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New 1,2,3,9-tetrahydro-4*H*-carbazol-4-one derivatives: Analogues of HEAT as ligands for the α_1-adrenergic receptor subtypes

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Abstract—With the aim to develop new ligands able to discriminate among the three subtypes of α_1-adrenergic receptors (α_{1A}-AR, α_{1B}-AR, and α_{1D}-AR), a series of new 1,2,3,9-tetrahydro-4*H*-carbazol-4-ones bearing a 3-[[[2-(4-hydroxyphenyl)ethyl]amino]methyl] or a 3-[[4-(2-substitutedphenyl)piperazin-1-yl]methyl] side chain were synthesized. The general structure of the new compounds is reminiscent of HEAT and RN5, two potent α_1-AR antagonists which show high affinities for all three α_1-AR subtypes. Some derivatives in which one ring of the tetrahydrocarbazolone system was opened were also prepared. Compounds were tested in radioligand binding assays on human cloned α_{1A}-AR, α_{1B}-AR, and α_{1D}-AR subtypes stably expressed in HEK293 cells. They showed moderate to good affinities, although their selectivity among the receptor subtypes hardly reached one order of magnitude.

1. Introduction

Adrenergic receptors (ARs) play a key role in the modulation of sympathetic nervous system activity and are classified into three different subfamilies (α_1-, α_2-, and β-ARs), on the basis of their pharmacology, amino acid sequence and signalling mechanisms.[1,2] They are membrane proteins and all belong to the super-family of G-protein-coupled receptors (GPCRs). α_1-ARs are therapeutically relevant because of their primary role in the cardiovascular system, notably in the control of the vascular tone. α_1-ARs are also widely distributed outside the cardiovascular system and are particularly abundant in organs such as brain, liver, spleen and prostate. Although the entire set of physiological roles in which α_1-ARs are involved is still not completely clear, α_1-ARs represent pharmacological targets for a number of drugs currently used in the treatment of two widely occurring diseases, hypertension and benign prostatic hyperplasia (BPH).[3–5]

α_1-ARs do not constitute a homogeneous receptor class and three different subtypes, namely α_{1A}-AR, α_{1B}-AR and α_{1D}-AR, have been identified, cloned and characterized so far.[6,7] They show preferential coupling to the G_q family of G-proteins leading to the inositol(1,4,5)triphosphate-mediated increase of intracellular Ca^{2+}. Each subtype shows a discrete tissue distribution suggesting specific physiological functions.[8] Recent approaches to the study of α_1-AR pharmacology have been based on transgenic knockout mice of α_{1A}-AR,[9] α_{1B}-AR[10] and α_{1D}-AR[11] as well as on the use of oligonucleotide microarray techniques;[12] such approaches have provided new valuable insights into the comparative analysis of functions mediated by each subtype. Thus, by using transgenic mice lacking α_{1B}-AR and/or α_{1D}-ARs, the differential contributions of these two subtypes in the control of blood pressure have been clarified.[13,14]

However, in the last two decades, the development of α_1-AR subtype-selective agonists and antagonists has also been of great importance for the advancement in understanding α_1-AR function. Their use has represented and still remains an invaluable approach, complementary to others, to the study of individual receptor subtypes. Through the years, medicinal chemistry has provided several selective molecules, especially for the α_{1A}-AR, which have been proved to be very useful experimental tools and, in some cases, potentially effective drugs.[15]

Keywords: α_1-adrenergic receptor ligands; α_1-adrenergic receptor subtypes; HEAT; 1,2,3,9-Tetrahydro-4*H*-carbazol-4-ones.
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Nevertheless, much research is still needed to discover new and more selective ligands, particularly for the α_{1B}-AR and α_{1D}-AR subtypes.

HEAT (**1**) and RN5 (**2**) (Chart 1) are two known α_1-AR antagonists with affinity values in the subnanomolar range.[16–19] From a structural point of view, HEAT (**1**) can be subdivided in a planar bicyclic system (α-tetralone) and a tyramine moiety held together with a methylene bridge. The protonatable nitrogen of the tyramine substructure represents the basic group which, in all known α_1-AR ligands, is essential for interaction with the receptor binding site. Similarly, in RN5 (**2**) a planar tricyclic pyrimido[5,4-*b*]indole moiety is linked, through an ethylene chain, to a phenylpiperazine (PP) portion bearing the essential protonatable nitrogen.

Although both **1** and **2** do not show appreciable selectivity among the α_1-AR subtypes, discrete modifications on their structures could provide new potential subtype-selective ligands.

On this basis, and with the aim to obtain subtype-selective molecules, we now report the synthesis and the binding properties to the three human cloned α_1-AR subtypes of a number of new 1,2,3,9-tetrahydro-4*H*-carbazol-4-one (TC) derivatives **16**–**20**. They are HEAT (**1**) analogues in which the α-tetralone moiety has been replaced by a tricyclic (TC) system which bears, at the 9-position, groups of different nature and shape. The larger aromatic plane of TC along with substituents at the 9-position could be further sites of interaction with the receptor binding sites and/or represent structural features inducing subtype-selectivity.

In these new compounds, the TC system can be considered an α-tetralone in which a pyrrole nucleus was inserted between the benzene and the cyclohexenone rings. A similar strategy had been successfully used in the design of RN5 (**2**), where the quinazoline-2,4-dione derivative SGB 1534 (**4**), one of the early α_1-AR ligands, was used as template (Chart 1).[18] In fact, in that case, the pyrimido[5,4-*b*]indole-2,4-dione system in RN5 (**2**) had been formally obtained by the insertion of a pyrrole ring between the benzene and the pyrimidine rings of the quinazoline-2,4-dione moiety of SGB 1534 (**4**).

In addition to compounds **16**–**20**, some other derivatives (**21**–**24**) in which the protonatable nitrogen is part of a 4-(2-substitutedphenyl)piperazin-1-yl substructure instead of a tyramine moiety were also prepared along with derivatives **26**–**28** lacking one of the six-membered rings of the TC system.

