Eur J Med Chem (1994) 29, 551–559

Synthesis and pharmacological evaluation of new indole derivatives structurally related to thymoxamine

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(Received 7 March 1994; accepted 14 April 1994)

Summary — The synthesis and pharmacological evaluation of a series of pyrrolidine analogues of thymoxamine allowed access to the basic SAR for the aromatic substitution pattern. The results confirm the relevance of the simultaneous presence of the hydroxy and methyl groups on the benzene ring and prompted us to prepare the corresponding indole congener. The principle of the phenol–indol bioisosterism was confirmed by the results obtained. The introduction of the *N*-(2-methoxyphenyl)piperazine moiety instead of pyrrolidine changed the receptor affinity profile and introduced a good uroselectivity.

thymoxamine / bioisosterism / indole / α-antagonist / uroselectivity

Introduction

Thymoxamine (**1**; moxisylyte, 4-[2-(dimethylamino)-ethoxy]-2-methyl-5-(1-methylethyl)phenyl acetate) is an α_1-receptor blocking agent, which has been used for many years for the treatment of vascular disorders [1–4]. Recently, pharmacological studies in dogs and rabbits [5–7] have shown that thymoxamine inhibits the urethral pressure increase induced by hypogastric nerve stimulation in a dose-dependent manner, but does not affect the increase in bladder pressure. Clinical trials have demonstrated its effectiveness in the urological field, especially regarding obstructive symptoms associated with benign prostatic hypertrophy [8, 9]. On the other hand, its scarce potency as an α_1-adrenoceptor antagonist and modest selectivity *in vivo* between the action on prostate smooth muscle and periferal vascular district cause a pharmacological action profile that is not as sharp and clean as would be desired.

The synthesis and pharmacological evaluation of a series of thymoxamine analogues characterized by substantial variations of the basic moiety of the molecule was recently reported [10–12] and 1-{2-[2-(1-methylethyl)-5-methylphenoxy]ethyl}pyrrolidine (compound **5** in table I and B 1007 in reference [12]) was indicated as the best compound with regard to potency and uroselectivity.

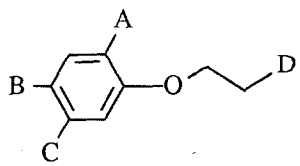
Due to our interest in the field of uroselective α_1-antagonists, we synthesized some pyrrolidinyl analogues of thymoxamine (**2–8**; see table I), focusing on modification of the aromatic substitution pattern. The affinity of these compounds and **1** for the α_1-receptor and for other members of the G-protein-coupled receptor superfamily [13] was checked by radioreceptor binding assays. Briefly, the obtained SARs indicated the relevance of the 1-methylethyl group at position 2 for a good affinity to the α_1-adrenoceptor and of the hydroxy and methyl groups at position 4 and 5 with respect to the aminoalkoxylic chain in determining receptor selectivity.

In the case of thymoxamine, it should be noted that the deacetylated compound corresponds to the active metabolite which is immediately formed after assumption of the drug (hereafter **1** is considered as a prodrug) [14–15].

In recent years, phenol–indole bioisosterism has been largely investigated as a means to discover more potent and selective pharmacologically active molecules. Moreover, this kind of isosterism offered a useful support in elucidating ligand–receptor interactions in the case of catechol or phenol amines, considering the ability of the indolic NH and the phenolic OH to form hydrogen bonds at the receptor site as a premise. Examples have been reported regarding potent indole congeners of dopamine agonist [16] or β-blocking agents such as labetalol [17].

Another role that could be played in maintaining bioisofunctionality in view of exchange of the phenol

Table I. Synthesized compounds **1–8** and their chemical properties.



Compd.	*A*	*B*	*C*	*D*	*Mp(°C) (Solvent)*	*Yield (%)*	*Molecular Formula*	*Elemental Analysis*
1	$(CH_3)_2CH$	$OCOCH_3$	CH_3	$N(CH_3)_2$	208-210[25][a] (EtOAc-EtOH)	50	$C_{16}H_{25}NO_3.HCl$	-
2	$(CH_3)_2CH$	$OCOCH_3$	CH_3	$N(CH_2)_4$[b]	182-183[12][a] (EtOAc-EtOH)	30	$C_{18}H_{27}NO_3.HCl$	-
3	$(CH_3)_2CH$	OH	CH_3	$N(CH2)_4$[b]	147-148[12][a] (EtOAc-EtOH)	60	$C_{16}H_{25}NO_2.HCl$	-
4	$(CH_3)_2CH$	OH	H	$N(CH_2)_4$[b]	130-132 (MeCN)	65	$C_{15}H_{23}NO_2.HCl$	C, H, N, Cl
5	$(CH3)_2CH$	H	CH_3	$N(CH_2)_4$[b]	157-158[12][a] (EtOAc)	55	$C_{16}H_{25}NO.HCl$	-
6	$(CH_3)_2CH$	H	H	$N(CH_2)_4$[b]	134-135 (Me_2CO)	48	$C_{15}H_{23}NO.HCl$	C, H, N, Cl
7	H	H	H	$N(CH_2)_4$[b]	157-159[26][c] (Me_2CO)	53	$C_{12}H_{17}NO.HCl$	C, H, N, Cl
8	CH_3	H	$(CH_3)_2CH$	$N(CH_2)_4$[b]	168-169 (Me_2CO)	62	$C_{16}H_{25}NO.HCl$	C, H, N, Cl

[a]Mp corresponds to that reported in the literature; [b]$N(CH_2)_4$ stands for 1-(pyrrolidinyl); [c]mp 154°C is reported in reference [26].

