to a lesser extent than its 7-chloro- (ΔMAP = –47.7 mmHg [48]) and 7-methyl- (ΔMAP = –49.7 mmHg, unpublished data) analogues.

Comparison of **6c** with **6d** or **8c** with **8d** reveals marked differences in cardiovascular properties that are in contradiction with the similar receptor affinity profiles. Another rule that can be seen in the **6b**–**6d** and **8b**–**8d** series of compounds is that C-6-substituted derivatives are more active than C-5-substituted ones. Indeed, **6b** was associated with relatively small MAP responses even though it showed moderately high affinity for α_2-ARs and I_1 imidazoline binding sites both of which are involved in central blood pressure regulation [73,74]. On the other hand, **8b** had no effects on MAP and HR, which is exceptional among the compounds studied.

Table 3.

Effects of compounds **6b**–**d** and **8b**–**d** at 0.1 mg/kg i.v. on mean arterial blood pressure (MAP) and heart rate (HR) in anesthetized rats.

compd	$+\Delta MAP_{max}$ [a,d] (t_{max}) [f]	$-\Delta MAP_{max}$ [b,d] (t_{max}) [f]	$-\Delta HR_{max}$ [c,d] (t_{max}) [f]	n [e]
6b	14.6 ± 5.9 (2) *%	–8.6 ± 2.3 (11) $!@	–61 ± 7 (5) *§	4
6c	24.8 ± 4.6 (3) *§	–36.0 ± 2.3 (35) *!	–118 ± 15 (2) *§^&	4
6d	21.1 ± 2.2 (4) *§	–59.4 ± 5.7 (33) *$@	–128 ± 9 (10) *§^&	4
8b	0.8 ± 1.0 (2)	–7.2 ± 1.6 (40) $!@	–12 ± 3 (38) ^&	4
8c	23.6 ± 3.6 (4) *§	–12.7 ± 1.8 (36) #$!@	–52 ± 9 (5) *§	5
8d [g]	17.7 ± 7.1 (1) *§	–29.5 ± 4.0 (34) *$!	–88 ± 21 (21) *§^	5
control [h]	0.4 ± 0.7	–2.7 ± 1.2 $!@	–9 ± 2 ^&	5

[a] The maximal hypertensive effect [mmHg] of a compound observed during 60 minutes after injection. [b] The maximal hypotensive effect [mmHg] of a compound observed during 60 minutes after injection. [c] The maximal bradycardic effect [bpm] of a compound observed during 60 minutes after injection. [d] Values given are means ± s.e.m from n independent experiments. [e] Number of experiments. [f] t_{max}: time (in minutes) after the injection when the maximal effect was observed. [g] The results have been published earlier in Sączewski J. et al. [48]. [h] Saline vehicle injection. (*) p <0.001, (#) p <0.05 vs control. (&) p <0.001 vs **6b**. ($) p<0.001 vs **6c**. (!) p <0.001 vs **6d**. (§) p <0.001, (%) p <0.05 vs **8b**. (^) p <0.001 vs **8c**. (@) p <0.001 vs **8d**.

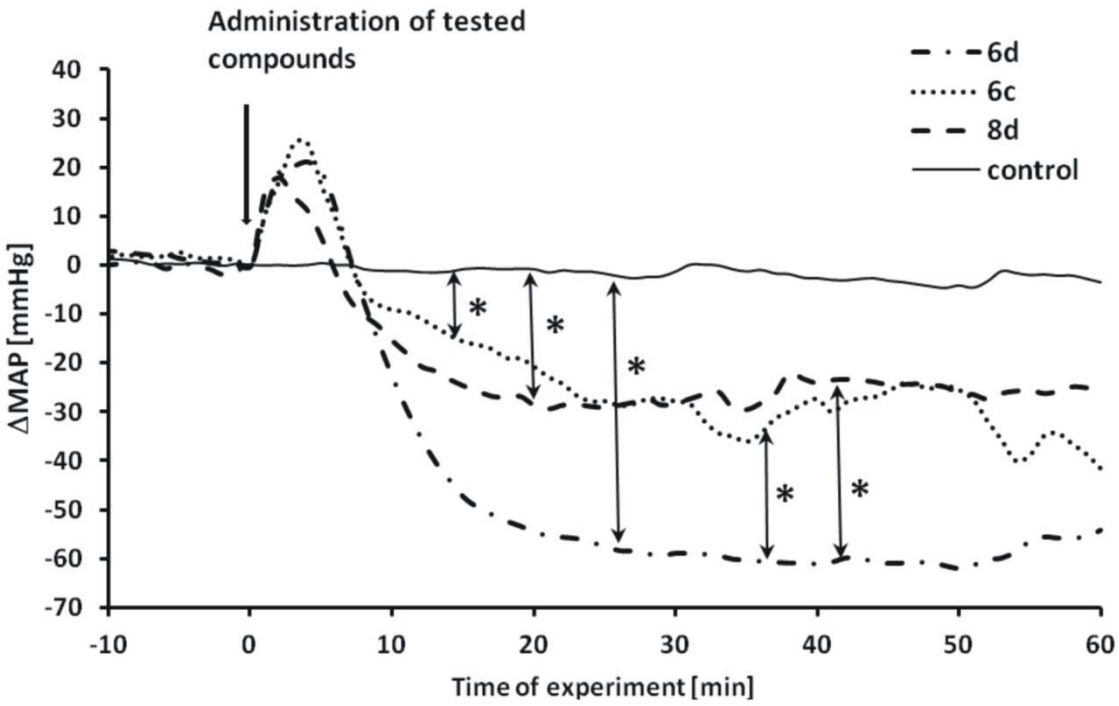


Fig. 2. Effects of 0.1 mg/kg of **6c**, **6d** and **8d** on MAP (ΔMAP calculated as the difference of MAP between sequential measurements and time 0 of the experiment) in anesthetized rats. The lines represent the mean values of ΔMAP for four or five animals. Comparisons were made using ANOVA with repeated measures and Fisher and Duncan tests. Significant differences (*) $p < 0.001$ were found for comparisons **6c** versus control, **6d** versus control, **8d** versus control, **6c** versus **6d** and **6d** versus **8d**.

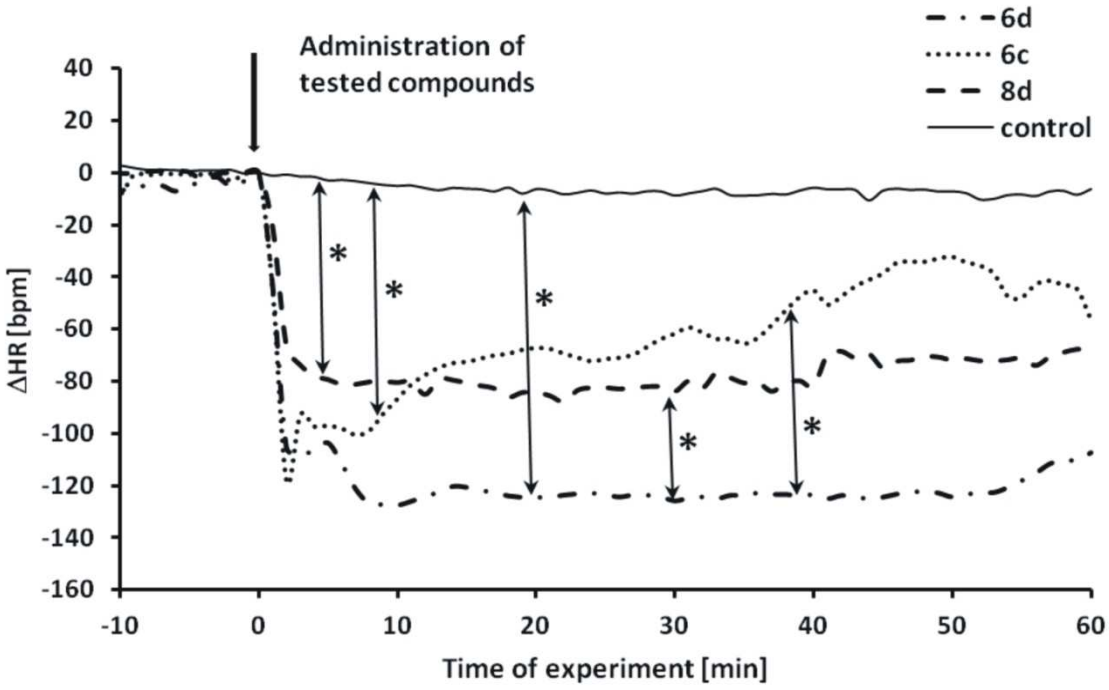


Fig. 3. Effects of 0.1 mg/kg of **6c**, **6d** and **8d** on HR (ΔHR calculated as the difference of HR between sequential measurements and time 0 of the experiment) in anesthetized rats. The lines represent mean values of ΔHR for four or five animals. Comparisons were made using ANOVA with repeated measures and Fisher and Duncan

tests. Significant differences (*) p <0.001 were found for comparisons **6c** versus control, **6d** versus control, **8d** versus control, **6c** versus **6d** and **6d** versus **8d**.

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

To explain the marked difference in the hemodynamic effects of compounds **6c** and **6d** that disagreed with their similar receptor affinity profiles, [^{35}S]GTPγS binding experiments were conducted to compare their agonist potency and intrinsic activity at recombinant human α_{2A}-ARs [75]. The intrinsic activity was measured relative to the full agonist norepinephrine. The results presented in **Table 4** indicate that, similarly to marsanidine, compounds **6c** and **6d** are only relatively weak partial agonists of human α_{2A}-ARs. It is however of note that **6d** is markedly more efficacious than **6c** which may at least in part account for its greater cardiovascular activity.

Table 4

Characterization of [^{35}S]GTPγS binding to CHO cell membranes expressing recombinant human α_{2A}-ARs: estimates of agonist potency (EC_{50}) and intrinsic activity relative to the natural full agonist norepinephrine. Values shown are means ± s.e.m. from 3 independent experiments for compounds **6c, 6d** and marsanidine and 19 independent experiments for norepinephrine.

