Eur J Med Chem (1997) 32, 339–342

Short communication

New 3(2*H*)-pyridazinone derivatives: synthesis and affinity towards α_1AR subtypes and $5HT_{1A}$ receptors

S Corsano[1], G Strappaghetti[1]*, A Leonardi[2], K Rhazri[1], R Barbaro[1]

[1]*Istituto di Chimica e Tecnologia del Farmaco, Università di Perugia, Via del Liceo 1, 06123 Perugia;*
[2]*Ricerca Sviluppo Farmaceutico, Recordati SpA, Via M Civitali 1, 20148 Milano, Italy*

(Received 28 May 1996; accepted 14 October 1996)

Summary — The synthesis and the evaluation of the radioreceptor binding affinity of a series of 3(2*H*)-pyridazinone derivatives is reported. Their affinity towards the α_1 receptor, three α_1-AR subtype ($\alpha_{1a}/\alpha_{1b}/\alpha_{1d}$) receptors and the $5HT_{1A}$ receptor has been determined. The results of the affinity on α_1-AR subtypes and the $5HT_{1A}/\alpha_1$ selectivity are discussed.

structure–activity relationship / α_1 and $5HT_{1A}$ receptor / pyridazinone derivative / arylpiperazine

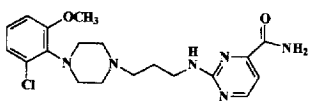
Introduction

Pharmacological evidence and recent molecular cloning studies have demonstrated that the α_1-AR is not a homogeneous population and three α_1-AR subtypes have been characterized by functional, radioligand binding and molecular biology techniques. These are usually referred to as α_{1A} (α_{1a}), α_{1B} (α_{1b}), and α_{1D} (α_{1d}) adrenoceptors where the lower case subscripts are used for recombinant receptors and the upper case subscripts for receptors in native tissues [1].

Recently, a great deal of attention has been paid towards obtaining α_1-antagonists with high selectivity towards only one type of subreceptor. This interest arises from the observation that only compounds with selectivity show activity on the lower tract of the urethra [2].

Therefore with the hope of obtaining compounds which are highly selective towards only one α_1 subreceptor, we synthesized compound **3** in which the 3(2*H*)-pyridazinone ring was linked in the 2-position with the 1-arylpiperazine through a chain of three carbon atoms, and in the 6-position with a phenyl group. The 3(2*H*)-pyridazinone ring was chosen since its derivatives show high adrenolytic activity, and since compounds like **1** [2] with a 1-phenyl-4-piperazin alkyl fragment show a high selectivity towards the α_{1a} subtype. Furthermore, we synthesized compound **7** in which the phenyl group was substituted with a furoylpiperazinyl group and compounds **4–6** in which the 3(2H)-pyridorinone ring was linked in 2-position with the differently substituted 1-arylpiferorine through a chain of two carbon atoms, since we were interested in determining the influence of these groups, present in many α_1 antagonists, towards the α_1 subreceptors.

It is well known that the α_1 adrenoceptor (α_1-AR) is a member of the super family of G-protein-coupled receptors as well as the $5HT_{1A}$ receptor. In spite of their completely distinct pharmacology, these receptors show common features in their binding sites [3, 4]. Therefore it was interesting to determine the affinity of all the compounds towards the $5HT_{1A}$ receptor and their selectivity ($5HT_{1A}/\alpha_1$). Finally, the pharmacological results obtained for compounds **3–7** were compared with the affinity towards α_1-AR subtypes and towards the $5HT_{1A}$ receptor found for compound **2**, which was synthesized previously in our laboratory [5].



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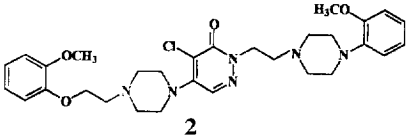