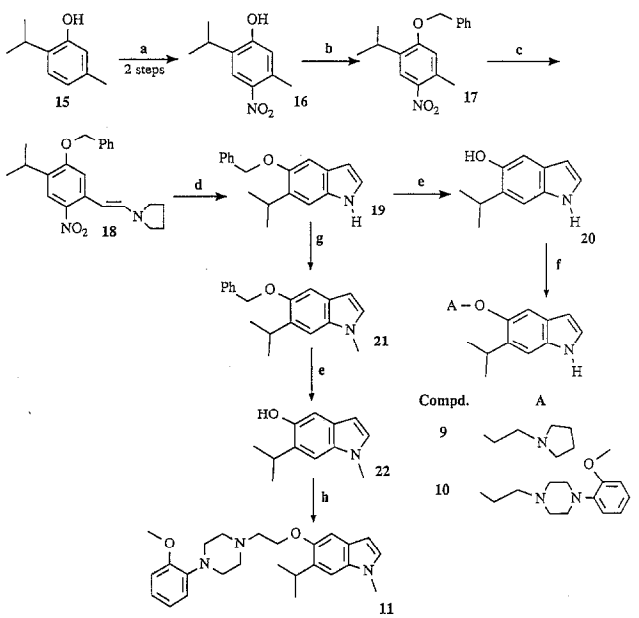
(or catechol) moiety with indole should be attributed to the electronic properties of the aromatic moiety [18], as reported for calcium entry blockers typified by verapamil [19].

It was on these premises that we carried out the bioisosteric replacement of the phenolic moiety of thymoxamine analogue **3** by synthesizing compound **9**, further modified to **10** and **11** (see scheme 1), with the aim of enhancing the affinity for the α_1-receptor and the tissue selectivity. In particular, selectivity for the prostatic urethra smooth muscle with regard to the vascular system was evaluated in a dog model similar to that described by Imagawa *et al* [20, 21]. In fact, it was shown that the prostate and the bladder neck are tissues particularly rich in the α_1-adrenoceptor [22]. Selective antagonism of this receptor population could provide benefits in treating pathological diseases that are dependent on the dynamic obstruction of the proximal urethral tract, such as in benign prostatic hypertrophy, or diseases due to neurological damage [23, 24].

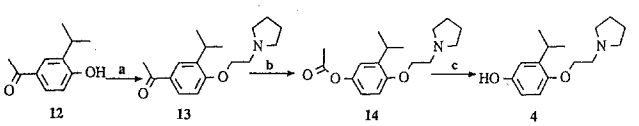
The aforementioned compounds were screened *in vitro* for binding affinity to α_1, α_2, $5HT_{1A}$, $5HT_2$ and D_2 receptors and for functional α_1-antagonism on rabbit urethral strips. The most interesting derivatives were tested also in the *in vivo* dog model for uroselectivity.

Chemistry

Compounds **1** [25], **2**, **3**, **5** [12] and **7** [26] are known and were prepared as described in the literature. Compounds **6** and **8** were synthesized from the corresponding substituted phenol by alkylation with 1-(2-chloroethyl)pyrrolidine under phase-transfer catalysis. As reported in scheme 2, compound **4** was prepared starting from 2-(1-methylethyl)-4-acetylphenol **12**



Scheme 1. a) 1. $NaNO_2$, AcOH, 98% H_2SO_4; 2. 70% HNO_3; b) BnCl, K_2CO_3, DMF; c) DMF-DMA, pyrrolidine, DMF; d) Raney Ni, $N_2H_4 \cdot H_2O$, THF/MeOH; e) H_2, Pd-C, MeOH; f, h) 1-(2-chloroethyl)pyrrolidine or 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperazine, K_2CO_3, DMF; g) CH_3I, KOH, TBBAC, toluene.



Scheme 2. a) 1-(2-Chloroethyl)pyrrolidine, 50% NaOH, TEBAC, CH_2Cl_2; b) TFA, MCPBA, $PhCH_3$; c) 1 N NaOH, EtOH.

which was alkylated by the same method as above to give the basic ether **13**. A Bayer–Villiger transposition carried out on **13** yielded the 4-acetoxy derivative **14**, which was hydrolyzed to give **4**. The chemical properties of compounds **1–8** are reported in table I.

The substituted indole ring was synthesized starting from thymol (**15**; scheme 1), which underwent nitrosation–oxidation reaction to give the 4-nitrophenol **16** [27], which in turn was etherified with benzyl chloride with almost quantitative yield. The benzyl ether **17** obtained was reacted (following the method described by Batcho and Leimgruber [28]) with dimethylformamide dimethyl acetal yielding the pyrrolidinylstyrene **18**. This was cyclized under reductive conditions with 80% hydrazine hydrate in methanol in the presence of Raney Ni to give 5-benzyloxy-6-(1-methylethyl)-indole **19**, which in turn was debenzylated with hydrogen and palladium catalysis to the 5-hydroxy derivative **20**.

Methylating **19** with methyl iodide and potassium hydroxide under phase-transfer catalysis yielded the *N*-methylindole **21**, which was deprotected in the same way as **19** to give **22**. By alkylating the hydroxy group of **20** and **22** with 1-(2-chloroethyl)pyrrolidine or 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperazine, the desired compounds **9–11** were obtained.

Attention was devoted to avoiding the use of excess HCl and exothermicity in the salification step of these compounds, in order to prevent formation of dimers and trimers of indole moieties, as reported in the literature [29].

Pharmacology

The affinity for the α_1, α_2, $5HT_{1A}$, $5HT_2$ and D_2 receptors was measured with radioligand binding assays technique using [^{3}H]prazosin, [^{3}H]rauwolscine, [^{3}H]8-OH-DPAT, [^{3}H]ketanserine and [^{3}H]spiperone, respectively. The results are reported in table II.

The functional α_1-adrenoceptor antagonist activity was determined by evaluating the compounds' ability to induce a rightward parallel shift of the concentration–response curve for the noradrenaline-induced contraction of rabbit urethral strips. The results obtained with selected compounds are listed in table III.

Table II. Receptor binding affinity of thymoxamine **1** and compounds **2–11**.