Compound	**pEC_{50}**	**Intrinsic activity** (% of norepinephrine)
Norepinephrine	6.89 ± 0.06	100
Marsanidine[a]	7.03 ± 0.29	14 ± 2
6c	7.41 ± 0.23	5.2 ± 2.9
6d	6.70 ± 0.33	18 ± 4

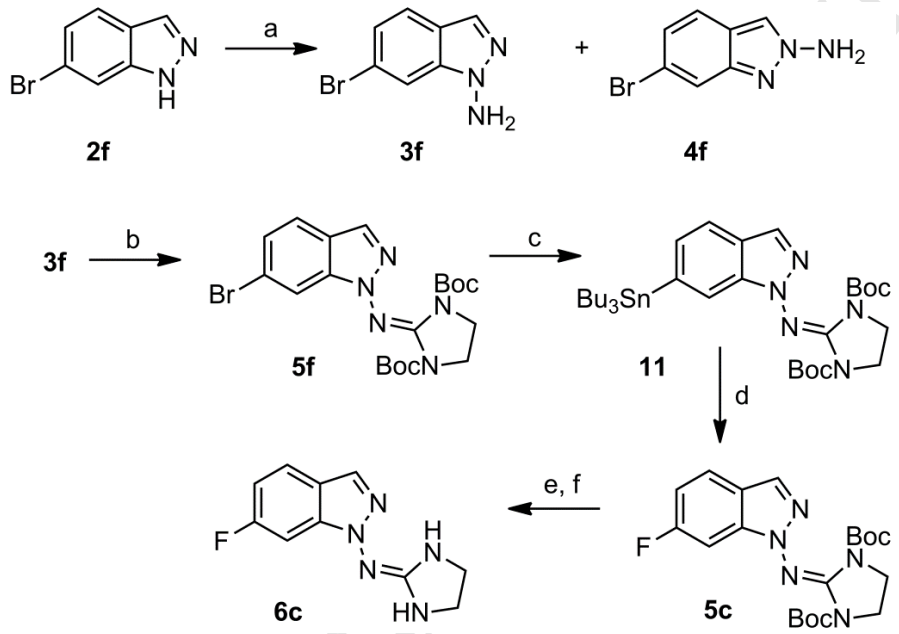
[a] The results have been published earlier in Sączewski F. et al. [46].

2.3. Procedure for late stage fluorination

A candidate for PET radiotracer development should be amenable to labelling with a positron emitter. Given the short half-lives of β^+-emitting radionuclides [76] their introduction into the target molecules should take place as late as possible in the course of the synthesis.

Fluorination of aromatic rings can be accomplished with aromatic nucleophilic substitution (SN_{AR}) [77–79]. This approach, though in many cases successful, requires aromatic moieties that carry good leaving groups and are suitably activated by the presence of electron-withdrawing substituents. The electron-rich indazole ring seems a poor substrate for SN_{AR}. The scarce reports [80–84] on SN_{AR} within the benzene portion of an indazole moiety concern molecules that have one or two nitro substituents functioning as activating or both activating and leaving groups. We therefore turned our attention to electrophilic fluorination methods and decided to explore the utility of site-selective fluorodestannylation reported by Ritter et al. [85] that uses Selectfluor as a fluorinating agent. The method seemed attractive since it yields the products in 20 minutes under mild conditions and is applicable to a wide scope of aromatic and heteroaromatic substrates. The potential usefulness of this method for ^{18}F-labelling was pioneered by Teare et al. [86] who developed the procedure for [^{18}F]Selectfluor radiosynthesis (using high specific activity [^{18}F]F_2) and demonstrated its usefulness for fluorodemetalation of variously substituted phenylstannanes [86,87]. Fluorination of 2-methyl-1-trimethylsilyloxytetral-1-ene with [^{18}F]Selectfluor yielded 2-[^{18}F]fluoro-2-methyl tetralone exhibiting specific activity of 20 GBq/μmol [86]. Based on the above, we have designed and tested the effectiveness of a potential procedure (**Scheme 3**) for the late stage fluorination of marsanidine leading to **6c**. In the first step we obtained 1-{[1,3-di(*tert*-butoxycarbonyl)imidazolidin-2-yl]imino}-6-

bromo-1*H*-indazole (**5f**) as a substrate for aryl stannane **11** synthesis according to published procedures [85]. The latter was treated with Selectfluor in the presence of silver triflate using conditions described by Ritter et al. [85]. The reaction, conducted for 20 minutes, yielded the desired **5c** together with 1-{[1,3-di(*tert*-butoxycarbonyl)imidazolidin-2-yl]imino}-1*H*-indazole (**12**) - a hydrodestannylation product - that could not be separated from **5c** by preparative thin layer chromatography. Formation of **12**-like side-products has been reported by Ritter et al. [85]. Then, however, deprotection of **5c** contaminated with **12** gave a mixture of marsanidine and **6c** that was separable by flash column chromatography. The desired compound **6c** was obtained from **11** with 16 % overall yield.



Scheme 3. The late stage fluorination leading to **6c**. Reagents and conditions: a) HOSA (2.6 molar equiv.), 6% $NaOH/H_2O$, EtOH, 55 °C, 20 min., then room temperature, 2 h, 46 % yield; b) *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., room temperature 5 days, 56 % yield; c) Sn_2Bu_6, $Pd[P(C_6H_5)_3]_4$, toluene, 100 °C, 24 h, 70 % yield; d) AgOTf (2 molar equiv.), Selectfluor (1.2 molar equiv.), acetone, room temperature, 20 min., 42 % yield; e) neat TFA, room temperature, 40 min.; f) 10 % $NaOH/H_2O$, 53 % yield.

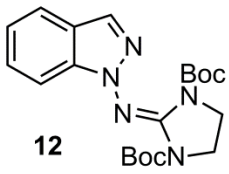


Fig. 4. The structure of **12**.

3. Conclusions

This study aimed to determine the influence of fluorination of the indazole nucleus on the pharmacological properties of **A** and **B**. Accordingly, the fluorinated compounds **6a–e** and **8a–c** were synthesized and evaluated for their affinity and selectivity towards α_1- and α_2-ARs as well as imidazoline I_1 and I_2 binding sites. In the case of compounds **6a–d**, decreased α_2-AR affinity as well as α_2/I_1 selectivity were observed.The results obtained for **8a–d** indicated that on fluorination the α_2-

AR-selectivity of **B** was lost due to markedly enhanced affinity for I_2 binding sites. From all of the compounds studied, **6c** and **6d** displayed the highest selectivity towards α_2-ARs. The in vivo cardiovascular effects of the high-affinity α_2-ARs ligands **6b–d**, **8b** and **8c** confirmed our previous observation that C-7 substitution of the indazole ring leads to congeners with relatively high hypotensive and bradycardic activity.

In the synthetic part of our studies, we have established the utility of silver-mediated fluorination of aryl stannanes [85] for the two-step preparation of **6c** from **11**. These results revealed that the reaction conditions and the chemical properties of Selectfluor [88,89] were tolerated by the iminoimidazolidine moiety. The present study thus widens the scope of the relatively new fluorination procedure developed by Ritter et al. [85].

4. Experimental section

4.1. Chemistry

Melting points were measured on a Boetius apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer FT-IR 1600 spectrometer. NMR spectra were recorded on a Varian Gemini 200 or a Varian Unity 500 apparatus. ^{1}H and ^{13}C NMR chemical shifts were measured relative to residual solvent signal at 7.26 ppm and 77.0 ppm ($CDCl_3$) or 2.50 ppm and 39.5 ppm (DMSO-d_6). Preparative thin layer chromatography was performed on silica gel 60 PF_{254} containing gypsum (Merck) with aid of Chromatotron® using the reported solvent systems. 5-Fluoro- and 6-bromo-1*H*-indazole were purchased from Sigma-Aldrich and used as obtained. *N,N'*-bis(*tert*-butoxycarbonyl)imidazolidine-2-thion [64], 2-(chloromethyl)-4,5-dihydro-1*H*-imidazole [65], 4-fluoro-1*H*-indazole [63] and 4,5,6,7-tetrafluoro-1*H*-indazole [90] were obtained according to published methods.

*4.1.1. General procedure for the synthesis of fluoro-1H-indazoles **2c**, **2d***

The title compounds were obtained by modifying a method described by Lukin et al. [63]. To the properly substituted 2-fluorobenzaldehyde (5 g, 35 mmol) was added hydrazine hydrate (12.9 g, 258 mmol, 12.5 ml). After stirring for 12–48 hours (TLC control) at reflux water (20 ml) was added and the resulting mixture was cooled to 5 °C. The precipitate was collected by vacuum filtration. The filtrate was extracted with dichloromethane and the residue after evaporation was combined with the precipitate.

*4.1.1.1. 6-Fluoro-1H-indazole (**2c**).* The combined solids were extracted with diethyl ether. The ethereal extracts were evaporated to dryness and the resulting yellow solid was purified by flash column chromatography (silica gel) eluting with dichloromethane and then chloroform. Yield: 1 g (20 %); mp 131–132 °C (lit. [91] 126 °C); IR (KBr, cm^{-1}) 3195, 3162, 3071, 3016, 2945, 2863, 1632, 1358, 1145, 947, 849; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.97 (dt, J_1=8.8 Hz, J_2=2.2 Hz, 1H), 7.15–7.20 (m, 1H), 7.72 (dd, J_1=8.8 Hz, J_2=5.2 Hz, 1H), 8.11 (1H, s), 10.98 (1H, s); ^{13}C NMR (50 MHz, $CDCl_3$) δ 95.8 (d, *J*=26 Hz), 111.8 (d, *J*=26 Hz), 120.2, 122.5 (d, *J*=11 Hz), 134.8, 140.5 (d, *J*=11 Hz); 162.7 (d, *J*=243.9 Hz, CF); Anal. calcd. for $C_7H_5FN_2$ (136.13): C, 61.76; H, 3.70; N, 20.58. Found: C, 61.94; H, 3.63; N, 20.79.