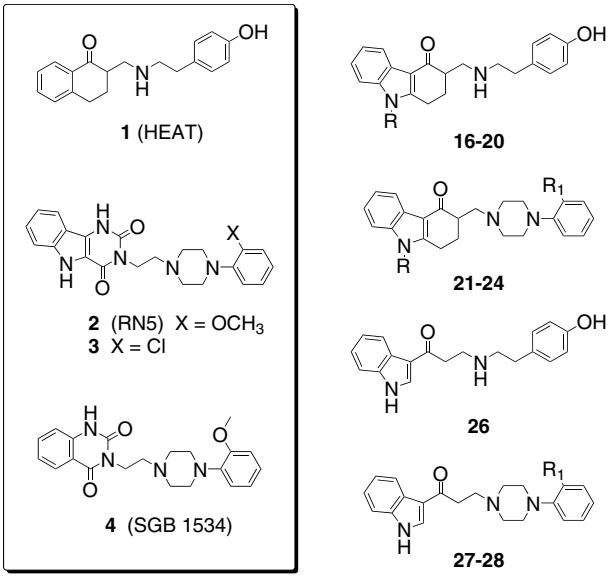


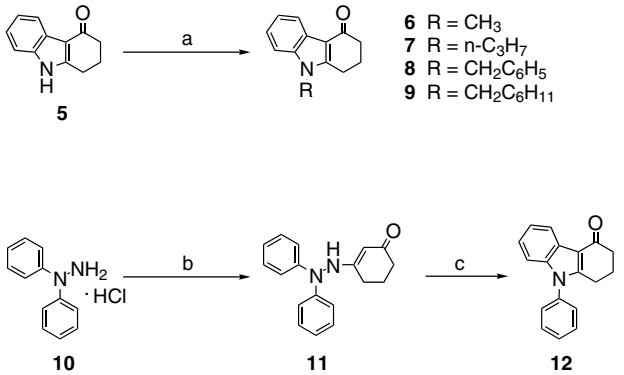
Chart 1.

2. Results and discussion

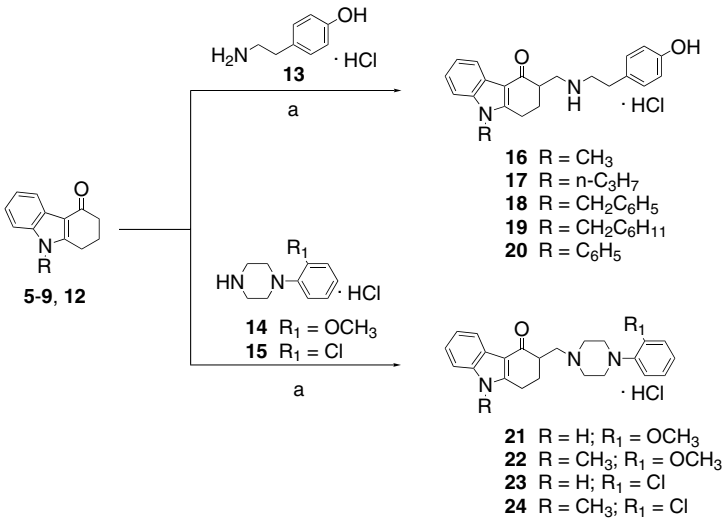
2.1. Synthesis

The preparation of title compounds is outlined in Schemes 1–3. 1,2,3,9-Tetrahydro-4*H*-carbazol-4-one **5**, obtained from phenylhydrazine and 1,3-cyclohexandione following the Fisher indole synthesis,[20] was N-alkylated by the suitable alkyl halide in dimethylformamide (DMF) in the presence of sodium hydride to give compounds **6**–**9** in good yields (Scheme 1). Some attempts to obtain 9-phenyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one **12** by a direct arylation of **5** were made on the basis of a previously reported N-arylation of the indole nucleus with bromobenzene in the presence of copper (I) bromide and sodium carbonate.[21] When we tried the same experimental conditions on carbazolone **5**, *N*-arylated derivative **12** was obtained only in very low yields. An alternative approach to the preparation of **12** had been reported in literature in a 26% yield.[22] It had been obtained, along with *N*-methyl[23] and *N*-benzyl derivatives **6** and **8**, by means of dehydrogenation with chloranil of the corresponding 9-substituted-1,2,3,4,5,6,7,8-octahydrocarbazol-4-ones. However, we synthesized **12** in comparable yields (23%) by ring closure of enehydrazine **11** in acetic acid and zinc chloride (Scheme 1).

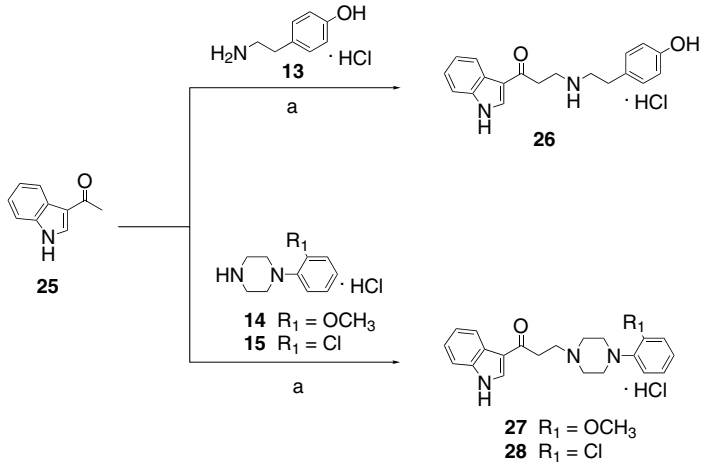
Successively, compounds **6**–**9** and **12** were caused to react with tyramine hydrochloride (**13**) in the presence of paraformaldehyde to give final compounds **16**–**20** as



Scheme 1. Reagents: (a) NaH 80%, alkyl halides, dry DMF; (b) 1,3-cyclohexanedione, EtOH/water, N_2; (c) $ZnCl_2$, AcOH.



Scheme 2. Reagents and condition: (a) paraformaldehyde, EtOH, reflux.



Scheme 3. Reagents and condition: (a) paraformaldehyde, EtOH, reflux.

hydrochlorides. In the same experimental conditions, reaction of carbazol-4-ones **5** and **6** with hydrochlorides of 1-(2-methoxyphenyl)piperazine (**14**) or 1-(2-chlorophenyl)piperazine (**15**) afforded hydrochlorides of Mannich bases **21**–**24** in good yields (Scheme 2).

Finally, reaction of 3-acetyl-1*H*-indole (**25**), commercially available, with paraformaldehyde and amines **13**, **14** or **15** led to final indole derivatives **26**–**28** (Scheme 3).