	IC_{50} (nM)				
Compd.	α_1	α_2	$5HT_2$	$5HT_{1A}$	D_2
1	934	7466	>10000	8675	>10000
2	445	6450	5480	>10000	>10000
3	109	5490	5092	>10000	>10000
4	1780	1586	-	3882	>10000
5	128	1769	2771	928	>10000
6	482	1094	814	530	3621
7	8377	839	>10000	4255	>10000
8	6310	1697	4833	130	>10000
9	63	700	1194	7315	9412
10	17	32	325	185	136
11	70	40	1391	147	91

Table III. *In vitro* and *in vivo* pharmacological tests on selected compounds.

Compd.	Rabbit urethra pA_2	Dog Model (N.A.) UP $ED_{50}(\mu g/kg)$	Dog Model (N.A.) DBP $ED_{25}(\mu g/kg)$
2	6.55		
3	7.12		
5	7.21	4.3	5.1
6	5.73		
9	7.48	14.8	10.4
10	7.22	8.7	74.7
11	6.65		
Prazosin	8.15	3.6	6.6
Phentolamine	7.72	12.6	124.4

The *in vivo* tissue selectivity was determined for the compounds having a good functional activity using a dog model derived from that described by Imagawa *et al* [20, 21]. The reduction of the diastolic blood pressure [ED_{25}] and the inhibition of urethral prostatic pressure [ED_{50}] after stimulation by intrarterial injection of noradrenaline were assessed. The results are reported in table III.

Results and discussion

As far as the *N*-(phenoxyethyl)pyrrolidine derivatives **1–8** are concerned, affinity for the α_1-receptor is the main property. Some SARs can be drawn for this subset: (i) pyrrolidine seems moderately better than dimethylamino group (compound **2** *vs* **1**); (ii) the presence of the 1-methylethyl group at position 2 is mandatory for a good affinity to the α_1-adrenoceptor (compound **6** *vs* **7** and **5** *vs* **8**); and (iii) the simultaneous presence of the 4-hydroxy and 5-methyl groups enhances the affinity for the α_1-adrenoceptor (compound **3** *vs* **4**) and confers selectivity for this receptor with regard to the 5-HT_{1A} receptor (compound **3** *vs* **5**).

In the aggregate, compounds **1–8** showed practically no affinity for the D_2 and 5-HT_2 receptors. Compounds **7** and **8** showed moderate affinity only for the α_2 and $5HT_{1A}$ receptors, respectively.

The compounds exhibiting a good affinity for the α_1-adrenoceptor (**2**, **3**, **5** and **6**) were also tested in the rabbit urethra test for assessing their functional antagonism to noradrenaline.

These compounds were competitive antagonists, compound **5** proving the most potent in this subset, in agreement with the radioreceptor-binding-affinity results and confirming that the presence of the phenolic group in these pyrrolidine derivatives is important in determining receptor selectivity but is not essential for potency at the α_1-adrenoceptor. Compound **5** was tested in the dog model for uroselectivity and, in contrast to what seen in the rabbit model [12], was found to be a potent α_1-antagonist both on the lower urinary tract and in lowering diastolic blood pressure; it is not uroselective (table III).

By comparing the results obtained *in vitro* with the phenol derivative **3** and its indole bioisoster **9**, we can conclude that a close similarity exists between the 2 structures, which gave practically the same pharmacological responses, perhaps with a tendency to higher potency for **9**. This compound was tested in the dog model, and proved not to be uroselective (table III).

Different results were obtained by inserting the *N*-(2-methoxyphenyl)piperazinyl moiety, instead of pyrrolidine, in compound **9** yielding **10**. This kind of basic moiety was introduced because of its presence in many different α_1-antagonists such as urapidil [30], 5-methylurapidil [31], and AR-C-239 [32].

On the other hand, the introduction of this moiety (or another similar moiety) on thymoxamine analogues was recently carried out [33], obtaining promising compounds with regard to potency and uroselectivity.

Compound **10** showed comparable potency to **9** at the α_1-receptor, both in the binding and in the functional assays. Moreover, it showed an appreciable affinity for the other investigated receptors, above all the α_2 one, and a noticeable uroselectivity; the dose active on prostatic urethra was about 9-fold lower than that inducing a 25% decrease of diastolic blood pressure (table III).

Finally, upon methylating the N-H indolic group of **10** (compound **11**), a slight decrease in α_1-affinity and antagonistic potency was achieved. This compound showed the same affinity as **10** for the α_2, $5HT_{1A}$ and D_2 receptors and slightly lower affinity for the $5HT_2$ site.

Conclusions

The comparison of the results obtained with the pyrrolidine derivatives **3** and **9** fully confirmed the concept of the phenol–indole bioisosterism, since these 2 compounds showed practically the same potency and selectivity for the α_1-adrenoceptor both in the radioreceptor and functional assay.

The introduction of a different basic residue, such as *N*-(2-methoxyphenyl)piperazine in compounds **10** and **11** probably led to a different kind of interaction with the receptor(s), where the N-H group, and in particular its hydrogen bonding generating capability, play a less determinant role, as confirmed by the presence of significant affinity for the other receptors investigated and by the small changes induced by its methylation.

Finally, compound **10** showed the same uroselectivity as that displayed by the molecules reported to be the most uroselective in the used dog model: phentolamine [5, 34] and tamsulosin [34].

Experimental protocols

Chemistry

Melting points (mp) were determined in open capillary tubes on a 535 Büchi apparatus and are uncorrected. IR spectra were taken on a Perkin–Elmer 287 spectrophotometer. ^{1}H-NMR spectra were recorded at 60 MHz on a Perkin–Elmer R24A and at 200 MHz on a Brucker AC-200 instrument, using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in δ units, and coupling constants are in Hertz. Flash chromatography was performed using 230–400 mesh silica gel and refers to the technique described by Still [35]. Column chromatography was performed using 70–230 mesh silica gel. Thin-layer chromatography (TLC) was performed on glass-backed silica-gel F-254 (0.25 mm thickness) plates. Elemental analyses indicated by the symbols of the elements were within ±0.4% of the theoretical values. Anhydrous dimethylformamide was obtained from drying on 3 Å molecular sieves.