*4.1.1.2. 7-Fluoro-1H-indazole (**2d**).* The combined solids were purified by flash column chromatography (silica gel) eluting with petroleum ether/acetone/ethyl acetate (100:4:4). Yield: 1.95 g (41 %); mp 124–125 °C (lit. [91] 120 °C); IR (KBr, cm^{-1}) 3193, 3145, 3018, 2960, 2854, 1589, 1522,

1357, 1241, 1059, 727; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.06–7.16 (m, 2H), 7.55–7.59 (m, 1H), 8.25 (d, *J*=3.3 Hz, 1H), 11.97 (br s); ^{13}C NMR (50 MHz, $CDCl_3$) δ 110.8 (d, *J*=15.7 Hz), 116.6 (d, *J*=4.4 Hz), 121.4 (d, *J*=5.2 Hz), 126.8, 130.0 (d, *J*=15.2 Hz), 134.9, 148.3 (d, *J*=248.0 Hz, CF). Anal. calcd. for $C_7H_5FN_2$ (136.13): C, 61.76; H, 3.70; N, 20.58. Found: C, 61.44; H, 3.93; N, 20.47.

4.1.2. General procedure for the preparation of 1-aminofluoro-1H-indazoles ***3a–e*** *and 1-amino-6-bromo-1H-indazole* ***3f***

The title compounds were obtained from the appropriate indazoles (10 mmol) using hydroxylamine-*O*-sulfonic acid (2.94 g, 26 mmol) in aqueous NaOH solution (2.2 g, 55 mmol in 34 ml of H_2O) and EtOH (9.6 ml) according to the procedure described by Adger et al. [92] To the aqueous-alcoholic solution of NaOH indazole was added and the resulting mixture was heated to 55 °C. HOSA was added in portions to keep the temperature at 55–57 °C. The reaction mixture was left to cool down to room temperature and then kept at this temperature for 1.5 h. The precipitate (1-amino-1*H*-indazole alone or its mixture with 2-amino-2*H*-indazole) was collected by vacuum filtration and if necessary subjected to flash column chromatography (silica gel, 1-amino-1*H*-indazole was eluted first) or recrystallized. The filtrate was extracted with dichloromethane and the residue after evaporation (the mixture of 1- and 2-aminoindazole and unreacted indazole) was separated by flash column chromatography (silica gel, 1-amino-1*H*-indazole was eluted first).

*4.1.2.1. 1-Amino-4-fluoro-1H-indazole (**3a**).* Eluted with CH_2Cl_2/petroleum ether (1:1). Yield 0.61 g (40 %); IR (KBr, cm^{-1}) 3326, 3210, 3093, 3057, 1654, 1577, 1514, 1385, 1225, 1000, 774, 683; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.26 (s, 2H), 6.71–6.81 (m, 1H), 7.24–7.37 (m, 2H), 7.92 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 105.2 (d, *J*=18.4 Hz), 106.0 (d, *J*=4.1 Hz), 112.6 (d, *J*=20 Hz), 127.3, 127.7 (d, *J*=7.6 Hz), 142.2 (d, *J*=8.5 Hz), 156.0 (d, *J*=252.4 Hz, CF). Anal. calcd. for $C_7H_6FN_3$ (151.14): C, 55.63; H, 4.00; N, 27.80. Found: C, 55.44; H, 3.92; N, 27.67.

*4.1.2.2. 1-Amino-5-fluoro-1H-idazole (**3b**).* Eluted with CH_2Cl_2/petroleum ether (2:1→5:1). Yield 0.68 g (45 %); IR (KBr, cm^{-1}) 3325, 3205, 1516, 1381, 1223, 856, 800, 575; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.36 (s, 2H), 7.17 (td, J_1=9.0 Hz, J_2=2.5 Hz, 1H), 7.24–7.30 (m, 1H), 7.53 (dd, J_1=9.0 Hz, J_2=4.2 Hz, 1H), 7.81 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 104.7 (d, *J*=24 Hz), 111.2 (d, *J*=9.6 Hz), 116.7 (d, *J*=26.7 Hz), 122.2 (d, *J*=10.5 Hz), 130.4 (d, *J*=5.7 Hz), 136.5, 158.2 (d, *J*=237 Hz, CF). Anal. calcd. for $C_7H_6FN_3$ (151.14): C, 55.63; H, 4.00; N, 27.80. Found: C, 55.67; H, 4.12; N, 27.74.

*4.1.2.3. 1-Amino-6-fluoro-1H-indazole (**3c**).* Eluted with chloroform. Yield 0.42 (30 %); mp 146–147 °C; IR (KBr, cm^{-1}) 3322, 3208, 1655, 1474, 1215, 848, 620; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.25 (s, 2H), 6.90 (td, J_1=9.0 Hz, J_2=2.0 Hz, 1H), 7.21 (dd, J_1=9.0 Hz, J_2=2.2 Hz, 1H), 7.58 (dd, J_1=9.0 Hz, J_2=5 Hz, 1H), 7.82 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 95.6 (d, *J*=27 Hz), 111.2 (d, *J*=26 Hz), 119.3, 122.3 (d, *J*=10.9 Hz), 131.2, 140 (d, *J*=10 Hz), 162.7 (d, *J*=244.2 Hz, CF). Anal. calcd. for $C_7H_6FN_3$ (151.14): C, 55.63; H, 4.00; N, 27.80. Found: C, 55.85; H, 3.83; N, 27.91.

*4.1.2.4. 1-Amino-7-fluoro-1H-indazole (**3d**).* Eluted with methylene chloride/petroleum ether (1:2→2:1). Yield 0.36 g (25 %); mp 84–86 °C; IR (KBr, cm^{-1}) 3326, 3210, 1581, 1318, 1223, 1180, 842, 731; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.47 (s, 2H), 6.98–7.09 (m, 2H), 7.37–7.43 (m, 1H), 7.84 (d, *J*=1.9 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 112.2 (d, *J*=16.7 Hz), 112.8, 117.1 (d, *J*=4.5 Hz), 121.8 (d, *J*=5.5 Hz), 127.0 (d, *J*=10 Hz), 131.5, 148.7 (d, *J*=249.5, CF). Anal. calcd. for $C_7H_6FN_3$ (151.14): C, 55.63; H, 4.00; N, 27.80. Found: C, 55.91; H, 4.13; N, 27.98.

*4.1.2.5. 1-Amino-4,5,6,7-tetrafluoro-1H-indazole (**3e**).* Eluted with petroleum ether/ethyl acetate (10:1→5:1). Yield 0.48 g (22 %); mp 135–136 °C; IR (KBr, cm^{-1}) 3316, 3206, 1559, 1496, 1355, 1160, 965; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.82 (s, 2H), 8.24 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 109.5 (dd, J_1=21.5, J_2=3.0 Hz), 127.4 (d, J=5.9 Hz), 129.9–130.3 (m), 131.6 (t, J=15.5 Hz), 134.9–135.3 (m), 136.07–136.91 (m), 139.8–140.2 (m), 141.2–141.8 (m). Anal. calcd. for $C_7H_3F_4N_3$ (205.11): C, 40.99; H, 1.47; N, 20.49. Found: C, 40.86; H, 1.62; N, 20.55.

*4.1.2.6. 1-Amino-6-bromo-1H-indazole (**3f**).* Obtained by crystallization of the crude product from methanol. Yield 0.99 g (46 %); mp 179–180 °C; IR (KBr, cm^{-1}): 3321, 1647, 1610, 1357, 1034, 905, 794, 615; ^{1}H NMR (200 MHz, DMSO-d_6) δ 6.48 (s, 2H), 7.23 (dd, J_1=8.3 Hz, J_2=1.7 Hz, 1H), 7.68 (d, J=8.3 Hz, 1H), 7.72 (s, 1H), 7.94 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 112.2, 119.5, 120.7, 122.5, 123.4, 129.6, 139.6; Anal. calcd. for $C_7H_6BrN_3$ (212,05): C, 39.65; H, 2.85; N, 19.82. Found: C, 39.42; H, 2.99; N, 19.71.

*4.1.3. General procedure for the synthesis of 1-{[1,3-di(tert-butoxycarbonyl)imidazolidin-2-yl]imino}-1H-indazoles **5a–f***

The title compounds were obtained according to the method described by F. Sączewski et al. [45]. To a stirred solution of the appropriate 1-amino-1*H*-indazole (0.5 g, 3.3 mmol), *N,N'*-bis(*tert*-butoxycarbonyl)imidazolidine-2-thion (1.5 g, 4.97 mmol) and triethylamine (1.16 g, 1.6 ml, 11.5 mmol) in DMF (5.5 ml) was added $HgCl_2$ (1.32 g, 4.97 mmol) at 0 °C. The reaction mixture was stirred for 20 min. at 0 °C and then for 5 days at room temperature. The resulting dark-grey/black reaction mixture was diluted with ethyl acetate (30 ml) and filtered through a celite pad. The filtrates were washed with brine (3×) and water (1×), dried over $MgSO_4$ and concentrated under vacuum. The viscous residue was separated through preparative thin layer chromatography. The unreacted *N,N'*-bis(*tert*-butoxycarbonyl)imidazolidine-2-thion was eluted first followed by the product.

*4.1.3.1. 4-Fluoro-1-{[1,3-di(tert-butoxycarbonyl)imidazolidin-2-yl]imino}-1H-indazole (**5a**)*. Eluted with petroleum ether/ethyl acetate (9:1→4:1). Yield 0.74 g (53 %); mp 118–119 °C; IR (KBr, cm^{-1}) 3008, 2976, 2933, 2892, 1724, 1711, 1653, 1629, 1368, 1314, 1147, 995, 781; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.05 (s, 9H), 1.58 (s, 9H), 3.81–4.02 (m, 4H), 6.74 (dd, J_1=7.7 Hz, J_2=9.2 Hz, 1H), 7.20–7.30 (m, 1H), 7.42 (dd, J_1=9.2 Hz, J_2=1.3 Hz, 1H), 7.91 (d, J=1 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 27.5, 28.4, 43.5, 44.1, 83.2, 83.4, 105.2 (d, J=18.4 Hz), 106.9 (d, J=4 Hz), 112.8 (d, J=24 Hz), 126.1, 127.5 (d, J=7.5 Hz), 140.1 (d, J=10 Hz), 143.9, 150.1, 150.3, 155.6 (d, J=251.4). Anal. calcd. for $C_{20}H_{26}FN_5O_4$ (419.45): C, 57.27; H, 6.25; N, 16.70. Found: C, 57.17; H, 6.54; N, 16.63.

