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Short communication

2-(Aminobenzyl)-1,2,3,4-tetrahydroisoquinolines: a new class of α_2-adrenergic receptor antagonists

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Summary — A new class of α_2-adrenergic receptor antagonists, the 2-(aminobenzyl)-1,2,3,4-tetrahydroisoquinolines **4** and their derivatives, is described. Two synthetic routes are reported. An investigation of the structure–activity relationships including various substitutions of the isoquinoline moiety and the benzyl group is discussed. The affinity and selectivity for both α_1- and α_2-adrenoceptors was defined by studying the displacement of [^{3}H]-prazosin (α_1-sites) and [^{3}H]-yohimbine (α_2-sites) from rat brain membranes. The 2-(2-amino-3,4-dimethoxybenzyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline **4a** presented affinity and selectivity close to yohimbine. In functional experiments the α-adrenoceptor blocking properties of **4a** have been evaluated on isolated rat aorta and on the twitch responses of the isolated rat vas deferens.

1,2,3,4-tetrahydroisoquinoline derivatives / synthesis / structure–affinity relationships / α-adrenoceptor antagonists

Introduction

During the past 20 years the α_2-adrenergic receptor has been the subject of much study and speculation [1]. A great number of physiological functions are mediated by α_2-adrenergic receptors. Their role in the treatment of variety of disease states is under investigation. Thus, an intense effort has been mounted to develop specific antagonists as potential therapeutic agents and many laboratories are focussed on this research [2–18].

There are many types of structures that have the capacity to block α_2-adrenergic receptors, and representative examples of the more important classes are shown in figure 1.

In the course of our investigation of isoquino[1,2-*b*]quinazoline derivatives [19, 20], we have discovered that substituted 2-(aminobenzyl-1,2,3,4-tetrahydroisoquinolines **4** are potent and selective α_2-adrenergic antagonists. We report here the synthesis, pharmacological evaluation and structure–affinity relationships of this structural class.

Chemistry

Compounds with the 2-benzyl-1,2,3,4-tetrahydroisoquinoline skeleton **4**, shown in table I, were prepared in the 2 following ways. The first route consisted of a cycloaddition reaction of 2-aminobenzaldehydes **1** with 3,4-dihydroisoquinolines **2** activated as hydrochlorides. The resulting tetracyclic isoquino[1,2-*b*]quinazolinium chlorides **3** were reacted with excess sodium borohydride to reduce the immonium double bond followed by ring opening to afford the 2-(2-aminobenzyl)isoquinolines **4** (scheme 1).

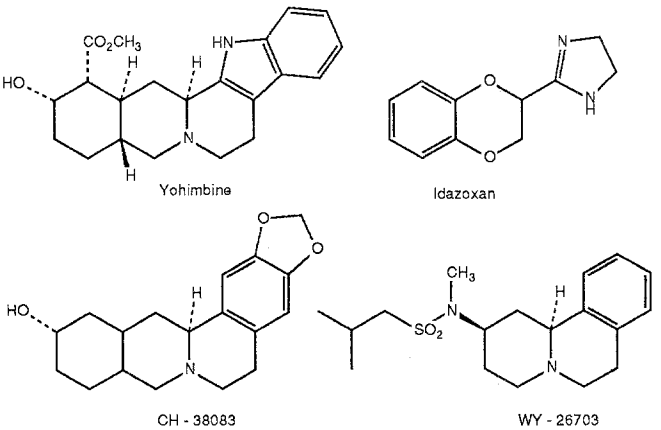
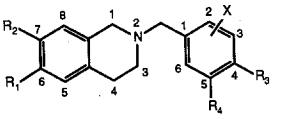
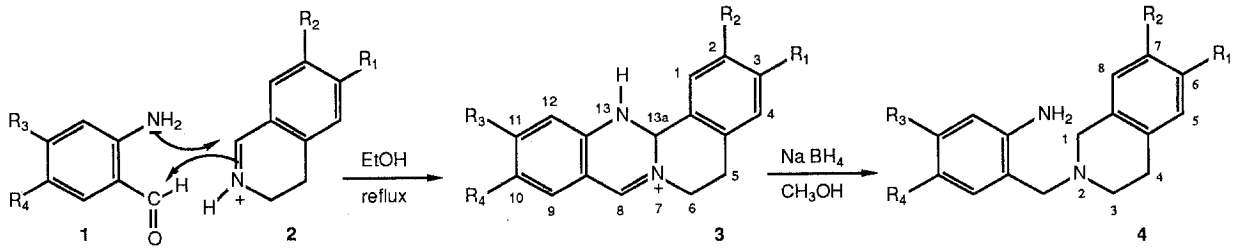