2.2. Binding studies

Title compounds **16**–**24** and **26**–**28** were tested in binding assays on human α_{1A}-AR, α_{1B}-AR and α_{1D}-AR subtypes stably expressed in HEK293 cells using [^{125}I]HEAT as radioligand. Affinities of tested compounds for the three cloned receptor subtypes are expressed as pK_i values and summarized in Table 1.

As a general trend, all tested compounds showed lower affinities than HEAT (**1**) and RN5 (**2**) for all three α_1-AR subtypes. In addition, their subtype-selectivity was generally less than one order of magnitude. Within this context, however, some structure–affinity relationships can be drawn.

Tyramine derivatives **16**–**20** displayed a slightly preferential affinity for the α_{1A}-AR subtype. From a chemical point of view, they differ from each other in the size of the substituent at the 9-position of the TC system, going from a simple methyl to the larger cyclohexyl or benzyl groups. Nevertheless, these differences in the substituent shape appear not to have great influence on affinity. In fact, methyl derivative **16** and the analogous compound **18**, bearing the bulkier benzyl moiety, displayed similar pK_i values at α_{1A}-AR (7.71 and 7.65), α_{1B}-AR (7.21 and 7.19) and α_{1D}-AR (7.39 and 7.52), respectively.

According to this trend, benzyl derivative **18** showed the same affinity as its cyclohexylmethyl analogue **19** at the α_{1A}-AR. However, for the other two α_1-AR subtypes, affinities of **19** were lower than those displayed by **18**. The benzyl and the cyclohexylmethyl groups have similar steric bulk but differ in aromaticity, which could be the discriminant feature. Thus, a nonaromatic cycloalkyl moiety seems to be less tolerated than an aromatic

Table 1. Binding properties of 1,2,3,9-tetrahydro-4*H*-carbazol-4-one (**16**–**24**) and 1*H*-indole derivatives (**26**–**28**)

Compound	pK_i (M)[a]		
	α_{1A}-AR	α_{1B}-AR	α_{1D}-AR
16	7.71 ± 0.11	7.21 ± 0.09	7.39 ± 0.10
17	7.87 ± 0.08	7.19 ± 0.13	7.43 ± 0.10
18	7.65 ± 0.06	7.19 ± 0.14	7.52 ± 0.06
19	7.64 ± 0.24	6.69 ± 0.20	6.57 ± 0.17
20	7.92 ± 0.17	7.78 ± 0.19	7.51 ± 0.21
21	7.51 ± 0.06	7.10 ± 0.21	7.88 ± 0.09
22	7.22 ± 0.08	6.76 ± 0.06	7.37 ± 0.14
23	7.08 ± 0.20	6.75 ± 0.16	7.30 ± 0.14
24	6.63 ± 0.17	6.37 ± 0.16	6.97 ± 0.16
26	7.10 ± 0.08	6.76 ± 0.16	7.10 ± 0.10
27	8.23 ± 0.10	7.39 ± 0.14	8.48 ± 0.09
28	7.87 ± 0.18	7.59 ± 0.19	7.95 ± 0.17
1 (HEAT)[b]	9.74	9.70	9.27
2 (RN5)[c]	9.57 ± 0.14	8.74 ± 0.14	9.44 ± 0.11

[a] Each value is the mean ± SE for data from three different experiments conducted in duplicate.
[b] Data from Ref. 7.
[c] Data from Ref. 19.

benzyl moiety by the α_{1B}-AR and α_{1D}-AR binding sites and, on the other hand, at the α_{1A}-AR subtype, this structural difference appears to have no influence on affinity. As a consequence of this behaviour, compound **19**, unlike **18**, shows a slight selectivity for the α_{1A}-AR subtype.

Derivative **20**, bearing a phenyl ring linked directly to the TC nitrogen, displayed comparable affinities at the three binding sites and, for the α_{1B}-AR receptor, presented the best pK_i value in the series.

In compounds **21**–**24**, a 4-(2-methoxyphenyl)piperazinyl (2-MeOPP) or a 4-(2-chlorophenyl)piperazinyl (2-ClPP) moiety was inserted in place of the tyramine moiety because the PP substructure represents the pharmacophoric portion in RN5 (**2**), in its analogue **3** (Chart 1), and in other α_1-AR antagonists.[15] Since in tyramine derivatives **16**–**20**, the nature of the substituent at the 9-position of the TC system had not greatly influenced affinity values, only phenylpiperazine derivatives with (**22** and **24**) or without (**21** and **23**) a simple methyl group were then synthesized.

When tested in binding assays, no sign of an appreciable subtype-selectivity emerged for any of them. In general, the lack of the substituent at the 9-position and the presence of a 2-methoxy group on the PP moiety gave compounds showing higher affinity values for all three α_1-ARs than analogues bearing a 9-methyl group or a 2-ClPP moiety (**21** vs **22**; **21** vs **23**; **22** vs **24**).

In order to have a rough idea of the fraction of positively ionized form of these ligands at the physiologic pH, a potentiometric measure of pK_a was performed for compounds **21** and **16**, taken as representatives of 2-MeOPP and tyramine derivatives prepared in this study, respectively. A pK_a value of 7.21 was estimated for **21**, whereas two different pK_a values (8.81 and 11.29) were obtained for **16** due to the presence of a phenolic group in addition to the protonated amine function. These data suggest that at physiologic pH (7.4) a reasonable fraction of both ligands should be in the positively ionized form which is supposed to interact with the receptor binding site.

In compounds **26**–**28**, a tyramine, a 2-MeOPP or a 2-ClPP moiety was linked, through a propanone chain, to the 3-position of an 1*H*-indole nucleus. These three compounds can be considered the 'open' analogues of derivatives **16**, **21** and **23** inasmuch as they lack part of the cyclohexenone ring of the TC system. Among them, tyramine derivative **26** showed lower affinity values than its analogue **16** for all three α_1-ARs, whereas the opposite was observed for the PP derivatives **27** and **28**. In fact, both these compounds displayed higher affinity for all α_1-AR subtypes than their TC analogues **21** and **23**.

In particular, 3-[4-(2-methoxyphenyl)piperazin-1-yl]-1-(indol-3-yl)propan-1-one **27** displayed the best affinity for the α_{1A}-AR ($pK_i = 8.23$) and the α_{1D}-AR ($pK_i = 8.48$) in the series and possesses an $\alpha_{1A/1D}$-AR selectivity over the α_{1B}-AR subtype of about one order of magnitude.