*4.1.3.2. 5-Fluoro-1-{[1,3-di(tert-butoxycarbonyl)imidazolidin-2-yl]imino}-1H-indazole (**5b**).* Eluted with petroleum ether followed by petroleum ether/ethyl acetate (9:1→7:1). Yield 0.5 g (36 %); mp 157–159 °C; IR (KBr, cm^{-1}) 2979, 2901, 1728, 1693, 1655, 1324, 1155, 1051, 848, 581; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.96 (s, 9H), 1.53 (s, 9H), 3.84 (br s, 4H), 7.29–7.33 (m, 1H), 7.46–7.56 (m, 2H), 7.94–7.96 (m, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 26.8, 27.7, 43.1, 43.8, 81.9, 82.0, 104.9 (d, J=24 Hz), 111.6 (d, J=10 Hz), 116.0 (d, J=28 Hz), 121.6 (d, J=11 Hz), 129.2 (d, J=5.5 Hz), 133.6, 144.0, 149.3, 149.5, 157.5 (d, J=240 Hz). Anal. calcd. for $C_{20}H_{26}FN_5O_4$ (419.45): C, 57.27; H, 6.25; N, 16.70. Found: C, 57.04; H, 7.34; N, 16.58.

*4.1.3.3. 6-Fluoro-1-{[1,3-di(tert-butoxycarbonyl)imidazolidin-2-yl]imino}-1H-indazole (**5c**).* Eluted with petroleum ether/ethyl acetate (8:1→4:1). Yield 1 g (70 %); mp 139–141 °C; IR (KBr, cm^{-1}) 3088, 2986,

2896, 1728, 1702, 1658, 1625, 1331, 1161, 1029, 952; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.96 (s, 9H), 1.52 (s, 9H), 3.83 (br s, 4H), 6.97–7.14 (m, 2H), 7.75–7.83 (m, 1H), 8.00 (d, *J*=1.2 Hz, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 26.9, 27.8, 43.2, 43.8, 81.9, 82.0, 94.9 (d, *J*=26.1 Hz), 110.4 (d, *J*=26.1 Hz), 118.8, 122.8 (d, *J*=10.9 Hz), 129.8, 136.5 (d, *J*=13.5 Hz), 144.1, 149.3, 149.5, 161.4 (d, *J*=242.4 Hz) Anal. calcd. for $C_{20}H_{26}FN_5O_4$ (419.45): C, 57.27; H, 6.25; N, 16.70. Found: C, 57.61; H, 6.43; N, 16.77.

*4.1.3.4. 7-Fluoro-1-{[1,3-di(tert-butoxycarbonyl)imidazolidin-2-yl]imino}-1H-indazole (**5d**).* Eluted with petroleum ether/ethyl acetate (10:1→4:1). Yield 0.6 g (42 %); mp 118–119 °C; IR (KBr, cm^{-1}) 2984, 1716, 1648, 1309, 1151, 1066, 729; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.01 (s, 9H), 1.56 (s, 9H), 3.80–4.00 (m, 4H), 6.94–7.04 (m, 2H), 7.36–7.41 (m, 1H), 7.85 (d, *J*=2.1 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 27.4, 28.3, 43.4, 44.1, 83.3, 83.5, 111.5 (d, *J*=17 Hz), 116.3 (d, *J*=5 Hz), 121.4 (d, *J*=6 Hz), 126.6 (d, *J*=4 Hz), 130.0, 144.6, 149.2 (d, *J*=252 Hz), 150.18, 150.2. Anal. calcd. for $C_{20}H_{26}FN_5O_4$ (419.45): C, 57.27; H, 6.25; N, 16.70. Found: C, 57.39; H, 6.03; N, 16.95.

*4.1.3.5. 4,5,6,7-Tetrafluoro-1-{[1,3-di(tert-butoxycarbonyl)imidazolidin-2-yl]imino}-1H-indazole (**5e**).* Eluted with petroleum ether/ethyl acetate (10:1→1:1). Yield: 0.047 g from 0.1 g of the substrate (20 %); mp 156–158 °C, IR (KBr, cm^{-1}) 2981, 2925, 1753, 1625, 1552, 1493, 1300, 1152, 946; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.52 (s, 18H), 3.73 (s, 4H), 7.96 (d, *J*=1.7 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.2, 39.8, 43.5, 44.03, 83.5, 149.1 (C=N), 150.6 (C=O) The other signals could not be detected due to their high multiplicity and thus low intensity. Anal. calcd. for $C_{20}H_{23}F_4N_5O_4$ (473.42): C, 50.74; H, 4.90; N, 14.79. Found: C, 50.36; H, 5.11; N, 14.66.

*4.1.3.6. 6-Bromo-1-{[1,3-di(tert-butoxycarbonyl)imidazolidin-2-yl]imino}-1H-indazole (**5f**).* Eluted with petroleum ether/ethyl acetate (20:1→5:1) and then crystallized from methanol. Yield 0.625 g (56 %); mp 133–135 °C; IR (KBr, cm^{-1}) 3108, 2983, 1740, 1709, 1302, 1154, 984, 763; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.99 (s, 9H), 1.53 (s, 9H), 3.84 (br s, 4H), 7.27 (dd, J_1=4.8 Hz, J_2=1.7 Hz, 1H), 7.61 (br s, 1H), 7.72 (d, *J*=8.3 Hz, 1H), 8.01 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 26.9, 27.8, 43.2, 43.8, 81.9, 82.0, 112.2, 119.7, 120.5, 122.7, 123.8, 129.7, 136.9, 144.4, 149.2, 149.4. Anal. calcd. for $C_{20}H_{26}BrN_5O_4$ (480.36): C, 50.01; H, 5.46; N, 14.58. Found: C, 50.20; H, 5.40; N, 14.81.

*4.1.4. General procedure for the synthesis of fluoro-1-[(imidazolidin-2-yl)imino]-1H-indazoles **6a–e** and their hydrochloride salts **7a–e**.*

A mixture of the appropriate fluoro-1-{[1,3-di(*tert*-butoxycarbonyl)imidazolidin-2-yl]imino}-1*H*-indazole (1.36 g, 3.24 mmol.) and 50% trifluoroacetic acid in dichloromethane (13 ml) was stirred for 2 h at room temperature. The mixture was evaporated to dryness and the resulting oily residue was treated with water (5 ml) and then made alkaline to pH≈11 with 10 % aqueous NaOH. The precipitated solid was collected by vacuum filtration, dried and crystallized from acetonitrile.

The free bases were then converted into their hydrochloride salts by adding 1.5 molar equiv. of the ethereal solution of hydrochloride (2.6 M) to the solution of the appropriate fluoro-1-[(imidazolidin-2-yl)imino]-1*H*-indazole in dichloromethane.

*4.1.4.1. 4-Fluoro-1-[(imidazolidin-2-yl)imino]-1H-indazole (**6a**).* Yield: 0.55 g (77 %); mp 204–205 °C; IR (KBr,cm^{-1}) 3227, 3173, 3008, 2875, 2830, 1636, 1622, 1513, 1403, 1288, 999, 778; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.61 (br s, 4H), 5.40 (br s, 1H), 6.40 (br s, 1H), 3.72 (ddd, J_1=10.2 Hz, J_2=7.1 Hz, J_3=1 Hz, , 1H), 7.17–7.34 (m, 2H), 7.92 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 42.4, 43.7, 104.8 (d, *J*=18.4 Hz), 106.7 (d, *J*=4.1 Hz), 124.9, 126.8 (d, *J*=7.7 Hz), 139.3, 155.9 (d, *J*=251.7 Hz), 162.2. Anal. calcd. for $C_{10}H_{10}FN_5$

(219.22): C, 54.79; H, 4.60; N, 31.95; Found: C, 54.61; H, 4.63; N, 32.12. *Hydrochloride* ***7a***. Mp 217–219 °C; IR (KBr, cm^{-1}) 3102, 2903, 2613, 1650, 1514, 1289, 995, 775. Anal. calcd. for $C_{10}H_{11}ClFN_5$ (255.68): C, 46.98; H, 4.34; N, 27.39. Found: C, 46.90; H, 4.16; N, 27.36.

*4.1.4.2. 5-Fluoro-1-[(imidazolidin-2-yl)imino]-1H-indazole (**6b**).* Yield: 0.45 g (63 %); IR (KBr, cm^{-1}) 3344, 3239, 3170, 2967, 2896, 1632, 1500, 1288, 1129, 796; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.59 (s, 4H), 5.50 (br s, 1H), 6.35 (br s, 1H), 7.09 (dt, J_1=8.8 Hz, J_2=2.2 Hz, 1H), 7.26 (dd, J_1=8.8 Hz, J_2=2.2 Hz, 1H), 7.46 (dd, J_1=9.2 Hz, J_2=4.4 Hz, 1H), 7.77 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 42.6 (br s), 104.3 (d, *J*=24 Hz), 111.6 (d, *J*=9.6 Hz), 116.0 (d, *J*=27.7 Hz), 128.3 (d, *J*=6 Hz), 133.8, 158.5 (d, *J*=238 Hz), 162.1. Anal. calcd. for $C_{10}H_{10}FN_5$ (219.22): C, 54.79; H, 4.60; N, 31.95; Found: C, 54.86; H, 4.23; N, 31.86. *Hydrochloride* ***7b***. Mp 215–217 °C; IR (KBr, cm^{-1}) 3118, 2933, 1655, 1626, 1513, 1215. Anal. calcd. for $C_{10}H_{11}ClFN_5$ (255.68): C, 46.98; H, 4.34; N, 27.39. Found: C, 47.12; H, 4.49; N, 27.22.