Fig 1. Examples of the more important classes of compounds blocking α_2-adrenergic receptors.

Table I. Chemical data of compounds **4a–m**, **7d–k** and **6m**.



Cpd No	R_1	R_2	R_3	R_4	X	*Synthetic route*	*Melting point (°C)*	*Formula*
4a	OCH_3	H	OCH_3	OCH_3	2-NH_2	a	101–2	$C_{19}H_{24}N_2O_3$
4b	OCH_3	OCH_3	OCH_3	OCH_3	2-NH_2	a	154	$C_{20}H_{26}N_2O_4$
4c	H	H	OCH_3	OCH_3	2-NH_2	a	106–8	$C_{18}H_{22}N_2O_2$
7d	OCH_3	OCH_3	H	H	2-NO_2	b	141–2	$C_{18}H_{20}N_2O_4$
4d	OCH_3	OCH_3	H	H	2-NH_2	a	145–6	$C_{18}H_{22}N_2O_2$
7e	OCH_3	H	H	H	2-NO_2	b	84–6	$C_{17}H_{13}N_2O_3$
4e	OCH_3	H	H	H	2-NH_2	b	75–6	$C_{17}H_{20}N_2O$
7f	H	H	Cl	H	2-NO_2	b	102–3	$C_{16}H_{15}ClN_2O_2$
4f	H	H	Cl	H	2-NH_2	b	100–1	$C_{16}H_{17}ClN_2$
7g	OCH_3	OCH_3	Cl	H	2-NO_2	b	120	$C_{18}H_{19}ClN_2O_2$
4g	OCH_3	OCH_3	Cl	H	2-NH_2	b	108–9	$C_{18}H_{21}ClN_2O_2$
7h	OCH_3	H	Cl	H	2-NO_2	b	112–3	$C_{17}H_{17}ClN_2O_3$
4h	OCH_3	H	Cl	H	2-NH_2	b	103–5	$C_{17}H_{19}ClN_2O$
7i	OCH_3	OCH_3	H	H	3-NO_2	b	133–4	$C_{18}H_{20}N_2O_4$
4i	OCH_3	OCH_3	H	H	3-NH_2	b	119–21	$C_{18}H_{22}N_2O_2$
7j	H	H	H	H	2-NO_2	b	78	$C_{16}H_{16}N_2O_2$
4j	H	H	H	H	2-NH_2	b	64–6	$C_{16}H_{18}N_2$
7k	OCH_3	OCH_3	NO_2	H	H	b	135–6	$C_{18}H_{20}N_2O_4$
4k	OCH_3	OCH_3	NH_2	H	H	b	123–4	$C_{18}H_{22}N_2O_2$
4l	OCH_3	OCH_3	H	H	H	b	210–2	$C_{18}H_{21}NO_2$, HCl
4m	H	H	H	H	H	b	218–20	$C_{16}H_{17}N$, HCl
6m		1,2,3,4-Tetrahydroisoquinoline				b	195–7	$C_9H_{11}N$, HCl



Scheme 1.

An alternative synthesis of compounds **4** was achieved following a classical method starting from nitrotoluenes. The methyl group was brominated with *N*-bromosuccinimide. The resulting nitrobenzyl bromides **5** were condensed with 1,2,3,4-tetrahydroisoquinolines **6** in refluxed ethanol. Nitrocompounds **7** were then reduced with zinc in acetic medium to give amino derivatives **4** (scheme 2).