*Synthesis of compounds **1**, **2**, **3**, **5** and **7***

Compound **1** was prepared by etherifying the nitrophenol **16** (scheme 1) with 2-chloroethyldimethylamine by phase-transfer catalysis following the method described for compounds **6** and **8** and reducing the nitro group to the amino group with Raney Ni and hydrazine hydrate. The subsequent de-diazohydroxylation yielded the corresponding phenol, which was finally acetylated with acetic anhydride in pyridine [25]. Compound **2** was prepared by acetylating compound **5** with acetic anhydride in toluene and carrying out Bayer–Villiger transposition on the obtained 1-{2-[4-acetyl-5-methyl-2-(1-methylethyl)phenoxy]ethyl}pyrrolidine (in analogy to the method for compound **14**, scheme 2) [12]. Compound **3** was synthesized by hydrolyzing **2** with 1 N NaOH in ethanol [12]. Compounds **5** and **7** were prepared on alkylating thymol or phenol, respectively, with 1-(2-chloroethyl)pyrrolidine by phase-transfer catalysis [12].

1-{2-[2-(1-Methylethyl)phenoxy]ethyl}pyrrolidine hydrochloride **6**

A mixture of 2-(1-methylethyl)phenol (20.43 g; 0.15 mol), 1-(2-chloroethyl)pyrrolidine hydrochloride (28.05 g; 0.165 mol), benzyl triethylammonium chloride (2.47 g; 0.011 mol), 50% NaOH (112.5 ml) and water (13.5 ml) in dichloromethane (225 ml) was vigorously stirred at reflux for 8 h.

The reaction mixture was cooled to 20°C and diluted with water (225 ml); the organic layer was recovered, washed with 0.5 N aqueous acetic acid, water, 1 N NaOH, water, dried on Na_2SO_4 and evaporated to dryness under reduced pressure. The solution of the residue in diethyl ether was decolorized with charcoal, filtered and excess ethereal HCl was added (pH 1). The title compound precipitated and was filtered and recrystallized from acetone. Yield: 19.45 g (48%); mp 134–135°C. IR (KBr): 3450, 2450, 1600, 1245 cm^{-1}. ^{1}H-NMR (DMSO–d_6) δ: 1.25 (d, 6H, $(CH_3)_2C$); 1.80–2.30 (m, 4H, pyrrolidine β CH_2); 3.20–3.90 (m, 7H, pyrrolidine α CH_2, CH_2N and $CH(CH_3)_2$); 4.60 (t, 2H, OCH_2); 7.10–7.65 (m, 4H, aromatics); 10.60–12.10 (bs, 1H, N^+H).

1-{2-[2-Methyl-5-(1-methylethyl)phenoxy]ethyl}pyrrolidine hydrochloride **8**

A mixture of 2-methyl-5-(1-methylethyl)phenol (4.5 g; 0.030 mol), NaOH (18 g; 0.450 mol), benzyl triethylammonium chloride (0.51 g; 0002 mol) in toluene (30 ml) and water (15 ml) was vigorously stirred at 65°C for 10 min. After this period a solution of 1-(2-chloroethyl)pyrrolidine hydrochloride (6.65 g; 0.039 mol) in water (7 ml) was added over 10 min.

After an additional 7 h stirring at 65°C, the mixture was diluted with water and toluene (100 + 100 ml) and extracted with toluene. After the usual procedure, the reaction crude was purified by column chromatography on silica gel (eluent: upper phase of *n*-butanol/water/acetic acid 40:50:10). The collected fractions were evaporated to dryness *in vacuo* and the residue was rinsed with water and 12.7 N NaOH and extracted with diethyl ether. The organic layer was dried on Na_2SO_4, filtered, decolorized with charcoal and acidified with ethanolic HCl. The precipitated solid was filtered and recrystallized from acetone yielding 5.3 g (63%) of **8**; mp 168–169°C. IR (Nujol): 2950, 2650, 2500, 1460, 1260 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.15 (d, 6H, $(CH_3)_2C$); 2.10 (s, 3H, phenyl CH_3); 1.30–2.30 (m, 4H, pyrrolidine β CH_2); 2.40–4.10 (m, 7H, pyrrolidine α CH_2, CH_2N, $CH(CH_3)_2$); 4.30–4.55 (m, 2H, CH_2O); 6.60–7.50 (m, 3H, aromatics); 11.30–12.50 (bs, 1H, N^+H).

1-{2-[4-Acetyl-2-(1-methylethyl)phenoxy]ethyl}pyrrolidine **13**

The title compound was obtained starting from 4-acetyl-2-(1-methylethyl)phenol (12.47 g; 0.07 mol) [36] following the procedure described for compound **6** and refluxing for 14 h. After the usual work-up, the residue was purified by flash chromatography eluting with a dichloromethane/95% ethanol (90:10) mixture. Evaporation *in vacuo* of the collected fractions gave crude **13**, which was dissolved in diethyl ether and washed with 1 N NaOH and water.

The organic layer was extracted with 1 N HCl and the acidic aqueous layer washed with ethyl acetate and diethyl ether, alkalinized with 1 N NaOH and extracted with diethyl ether. The ethereal layer was washed with water, dried on Na_2SO_4 and evaporated to dryness under reduced pressure. Yield: 12.7 g (66%); oil. IR (film): 2950, 1680, 1600, 1250 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (d, 6H, $(CH_3)_2C$); 1.70–2.00 (m, 4H, pyrrolidine β CH_2); 2.60 (s, 3H, CH_3CO); 2.60–2.90 (m, 4H, pyrrolidine α CH_2); 3.05 (t, 2H, CH_2N); 3.10–3.70 (m, 1H, $CH(CH_3)_2$); 4.30 (t, 2H, CH_2O); 7.05 (d, 1H, aromatic at position 6); 8.00 (dd, 1H, aromatic at position 5); 8.05 (s, 1H, aromatic at position 3). Anal $C_{17}H_{25}NO_2$ (C, H, N).