*4.1.4.3. 6-Fluoro-1-[(imidazolidin-2-yl)imino]-1H-indazole (**6c**).* Yield: 0.52 g (73 %); mp 182–184 °C; IR (KBr, cm^{-1}) 3248, 3162, 2999, 2886, 2808, 1635, 1618, 1473, 1289, 1203, 952, 839. ^{1}H NMR (DMSO-d_6, 200 MHz) δ 3.38 (br s), 6.48 (br s, 1H), 6.68 (br s, 1H), 6.89–7.02 (m, 2H), 7.73 (dd, J_1= 5.1 Hz, J_2= 8.3 Hz, 1H), 7.91 (s, 1H). ^{13}C NMR (DMSO-d_6, 50 MHz) δ 42.6, 43.7, 95.7 (d, *J*=26.3 Hz), 110.5 (d, *J*=26.5 Hz), 119.6, 123.4 (d, *J*=11.1 Hz), 129.2, 136.7, 161.8 (d, *J*=240.8 Hz), 165.1. Anal. calcd. for $C_{10}H_{10}FN_5$ (219.22): C, 54.79; H, 4.60; N, 31.95; Found: C, 54.91; H, 4.66; N, 31.73. *Hydrochloride* ***7c***. Mp 202–204 °C; IR (KBr, cm^{-1}) 3194, 2926, 1648, 1624, 1477, 1215, 950. Anal. calcd. for $C_{10}H_{11}ClFN_5$ (255.68): C, 46.98; H, 4.34; N, 27.39. Found: C, 46.99; H, 4.16; N, 27.51.

*4.1.4.4. 7-Fluoro-1-[(imidazolidin-2-yl)imino]-1H-indazole (**6d**).* Yield 0.49 g (70 %); mp 253–255 °C; IR (KBr, cm^{-1}) 3243, 3167, 3060, 3012, 2878, 2842, 1632, 1519, 1289, 1214, 1069, 729; H^1 NMR (DMSO-d_6, 200 MHz) δ 3.31 (br s, 4H), 6.38 (br s, 1H), 6.72 (br s, 1H), 6.95–7.09 (m, 2H), 7.51 (d, *J*=7.8 Hz, 1H), 7.95 (d, *J*=2.0 Hz, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 41.3, 42.2, 109.4 (d, *J*=16.8 Hz), 116.2 (d, *J*=4.5 Hz), 119.5 (d, *J*=5.4 Hz), 125.5 (d, *J*=4.2 Hz), 128.2, 147.5 (d, *J*=249 Hz), 164.8. Anal. calcd. for $C_{10}H_{10}FN_5$ (219.22): C, 54.79; H, 4.60; N, 31.95; Found: C, 55.10; H, 4.28; N, 32.16. *Hydrochloride* ***7d***. Mp 228–230 °C; IR (KBr, cm^{-1}) 3297, 3099, 2888, 1655, 1628, 1214, 1070, 859, 734. Anal. calcd. for $C_{10}H_{11}ClFN_5$ (255.68): C, 46.98; H, 4.34; N, 27.39. Found: C, 46.73; H, 4.50; N, 27.14.

*4.1.4.5. 4,5,6,7-Tetrafluoro-1-[(imidazolidin-2-yl)imino]-1H-indazole (**6e**).* Obtained from 0.047 g (0.1 mmol) of **5e** and 0.4 ml of 50% TFA in CH_2Cl_2. Yield 0.024 g (88 %); mp 210–212 °C; IR (KBr, cm^{-1}) 3332, 3254, 3184, 1634, 1547, 1489, 1345, 955; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.64 (br s, 4H), 5.02 (br s, 1H), 6.18 (br s, 1H), 7.95 (d, *J*=1.5 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 42.2 (br s), 43.6 (br s), 162.2 (d, *J*=6.5 Hz), 137.3, 162.4. The other signals could not be detected due to their high multiplicity and thus low intensity. Anal. calcd. for $C_{10}H_7F_4N_5$ (273.19): C, 43.96; H, 2.58; N, 25.64. Found: C, 44.13; H, 2.37; N, 25.79. *Hydrochloride* ***7e***. Mp 216–218 °C; IR (KBr, cm^{-1}) 3075, 3003, 2876, 1648, 1533, 1488, 1349, 944. Anal. calcd. for $C_{10}H_8ClF_4N_5$ (309.65): C, 38.79; H, 2.60; N, 22.62. Found: C, 38.88; H, 2.64; N, 2.37.

*4.1.5. The general procedure for the synthesis of fluoro-1-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-1H-indazoles **8a–c** and their hydrochloride salts **10a–c**.*

The title compounds were obtained according to a method described by J. Sączewski et al. [48]. To the stirred solution of properly substituted fluoroindazole (0.5 g, 3.7 mmol) in anhydrous THF (5 ml) sodium hydride (0.22 g, 5.5 mmol, 60 % oil dispersion) was added in one portion. After 15 min.

freshly prepared 2-(chloromethyl)-4,5-dihydro-1*H*-imidazole was added and the reaction mixture was stirred at room temperature for 12 hours. After this time the reaction was quenched with water (10 ml). The layers were separated and the aqueous one was extracted with dichloromethane (3×10 ml). The combined organic layers were dried (Na_2SO_4) and evaporated under vacuum. The oily residue thus obtained was purified by preparative thin layer chromatography eluting first with ethyl acetate and then with ethyl acetate/methanol/triethylamine 50:5:3. The *N*1-alkylated products were eluted first, while the *N*2-alkylated ones had considerably lower Rf and were not isolated in pure form.

Compounds **8a**–**8c** were then converted into their hydrochloride salts **10a**–**10c** by adding 1.5 molar equiv. of the ethereal solution of hydrochloride (2.6 M) to the solution of the appropriate fluoro-1-[(4,5-dihydro-1*H*-imidazol-2-yl)methyl]-1*H*-indazole in dichloromethane.

4.1.5.1. 4-*Fluoro-1-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-1H-indazole (**8a**)*. Yield (0.2 g, 25%); mp 96–97 °C, IR (KBr, cm^{-1}) 3258, 2938, 1612, 1493, 1238, 1188, 1000, 779; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.60 (s, 4H), 4.48 (br s, 1H), 5.21 (s, 2H), 6.80–6.86 (m, 1H), 7.30–7.36 (m, 2H), 8.11 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 47.9, 50.1, 105.5, 105.6 (d, *J*=20.8 Hz), 113.9 (d, *J*=4.7 Hz), 128.2 (d, *J*=7.8 Hz), 155.9 (d, *J*=253.1 Hz), 162.7. Anal. calcd. for $C_{11}H_{11}FN_4$ (218.23): C, 60.54; H, 5.08; N, 25.67. Found: C, 60.66; H, 5.15; N, 25.31. *Hydrochloride **10a***. Mp 197–199 °C; IR (KBr, cm^{-1}) 3411, 3339, 3074, 2960, 1634, 1242, 779. Anal. calcd. for $C_{11}H_{12}ClFN_4$ (254.69): C, 51.87; H, 4.75; N, 22.00. Found: C, 51.93; H, 4.63; N, 22.26.

*4.1.5.2. 5-Fluoro-1-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-1H-indazole (**8b**).* Yield (0.2 g, 25 %); mp 103–105 °C; IR (KBr, cm^{-1}) 3164, 3129, 2946, 2874, 1618, 1508, 1216, 847, 799; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.59 (s, 4H), 5.14 (br s, 1H), 5.20 (s, 2H), 7.13–7.23 (m, 1H), 7.31–7.37 (m, 1H), 7.44–7.50 (m, 1H), 7.98 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 47.8, 49.9, 105.2 (d, *J*=23.6 Hz), 110.9 (d, *J*=9.7 Hz), 116.8 (d, *J*=27.8), 124.3 (d, *J*=10.2), 134.1 (d, *J*=50.6 Hz), 137.0, 158.3 (d, *J*=239), 163.0. Anal. calcd. for $C_{11}H_{11}FN_4$ (218.23): C, 60.54; H, 5.08; N, 25.67. Found: C, 60.58; H, 4.90; N, 25.80. *Hydrochloride **10b***. Mp 257–260 °C; IR (KBr, cm^{-1}) 3059, 2949, 2918, 1623, 1508, 1290, 1210, 851; Anal. calcd. for $C_{11}H_{12}ClFN_4$ (254.69): C, 51.87; H, 4.75; N, 22.00. Found: C, 52.02; H, 4.58; N, 21.79.

*4.1.5.3. 6-Fluoro-1-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-1H-indazole (**8c**).* Yield (0.4 g, 41 %); mp 114–116 °C; IR (KBr, cm^{-1}) 3093, 2931, 2862, 1620, 1505, 1477, 1247, 955; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.59 (s, 4H), 4.65 (br s, 1H), 5.14 (s, 2H), 6.95 (td, J_1=9.3, J_2=2.4 Hz, 1H), 7.16 (d, *J*=9.3 Hz, 1H), 7.67 (dd, J_1=8.8 Hz, J_2=5.4, 1H), 8.01 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 47.7, 50.0, 95.3 (d, *J*=26.7), 111.4 (d, *J*=26.1 Hz), 121.1, 134.6, 140.3, 162.6 (d, *J*=246.4), 162.8. Anal. calcd. for $C_{11}H_{11}FN_4$ (218.23): C, 60.54; H, 5.08; N, 25.67. Found: C, 60.71; H, 5.31; N, 25.49. *Hydrochloride **10c***. Mp 270–272 °C; IR (KBr, cm^{-1}) 3053, 2946, 1620, 1249, 847. Anal. calcd. for $C_{11}H_{12}ClFN_4$ (254.69): C, 51.87; H, 4.75; N, 22.00. Found: C, 51.52; H, 4.84; N, 21.72.