The latter scheme was suitable for the introduction of varied groups in the benzyl ring in order to explore their structure–activity relationships. The importance of the benzyl and the amino groups was examined. In addition, what role substituents on the 2 aromatic rings of 2-benzyl-1,2,3,4-tetrahydroisoquinoline play in the affinity and selectivity of adrenergic receptors was investigated.

Biological results and discussion

Relative affinities of the aminocompounds **4a–m**, their nitro precursors **7d–k** and the 1,2,3,4-tetrahydroisoquinoline **6m** for the α-adrenoceptors were determined by measuring radioligand displacement from binding sites of rat central cortex. α_1- and α_2-adrenoceptor binding constants were obtained by studying the displacement of [^{3}H]-prazosin (α_1-sites) and [^{3}H]-yohimbine (α_2-sites). The results of the affinity evaluation are given in table II.

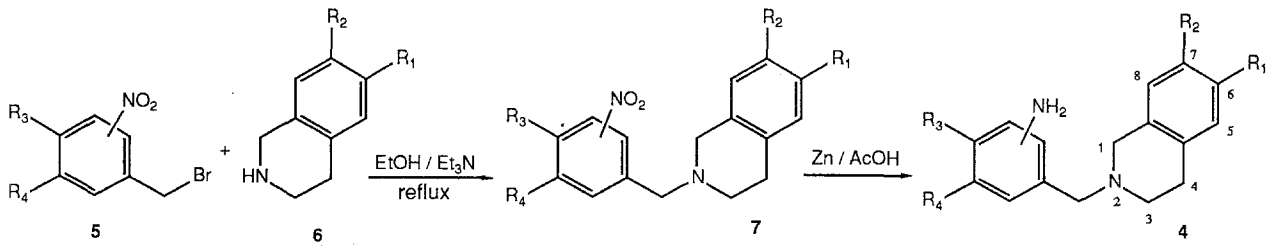
In order to determine the structural features involved in the activity, the first approach was to successively evaluate the effect of the 1,2,3,4-tetrahydroisoquinoline **6m** devoid of the 2-benzyl ring, then the 2-benzyl analogue **4m** and the 2-(2-aminobenzyl) derivative **4j**. With regard to results, it appears that the isoquinoline ring is responsible for the activity. Substitution of the nitrogen atom by the benzyl group produced a significant increase in affinity. Furthermore, introduction of the amino group reinforced this effect. In the search for further structure–activity relationships, we investigated different ways of varying the substitution of **4j** on the isoquinoline moiety and on the aminobenzyl ring.

Table II. Pharmacological activity in binding study of compounds **4a–m**, **7d–k** and **6m** compared to prazosin and yohimbine.

Compound No	$K_i\ \alpha_1$ *(nM)*	$K_i\alpha_2$ *(nM)*	*Selectivity*[a]
4a	993 ± 50	63 ± 20	15.7
4b	2939 ± 400	811 ± 120	3.6
4c	3224 ± 70	220 ± 80	14.7
7d	> 10 000	1600 ± 400	–
4d	3411 ± 280	1714 ± 300	2.0
7e	> 10 000	929 ± 40	–
4e	> 10 000	2900 ± 990	–
7f	> 10 000	2738 ± 1000	–
4f	> 10 000	1400 ± 400	–
7g	> 10 000	3367 ± 1200	–
4g	2589 ± 500	1870 ± 700	1.4
7h	> 10 000	2230 ± 600	–
4h	1797 ± 350	155 ± 35	11.6
7i	> 10 000	721 ± 200	–
4i	4090 ± 800	1041 ± 140	3.9
7j	> 10 000	1377 ± 370	–
4j	6300 ± 1200	410 ± 90	15.4
7k	> 10 000	677 ± 110	–
4k	3081 ± 120	370 ± 30	8.3
4l	> 10 000	775 ± 240	–
4m	8025 ± 800	1185 ± 230	6.8
6m	> 10 000	2500 ± 400	–
Prazosin	0.15	374	0.0004
Yohimbine	347	17	20.4

[a]Selectivity was estimated from the ratio $K_i\ \alpha_1/K_i\ \alpha_2$.