1-{2-[4-Acetoxy-2-(1-methylethyl)phenoxy]ethyl} pyrrolidine **14**

Trifluoroacetic acid (17.25 g; 0.1 mol) followed by 90% 3-chloroperbenzoic acid (10.12 g; 0.053 mol) were added at 10–15°C to a solution of compound **13** (12.1 g; 0.040 mol) in toluol (75 ml). Stirring was continued for 48 h at the same temperature. The reaction mixture was cooled to 0–4°C and treated with 5% NH_4OH (94 ml). After 10 min, the organic layer was separated and the aqueous layer was extracted with

toluol. The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated to dryness under reduced pressure. Yield: 10.32 g (88.5%); oil. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (d, 6H, $(CH_3)_2C$); 1.60–1.95 (m, 4H, pyrrolidine β CH_2); 2.20 (s, 3H, CH_3CO); 2.45–3.00 (m, 6H, pyrrolidine α CH_2, CH_2N); 2.90–3.50 (m, 1H, $CH(CH_3)_2$); 4.05 (t, 2H, CH_2O); 6.60–6.80 (m, 3H, aromatics). **14** was used without further purification in the next step.

*1-{2-[4-Hydroxy-2-(1-methylethyl)phenoxy]ethyl}pyrrolidine hydrochloride **4***

A mixture of compound **14** (9.32 g; 0.032 mol) and 1 N NaOH (35.2 ml) in ethanol (35 ml) was stirred at 20–25°C for 24 h. Organic solvent was removed by evaporation *in vacuo* and the residue diluted with water and extracted with diethyl ether. The combined organic layers were washed with brine, dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The oily residue was flash chromatographed eluting with a dichloromethane/methanol (85:15) mixture. The collected fractions were evaporated to dryness and the residue taken up with diethyl ether, which was washed with water, dried and distilled off, yielding compound **4** as the base, mp 77–80°C. IR (Nujol): 3160, 2950, 2480, 1500, 1460, 1210 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (d, 6H, $(CH_3)_2C$); 1.80–2.10 (m, 4H, pyrrolidine β CH_2); 2.80–3.30 (m, 6H, pyrrolidine α CH_2 and CH_2N); 3.20–3.80 (m, 1H, $CH(CH_3)_2$); 4.35 (t, 2H, OCH_2); 6.90–7.25 (m, 3H, aromatics). Anal $C_{15}H_{23}NO_2$ (C, H, N).

A slight excess of 5 N HCl in ethanol was added to a solution of the base in diethyl ether. The precipitate was recovered by filtration and recrystallized from acetonitrile. Yield: 5.98 g (65%) of **4**; mp 130–132°C.

*4-Benzyloxy-2-methyl-5-(1-methylethyl)nitrobenzene **17***

To a solution of 5-methyl-2-(1-methylethyl)-4-nitrophenol (compound **16**; scheme 1; [27]; 20 g; 0.102 mol) in anhydrous dimethylformamide (128 ml), K_2CO_3 (14.9 g; 0.107 mol) and benzyl chloride (15.3 ml; 0.133 mol) were added. The reaction mixture was stirred at 90°C for 3.5 h, cooled to 20°C, poured into water and filtered. The precipitated solid was purified by flash chromatography eluting with a benzene/ethyl acetate (96:4) mixture to give 28 g (96%) of **17**; mp 90–91°C (petroleum ether). IR (Nujol): 3000, 1620, 1580, 1500, 1340, 1260 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (d, 6H, $(CH_3)_2C$); 2.40 (s, 3H, phenyl CH_3); 2.90–3.40 (m, 1H, $CH(CH_3)_2$); 5.00 (s, 2H, CH_2O); 6.50 (s, 1H, aromatic at position 6); 7.10 (m, 5H, benzyl ring aromatics); 7.70 (s, 1H, aromatic at position 3). Anal $C_{17}H_{19}NO_3$ (C, H, N).

*(E)-1-{2-[(5-Benzyloxy)-4-(1-methylethyl)-2-nitrophenyl]ethenyl}-pyrrolidine **18***

To a solution of compound **17** (22 g; 0.077 mol) in anhydrous dimethylformamide (57 ml), dimethylformamide dimethyl acetal (15.4 ml; 0.115 mol) and pyrrolidine (9.65 ml; 0.115 mol) were added and the resulting red solution was stirred for 4 h at 110°C. After this period the volume of the solution was concentrated to 1/3 of the initial volume by distillation under reduced pressure (70°C; 20 mmHg) and cooled to 5°C for 24 h. The precipitate was filtered and recrystallized from methanol to give 25.6 g of compound **18** (90%); mp 114–115°C. IR (KBr): 2950, 2850, 1640, 1600, 1580, 1240 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.15 (d, 6H, $(CH_3)_2C$); 1.70–2.00 (m, 4H, pyrrolidine β CH_2); 3.00–3.55 (m, 5H, pyrrolidine α CH_2 and $CH(CH_3)_2$); 5.00 (s, 2H, CH_2O); 5.80 (d, 1H, J = 16 Hz, CH=CHN); 6.60 (s, 1H, aromatic at position 6); 6.90 (d, 1H, J = 16 Hz, =CHN); 7.20– 7.25 (m, 5H, benzyl ring aromatics); 7.60 (s, 1H, aromatic at position 3). Anal $C_{22}H_{26}N_2O_3$ (C, H, N).