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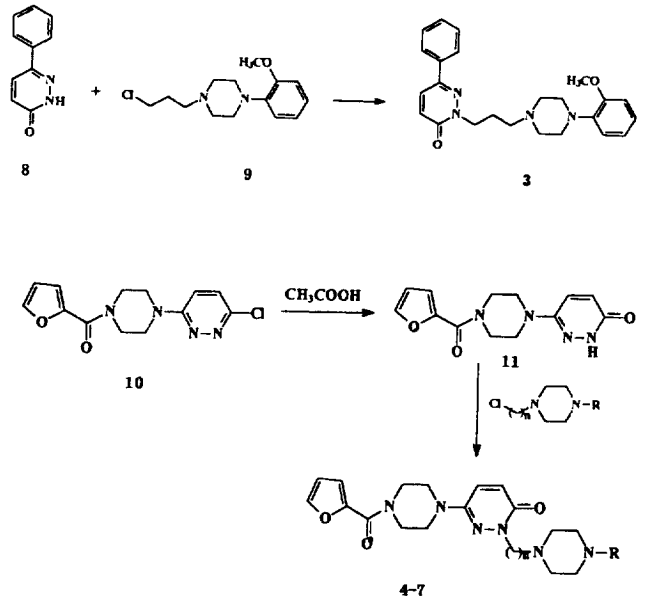
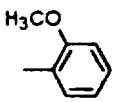
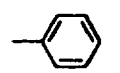
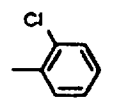
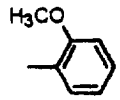
*Correspondence and reprints
Dedicated to Professor Mario Piattelli on the occasion of his 70th birthday.

Chemistry

Compound **3** was prepared by alkylation of 6-phenyl-3(2*H*) pyridazinone **8** with 4-(2-methoxyphenyl)-1-(3-chloropropyl)piperazine **9** in dry methanol and potassium hydroxide pellets. Compound **11** was prepared by alkylation of 1-(2-furoyl)piperazine with 3,6-dichloropyridazine (compound **10**) followed by hydrolysis with glacial acetic acid. Starting from compound **11**, by alkylation with the appropriate 1-aryl-piperazinyl alkyl halide in dry ethanol and sodium hydroxide pellets in equimolar ratio, the corresponding compounds **4–7** were obtained (scheme 1).



Compound	*n*	*R*
4	**2**	
5	**2**	
6	**2**	
7	**3**	

Scheme 1.

Results and discussion

From the pharmacological results reported in tables I and II, compound **3**, in which a [4-(2-methoxyphenyl)-1-piperazinyl]propyl system is linked in the 2-position and a phenyl ring in the 6-position of the pyridazinone ring, shows a higher affinity towards the α_1 adrenoceptor (table II), particularly towards the α_{1a} subtype (table I) compared with compound **7** in which the phenyl group was substituted by the 4-(2-furoyl)-1-piperazinyl system. Compound **4**, obtained by shortening the alkyl chain linking the 6-[4-(2-furoyl)-1-piperazinyl]-3-(2*H*) pyridazinone to the 4-(2-methoxyphenyl)-1-piperazine moiety of compound **7** by one unit, shows a slight increase in affinity towards the α_{1a} subtype with an increase in selectivity towards the α_{1b} subtype. Furthermore, compounds **4** and **7** have a similar affinity towards the α_{1b} subtype.

In compounds **5** and **6**, in which the methoxy group was eliminated or substituted with a chlorine atom respectively, both affinity and selectivity decreased.

Table I. Affinity towards different α_1 adrenoceptor subtypes.

Compound	α_{1a} K_i *(nM)*	α_{1b} K_i *(nM)*	α_{1d} K_i *(nM)*
3	1.8	47.1	2.1
4	4.7	793.5	11.5
5	94.6	111.6	445.9
6	11.4	41.9	4.9
7	24.5	734.4	18.3
5-Methylurapidil	2.0	775.0	27.4

Table II. Affinity towards α_1 and $5HT_{1A}$ receptor types.

Compound	α_1 K_i *(nM)*	$5HT_{1A}$ K_i *(nM)*	*Ratio* K_i/ $5HT_{1A/\alpha 1}$
3	17.5	60.0	3.42
4	282.8	5825.2	20.60
5	1261.9	6427.6	5.09
6	176.3	731.7	4.15
7	118.0	2292.3	19.42
5-Methylurapidil	28.0	1.2	0.04

In table III, the affinity of pyridazinone derivative **2** towards α_1 subtypes ($\alpha_{1a}/\alpha_{1b}/\alpha_{1d}$) and $5HT_{1A}$ receptors is reported. These pharmacological results can be compared with the pharmacological data obtained for compounds **3–7** to show that compounds **2** and **4** possess a similar affinity towards α_{1a} and α_{1d}, while the affinity towards α_{1b} is higher for compound **2**. Therefore the substitution of the 4-(2-methoxyphenoxyethyl)-1-piperazinyl fragment (compound **2**) with the 1-(2-furoyl)piperazine group (compound **4**) increases the selectivity.

The affinity of all the compounds towards the $5HT_{1A}$ receptor and $5HT_{1A}/\alpha_1$ selectivity was also determined (table II). These compounds show a low affinity for this receptor, except compound **3**; the highest selectivity ($5HT_{1A}/\alpha_1$) was found for compounds **4**.