Recently, a homology model of α_{1A}-AR based on structure of bovine rhodopsin was described.[24] In GPCRs for biogenic amines, it is generally accepted that the ligand binding site is located within the bundle formed by seven transmembrane domains (TM 1–7) and that an acidic residue in TM 3 is involved in binding the protonatable amine group of the ligand. In the case of human α_{1A}-AR sequence, this residue is represented by Asp106. According to the above-quoted model, Asp106 in TM 3 can be considered the central anchoring point for ligands, dividing the receptor binding site into two different subpockets. One is defined by TM 4–7 and the other by TM 1–3 and 7. It was also reported that α_{1A}-AR ligands containing the 2-MeOPP moiety probably fit the binding site arranging the 2-methoxyphenyl group in the subpocket defined by TM 4–7.[24]

On these bases, a putative binding mode of 2-MeOPP derivative **27** at the α_{1A}-AR can be supposed to take place in a similar way: an electrostatic interaction occurs between the central ionized amine group of the ligand and Asp106 side chain, with the 2-methoxyphenyl group addressing the subpocket defined by TM 4–7 and the 1-(indol-3-yl)propan-1-one moiety extending to the other. It is noteworthy that 1,2,3,9-tetrahydro-4*H*-carbazol-2-one derivative **21**, the analogue of **27** in which part of the propanone chain has been incorporated into a ring, showed 5-fold lower α_{1A}-AR affinity than **27**. It could be argued that, in **21**, the presence of an additional ring or the reduced flexibility of the 1,2,3,9-tetrahydro-4*H*-carbazol-2-one system compared to the 1-(indol-3-yl)propan-1-one moiety induces a less favourable fit with the subpocket defined by the TM 1–3 and 7.

3. Conclusions

With the aim to develop new ligands able to discriminate among the three α_1-AR subtypes, a series of new compounds structurally related to the potent antagonist HEAT (**1**) was prepared and tested in binding assays on human cloned α_{1A}-AR, α_{1B}-AR and α_{1D}-AR subtypes stably expressed in HEK293 cells.

The replacement, in the HEAT structure, of the bicyclic α-tetralone moiety with a larger tricyclic TC system led to derivatives **16**–**20** which showed reduced affinity for all three subtypes. A slight preference for the α_{1A}-AR over the other two receptors was displayed by some of them; however, this subtype-selectivity reached, at the most, one order of magnitude in 9-cyclohexylmethyl derivative **19**.

These results indicate that the α-tetralone moiety is a critical determinant for the high affinity of HEAT at α_1-ARs and its enlargement to the TC system is detrimental for the stability of the receptor–ligand complex.

The successive replacement of the tyramine moiety with a PP substructure, the pharmacophoric portion of RN5 (**2**) and other α_1-AR ligands, gave derivatives **21**–**24** which displayed moderate affinity and no appreciable subtype-selectivity.

Finally, opening of the TC system afforded the more flexible indole derivatives **26**–**28**. Among them, compound **27**, bearing the 2-MeOPP moiety, was endowed with good affinity in the nanomolar range and a preferential $\alpha_{1A/1D}$-AR affinity over the α_{1B}-AR subtype.

4. Experimental

4.1. Chemistry: General methods

Melting points were determined using a Gallenkamp apparatus with a digital thermometer MFB-595 in glass capillary tubes and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer FT IR 1600 spectrometer in KBr disks. Elemental analyses for C, H and N were within ±0.4% of theoretical values and were performed on a Carlo Erba Elemental Analyzer Mod. 1108 apparatus. ^{1}H NMR spectra were recorded on a Varian Inova Unity 200 spectrometer (200 MHz for ^{1}H NMR and 50 MHz for ^{13}C NMR) in DMSO-d_6 solution. Chemical shifts are given in δ values (ppm), using tetramethylsilane as the internal standard; coupling constants (J) are given in Hz. Signal multiplicities are characterized as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad signal). All the synthesized compounds were tested for purity on TLC (aluminium sheet coated with silica gel 60 F_{254}, Merck) and visualized by UV (λ = 254 and 366 nm). All chemicals and solvents were of reagent grade and were purchased from commercial vendors.

4.1.1. 9-Propyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one (7). This procedure is presented as an example for the synthesis of compounds **6**–**8**. To a mixture of 1,2,3,9-tetrahydro-4*H*-carbazol-4-one (**5**) (1.00 g, 5.39 mmol) in dry DMF (8 mL), NaH (80% m/m suspension in mineral oil) (0.24 g, 8.00 mmol) was added in a single portion under stirring. After the evolution of hydrogen ceased, 1-iodopropane (0.91 g, 5.36 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. Then the mixture was poured in ice water (50 mL) and stirred for 2 h. The solid was filtered off, washed with water and dried. Recrystallization from EtOH/water (1:1, v/v) gave **7** (0.90 g, 73%) as a pure solid: mp 149–151 °C; IR (KBr, selected lines) cm^{-1} 3111, 2954, 1701, 1662, 1549, 1485, 1234, 973, 743, 611; ^{1}H NMR (DMSO-d_6) δ 8.06–7.97 (m, 1H, aromatic), 7.61–7.52 (m, 1H, aromatic), 7.30–7.12 (m, 2H, aromatic), 4.16 (t, J = 7.2 Hz, 2H, NCH_2), 3.00 (t, J = 6.2 Hz, 2H, $COCH_2$), 2.44 (t, J = 6.0 Hz, 2H, $COCH_2CH_2CH_2$), 2.22–2.02 (m, 2H, $COCH_2CH_2$), 1.84–1.61 (m, 2H, NCH_2CH_2), 0.88 (t, J = 7.4 Hz, 3H, $NCH_2CH_2CH_3$). Anal. ($C_{15}H_{17}NO$) C, H, N.

4.1.2. 9-Methyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one (6). The same procedure, as described for the synthesis of **7**, was followed using iodomethane. Recrystallization from EtOH gave **6** (65%) as a pure solid: mp 192–193 °C; IR (KBr, selected lines) cm^{-1} 3048, 2947, 1631, 1605, 1473, 1317, 1131, 1090, 956, 747, 548; ^{1}H NMR (DMSO-d_6) δ 8.04–7.96 (m, 1H, aromatic), 7.60–7.48 (m, 1H, aromatic), 7.30–7.11 (m, 2H, aromatic), 3.72 (s, 3H, NCH_3), 2.98 (t, J = 6.2 Hz, 2H, $COCH_2$), 2.42 (t, J = 5.6 Hz, 2H, $COCH_2CH_2CH_2$), 2.22–2.02 (m, 2H, $COCH_2CH_2$). Anal. ($C_{13}H_{13}NO$) C, H, N.