*5-Benzyloxy-6-(1-methylethyl)indole **19***

To a suspension of compound **18** (7.88 g; 0.021 mol) in tetrahydrofuran (70 ml) and methanol (70 ml), under N_2 atmosphere, wet Raney Ni (1.0 g) and 98% hydrazine hydrate (1.55 ml, 0.032 mol) were added. The mixture was stirred at 45°C for 4.5 h. During this period, 98% hydrazine hydrate (2 x 1.55 ml; 0.065 mol) was added at room temperature after 0.5 and 1.5 h. The mixture was filtered from catalyst and the solvents evaporated *in vacuo*. The crude product was purified by flash chromatography eluting with a petroleum ether/ethyl acetate (92:8) mixture to give 4.0 g (70%) of compound **19**; mp 113–114°C (cyclohexane). IR (KBr): 3400, 2950, 1480, 1460, 1300, 1160 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (d, 6H, $(CH_3)_2C$); 3.51 (m, 1H, $CH(CH_3)_2$); 5.12 (s, 2H, CH_2); 6.40–6.46 (m, 1H, indole H_3); 7.10 (dd, 1H, indole H_2); 7.15 (s, 1H, indole H_4); 7.24 (s, 1H, indole H_7); 7.25–7.55 (m, 5H, benzyl ring aromatics); 7.95 (bs, 1H, NH). Anal $C_{18}H_{19}NO$ (C, H, N).

*5-Hydroxy-6-(1-methylethyl)indole **20***

A mixture of compound **19** (9.0 g; 0.024 mol) and Pd/C 10% (6.9 g) in methanol (450 ml) was hydrogenated at room temperature (hydrogen pressure = 25 psi) for 4 h. The catalyst was filtered off and the solvent was evaporated *in vacuo*. The crude was purified by flash chromatography eluting with a petroleum ether/ethyl acetate (85:15) mixture to give 5.6 g (94%) of compound **20**; mp 88–89°C (cyclohexane). IR (KBr): 3500, 3400, 1580, 1460, 1300, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (d, 6H, $(CH_3)_2C$); 3.32 (m, 1H, $CH(CH_3)_2$); 4.54 (bs, 1H, OH); 6.35–6.40 (m, 1H, indole H_3); 6.96 (s, 1H, indole H_4); 7.10 (dd, 1H, indole H_2); 7.24 (s, 1H, indole H_7); 7.94 (bs, 1H, NH). Anal $C_{11}H_{13}NO$ (C, H, N).

*5-Benzyloxy-1-methyl-6-(1-methylethyl)indole **21***

To a solution of compound **19** (5.16 g; 0.019 mol) in toluene (48 ml), KOH powder (4.34 g; 0.078 mol), benzyl tributylammonium chloride (1.21 g; 0.004 mol) and methyl iodide (1.82 ml; 0.029 mol) were added. The mixture was stirred for 4 h at 60°C, cooled to room temperature, poured into water and extracted with ethyl acetate. The organic layer was washed with water, dried (Na_2SO_4) and evaporated to dryness. The crude product was purified by flash chromatography, eluting with a petroleum ether/ethyl acetate (97:3) mixture to give 4.59 g (85%) of **21**; mp 54–55°C (methanol); IR (KBr): 2950, 1560, 1490, 1240, 1160 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (d, 6H, $(CH_3)_2C$); 3.20–3.60 (m, 1H, $CH(CH_3)_2$); 3.65 (s, 3H, NCH_3); 5.00 (s, 2H, OCH_2); 6.25 (d, 1H, indole H_3); 6.60–6.75 (m, 1H, indole H_2); 6.90–7.10 (m, 2H, indole H_4 and H_7); 7.10–7.50 (m, 5H, benzyl ring aromatics). Anal $C_{19}H_{21}NO$ (C, H, N).

*5-Hydroxy-1-methyl-6-(1-methylethyl)indole **22***

To a solution of compound **21** (5.42 g; 0.019 mol) in methanol (250 ml), 10% Pd/C (2.5 g) was added and the mixture was stirred in a H_2 atmosphere (H_2 pressure = 23–25 psi) at room temperature for 2 h. The catalyst was filtered off and the crude product, after evaporation to dryness, was purified by flash chromatography, eluting with ethyl acetate/*n*-hexane (11:89), to give 2.66 g (73%) of compound **22**; mp 78–79°C (*n*-hexane). IR (KBr): 3520, 2950, 1510, 1480, 1240, 1160 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (d, 6H, $(CH_3)_2C$); 3.10–3.50 (m, 1H, $CH(CH_3)_2$); 3.60 (s, 3H, CH_3N); 4.50 (bs, 1H, OH); 6.20–6.40 (m, 1H, indole H_3); 6.70–7.00 (m, 3H, indole H_2, H_4, H_7). Anal $C_{12}H_{15}NO$ (C, H, N).

*6-(1-Methylethyl)-5-[2-(1-pyrrolidinyl)ethoxy]indole hydrochloride **9***

To a solution of compound **21** (3.2 g; 0.018 mol) in anhydrous dimethylformamide (37 ml) under a N_2 stream, were added

K_2CO_3 (8.1 g; 0.059 mol) and 1-(2-chloroethyl)pyrrolidine (3.7 g; 0.028 mol). The resulting mixture was stirred in the dark for 6 h at 60°C, cooled to room temperature, poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried (Na_2SO_4) and the solvent evaporated. The crude was purified by flash chromatography eluting with a dichloromethane/methanol (95:5) mixture to give the base of compound **9**. This was dissolved in methanol and one equivalent of 0.45 N ethereal HCl added. The solvents were evaporated and the residue was treated with diethyl ether. Evaporation of diethyl ether gave a solid which was crystallized from acetone to give 2.72 g (49%) of **9**; mp 185–186°C. IR (KBr): 3100, 2950, 2600, 2500, 1460, 1300, 1160 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 1.15 (d, 6H, $(CH_3)_2C$); 1.75–2.15 (m, 4H, pyrrolidine β CH_2); 3.05–3.50 (m, 7H, pyrrolidine α CH_2, CH_2N and $CH(CH_3)_2$); 4.10 (t, 2H, CH_2O); 6.05–6.15 (m, 1H, indole H_3); 6.70–7.00 (m, 3H, indole H_2, H_4, H_7); NH and N$^+$H are displayed as CD_3OH at 4.60 δ. Anal $C_{17}H_{24}N_2O$•HCl (C, H, N, Cl).