*4.1.6. The procedure for fluorination leading from **5f** to **6c***

*4.1.6.1. 1-{[1,3-di(tert-butoxycarbonyl)imidazolidin-2-yl]imino}-6-(tributylstannyl)indazole (**11**)*

The title compound was prepared analogously to a method described by Ritter et al. [85]. To **5f** (480 mg, 1.00 mmol, 1.00 molar equiv.) and tetrakis(triphenylphosphine)palladium (116 mg, 0.1 mmol, 10 mol%) dissolved in toluene (10 ml) was added bis(tri-n-butyltin) (1.01 ml, 2.00 mmol, 2.00 molar equiv.) under argon. After stirring for 24 h at 100 °C under argon atmosphere, the reaction mixture was cooled to room temperature and concentrated under vacuum. The residue was purified

by flash column chromatography (silica gel) eluting with petroleum ether/acetone/ethyl acetate (100:5:5) to afford 480 mg of the title compound as a colourless oil (yield 70 %). IR (oil fim, cm^{-1}) 2957, 2928, 2871, 1719, 1651, 1313, 1153, 759; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*=7.2 Hz, 9H), 0.98 (s, 9H), 1.04–1.12 (m, 6H), 1.23–1.38 (m, 6H), 1.41–1.67 (m, 6H), 1.60 (s, 9H), 3.85 (m, 4H), 7.19 (d, *J*=7.8 Hz, 1H), 7.60 (d, *J*=7.8 Hz, 1H), 7.72 (s, 1H), 7.83 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 9.7, 13.7, 27.2, 27.4, 28.3, 29.1, 43.1, 43.7, 82.8, 83.0, 118.2, 119.3, 122.0, 128.0, 129.5, 137.5, 140.2, 142.5, 150.5 (two overlapping signals). Anal. calcd. for $C_{32}H_{53}N_5O_4Sn$ (690.50): C, 55.66; H, 7.74; N, 10.14. Found: C, 55.48; H, 7.91; N, 9.85.

*4.1.6.2. 6-Fluoro-1-[(imidazolidin-2-yl)imino]indazole (**6c**) from **11***

The multistep synthesis was accomplished using the procedures desribed by Ritter et al. [85] and F. Sączewski et al. [45]. To 1-{[1,3-di(*tert*-butoxycarbonyl)imidazolidin-2-yl]imino}-6-(tributylstannyl)indazole (**11**) (0.4 g, 0.58 mmol, 1.00 molar equiv.) in acetone (12 ml) at room temperature under argon atmosphere was added silver triflate (0.30 g, 1.16 mmol, 2.00 molar equiv.) and 1-(chloromethyl)-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(trifluoroborate) (0.25 g, 0.696 mmol, 1.20 molar equiv.). The reaction mixture was stirred for 20 min. at room temperature, filtered through a celite pad and evaporated to dryness. The residue was suspended in dichloromethane and washed with water. The organic phase was dried ($MgSO_4$), concentrated under vacuum and subjected to preparative thin layer chromatography eluting with petroleum ether/ethyl acetate (6:1→1:1) to afford a 2:1 (according to ^{1}H NMR) mixture (110 mg) of **5c** and 1-{[1,3-di(*tert*-butoxycarbonyl)imidazolidin-2-yl]imino}indazole (**12**) as a white solid. The mixture (110 mg) was added to trifluoroacetic acid (0.9 ml), stirred for 40 min. at room temperature and then evaporated to dryness. The residue was treated with water (1 ml) and then made alkaline to pH≈11 with 10 % aqueous NaOH. The precipitated solid (40 mg) was extracted to methylene chloride (3×10 ml). The organic phase was dried ($MgSO_4$), concentrated under vacuum and subjected to flash column chromatography (Silica gel 60, less than 0.063 mm) eluting with ethyl acetate/methanol/triethylamine (50:1:1). 6-fluoro-1-[(imidazolidin-2-yl)imino]indazole (**6c**) was eluted first (22 mg, yield 16 %) followed by 1-[(imidazolidin-2-yl)imino]indazole (**6**) (8 mg, yield 5 %, mp 175–177 °C, lit. 172–174 °C [45]). For the spectroscopic data of **6c** see 4.1.4.3.

4.2 Pharmacological methods

4.2.1. Radioligand binding assays

4.2.1.1. I_1-Imidazoline site binding assay

Kidneys were obtained *post mortem* from male Sprague-Dawley rats (250–280 g) and crude P2 membranes prepared according to Lione et al. [93]. Binding of [^{3}H]clonidine (3 nM, Perkin–Elmer) was investigated in the presence of 10 µM rauwolscine to preclude binding to α_2-ARs. 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 [94]. Membrane aliquots (400 µl, 0.2–0.5 mg protein) were incubated with 11 concentrations of the test compounds over the range 0.1 nM–100 µM. Incubations were carried out in 50 mM Tris–HCl buffer (pH 7.4) at room temperature for 45 min. Bound radioligand and free radioactivity were separated by rapid filtration through pre-soaked (0.5 % polyethyleneimine) glass-fibre filters (Whatman GFB). Trapped radioligand was determined by liquid scintillation counting and the data were analysed with GraphPad Prism version 4.03 for Windows

(GraphPad Software, San Diego, CA, USA) to yield IC_{50} values (the concentration of tested ligand that displaces 50 % of specifically bound [^{3}H]clonidine).

4.2.1.2. α_1-AR, α_2-AR and I_2-imidazoline site binding assays

Brains were obtained *post mortem* from male Sprague-Dawley rats (250–280 g) and crude P2 membranes were prepared [93]. Membrane aliquots (400 µl, 0.2–0.3 mg protein) were incubated with 11 concentrations of the tested compounds over the range 0.1 nM–100 µM in the presence of the selective I_2 binding site radioligand [^{3}H]-2BFI (2-(benzofuranyl)-2-imidazoline) [95] (1 nM), α_1-AR antagonist radioligand [^{3}H]prazosin (1 nM) or α_2-AR antagonist radioligand [^{3}H]RX821002 (2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1*H*-imidazole) (1 nM) in a final volume of 500 µl. Non-specific binding was determined using 10 µM BU224 (2-(4,5-dihydroimidazol-2-yl)quinoline) [96] for I_2 binding, 10 µM phenylephrine for α_1-ARs and 10 µM rauwolscine to define α_2-AR binding. Incubations were performed in triplicate at room temperature and were allowed to reach equilibrium (45 min). Bound and free radioactivity were separated by rapid filtration through pre-soaked (0.5 % polyethyleneimine) glass-fibre filters (Whatman GF/B). Filters were then washed twice with 5 ml of ice-cold buffer and membrane-bound radioactivity remaining on the filters was determined by liquid scintillation counting. The data were analyzed by iterative non-linear regression analysis with GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, CA, USA). Each experiment was analyzed individually and equilibrium dissociation constants (K_i) were determined by the method of Cheng and Prusoff [97]. 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.

4.2.1.3. α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor binding assays

Adherent Chinese hamster ovary (CHO) cells (K1 strain) (American Type Culture Collection, Manassas, VA, USA), stably expressing cDNAs encoding each of the human α_2-AR subtypes were produced and treated as described by Pohjanoksa et al. [98]. Receptor density and the affinity of the subtype-nonselective radioligand [^{3}H]RS-79948-197 were determined for each human α_2-AR subtype with saturation binding assays, as described previously by Fallarero et al. [99]. The K_d and B_{max} values were as follows: α_{2A}, 0.20 ± 0.04 nM; α_{2B}, 0.33 ± 0.04 nM and α_{2C}, 0.18 ± 0.02 nM; and α_{2A}, 4.2 ± 0.3 pmol/mg protein; α_{2B}, 9.6 ± 2.0 pmol/mg protein and α_{2C}, 8.6 ± 1.1 pmol/mg protein. Competition binding assays were implemented using a MultiScreen Vacuum Manifold system (Millipore Corporation, Bedford, MA, USA) with Millipore MultiScreen MSFBN 96-well glass fibre filtration plates. The experiments were performed in a total assay volume of 180 µl (in 50 mM potassium phosphate buffer, pH 7.4) using 0.2 nM [^{3}H]RS-79948-197 ((8a*R*,12a*S*,13a*S*)-5,8,8a,9,10,11,12,12a,13,13a-dechydro-3-methoxy-12-(ethylsulfonyl)-6*H*-isoquino[2,1-*g*][1,6]naphthyridine; GE Healthcare, London, U.K.), 8 serial dilutions of the competitor ligands and crude cell membrane preparations containing 6-10 µg of protein per sample. Non-specific binding was determined in parallel wells in the presence of 100 µM oxymetazoline. Bound radioactivity was measured with a Wallac 1450 MicroBeta scintillation counter (PerkinElmer Wallac, Turku, Finland). All experiments were performed in duplicate and repeated at least three times. The apparent affinity (apparent K_i) of each ligand was determined using nonlinear regression analysis (GraphPad Prism), assuming one-site binding. For conversion of IC_{50} estimates to K_i values, the Cheng-Prusoff equation was applied [97].

4.2.2. In vivo cardiovascular effects

Male Wistar rats, weighing 200–290 g, were purchased from the Animal House of the Medical University of Gdańsk, Poland. All in vivo experiments were approved by the Local Ethical Committee on Animal Experiments. The animals were fed commercial rodent chow (Labofeed-B, Poland). Tap water was available ad libitum. Rats were anaesthetized by i.p. injection of thiopental (Sandoz, Austria) at 70 mg/kg body weight and anaesthesia was maintained by thiopental supplementation (30 µg/kg/min). The animals were placed on a heated table, and body temperature was maintained between 36 and 37 °C. Tracheotomy was performed. Catheters were inserted into the carotid artery for monitoring of MAP and HR, into a jugular vein for infusions, and into the bladder for free diuresis. After all surgical procedures, a 40 min recovery period was allowed to establish a stable baseline. The rats were infused with isotonic saline (Fresenius Kabi, Poland) supplemented with thiopental at a rate of 1.2 ml/h. After 40 min of saline infusion, the tested compound was administered as a 100 µl bolus through the venous catheter at doses of 0.1 mg/kg. The time of administration of a compound was recorded as "time 0". MAP and HR were monitored directly and sampled continuously at 100 Hz as described previously [72], using Biopac Systems, Inc., Model MP 100 (Goleta, CA, USA). The recorded results were elaborated with the help of the ACQKnowledge (Goleta, CA, USA) analysis system and were selected, scaled and filtered to remove accidental signal disturbances. The recorded time domain transient data are presented as graphs with the help of Excel (Microsoft, USA).