4.1.3. 9-Phenylmethyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one (8). The same procedure, as described for the synthesis of **7**, was followed using chloromethylbenzene. Recrystallization from EtOH/water (1:1, v/v) gave **8** (39%) as a pure solid: mp 148–150 °C; IR (KBr, selected lines) cm^{-1} 3033, 2937, 1635, 1528, 1463, 1357, 1130, 953, 744, 696, 556; ^{1}H NMR (DMSO-d_6) δ 8.10–7.99 (m, 1H, aromatic), 7.61–7.46 (m, 1H, aromatic), 7.43–7.08 (m, 2H + 5H, aromatic), 5.50 (s, 2H, NCH_2), 2.99 (t, J = 6.0 Hz, 2H, $COCH_2$), 2.46 (t, J = 6.0 Hz, 2H,

$COCH_2CH_2CH_2$), 2.22–2.04 (m, 2H, $COCH_2CH_2$). Anal. ($C_{19}H_{17}NO$) C, H, N.

4.1.4. 9-Cyclohexylmethyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one (9). To a mixture of **5** (1.00 g, 5.39 mmol) in dry DMF (8 mL), NaH (80% m/m suspension in mineral oil) (0.24 g, 8.00 mmol) was added in a single portion under stirring. After the evolution of hydrogen ceased, cyclohexylmethyl bromide (0.95 g, 5.39 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. Then the mixture was poured in ice water (50 mL) and extracted with ethyl acetate (3× 25 mL). The combined organic layers were dried over anhydrous sodium sulfate and the solvent evaporated in vacuo. The resulting crude oil (0.30 g), containing **9**, was successively used for the synthesis of **19** without further purification.

4.1.5. 3-(*N*′,*N*′-Diphenylhydrazino)-2-cyclohexen-1-one (11). A solution of 1,1-diphenylhydrazine hydrochloride (**10**) (10.0 g, 45.3 mmol) in EtOH (175 mL) was added, dropwise under nitrogen, to a well-stirred solution of 1,3-cyclohexandione (5.08 g, 45.3 mmol) in water (700 mL) at room temperature. Successively, the mixture was stirred for 30 min and the solid was filtered off, washed with water and dried. Recrystallization from EtOH/water (1:2, v/v) afforded **11** as a pure solid (9.00 g, 78%): mp 165–167 °C; IR (KBr, selected lines) cm^{-1} 3176, 1584, 1491, 1363, 1187, 1141, 749, 694; ^1H NMR (DMSO-d_6) δ 9.70 (br s, 1H, NH which exchanges with D_2O), 7.50–7.22 (m, 4H, aromatic), 7.22–6.91 (m, 6H, aromatic), 5.13 (s, 1H, COCH), 2.43 (t, J = 5.6 Hz, 2H, $COCH_2$), 2.12 (t, J = 6.4 Hz, 2H, $COCH_2CH_2CH_2$), 2.01–1.82 (m, 2H, $COCH_2CH_2$). Anal. ($C_{18}H_{18}N_2O$) C, H, N.

4.1.6. 9-Phenyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one (12). Compound **11** (9.00 g, 32.33 mmol) was added to a hot solution of zinc chloride (32.30 g, 237 mmol) in glacial acetic acid (40 mL). The mixture was stirred at 100 °C for 4 h. After the mixture was cooled, the precipitate was filtered off, washed with water and dried. The crude solid was purified by flash chromatography using ethyl acetate/cyclohexane (1:1, v/v) as eluent. The homogeneous fractions were evaporated in vacuo to afford **12** as a pure solid (1.94 g, 23%): mp 141–143 °C (literature[22] mp 171 °C); IR (KBr, selected lines) cm^{-1} 1718, 1654, 1498, 1455, 751, 697; ^1H NMR (DMSO-d_6) δ 8.14–8.07 (m, 1H, aromatic), 7.72–7.52 (m, 5H, aromatic), 7.33–7.12 (m, 3H, aromatic), 2.84 (t, J = 5.8 Hz, 2H, $COCH_2$), 2.51 (t, J = 6.4 Hz, 2H, $COCH_2CH_2CH_2$), 2.23–2.05 (m, 2H, $COCH_2CH_2$); ^1H NMR ($CDCl_3$) δ 8.40–8.25 (m, 1H, aromatic), 7.65–7.10 (m, 7H, aromatic), 2.82 (t, J = 6.2 Hz, 2H, $COCH_2$), 2.63 (t, J = 6.0 Hz, 2H, $COCH_2CH_2CH_2$), 2.31–2.12 (m, 2H, $COCH_2CH_2$). Anal. ($C_{18}H_{15}NO$) C, H, N.

4.1.7. 3-[[[2-(4-Hydroxyphenyl)ethyl]amino]methyl]-9-methyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one hydrochloride (16). This procedure is presented as an example for the synthesis of compounds **16**–**20**. Tyramine hydrochloride (**13**) (0.43 g, 2.50 mmol) was dissolved in absolute EtOH (10 mL) by gentle heating. After being cooled, **6** (0.50 g, 2.50 mmol), and paraformaldehyde (0.07 g, 2.50 mmol) were added to the mixture which was then refluxed for 10 h. After the reaction mixture was cooled, the precipitate was filtered off, washed with diethyl ether and triturated in acetone. The solid was filtered off, dried and recrystallized from EtOH/diethyl ether (1/2, v/v) to give **16** (0.20 g, 21%) as a pure product: mp 216–218 °C; IR (KBr, selected lines) cm^{-1} 3242, 2939, 2787, 1613, 1516, 1481, 1468, 1262, 833, 756; ^1H NMR (DMSO-d_6) δ 9.41 (s, 1H, OH which exchanges with D_2O), 8.06–7.94 (m, 1H, aromatic), 7.62–7.51 (m, 1H, aromatic), 7.34–7.18 (m, 2H, aromatic), 7.17–7.02 (m, 2H, aromatic), 6.81–6.67 (m, 2H, aromatic), 3.75 (s, 3H, NCH_3), 3.55–3.39 (m, 1H, COCH), 3.28–2.81 (m, 8H, CH_2), 2.55–2.29 (m, 1H, $COCHC\mathit{H}_A\mathrm{H}_B\mathrm{CH}_2$), 2.12–1.86 (m, 1H, $COCHCH_A\mathit{H}_B\mathrm{CH}_2$); ^{13}C NMR (DMSO-d_6) δ 192.30, 156.06, 153.36, 137.55, 129.62, 127.21, 124.12, 122.84, 122.38, 120.06, 115.43, 115.34, 110.48, 48.81, 47.25, 42.38, 30.54, 29.92, 26.93, 20.97. Anal. ($C_{22}H_{25}ClN_2O_2$) C, H, N.