Any modification in the affinity was observed when 2 methoxy groups were introduced at the C_6 and C_7 positions of the isoquinoline part ($R_1 = R_2 = CH_3O$). But the presence of only 1 methoxy group at the C_6 position ($R_1 = CH_3O$) increased the α_2-affinity (**4a** *versus* **4b** and **4h** *versus* **4g**), whereas no change in potency for α_1-sites appeared. On the contrary, the introduction of 2 methoxy groups in the benzylic ring enhanced the affinity and selectivity for the α_2-sites (**4a** *versus* **4e** and **4c** *versus* **4j**). Adding of an electron-withdrawing group, like the chlorine atom of **4f–h** instead of the 2 methoxy groups, led to a decrease in the α_1-affinity (**4a** *versus* **4h** and **4c** *versus* **4f**).



Scheme 2.

We also investigated the displacement of the amino group to the *meta* (**4i**) or *para* (**4k**) positions. The *meta* position did not affect the affinity (**4d** *versus* **4i**). However, the *para* position increased the α_2-affinity (**4d** *versus* **4k**), probably due to an isosteric effect of the aromatic amine instead of the methoxy group.

The replacement of the amino by a nitro group, **7e–k**, was also investigated. In this series the selectivity was considerably increased by the lack of α_1-affinity. However, on the whole, these compounds exhibited a lower level of α_2-affinity with respect to the parent compound **4a**.

Compound **4a** is a potent competitor of [^{3}H]-prazosin and [^{3}H]-yohimbine binding to α_1- and α_2-adrenoceptor binding sites respectively in rat cortex membranes. A marked difference in the selectivity of **4a** for either α_1- or α_2-adrenoceptor binding sites is observed. **4a** is 15-fold more selective for the α_2-adrenoceptor than for the α_1-adrenoceptor. Its affinity for α_2- binding sites is similar to those observed for yohimbine.

In preliminary studies (data non shown), compound **4a** was unable to displace [^{3}H]-dexetimine (D_2 dopaminergic ligand), [^{3}H]-ketanserine (serotoninergic ligand), [^{3}H]-pyrilamine (H_1 histamine ligand) and [^{3}H]-nitrendipine (dihydropyridine ligand) respectively from their binding sites at concentrations < 100 μM.

In isolated rat aortic rings, compound **4a** inhibited the agonist activity of BHT-920, producing parallel shifts to the right of the concentration–response curves without affecting the maximal response. **4a** presented no partial agonist effect, whatever the concentration used. These experiments demonstrated the α_2-blocking activity of **4a**. The K_b value was $\approx$ 10 μM.

In order to determine the potency and selectivity of compound **4a** on postjunctional α_1- and prejunctional α_2-adrenoceptors, functional studies were performed on rat vas deferens. The effects of **4a** on twitch responses of field-stimulated or on phenylephrine-induced contractions were measured. Clonidine produced a dose-dependent inhibition of the twitch response of field-stimulated vas deferens. EC_{50} (concentration inducing 50% maximal response) value of 5.72 $\pm$ 0.05 nM was determined. **4a**, yohimbine and prazosin inhibited the agonist activity of clonidine, producing parallel shifts to the right of the concentration–response curves. The slopes of the Schild plots were close to unity for **4a** and yohimbine indicating a competitive antagonism quantified by pA_2 values reported in table III. The Schild plot slope was statistically different from unity for prazosin. In the latter case the potency of the antagonism was evaluated by the apparent dissociation constant (–log K_b).

The postsynaptic α_2-adrenoceptor antagonistic potency of **4a** compared to prazosin and yohimbine was determined on phenylephrine-induced contractions of rat vas deferens (table III). Parallel displacement of phenylephrine concentration–response curves were produced only by yohimbine and prazosin, **4a** inducing an apparent non competitive antagonism (Schild plot slope significantly different from unity).