Compounds **4** in particular show an α_1-AR receptor binding profile similar to the α_{1a}-selective antagonist 5-methylurapidil, with the remarkable advantage of a more than 1000-fold selectivity when the α_{1a} and 5-HT_{1A} receptors are considered.

In conclusion, these results provide more information on the affinity and selectivity of new pyridazinone derivatives towards the α_1-AR subtype and on the 5-HT_{1A} receptors.

Table III. Affinity towards subtypes $\alpha_{1a}/\alpha_{1b}/\alpha_{1d}$ and $5HT_{1A}$ receptors for compound **2**.

Compound	K_i *(nM)*[a]			
	α_{1a}	α_{1b}	α_{1d}	$5HT_{1A}$
2	7.45	130	31	270
Prazosin	0.2	0.5	0.3	–
Trazodone	–	–	–	120

[a]The K_i binding data were calculated as described by De Blasi [13].

Experimental protocols

Biological methods

Radioligand binding assay at cloned bovine α_{1a} and hamster α_{1b} adrenoceptors expressed in COS-7 cells

[^{3}H]Prazosin binding to cloned α_1 adrenoceptor subtypes was perfomed in COS-7 cells (CV-1 monkey kidney epithelial cells) transiently expressing bovine α_{1a} and hamster α_{1b} adrenoceptors. Construction and transfection of individual α_1 adrenoceptor subtypes were carried out by S Cotecchia [6], as previously described [7]. COS-7 cell membranes (35 μg proteins) were incubated in 50 mm Tris–HCl pH 7.4, containing 10 μg pargyline and 0.1% ascorbic acid, with 0.1–0.4 nM [^{3}H]prazosin, in a final volume of 0.22 mL for 30 min at 25 °C, in the absence or the presence of competing drugs. Non-specific binding was determined in the presence of 100 μM phentolamine.

The incubation was stopped by addition of ice-cold Tris–HCl buffer and rapid filtration through 0.2% polyethyleneimine pretreated Whatman GF/B or Schleicher and Schuell GF 52 filters.

Radioligand binding assay at cloned α_{1d} adrenoceptors expressed in CHO cells

Binding to cloned human α_{1d} adrenoceptors was performed in membranes from CHO cells (Chinese hamster overy cells) transfected by electroporation with DNA expressing the gene encoding the α_{1d} adrenoceptor subtype. Cloning and stable expression of the human α_{1d} adrenoceptor gene was performed as previously described [8].

CHO cell membranes (30 μg proteins) were incubated in 50 mM Tris–HCl, pH 7.4, with 0.1–0.4 nM [^{3}H]prazosin, in a final volume of 1.02 mL for 30 min at 25 °C, in the absence or the presence of competing drugs. Non-specific binding was determined in the presence of 10 μM phentolamine.

The incubation was stopped by addition of ice-cold Tris–HCl buffer and rapid filtration through 0.2% polyethyleneimine pretreated Whatman GF/B or Schleicher and Schuell GF 52 filters.

Radioligand binding assay on native receptors

Radioligand binding studies on α_1 adrenergic and 5-HT_{1A} serotoninergic receptors were performed as previously described [9, 10] with minor modifications.

Membrane preparation. Male rats were killed by cervical dislocation and cerebral cortex (α_1 adrenergic receptor) and hyppocampus (5-HT_{1A} serotoninergic receptor) immediately frozen and stored at –70 ° C until use. Tissues were homogenized (2 x 20 esc) in 50 vol of cold Tris–HCl, pH 7.4, using a Politron homogenizer (speed 7). Homogenates were centrifuged at 49 000 *g* for 10 min, resuspended in 50 vol of the same buffer, incubated at 37 °C for 15 min and centrifuged and resuspended twice more. The final pellets were suspended in 100 vol of Tris–HCl buffer pH 7.4, containing 10 μM pargyline and 0.1% ascorbic acid.

Binding assay. Membranes were incubated with 0.2–0.4 nM [^{3}H]prazosin (α_1 adrenergic receptor) or 1 nM [^{3}H]8-OH-DPAT (5-HT_{1A}) in a final vol of 1 mL for 30 min at 25 °C, in the absence or the presence of competing drugs. Non-specific binding was determined in the presence of 10 μM phentolamine (α_1) or 10 μM 5-HT (5-HT_{1A}). The incubation was stopped by addition of ice-cold Tris–HCl buffer and rapid filtration through 0.2% polyethyleneimine pretreated Whatman GF/B or Schleicher and Schuell GF 52 filters.