4.1.8. 3-[[[2-(4-Hydroxyphenyl)ethyl]amino]methyl]-9-(1-propyl)-1,2,3,9-tetrahydro-4*H*-carbazol-4-one hydrochloride (17). The same procedure, as described for the synthesis of **16**, was followed using compound **7** as starting material. Recrystallization from EtOH gave **17** (10%) as a pure product: mp 198–200 °C; IR (KBr, selected lines) cm^{-1} 3293, 2952, 2814, 1627, 1516, 1436, 1268, 1209, 831, 751; ^1H NMR (DMSO-d_6) δ 9.39 (s, 1H, OH which exchanges with D_2O), 8.09–7.97 (m, 1H, aromatic), 7.70–7.56 (m, 1H, aromatic), 7.35–7.17 (m, 2H, aromatic), 7.17–7.00 (m, 2H, aromatic), 6.84–6.68 (m, 2H, aromatic), 4.20 (t, J = 6.8 Hz, 2H, NCH_2), 3.55–3.39 (m, 1H, COCH), 3.29–2.82 (m, 8H, CH_2), 2.46–2.28 (m, 1H, $COCHC\mathit{H}_A\mathrm{H}_B\mathrm{CH}_2$), 2.12–1.88 (m, 1H, $COCHCH_A\mathit{H}_B\mathrm{CH}_2$), 1.87–1.65 (m, 2H, NCH_2CH_2), 0.90 (t, J = 7.2 Hz, 3H, $NCH_2CH_2CH_3$); ^{13}C NMR (DMSO-d_6) δ 192.55, 156.06, 152.95, 136.93, 129.64, 127.21, 124.23, 122.89, 122.35, 120.18, 115.34, 110.78, 110,56, 48.79, 47.26, 44.57, 42.39, 30.60, 27.10, 22.54, 21.10, 11.09. Anal. ($C_{24}H_{29}ClN_2O_2$) C, H, N.

4.1.9. 3-[[[2-(4-Hydroxyphenyl)ethyl]amino]methyl]-9-phenylmethyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one hydrochloride (18). The same procedure, as described for the synthesis of **16**, was followed using compound **8** as starting material. Recrystallization from 2-propanol gave **18** (9%) as a pure product: mp 208–210 °C; IR (KBr, selected lines) cm^{-1} 3100, 2930, 2681, 1615, 1516, 1457, 1262, 1127, 1075, 832; ^1H NMR (DMSO-d_6) δ 9.40 (s, 1H, OH which exchanges with D_2O), 8.11–7.98 (m, 1H, aromatic), 7.63–7.49 (m, 1H, aromatic), 7.44–7.14 (m, 2H + 5H, aromatic), 7.14–7.02 (m, 2H, aromatic), 6.82–6.67 (m, 2H, aromatic), 5.53 (s, 2H, NCH_2), 3.55–3.39 (m, 1H, COCH), 3.29–2.83 (m, 8H, CH_2), 2.46–2.30 (m, 1H, $COCHC\mathit{H}_A\mathrm{H}_B\mathrm{CH}_2$), 2.15–1.90 (m, 1H, $COCHCH_A\mathit{H}_B\mathrm{CH}_2$). Anal. ($C_{28}H_{29}ClN_2O_2 \cdot \frac{1}{2}H_2O$) C, H, N.

4.1.10. 9-Cyclohexylmethyl-3-[[[2-(4-hydroxyphenyl)-ethyl]amino]methyl]-1,2,3,9-tetrahydro-4*H*-carbazol-4-one hydrochloride (19). The same procedure, as described for the synthesis of **16**, was followed using compound **9** as starting material. Recrystallization from 2-propanol gave

19 (10%) as a pure product: mp 194–195 °C; IR (KBr, selected lines) cm^{-1} 3245, 2927, 2850, 1611, 1516, 1453, 1263, 832, 759; ^1H NMR (DMSO-d_6) δ 9.41 (s, 1H, OH which exchanges with D_2O), 8.10–7.95 (m, 1H, aromatic), 7.66–7.53 (m, 1H, aromatic), 7.35–7.16 (m, 2H, aromatic), 7.15–6.98 (m, 2H, aromatic), 6.85–6.68 (m, 2H, aromatic), 4.07 (d, $J = 7.0$ Hz, 2H, NCH_2), 3.58–3.45 (m, 1H, COCH), 3.28–2.85 (m, 8H, CH_2), 2.46–2.29 (m, 1H, $COCHCH_AH_BCH_2$), 2.13–1.92 (m, 1H, $COCHCH_AH_BCH_2$) 1.92–1.40 (m, 6H, cyclohexyl), 1.34–0.91 (m, 5H, cyclohexyl). Anal. ($C_{28}H_{35}ClN_2O_2 \cdot \frac{1}{2}H_2O$) C, H, N.

4.1.11. 3-[[[2-(4-Hydroxyphenyl)ethyl]amino]methyl]-9-phenyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one hydrochloride (20). The same procedure, as described for the synthesis of **16**, was followed using compound **12** as starting material. Recrystallization from 2-propanol/diethyl ether (1:1, v/v) gave **20** (10%) as a pure product: mp 215 °C; IR (KBr, selected lines) cm^{-1} 3292, 2940, 1650, 1614, 1509, 1437, 1214, 759; ^1H NMR (DMSO-d_6) δ 9.39 (s, 1H, OH which exchanges with D_2O), 8.16–8.05 (m, 1H, aromatic), 7.75–7.53 (m, 5H, aromatic), 7.37–7.13 (m, 3H, aromatic), 7.13–6.98 (m, 2H, aromatic), 6.81–6.68 (m, 2H, aromatic), 3.59–3.40 (m, 1H, COCH), 3.27–2.72 (m, 8H, CH_2), 2.44–2.24 (m, 1H, $COCHC\mathit{H}_AH_BCH_2$), 2.14–1.90 (m, 1H, $COCHCH_AH_B\mathit{CH}_2$). Anal. ($C_{27}H_{27}ClN_2O_2 \cdot H_2O$) C, H, N.