The experiments performed on the rat vas deferens demonstrate the α-blocking activity of compound **4a** and confirm that it is a selective competitive antagonist of the α_2-adrenoceptors. **4a** is shown to be more potent at presynaptic α_2 than at the postsynaptic α_1-adrenoceptors in the vas deferens, since **4a** presents an antagonistic effect on phenylephrine-induced contractions at higher concentrations. The affinity of **4a** for the postsynaptic α_1-adrenoceptor as measured by ability to block phenylephrine-mediated constrictor response in rat vas deferens (apparent $K_b \approx$ 3 μM) is much lower than for the presynaptic α_2-adrenoceptor as measured by potency to antagonize clonidine-induced inhibition of the twitch responses of field-stimulated rat vas deferens (apparent $K_b \approx$ 0.2 μM). The α_2-selectivity is confirmed by this functional study on rat vas deferens which presents dissociable α_1- and α_2-adrenoceptor-mediated effects. The apparent affinity for α_2-adrenoceptors estimated on functional studies in rat vas deferens is in good agreement with that measured in binding experiments.

Table III. Antagonism results of compound **4a** compared to prazosin and yohimbine on isolated rat vas deferens.

Compound	*Antagonism of the clonidine effect*		*Antagonism of the phenylephrine effect*	
	pA_2	*Slope*	pA_2	*Slope*
4a	6.7	1.05	5.54[a]	–
Yohimbine	7.59	1.15	6.08	0.85
Prazosin	5.14[a]	–	8.34	0.99

[a]–log K_b; slope significantly different from unity.

Conclusion

Thus, the major finding of this study is to describe a new class of α_2-adrenergic receptor antagonists possessing the 2-(aminobenzyl)-1,2,3,4-tetrahydroisoquinoline skeleton. Among the compounds tested, **4a** appears to be a novel α_2-antagonist, devoid of partial agonist activity as observed for other α_2-antagonists such as idazoxan, without affinity for dopamine, serotonin, histamine receptor and the dihydropyridine sites. An interesting α_1-/α_2-selectivity is observed for this derivative with a chemical structure which is different from that of other α_2-antagonist families.

Experimental protocols

Chemistry

Melting points (mp) were determined on Kofler hot stage apparatus and are uncorrected. Proton NMR spectra were recorded on a Brucker AC 200 spectrometer. Infrared spectra were performed on a Beckman IR 4230 spectrometer. All new substances exhibited spectroscopic data consistent with the assigned structures. Analyses indicated by elemental symbols were within 0.4% of the theoretical values and were performed by the Central Service of Microanalyses in Vernaison. All TLC were performed on Merck silica gel F-254 plates (chloroform/ethyl acetate/triethylamine, 25:25:1).

The experimental procedures have been detailed as follows:

*Route a – synthesis of **4a***

*3,10,11-Trimethoxy-5,6,13,13a-tetrahydroisoquino[1,2-b]-quinazolinium chloride **3a**.* A mixture of 2-amino-4,5-dimethoxybenzaldehyde **1a** [21] (1.50 g, 8.3 mmol), 6-methoxy-3,4-dihydroisoquinoline hydrochloride **2a** [22] (1.64 g, 8.3 mmol) and absolute ethanol (50 ml) were refluxed under stirring for 1 h. After cooling and addition of ether (50 ml), the solid which precipitated was collected, washed with ether and recrystallized from ethanol. Yield: 2.51 g (84%); mp: 264°C decomp IR ($CHCl_3$) cm^{-1}: 3440 (ν_{NH}); 1625 ($\nu_{CH=N^+}$). ^{1}H-NMR ($CDCl_3$) δ: 2.75 (m, 2H, H_5); 3.80 (s, 3H, OCH_3 at C_3); 3.90 (s, 3H, OCH_3 at C_{10}); 3.95 (m, 2H, H_6); 4.10 (s, 3H, OCH_3 at C_{11}); 5.60 (s, 1H, H_{13a}); 6.45 (s, 1H, H_{12}); 6.80 (s, 1H, H_9); 7.15 (m, 3H, $H_{1,2,4}$); 8.75 (s, 1 H, H_8); 9.20 (s, 1 H, NH).