ANOVA was performed for ΔMAP and ΔHR, calculated as the difference in MAP and in HR from baseline measurements ("time 0") for each group, as described previously [72]. This allowed for direct comparison of responses to treatment between groups. Data were analyzed by ANOVA for repeated measurements, using Statistica StatSoft software (StatSoft, Inc., Tulsa, OK, USA), after test compound or vehicle administration. When a treatment effect was significant, *post hoc* comparisons were performed using Fisher's test. A value of $p < 0.05$ was considered statistically significant.

4.2.3. In vitro estimation of agonist potency and efficacy

4.2.3.1. Cell culture

CHO cells stably expressing human α_{2A}-ARs were cultured as described by Pohjanoksa et al. [98]. Before the functional [^{35}S]GTPγS binding assays, the cultured cells were tested for their capacity to bind the α_2-AR antagonist radioligand [^{3}H]RS-79948-197. Confluent cells were harvested into chilled phosphate-buffered saline, pelleted and frozen at –70 °C.

4.2.3.2. 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 8000 rpm). The homogenate was centrifuged at 180 g for 15 min to remove cell nuclei, unbroken cells and aggregates. The supernatants were pooled and centrifuged at 50,200 g for 30 min. The pellet was washed with TE buffer (10 mM Tris, 0.1 mM EDTA) and re-centrifugated as above. The membranes were then suspended in TE buffer, aliquoted and stored at –70 °C until used. Protein concentrations were determined with the method of Bradford [100] using bovine serum albumin as reference.

4.2.3.4. [^{35}S]GTPγS binding assay

Agonist-induced stimulation of [^{35}S]GTPγS binding was measured essentially as described previously [75]. 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. Samples containing 5 µg of membrane protein were incubated with 7 or 8 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. 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 norepinephrine).

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Table 1.

Binding affinity data for marsanidine, **B**, **6a–e** and **8a–d**

Compd	α_1 K_i (nM)[a,c]	α_2 K_i (nM)[a,c]	I_1 IC_{50} (nM)[b,c]	I_2 K_i (nM)[a,c]
marsanidine[d]	nd[f]	14.05 ± 2.7	54550 ± 16730	16900 ± 5900
6a	3807 ± 1342	416.0 ± 16.2	14776 ± 7893	6177 ± 4962
6b	2320 ± 293	64.33 ± 4.39	91.65 ± 16.54	5703 ± 2656
6c	918.3 ± 52.6	26.20 ± 6.30	20675 ± 20513	21967 ± 3735
6d	1625 ± 475.2	30.97 ± 1.93	7740 ± 5522	348833 ± 306639
6e	19793 ± 18003	3717 ± 220.2	5827 ± 3402	22733 ± 817.2
B[e]	272 ± 42	18.5 ± 5.9	19400 (n=1)[g]	42200 (n=1)[g]
8a	534.7 ± 118.4	187.3 ± 40.26	1765 ± 1200	21.87 ± 4.703
8b	476.3 ± 267.5	32.17 ± 9.60	2419 ± 2057	10.38 ± 5.44
8c	518.3 ± 159.9	29.73 ± 3.04	33456 ± 24023	8.193 ± 3.43
8d[e]	107 (n=1)[g]	12.6 (n=1)[g]	16400 (n=1)[g]	156 (n=1)[g]

[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 tested compounds to compete with [^{3}H]prazosin, [^{3}H]RX821002 or [^{3}H]2BFI binding to rat brain membranes. [b] Molar concentration of the tested compounds that displaces 50% of specifically bound [^{3}H]clonidine in rat kidney membranes in the presence of rauwolscine (I_1 imidazoline binding sites). [c] Values given are means ± s.e.m. from 3–4 independent experiments. [d] The results have been published earlier in Sączewski F. et al. [45]. [e] The results have been published earlier in Sączewski J. et al. [48]. [f] nd: not determined. [g] n: number of experiments.

Table 2.

Binding affinities of **6c** and **6d** obtained with [^{3}H]RS-79948-197 to human α_2-AR subtypes expressed in CHO cell membranes. Results expressed as K_i (nM) and their 95 % confidence intervals of three independent experiments.

		Human	
Compd	α_{2A}-AR	α_{2B}-AR	α_{2C}-AR
marsanidine	52 (39–70)	79 (63–99)	640 (520–780)***
6c	33 (27–41)	72 (54–92)**	600 (510–710)***
6d	65 (54–77)	158 (130–190)**	1200 (960–1400)**

Statistical significance of differences from the α_{2A}-AR is shown by symbols: **P<0.01; ***P<0.001 tested with unpaired t-tests.

Table 3.

Effects of compounds **6b**–**d** and **8b**–**d** at 0.1 mg/kg i.v. on mean arterial blood pressure (MAP) and heart rate (HR) in anesthetized rats.

compd	$+\Delta MAP_{max}$[a,d] (t_{max})[f]	$-\Delta MAP_{max}$[b,d] (t_{max})[f]	$-\Delta HR_{max}$[c,d] (t_{max})[f]	n[e]
6b	14.6 ± 5.9 (2) *%	–8.6 ± 2.3 (11) $!@	–61 ± 7 (5) *§	4
6c	24.8 ± 4.6 (3) *§	–36.0 ± 2.3 (35) *!	–118 ± 15 (2) *§^&	4
6d	21.1 ± 2.2 (4) *§	–59.4 ± 5.7 (33) *$@	–128 ± 9 (10) *§^&	4
8b	0.8 ± 1.0 (2)	–7.2 ± 1.6 (40) $!@	–12 ± 3 (38) ^&	4
8c	23.6 ± 3.6 (4) *§	–12.7 ± 1.8 (36) #$!@	–52 ± 9 (5) *§	5
8d[g]	17.7 ± 7.1 (1) *§	–29.5 ± 4.0 (34) *$!	–88 ± 21 (21) *§^	5
control[h]	0.4 ± 0.7	–2.7 ± 1.2 $!@	–9 ± 2 ^&	5

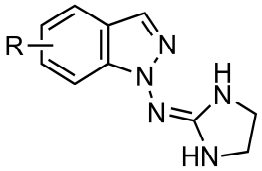
[a] The maximal hypertensive effect [mmHg] of a compound observed during 60 minutes after injection. [b] The maximal hypotensive effect [mmHg] of a compound observed during 60 minutes after injection. [c] The maximal bradycardic effect [bpm] of a compound observed during 60 minutes after injection. [d] Values given are means ± s.e.m from n independent experiments. [e] Number of experiments. [f] t_{max}: time (in minutes) after the injection when the maximal effect was observed. [g] The results have been published earlier in Sączewski J. et al. [48]. [h] Saline vehicle injection. (*) p <0.001, (#) p <0.05 vs control. (&) p <0.001 vs **6b**. ($) p<0.001 vs **6c**. (!) p <0.001 vs **6d**. (§) p <0.001, (%) p <0.05 vs **8b**. (^) p <0.001 vs **8c**. (@) p <0.001 vs **8d**.

Table 4

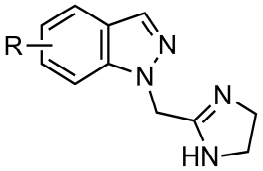
Characterization of [^{35}S]GTPγS binding to CHO cell membranes expressing recombinant human α_{2A}-ARs: estimates of agonist potency (EC_{50}) and intrinsic activity relative to the natural full agonist norepinephrine. Values shown are means ± s.e.m. from 3 independent experiments for compounds **6c, 6d** and marsanidine and 19 independent experiments for norepinephrine.

Compound	**pEC_{50}**	**Intrinsic activity** (% of norepinephrine)
Norepinephrine	6.89 ± 0.06	100
Marsanidine[a]	7.03 ± 0.29	14 ± 2
6c	7.41 ± 0.23	5.2 ± 2.9
6d	6.70 ± 0.33	18 ± 4

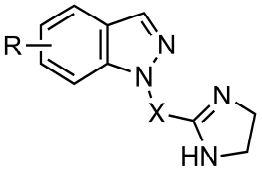
[a] The results have been published earlier in Sączewski F. et al. [46].



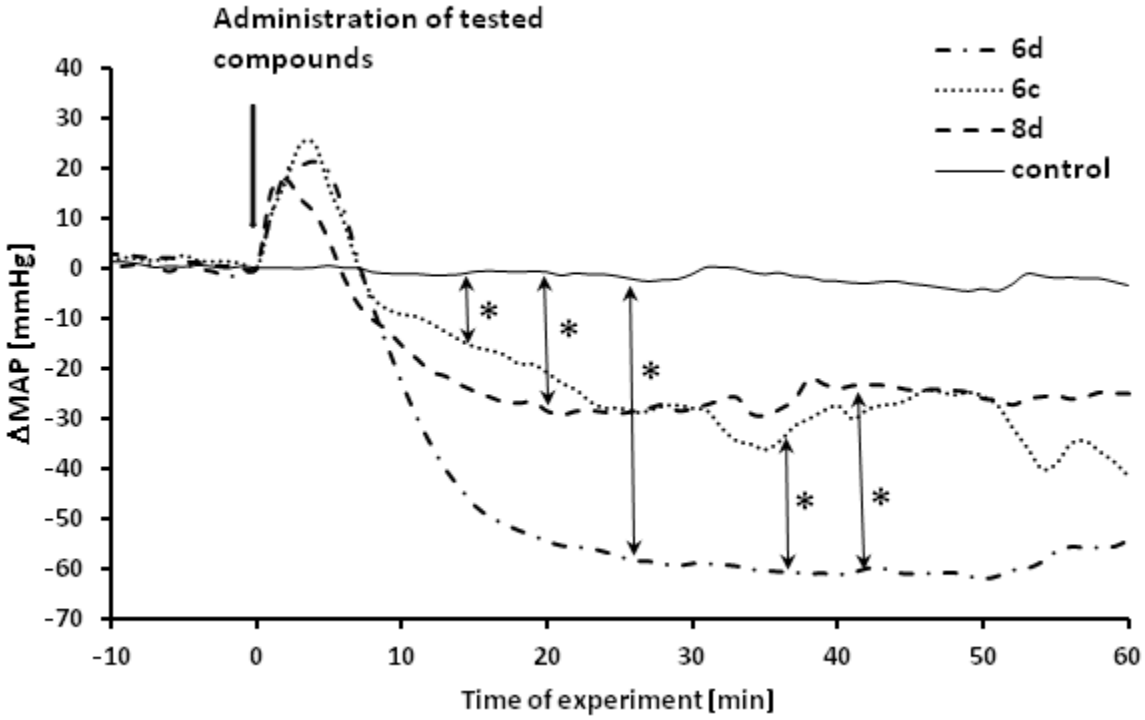
A
R= H (marsanidine), alkyl, alkoxyl, Cl