4.1.12. 3-[[4-(2-Methoxyphenyl)piperazin-1-yl]methyl]-1,2,3,9-tetrahydro-4*H*-carbazol-4-one hydrochloride (21). This procedure is presented as an example for the synthesis of compounds **21**–**24**. 1-(2-Methoxyphenyl)piperazine hydrochloride (**14**) (0.37 g, 1.62 mmol) was solubilized in absolute EtOH (7 mL) by gentle heating. After being cooled, **5** (0.30 g, 1.62 mmol), and paraformaldehyde (0.05 g, 1.62 mmol) were added to the mixture which was then refluxed for 10 h. After the reaction mixture was cooled, the precipitate was filtered off, washed with cold EtOH and dried. Recrystallization from EtOH/diethyl ether (1/5, v/v) gave **21** (0.22 g, 32%) as a pure product: mp 206–208 °C; IR (KBr, selected lines) cm^{-1} 3145, 2918, 2837, 2572, 1614, 1500, 1452, 1242, 1176, 1023, 744; ^1H NMR (DMSO-d_6) δ 12.20 (br s, 1H, carbazole NH which exchanges with D_2O), 10.18 (br s, 1H, NH which exchanges with D_2O), 8.06–7.93 (m, 1H, aromatic), 7.52–7.36 (m, 1H, aromatic), 7.26–7.10 (m, 2H, aromatic), 7.09–6.85 (m, 4H, aromatic), 3.81 (s, 3H, OCH_3), 3.76–3.60 (m, 2H, CH_2), 3.58–3.44 (m, 2H, CH_2), 3.42–3.32 (m, 1H, COCH), 3.32–2.98 (m, 8H, CH_2), 2.50–2.36 (m, 1H, $COCHC\mathit{H}_AH_BCH_2$), 2.20–1.92 (m, 1H, $COCHCH_AH_B\mathit{CH}_2$); ^{13}C NMR (DMSO-d_6) δ 192.22, 152.51, 151.85, 139.44, 136.11, 124.48, 123.47, 122.85, 121.94, 120.90, 120.03, 118.28, 111.98, 111.83, 110.88, 56.44, 55.42, 51.91, 46.69, 40.96, 28.53, 22.17. Anal. ($C_{24}H_{28}ClN_3O_2$) C, H, N.

4.1.13. 3-[[4-(2-Methoxyphenyl)piperazin-1-yl]methyl]-9-methyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one hydrochloride (22). The same procedure, as described for the synthesis of **21**, was followed using compound **6** as starting material. Recrystallization from 2-propanol gave **22** (21%) as a pure product: mp 188–190 °C; IR (KBr, selected lines) cm^{-1} 3448, 3044, 2938, 2827, 2625, 1643, 1500, 1475, 1232, 1121, 1026, 755; ^1H NMR (DMSO-d_6) δ 10.53 (br s, 1H, NH which exchanges with D_2O), 8.10–7.95 (m, 1H, aromatic), 7.65–7.49 (m, 1H, aromatic), 7.35–7.16 (m, 2H, aromatic), 7.12–6.86 (m, 4H, aromatic), 3.81 (s, 3H, OCH_3), 3.76 (s, 3H, NCH_3), 3.74–3.58 (m, 2H, CH_2), 3.58–3.42 (m, 2H, CH_2), 3.42–2.95 (m, 1H + 8H, COCH + CH_2), 2.67–2.51 (m, 1H, $COCHC\mathit{H}_AH_BCH_2$), 2.20–1.92 (m, 1H, $COCHCH_AH_B\mathit{CH}_2$). Anal. ($C_{25}H_{30}ClN_3O_2$) C, H, N.

4.1.14. 3-[[4-(2-Chlorophenyl)piperazin-1-yl]methyl]-1,2,3,9-tetrahydro-4*H*-carbazol-4-one hydrochloride (23). The same procedure, as described for the synthesis of **21**, was followed using 1-(2-chlorophenyl)piperazine hydrochloride **15**. Recrystallization from MeOH gave **23** (13%) as a pure product: mp 204–205 °C; IR (KBr, selected lines) cm^{-1} 3446, 3069, 2940, 2846, 2579, 1647, 1582, 1468, 1228, 1176, 1038, 766; ^1H NMR (DMSO-d_6) δ 12.21 (br s, 1H, carbazole NH which exchanges with D_2O), 10.30 (br s, 1H, NH which exchanges with D_2O), 8.07–7.91 (m, 1H, aromatic), 7.58–7.05 (m, 7H, aromatic), 3.89–3.62 (m, 2H, CH_2), 3.60–3.01 (m, 1H + 10H, COCH + CH_2), 2.50–2.38 (m, 1H, $COCHC\mathit{H}_AH_BCH_2$), 2.22–1.94 (m, 1H, $COCHCH_AH_B\mathit{CH}_2$). Anal. ($C_{23}H_{25}Cl_2N_3O$) C, H, N.

4.1.15. 3-[[4-(2-Chlorophenyl)piperazin-1-yl]methyl]-9-methyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one hydrochloride (24). The same procedure, as described for the synthesis of **21**, was followed using compounds **6** and **15** as starting materials. Recrystallization from MeOH gave **24** (25%) as a pure product: mp 193 °C; IR (KBr, selected lines) cm^{-1} 3045, 2925, 2625, 1646, 1477, 1126, 1040, 759; ^1H NMR (DMSO-d_6) δ 10.37 (br s, 1H, NH which exchanges with D_2O), 8.09–7.95 (m, 1H, aromatic), 7.65–7.52 (m, 1H, aromatic), 7.51–7.05 (m, 6H, aromatic), 3.77 (s, 3H, NCH_3), 3.74–3.58 (m, 2H, CH_2), 3.56–2.96 (m, 1H + 10H, COCH + CH_2), 2.64–2.50 (m, 1H, $COCHC\mathit{H}_AH_BCH_2$), 2.20–1.92 (m, 1H, $COCHCH_AH_B\mathit{CH}_2$). Anal. ($C_{24}H_{27}Cl_2N_3O$) C, H, N.

