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ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY · MAY 2006

Impact Factor: 2.79 · DOI: 10.1016/j.bmc.2005.12.009 · Source: PubMed

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Synthesis of new piperazine–pyridazinone derivatives and their binding affinity toward α_1-, α_2-adrenergic and 5-HT_{1A} serotoninergic receptors

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Received 5 August 2005; revised 25 November 2005; accepted 2 December 2005
Available online 20 December 2005

Abstract—We report the design and synthesis of a new class of piperazine-pyridazinone analogues. The arylpiperazine moiety, the length of the spacer, and the terminal molecular fragment were varied to evaluate their influence in determining the affinity of the new compounds toward the α_1-adrenergic receptor (α_1-AR), α_2-adrenergic receptor (α_2-AR), and the 5-HT_{1A} serotoninergic receptor (5-HT_{1A}R). Biological data showed that most of the compounds have an α_1-AR affinity in the nanomolar or subnanomolar range, while affinity toward the other two receptors was lower in most cases. However, several of the tested compounds also showed very good (in the nanomolar range) or moderate affinity toward the 5-HT_{1A}R subtype.

1. Introduction

Although in the past decade, pharmaceutical research has focused the attention on the development of subtype selective ligands, comparable efforts have been made to discover ligands selective for α_1-AR over other related receptors, such as α_2-AR, and the 5-HT_{1A}R (showing 45% of structural similarity). Among compounds that showed high affinity toward α_1-AR, relevant attention has been devoted to molecules containing an arylpiperazinyl moiety as an essential pharmacophoric portion.[1,2]

Our efforts in the field of α_1-AR antagonists led recently to the identification of a pharmacophoric model (Fig. 1) characterized by five chemical features corresponding to three hydrophobic substituents (HY1–3), one hydrogen bond acceptor moiety (HBA), and a positively ionizable group (PI).[3] In addition, suggestions from either a pharmacophore-based database search[3] or the analysis of the superposition mode of many α_1-AR antagonists into the pharmacophoric model contributed to the optimization of the arylpiperazinylalkylpyridazinone scaffold to obtain several new compounds with affinity values in the

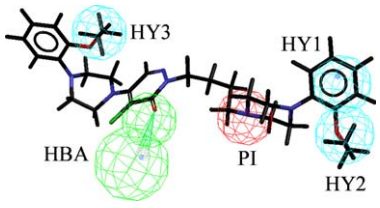

Figure 1. Superposition pathway of compound **16** into the five feature pharmacophoric model for α_1-AR antagonists. The *o*-ethoxyphenyl group of the arylpiperazine moiety is embedded into the HY1–HY2 hydrophobic system, while the most basic nitrogen atom of the piperazine ring corresponds to the positively ionizable feature, PI. On the other hand, the hydrogen bond acceptor group HBA is represented by the carbonyl of the pyridazinone nucleus, while the hydrophobic region HY3 is filled by the ethoxy group of the terminal molecular portion. Features are color coded: blue for hydrophobics (HY), red for positively ionizable groups (PI), and green for hydrogen bond acceptor groups (HBA).

Keywords: α-Adrenergic receptor ligands; Piperazine–pyridazinones; Arylalkylpiperazines; 5-HT_{1A} receptor ligands.
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doi:10.1016/j.bmc.2005.12.009

subnanomolar range and appreciable selectivity toward both α_2-AR and 5-HT_{1A}R.[4] Moreover, the analysis of the superposition mode of arylpiperazine derivatives (a highly populated class of α_1-AR antagonists) reported by our and other research groups suggested that the pharmacophoric model can be divided in two parts,[1–3] constituted by the arylpiperazine moiety and by a terminal heterocyclic molecular fragment, held at the appropriate distance by an alkylen chain that serves as a spacer. The HY1 and HY2 features were usually found to be filled by the substituted phenyl ring of the arylpiperazine moiety, while the latter satisfied the positively ionizable group of the pharmacophore (PI) with its more basic nitrogen atom. Since the polymethylene spacer did not contact any of the pharmacophoric features, it could be thought of as a geometric constraint able to appropriately separate the arylpiperazine moiety from the terminal group of α_1-AR antagonists. The latter is required to possess a hydrogen bond acceptor moiety (often represented by a carbonyl group) and a hydrophobic terminus at the edge of the molecule opposite to the arylpiperazine group, matching the HBA and HY3 features of the pharmacophoric model, respectively.

With the aim of further investigating the relationships between structure and affinity of arylpiperazine congeners, we report here the design and synthesis of new compounds, along with their affinity data toward α_1-AR, α_2-AR, and 5-HT_{1A}R. Such compounds were designed on the basis of a previous SAR analysis suggesting that (i) *ortho* alkoxy substituents at the phenyl ring attached to the piperazine nucleus of the arylpiperazine moiety were optimal for affinity, and *meta* substituents were also tolerated, with a drop in affinity. Regarding the size of such an *ortho* substituent, bulkier groups led to improved affinity.[5] (ii) A linear (unbranched) polymethylene spacer with four- or seven-carbon atoms was found to be the best spacer to bring the arylpiperazine moiety at the due distance from the terminal heterocyclic group.[5] (iii) A phenylpiperazinylpyridazinone moiety was inserted as the terminal fragment instead of the previously investigated phenoxyethylpiperazinylpyridazinone group characterized by an 'extra-size' portion.[3]

Based on the suggestions reported above, compounds **5**–**19** were designed. In principle, all the new compounds were characterized by chemical structures able to fill the five features of the pharmacophoric model. Regarding their orientation with respect to the pharmacophore, the new compounds showed a great similarity with arylpiperazines used to generate the pharmacophore itself. In detail, the arylpiperazine moiety of the ligands matched the HY1–HY2–PI system of the pharmacophore, as depicted in Figure 1 for compound **16**, taken as a representative example for the whole set of new compounds. Moreover, the carbonyl group of the pyridazinone moiety usually corresponded to the hydrogen bond acceptor group. However, because of a 180 degree rotation of the pyridazinone nucleus, the N1 nitrogen atom was sometimes allowed to interact with HBA. Finally, different chemical substituents were found to be able to fill the HY3 feature.