*2-(2-Amino-4,5-dimethoxybenzyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline **4a**.* To a methanolic solution (50 ml) of **3a** (2.50 g, 6.9 mmol) sodium borohydride (0.2 g, 5.3 mmol) was added portionwise under continuous stirring. After 30 min the solvent was removed *in vacuo*. The resulting oil was dissolved in chloroform (50 ml). The organic phase was washed with water, dried over magnesium sulfate, filtered and evaporated under reduced pressure. The solid residue was recrystallized from ethanol/ether. Yield: 1.25 g (55%); mp: 101–102°C. IR (KBr) cm^{-1}: 3340 and 3230 (ν_{NH_2}). ^{1}H-NMR ($CDCl_3$) δ: 2.70 (m, 4H, $H_{3,4}$); 3.45 (s, 2H, H_1 or benzylic CH_2); 3.60 (s, 2H, benzylic CH_2 or H_1); 3.75 (s, 3H, OCH_3 at C_6); 3.80 (s, 6H, 2 benzylic OCH_3); 4.50 (s, 2H, NH_2); 6.25–6.70 (m, 5H, ArH). Anal $C_{19}H_{24}N_2O_3$ (C, H, N).

*Route b – synthesis of **4h***

4-Chloro-2-nitrobenzyl bromide. 4-Chloro-2-nitrotoluene (2.5 g, 14.5 mmol), *N*-bromosuccinimide (3 g, 16.7 mmol), benzoyl peroxide (0.1 g) and CCl_4 (50 ml) were heated under reflux for 6 h. After cooling the mixture was filtered and the filtrate evaporated to dryness under reduced pressure. The residual oil was used in the following step without further purification. Yield: 3.58 g (98%). IR ($CHCl_3$) cm^{-1}: 1555 (ν_{NO_2}). ^{1}H-NMR ($CDCl_3$) δ: 4.78 (s, 2H, CH_2Br); 7.40 (m, 3H, Ar-H).

*2-(4-Chloro-2-nitrobenzyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline **7h**.* A mixture of 4-chloro-2-nitrobenzyl bromide (3.5 g, 13.9 mmol), 6-methoxy-1,2,3,4-tetrahydroisoquinoline **6h** [22] (2.3 g, 14.1 mmol), absolute ethanol (30 ml) and triethylamine (1.75 g, 34.7 mmol) was stirred at room temperature for 3 h. The resulting compound **7h** was slowly precipitated. It was collected by filtration and recrystallized from ethanol. Yield: 3.16 g (68%); mp: 112–113°C. IR ($CHCl_3$) cm^{-1}: 1540 (ν_{NO_2}). ^{1}H-NMR ($CDCl_3$) δ: 3.05 (m, 4H, $H_{3,4}$); 3.70 (s, 2H, benzylic CH_2); 3.80 (s, 2H, H_1); 7.20 (m, 3H, $H_{5,7,8}$); 7.50 (dd, 1H, $J_{5,6}$ = 8.0 Hz, $J_{3,5}$ = 2.6 Hz, $H_{5'}$); 7.75 (d, 1H, $J_{5,6}$ = 8.0 Hz, $H_{6'}$); 7.85 (d, 1H, $J_{3,5}$ = 2.6 Hz, $H_{3'}$).

*2-(2-Amino-4-chlorobenzyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline **4h**.* Nitrocompound **7h** (3 g, 9.0 mmol) was dissolved in 75% aqueous acetic acid (30 ml) and zinc powder (5 g, excess) was added. The suspension was stirred and heated at 50–60°C for 2 h. The excess zinc was then filtered and the filtrate concentrated under reduced pressure. The residue was made alkaline with NH_4OH and extracted with 3 portions of CH_2Cl_2 (120 ml). The combined organic phases were washed with water, dried over magnesium sulfate and evaporated *in vacuo* to yield a crude solid which was dissolved in ethanol saturated with anhydrous gaseous hydrochloric acid. The precipitated dihydrochloride salt **4h** was recrystallized from ethanol. Yield: 2.52 g (75%); mp: 210°C. IR ($CHCl_3$) (base) cm^{-1}: 3470 and 3320 (ν_{NH_2}). ^{1}H-NMR ($CDCl_3$) (base) δ: 3.00 (m, 4H, $H_{3,4}$); 3.85 (s, 2H, benzylic CH_2); 4.10 (s, 3H, OCH_3); 4.30 (s, 2H, H_1); 4.50 (s, 2H, NH_2); 6.75 (m, 6H, ArH). Anal $C_{17}H_{19}ClN_2O$ (C, H, N).