5-{2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethoxy}-6-(1-methylethyl)indole hydrochloride **10**

To a solution of compound **20** (1.95 g; 0.011 mol) in anhydrous dimethylformamide (22.5 ml), were added K_2CO_3 (4.97 g; 0.036 mol) and 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperazine (4.23 g; 0.017 mol). The resulting mixture was stirred for 6 h at 60°C, cooled to room temperature, poured into water and extracted with ethyl acetate. The organic layer was washed with water, dried on Na_2SO_4 and the solvent was evaporated *in vacuo*. The crude was purified by flash chromatography eluting with a *n*-hexane/ethyl acetate (7:3) mixture to give 3.37 g (77%) of the base of **10**. This base was dissolved in diethyl ether (10 ml) and 0.38 N ethereal HCl (22.53 ml) was added. The precipitate was filtered and crystallized from acetone to give 2.08 g (43%) of **10**; mp 176–177°C. IR (KBr): 3400, 3300, 2950, 2550, 2450, 1500, 1480, 1300, 1240, 1160 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 1.25 (d, 6H, $(CH_3)_2C$); 3.00–3.60 (m, 11H, 5 x CH_2 and $CH(CH_3)_2$); 3.70 (s, 3H, CH_3O); 4.15–4.40 (m, 2H, CH_2O); 6.15–6.25 (m, 1H, indole H_3); 6.60–7.10 (m, 7H, remaining indole and phenyl ring aromatics); NH and N$^+$H are displayed as CD_3OH at 4.6 δ. Anal $C_{24}H_{31}N_3O_2$•HCl (C, H, N, Cl).

5-{2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethoxy}-1-methyl-6-(1-methylethyl)indole hydrochloride emihydrate ***11***

To a solution of compound **22** (3.0 g; 0.016 mol) in anhydrous dimethylformamide (32 ml), were added K_2CO_3 (7.0 g; 0.051 mol) and 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperazine (6.06 g; 0.024 mol). The mixture was stirred for 6 h at 60°C, cooled to room temperature, poured into water and extracted with ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4 and the solvent was evaporated *in vacuo*. The crude was purified by flash chromatography eluting with a dichloromethane/ethyl acetate (85:15) mixture and crystallized from ethanol to give 2.60 g of the base of **11** (40%). This base was dissolved in diethyl ether/dichloromethane and added with 0.35 N ethereal HCl (18.3 ml). The solvents were evaporated *in vacuo* and the residue was crystallized from ethanol to give 1.8 g (25%) of **11**; mp 181–182°C. IR (KBr): 3400, 3250, 2950, 2450, 1500, 1450, 1280, 1260, 1180 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (d, 6H, $(CH_3)_2C$); 3.10–3.50 (m, 13H, 5 x CH_2N, $CH(CH_3)_2$, H_2O, N$^+$H); 3.60 (s, 3H, NCH_3); 3.70 (s, 3H, OCH_3); 4.30–4.60 (m, 2H, OCH_2); 6.10–6.20 (m, 1H, indole H_3); 6.60–7.00 (m, 7H, remaining indole and phenyl ring aromatics). Anal $C_{25}H_{33}N_3O_2$• HCl•0.5H_2O (C, H, N, Cl, H_2O).

Pharmacology

Animals

Male Sprague–Dawley rats, male New Zealand White rabbits, and male beagle dogs were used in these experiments. Animals were housed with free access to food and water and maintained on forced light–dark cycles until the day of experiment.

In vitro experiments

[^{3}H]Prazosin binding (α_1 receptors). Rat cerebral cortices were homogenized in 50 volumes of original wet weight of ice-cold 50 mM Tris-HCl buffer pH 7.4. The homogenates were centrifuged at 48 000 *g* for 10 min, and the pellets were resuspended in the same volume of ice-cold buffer, centrifuged and resuspended 2 more times. The final pellets obtained were resuspended in the same volume of buffer and incubated according to the conditions reported in table IV.

[^{3}H]Rauwolscine binding (α_2 receptors). General procedures were the same described for the [^{3}H]prazosin binding. The pellets were incubated for 15 min at 37°C and then centrifuged and washed twice before use.

[^{3}H]Spiperone binding (D_2 receptors). Rat striata were homogenized in 20 volumes of original wet weight of ice-cold 50 mM Tris-HCl buffer pH 7.4 and diluted to 30 volumes of

Table IV. Receptor binding studies: details of methods.

Conditions	*α_1-adrenergic* *[^{3}H]prazosin*	*α_2-adrenergic* *[^{3}H]rauwolscine*	*D_2-dopaminergic* *[^{3}H]spiperone*	*5-HT_{1A}-serotoninergic* *[^{3}H]8-OH-DPAT*	*5-HT_2-serotoninergic* *[^{3}H]ketanserine*
Ligand preparation (nM)	0.35	1.0	0.3	1.0	1.0
cmp[a]	1 ml (10 mg/ml)	1 ml (10 mg/ml)	1 ml (5 mg/ml)	1 ml (10 mg/ml)	1 ml (0.3 mg/ml)
Incubation buffer[b]	Tris HCl 50 mM pH 7.4	Tris HCl 50 mM pH 7.4	Tris HCl 50 mM pH 7.4	Tris HCl 50 mM pH 7.4	Tris HCl 50 mM pH 7.7
Non-specific binding	Prazosin 2 μM	Phentolamine 10 μM	Butaclamol 1 μM	5-HT 10 μM	Ketanserine 2 μM
Incubation	25°C 30 min	25°C 30 min	37°C 15 min	25°C 30 min	37°C 20 min

[a]Crude membrane preparation; [b]containing ascorbic acid 1% and pargiline 10 μM.

original weight with the same buffer. The homogenates were centrifuged at 48 000 *g* for 10 min, and the pellets were resuspended in 200 volumes of ice-cold buffer. The final membranes preparation obtained was incubated according to the conditions reported in table IV.