4.1.16. 3-[[2-(4-Hydroxyphenyl)ethyl]amino]-1-(indol-3-yl)propan-1-one hydrochloride (26). This procedure is presented as an example for the synthesis of compounds **26**–**28**. Tyramine hydrochloride (**13**) (0.54 g, 3.14 mmol) was dissolved in absolute EtOH (15 mL) by gentle heating. After being cooled, 3-acetyl-1*H*-indole (**25**) (0.50 g, 3.14 mmol) and paraformaldehyde (0.09 g, 3.14 mmol) were added to the mixture which was then refluxed for 10 h. After the reaction mixture was cooled, the precipitate was filtered off, washed with EtOH and triturated twice in acetone. The solid was filtered off, dried and recrystallized from DMF/ethyl acetate (1/3, v/v) to give **26** (0.13 g, 12%) as a pure product: mp 206–207 °C; IR (KBr, selected lines) cm^{-1} 3262, 3093, 2929, 2784, 1615, 1516, 1439, 1242, 1141, 742; ^1H NMR (DMSO-d_6) δ 12.21 (br s, 1H, indole NH which exchanges with D_2O), 9.42 (s, 1H, OH which exchanges with D_2O), 9.10 (br s, 2H, NH which exchanges with D_2O), 8.41 (s, 1H, indole), 8.46–8.35 (m, 1H, indole), 7.60–7.43 (m, 1H, indole), 7.34–7.14 (m, 2H, indole), 7.13–6.98

(m, 2H, aromatic), 6.84–6.65 (m, 2H, aromatic), 3.40–3.22 (m, 4H, CH_2), 3.21–3.05 (m, 2H, CH_2), 2.97–2.76 (m, 2H, CH_2). Anal. ($C_{19}H_{21}ClN_2O_2 \cdot \frac{1}{2}H_2O$) C, H, N.

4.1.17. 3-[4-(2-Methoxyphenyl)piperazin-1-yl]-1-(indol-3-yl)propan-1-one hydrochloride (27). The same procedure, as described for the synthesis of **26**, was followed using compound **14** as starting material. Recrystallization from DMF/ethyl acetate (1/3, v/v) gave **27** (63%) as a pure product: mp 220–222 °C; IR (KBr, selected lines) cm^{-1} 3128, 2926, 2672, 1647, 1496, 1439, 1242, 1148, 754; ^1H NMR (DMSO-d_6) δ 12.22 (br s, 1H, indole NH which exchanges with D_2O), 10.97 (br s, 1H, NH which exchanges with D_2O), 8.48 (s, 1H, indole), 8.26–8.12 (m, 1H, indole), 7.57–7.43 (m, 1H, indole), 7.32–7.14 (m, 2H, indole), 7.10–6.84 (m, 4H, aromatic), 3.80 (s, 3H, OCH_3), 3.74–3.42 (m, 8H, CH_2), 3.40–2.95 (m, 4H, CH_2). Anal. ($C_{22}H_{26}ClN_3O_2$) C, H, N.

4.1.18. 3-[4-(2-Chlorophenyl)piperazin-1-yl]-1-(indol-3-yl)propan-1-one hydrochloride (28). The same procedure, as described for the synthesis of **26**, was followed using compound **15** as starting material. Recrystallization from DMF gave **28** (22%) as a pure product: mp 227–228 °C; IR (KBr, selected lines) cm^{-1} 3129, 2922, 2674, 2578, 1651, 1522, 1438, 1242, 1148, 940, 754; ^1H NMR (DMSO-d_6) δ 12.22 (br s, 1H, indole NH which exchanges with D_2O), 11.02 (br s, 1H, NH which exchanges with D_2O), 8.48 (s, 1H, indole), 8.28–8.13 (m, 1H, indole), 7.60–7.05 (m, 3H + 4H, indole + aromatic), 3.83–3.06 (m, 12H, CH_2). Anal. ($C_{21}H_{23}Cl_2N_3O$) C, H, N.

4.2. Binding experiments on human cloned α_1-AR subtypes

4.2.1. Transfection and cell culture. HEK293 cells were transfected with the constitutively active pRSVICAT vectors containing the human α_{1A}-AR,[25] α_{1B}-AR,[26] or α_{1D}-AR[27] cDNAs by calcium phosphate transfection.[28] Cells were propagated for several weeks in the presence of 400 μg/mL geneticin, and subclones screened by radioligand binding for high receptor expression. Transfected HEK293 cells were propagated in 75 cm^2 flasks at 37 °C in a humified 5% CO_2 incubator in Dulbecco's modified Eagle's medium containing 4.5 g/L glucose, 1.4% glutamine, 20 mM HEPES, 100 mg/L streptomycin, 10^5 U/L penicillin and 10% calf serum. The cells were detached by trypsinization and subcultured at a ratio of 1:4 upon reaching confluency.

4.2.2. Radioligand binding. Confluent 100-mm plates were washed with phosphate-buffered saline (18 mM Na_2HPO_4, 2 mM NaH_2PO_4 and 154 mM NaCl, pH 7.6) and harvested by scraping. Cells were collected by centrifugation and homogenized with a Polytron. Cell membranes were collected by centrifugation at 30,000*g* for 10 min and resuspended by homogenization. Receptor density was determined by saturation analysis of the α_1-AR specific antagonist radioligand [^{125}I]HEAT (20–800 pM).[29] For analysis of competition by selective drugs, 50 pM radioligand was used. Curves were analyzed by nonlinear regression analysis using GraphPad Prism.[30] Nonspecific binding was determined in the presence of 10 μM phentolamine.

4.3. pK_a determination

pK_a values for compounds **16** and **21** (as hydrochlorides) were evaluated by potentiometric determination following a literature method[31] and using an automatic titrator (Crison Compact Titrator) equipped with a combined glass pH electrode (Crison 52-02). Briefly, titrations were performed with standardised 0.1 mol/L sodium hydroxide by constant volume addition of 0.03 mL at 25 ± 1 °C under a nitrogen atmosphere and magnetic stirring. Each compound (40 mg) was dissolved in 40 mL of a methanol/water (1:3) mixture containing 0.1 M potassium chloride. A pK_a value was calculated from several points (8–10) of the titration curve using the following relationship: $pK_a = pH_d + \log[(V_{eq} - V_d)/V_d]$, where V_{eq} is the volume titrant used at the equivalence point and V_d and pH_d are the volume titrant and pH value at the datapoint, respectively. pK_a values from different datapoints of each titration curve were averaged and the standard deviation was estimated at 0.02.

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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.2006.04.002.

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