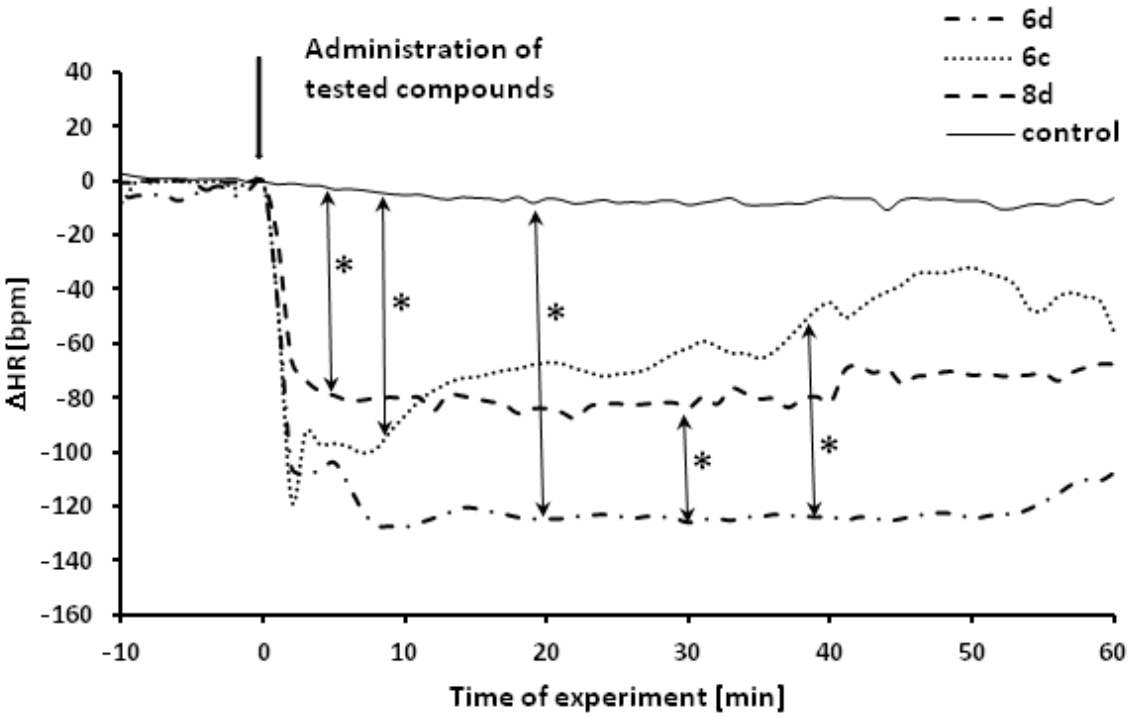


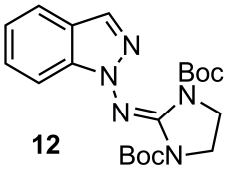
B
R= H, alkyl, alkoxyl, Cl



6a-e X= NH, R= F
8a-d X= CH, R= F







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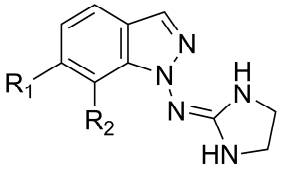


Biological activity *in vitro*:

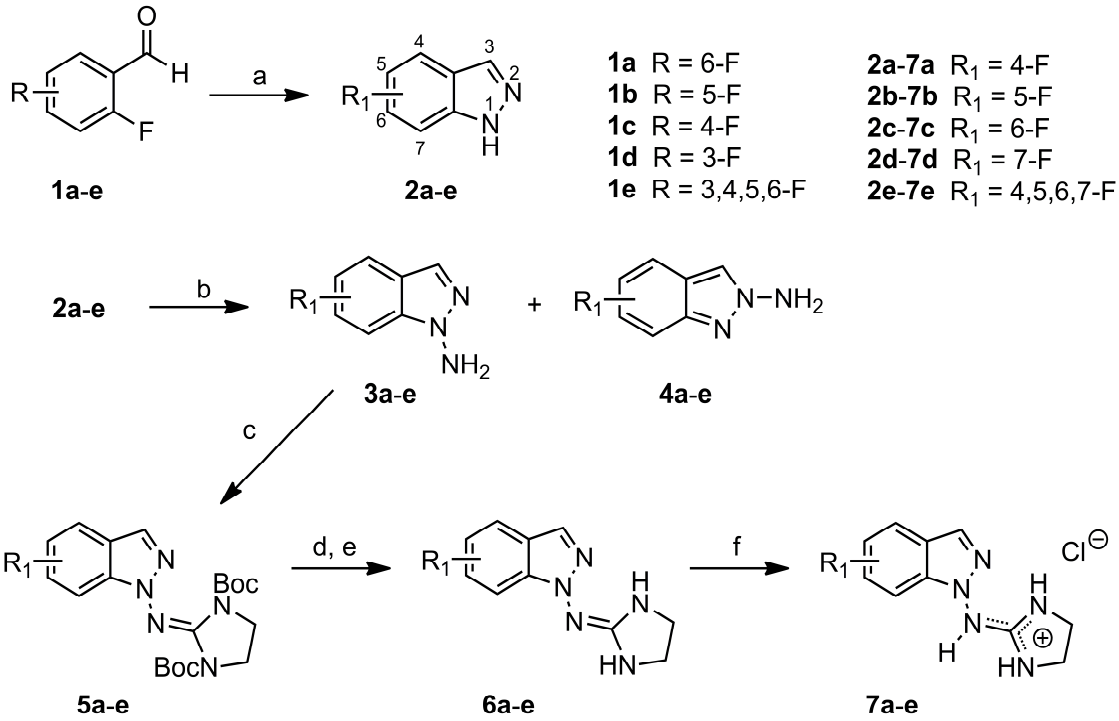
R_1 = F, R_2 = H : $\alpha_2 K_i$ = 26.2 nM, I_1 IC_{50} = 20.7 μM

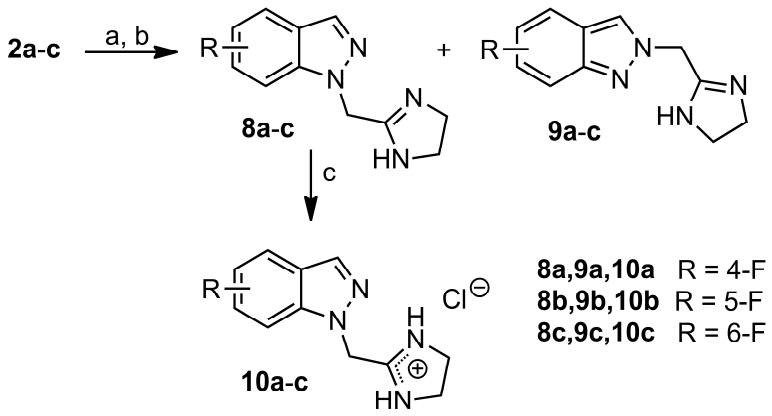
R_1 = H, R_2 = F : $\alpha_2 K_i$ = 30.9 nM, I_1 IC_{50} = 9.89 μM

Circulatory effects in rats at dose 0.1 mg/kg i.v.

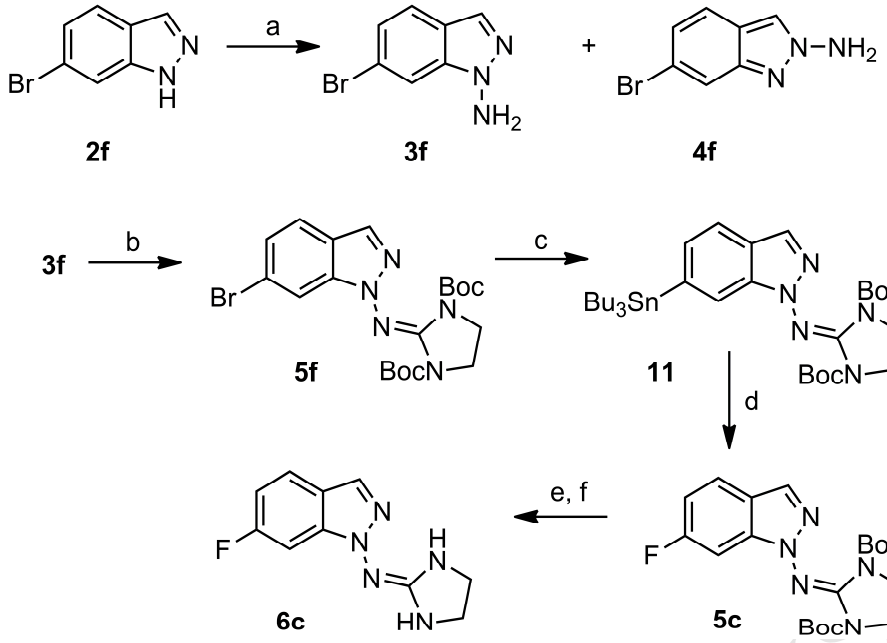
R_1 = F, R_2 = H : ΔMAP = -36 mmHg, ΔHR = -118 bpm

R_1 = H, R_2 = F : ΔMAP = -59 mmHg, ΔHR = -128 bpm





8a,9a,10a R = 4-F
8b,9b,10b R = 5-F
8c,9c,10c R = 6-F



Fluorinated marsanidine analogues were assessed for their pharmacological properties.

Two selective fluorine-containing α_2-adrenoceptor ligands were identified.

A fluorination method potentially useful for ^{18}F-labelling of marsanidine was developed.