[^{3}H]8-OH-DPAT binding (5-HT$_{1A}$ receptors). Rat hyppocampa were homogenized in 50 volumes of original wet weight of ice-cold 50 mM Tris-HCl buffer pH 7.4. The homogenates were centrifuged at 48 000 *g* for 10 min, and the pellets were resuspended in the same volume of ice-cold buffer, incubated for 10 min at 37°C, centrifuged and resuspended 2 more times. The final pellets obtained were resuspended in the same volume of buffer and incubated according to the conditions reported in table IV.

[^{3}H]Ketanserine binding (5-HT$_2$ receptors). Rat cerebral cortices were homogenized in 50 volumes of original wet weight of ice-cold 50 mM Tris-HCl buffer pH 7.7. The homogenates were centrifuged at 48 000 *g* for 10 min, and the pellets were resuspended in the same volume of ice-cold buffer, incubated for 10 min at 37°C, centrifuged and resuspended 2 more times. The final pellets obtained were resuspended in the same volume of buffer and incubated according to the conditions reported in table IV.

The incubation was terminated after the appropriate time (see table IV) by rapid filtration through Whatman GF/B filters using a Brandel cell harvester. The filters were washed twice with 15 ml of ice-cold buffer (see table IV). The radioactivity retained on the filters was determined by liquid scintillation counting. Non-specific binding (which generally amounted to 10–30%) was evaluated by adding high concentrations of the specific displacers. All compounds were initially tested at 1×10^{-4} M concentration, and, in the presence of significant displacing activity, a complete competition curve was performed. All samples were run in triplicate. The competition curves were analyzed, to evaluate the IC_{50} values, by non-linear curve fitting of the logistic equation according to the method reported by De Lean *et al* [37], utilizing the Allfit program (from NIH) written for the IBM pc.

Functional α_1-antagonistic activity. The effects of the compounds on noradrenaline-induced contractions of rabbit urethra were evaluated according to the method of Honda *et al* [38] with some modifications. Briefly, rabbits were killed by cervical dislocation and the urethra (starting from the trigone) removed. The strips' preparation from each urethra was made in Krebs bicarbonate buffer containing (mM): 118.4 NaCl; 4.7 KCl; 2.5 $CaCl_2$; 1.2 KH_2PO_4; 1.2 $MgSO_4$; 25 $NaHCO_3$; and 11.1 glucose; equilibrated with 95% O_2:5% CO_2. The Krebs solutions also contained 0.1 μM desmethylimipramine and 1 μM corticosterone to block neuronal and extraneuronal uptake of noradrenaline, 1 μM (+)-propranolol to block β-adrenoceptors, and 0.1 μM yohimbine to block α_2-adrenoceptors.

Preparations were suspended in 20 ml organ baths containing Krebs solution maintained at 37°C, and attached to isotonic transducers. The strips were stretched to a resting tension of 1 g. Tissues were allowed to equilibrate for 60 min and primed with 10 μM noradrenaline. After washing, the preparations were equilibrated for another 30 min and 2 cumulative concentration–response curves for noradrenaline were generated (generally from 1×10^{-8} to 1×10^{-4} M), followed by another 30 min washing and equilibration. Compounds were then added, and, after 30 min, the concentration–response curves for noradrenaline were repeated. Experimental groups consisted of 2–4 preparations taken from different animals for each concentration of drug tested. The dose–response curves were analyzed by non-linear curve fitting of the logistic equation according to the method reported by De Lean *et al* [37], utilizing the Allfit program (from NIH) written for the IBM pc. Schild-plot parameters (pA_2) were evaluated by linear regression analysis.

In vivo experiments

Effect on urethral contractility and blood pressure in anaesthetized dogs. The experiments were performed according to the method of Imagawa *et al* [20, 21], with substantial modifications. Adult dogs, weighing 8–10 kg, were anaesthetized with pentobarbital sodium (30 mg/kg iv and 2 mg/kg/h iv), incubated and spontaneously ventilated with room air. In order to monitor the systemic blood pressure (BP), a polyethylene (PE) catheter was introduced into the aortic arc through the right common carotid artery.

A collateral of the left femoral vein was cannulated for infusion of the anaesthetic, and the right femoral vein was cannulated for administration of the compounds. For intrarterial injection of noradrenaline, a PE catheter was introduced into the lower portion of abdominal aorta *via* the right external iliac artery. Through such procedure, noradrenaline is selectively distributed to the lower urinary tract. *Via* a midline laparatomy, the urinary bladder and proximal urethra were exposed. In order to prevent filling of the bladder, the 2 ureters were cannulated and urine was let outside. In order to record the prostatic urethral pressure, a Mikro-tip catheter (6 F) was introduced into the bladder *via* the external urethral meatus, and withdrawn until the pressure transducer was positioned in the prostatic urethra. A ligature was secured between the neck of the bladder and urethra to isolate the response of the latter and avoid any interaction with the bladder. Another ligature was put around the Mikro-tip catheter at the external urethral meatus, to secure the catheter itself. After a stabilizing period following the surgical procedure (30 min), in which arterial and prostatic urethral pressure were continuously monitored as basal values, intrarterial administration of noradrenaline was made at intervals of 10 min. The dose of noradrenaline used was so as to produce an increase of at least 100% in urethral pressure (usually 0.5–2 μg/kg). The test compounds were iv administered in a cumulative manner with intervals of 15–20 min between administrations. Intrarterial injections of noradrenaline were repeated approximately 5 min after every dosing of test compound. Dose–response curves were constructed by computing the percent inhibition to increase in urethral pressure (noradrenaline-induced), and the percentage fall in blood pressure produced by the test compound. ED_{25} for diastolic blood pressure (dose inducing 25% decrease) and ED_{50} (dose inducing 50% inhibition of noradrenaline-induced increase in urethral pressure) values were computed by means of linear regression analysis.

Acknowledgment

The authors wish to thank R Cappelletti for the NMR spectra.

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