Pharmacology

α_1- and α_2-binding assay procedure

Rat brain membranes were prepared as described by Greengrass and Bremmer [23] with some modifications [24].

Male Wistar rats weighing 250–300 g were killed by decapitation and the brain quickly removed and dissected. The cortex was homogenized in 20 vol (v/w) of ice-cold Tris–buffer (50 mmol Tris–HCl, pH 7.5 at 25°C) with an Ultraturax tissue grinder at maximal speed for 30 s. The homogenate was centrifuged at 35 000 *g* for 10 min at 4°C, the pellet resuspended in 20 vol ice-cold Tris–buffer and recentrifuged as before. The resultant pellet was resuspensed at a protein concentration of 1 mg per ml in Tris–buffer.

Binding assays were run by incubating 500 μl rat brain membrane suspensions at 25°C for 30 min with 0.2 mmol [^{3}H]-prazosin, an α_1 selective radioligand (NEN, France, spec act 644 GBq $mmol^{-1}$), or 20 min with 0.2 mmol [^{3}H]-yohimbine, an α_2-selective radioligand (NEN, France, spec act 3126 GBq $mmol^{-1}$), in a total vol of 1 ml Tris–buffer. The inhibition of the specific binding of both radioligands was determined in the presence of various concentrations of unlabelled competing drugs. Incubations were determined by rapid filtration through Whatman GF/B glass fiber filters (Whatman, Maidstone, UK)

in vacuo. The filters were rinsed 4 times with 5 ml ice-cold Tris-buffer, transferred to vials containing 8 ml Aquassure (NEN, France) and counted in liquid scintillation counter.

Non specific binding was determined in the presence of 10 μmol phentolamine and specific binding was defined as the difference between total and specific binding. In displacement experiments, the IC_{50} (concentration of competitor which displaced 50% of the maximal specific binding) and the slope factor of the displacement curve were calculated using a computerised iterative non-linear curve fitting program [25]. The equilibrium inhibition constant (K_i) of each competitor was calculated using the formula of Cheng and Prussof, $K_i = IC_{50}/1 + L/K_D$, where L is the radioligand concentration used in the experiment [26]. Each result is given as the mean ± SE of 3 experiments, each performed in triplicate.

Isolated rat aorta

Male Wistar rats (15–17-wk old) were killed by cervical dislocation. Endothelium-free rings of thoracic aorta were dissected out as previously described [27]. The rings were mounted in a 10-ml organ bath containing Krebs solution (composition in mM: NaCl 112, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1, $MgSO_4$ 1.2, $CaCl_2$ 1.25 and glucose 11) maintained at 37°C and bubbled with a mixture of 95% O_2 and 5% CO_2. Isometric responses were recorded. Following a rest period of 120 min, 2 cumulative concentration–effect curves to BHT-920 (6-allyl-2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo[4,5-*d*]azepine) were established before and after adding compound **4a** (10 min before the agonist cumulative doses). The apparent dissociation constant (–log K_b) was calculated according to Furchgott [28] to determine the α-antagonistic potency.

Isolated rat vas deferens

Rat vasa deferentia were dissected from the surrounding tissues and mounted in a 50-ml chamber containing Krebs solution at 37°C, aerated throughout the experiment with 5% CO_2 in O_2 as described by Vizi *et al* [29]. When presypnatic α_2-adrenoceptor antagonist studies were performed, field stimulation (0.2 Hz, 2 ms impulse duration, 30 V) was applied by a stimulator (Bioscience stimulator 100) and clonidine was used as an α_2-agonist to inhibit twitch responses in a concentration-dependent manner. When postsynaptic α_1-adrenoceptor antagonists were analysed, the log concentration–response curves to phenylephrine were constructed before and after antagonist addition (10 min before agonist cumulative doses). The potencies of antagonist were expressed as pA_2, calculated from Schild plots according to Arunlakshana and Schild [30]. When the Schild plot slope was different from unity, the apparent K_b was calculated.

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