Chemistry

Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. The NMR spectra were recorded with a Bruker AC 200 MHz instrument in the solvent indicated below. The chemical shift values (ppm) are relative to tetramethylsilane as the internal standard. Elemental analyses are within ± 0.4% of the theoretical values. Precoated Kieselgel 60 F_{254} plates (Merck) were used for TLC.

2-{[4-(2-Methoxyphenyl)-1-piperazinyl]propyl}-6-phenyl-3(2H)-pyridazinone ***3***

To 50 mL of dry methanol was added 0.17 g (3.1 x 10^{-3} mol) of potassium hydroxide pellets and 0.5 g (2.9 x 10^{-3} mol) of 6-phenyl-3(2*H*)-pyridazinone **8** [11]. The mixture was refluxed for 15–20 min, and then 0.76 g (2.9 x 10^{-3} mol) of 4-(2-methoxyphenyl)-1-(3-chloropropyl)piperazine **9** [12] was added, and was refluxed under stirring for 8 h. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography using CH_2Cl_2/EtOH (96:4) as eluent, giving a dense oil. Yield: 40%. ^{1}H-NMR ($CDCl_3$) δ: 2.1–2.3 (2H, m, CH_2), 2.55 (2H, t, J = 6 Hz, CH_2), 2.65–2.75 (4H, m, H-piperazinic), 3.05–3.15 (4H, m, H-piperazinic), 3.85 (3H, s, OCH_3), 4.4 (2H, t, J = 6 Hz, CH_2), 6.8–7.05 (5H, m, 4H-aromatic, 1H-pyridazinonic), 7.4–7.5 (3H, m, H-aromatic), 7.65 (1H, d, J = 9.5 Hz, H-pyridazinonic), 7.75–7.85 (2H, m, H-aromatic). The corresponding hydrochloride had mp = 166–170 °C.

General method for compounds ***4–7***

2-{[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl}-6-[4-(2-furoyl)-1-piperazinyl]-3(2H)-pyridazinone ***4***. To 20 mL of dry ethanol was added 0.16 g (4.0 x 10^{-3} mol) of sodium hydroxide pellets and 1.1 g (4.0 x 10^{-3} mol) of 6-[4-(2-furoyl)-1-piperazinyl]-3(2*H*)-pyridazinone **11**; this mixture was refluxed for 30 min. Then 1.0 g (4.0 x 10^{-3} mol) of the 4-(2-methoxyphenyl)-1-(2-chloroethyl)piperazine [12] dissolved in dry ethanol was added, and this mixture was refluxed under stirring for 5 h. After evaporation under reduced pressure, the residue was purified by chromatography silica gel using as eluent a stepwise gradient of ethanol (0–3%) in CH_2Cl_2. Yield: 40%. ^{1}H-NMR ($CDCl_3$) δ: 2.7–2.9 (6H, m, 4H-piperazinic, CH_2), 3.0–3.15 (4H, m, H-piperazinic), 3.3–3.4 (4H, m, H-piperazinic), 3.9 (3H, s, OCH_3), 3.95–4.1 (4H, m, H-piperazinic), 4.3 (2H, t, J = 6 Hz, CH_2), 6.5 (1H, m, H-furanic), 6.8–7.1 (7H, m, 4H-aromatic, 2H-pyridazinonic, 1H-furanic), 7.5 (1H, s, H-furanic). The corresponding hydrochloride had mp = 65–68 °C.

2-{[4-(Phenyl)-1-piperazinyl]ethyl}-6-[4-(2-furoyl)-1-piperazinyl]-3(2H)-pyridazinone ***5***. This compound was prepared by alkylation of **11** with 4-(phenyl)-1-(2-chloroethyl)piperazine [12], purified by chromatography silica gel using CH_2Cl_2/EtOH (95:5) as eluent, and a dense oil was obtained. Yield: 40%. ^{1}H-NMR ($CDCl_3$) δ: 2.65–2.75 (4H, m, 4H-piperazinic), 2.85 (2H, t, J = 6 Hz, CH_2), 3.15–3.25 (4H, m, H-piperazinic), 3.3–3.4 (4H, m, H-piperazinic), 3.8–3.9 (4H, m, H-piperazinic), 4.2 (2H, t, J = 6 Hz, CH_2), 6.5 (1H, m, H-furanic), 6.8–6.95 (5H, m, 5H-aromatic), 7.0–7.1 (2H, m, H-aromatic), 7.2–7.3 (1, m, H-aromatic), 7.5 (1H, s, H-furanic). The corresponding hydrochloride had mp = 115–120 °C.

2-{[4-(2-Chlorophenyl)-1-piperazinyl]ethyl}-6-[4-(2-furoyl)-1-piperazinyl]-3(2H)-pyridazinone ***6***. This compound was prepared by the same procedure described above, purified by chromatography silica gel using as eluent a stepwise gradient of EtOH (0–5%) in CH_2Cl_2; a dense oil was obtained. Yield: 45%. ^{1}H-NMR ($CDCl_3$) δ: 2.7–2.9 (6H, m, 4H-piperazinic, CH_2), 3.05–3.15 (4H, m, H-piperazinic), 3.3–3.4 (4H, m, H-piperazinic), 3.85–3.95 (4H, m, H-piperazinic), 4.25 (2H, t, J = 6 Hz, CH_2), 6.5 (1H, m, H-furanic), 6.9–7.4 (7H, m, 4H-aromatic, 2H-pyridazinonic, 1H-furanic), 7.5 (1H, s, H-furanic). The corresponding hydrochloride had mp = 125–128 °C.

2-{[4-(2-Methoxyphenyl)-1-piperazinyl]propyl}-6-[4-(2-furoyl)-1-piperazinyl]-3(2H)pyridazinone ***7***. This compound was prepared by alkylation of **11** with 4-(2-methoxyphenyl)-1-(3-chloropropyl)piperazine [12], using the method described above, purified by chromatography silica gel using as eluent a stepwise gradient of EtOH (0–10%) in CH_2Cl_2. Yield: 40%. ^{1}H-NMR ($CDCl_3$) δ: 1.9–2.1 (2H, m, CH_2), 2.5 (2H, t, J = 6 Hz, CH_2), 2.6–2.7 (4H, m, 4H-piperazinic), 3.05–3.15 (4H, m, H-piperazinic), 3.3–3.5 (4H, m, 4H-piferorine), 3.9 (3H, s, OCH_3), 3.9–4.0 (4H, m, H-piperazinic), 4.15 (2H, t, J = 6 Hz, CH_2), 6.5 (1H, m, H-furanic), 6.8–7.0 (5H, m, 4H-aromatic, 1H-pyridazinonic), 7.05–7.15 (2H, m, 1H-pyridazinonic, 1H-furanic), 7.5 (1H, s, H-furanic). The corresponding hydrochloride had mp = 110-115 °C.

3-Chloro-6-[4-(2-furoyl)-1-piperazinyl]piridazine ***10***

A mixture of 4.9 g (2.7 x 10^{-2} mol) of 1-(2-furoyl)piperazine and 4.47 g (3.0 x 10^{-2} mol) of 3,6-dichloropiridazine in 2-butanone containing 10 g of anhydrous potassium carbonate, was heated under reflux with stirring for 17 h. The mixture was filtered hot, and the filtrate was evaporated under reduced pressure. The resulting product was purified by flash chromatography using CH_2Cl_2/EtOH (95:5). Yield: 56%. Mp = 138–142 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.7–3.8 (4H, m, H-piperazinic), 3.85–3.95 (4H, m, H-piperazinic), 6.5 (1H, m, H-furanic), 6.9 (1H, d, 1H-pyridazine), 7.1 (1H, m, 1H-furanic), 7.3 (1H, d, H-pyridazine), 7.5 (1H, s, H-furanic).

6-[4-(2-Furoyl)-1-piperazinyl]-3(2H) pyridazinone ***11***

A solution of 2 g (5.1 x 10^{-3} mol) of **10** in 30 mL of glacial acetic acid was refluxed for 6 h. The acetic acid was removed under reduced pressure, and the residue dissolved in water and extracted with CH_2Cl_2. The organic phase, dry on Na_2SO_4, was evaporated under reduced pressure. The residue was purified by gel chromatography using as eluent a stepwise gradient of EtOH (0–10%) in CH_2Cl_2. Yield: 40%. Mp = 212–215 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.25–3.4 (4H, m, H-piperazinic), 3.85–3.95 (4H, m, H-piperazinic), 6.5 (1H, m, H-furanic), 6.85 (1H, d, J = 9.5 Hz, 1H-pyridazinonic), 7.1 (1H, m, 1H-furanic), 7.15 (1H, d, J = 9.5 Hz, H-pyridazinonic), 7.5 (1H, s, H-furanic), 10.4 (1H, s, NH-pyridazinonic).

Acknowledgment

This work was supported by MURST (the Ministry of University and Scientific Research).

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