2. Chemistry

Compounds **5**–**19** were synthesized as shown in Schemes 1 and 2.

4-Chloro-5-[4-(2-furoyl)piperazin-1-yl]pyridazin-3(2*H*)-one (**20**)[3] and 4-chloro-5-[4-(2-ethoxyphenyl)piperazin-1-yl]pyridazin-3(2*H*)-one (**21**) were obtained by condensation of 4,5-dichloropyridazin-3(2*H*)-one with 1-(2-furoyl)piperazine and 1-(2-ethoxyphenyl)piperazine, respectively, in ethanol and Et_3N.

Condensation of **20** with 1,4-dibromobutane led to **22**[3] that was in turn treated with an appropriate piperazine (1-(2-isopropoxyphenyl)piperazine,[6] 1-(2-ethoxyphenyl)piperazine, 1-(2,3-dihydrobenzo[1,4]dioxin-2-yl-methyl)piperazine,[7] 2-piperazin-1-yl-pyrimidine, and 1-(3-chlorophenyl)piperazine) in acetonitrile, in the presence of dry potassium carbonate at reflux (Method A), to give the final products **5**–**9**. Following the same procedure, compound **10** was obtained with 1-(3-trifluoromethylphenyl)piperazine using isoamyl alcohol to increase the final yield.

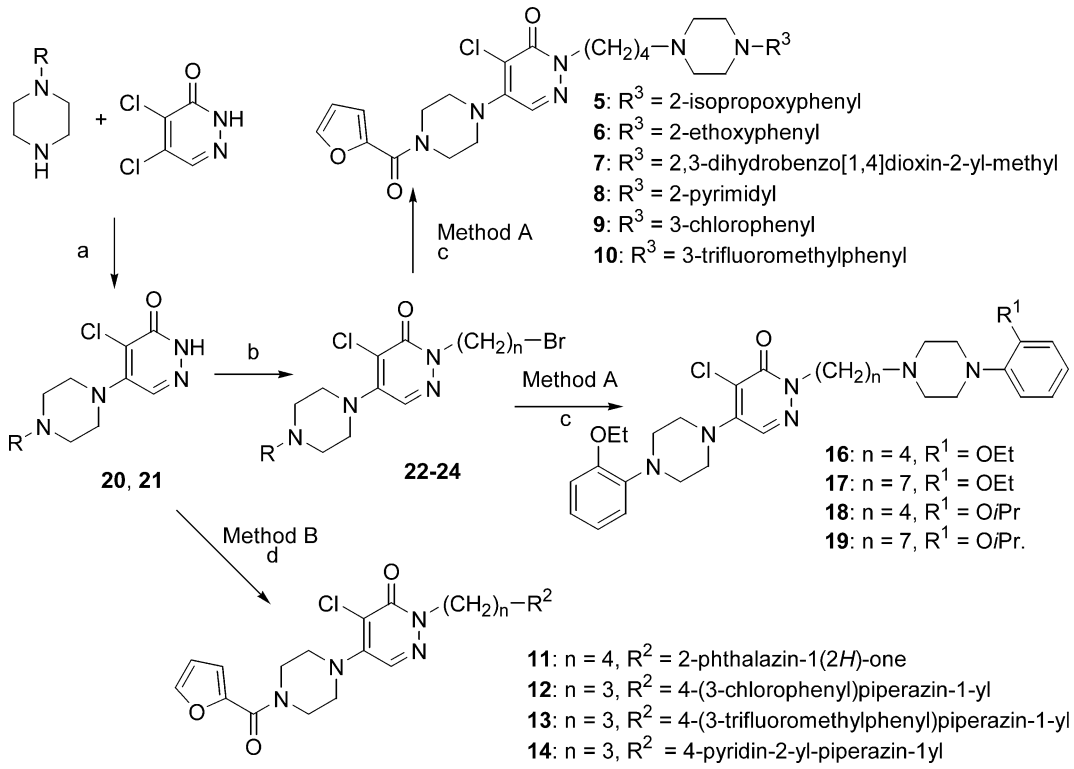
In a similar way, alkylation of **21** with 1,4-dibromobutane or 1,7-dibromoheptane in acetone, in the presence of dry potassium carbonate, gave intermediates **23** and **24**, respectively, which in turn were converted by reaction with the appropriate piperazine into the final compounds **16**–**17** and **18**–**19**, following Method A, in isoamyl alcohol, acetone or acetonitrile, respectively.

Compounds **11**–**14** have been synthesized by alkylation of **20** in acetone, in the presence of dry potassium carbonate at reflux, with 2-(4-bromobutyl)phthalazin-1(2*H*)-one (**25**), 1-(3-bromopropyl)-4-(3-chlorophenyl)piperazine (**26**), 1-(3-chloropropyl)-4-(3-trifluoromethylphenyl)piperazine (**27**), and 1-(3-chloropropyl)-4-pyridin-2-yl-piperazine (**28**), respectively (Method B). Compounds **26**–**28** were in turn prepared according to the procedure reported by Bourdais.[8]

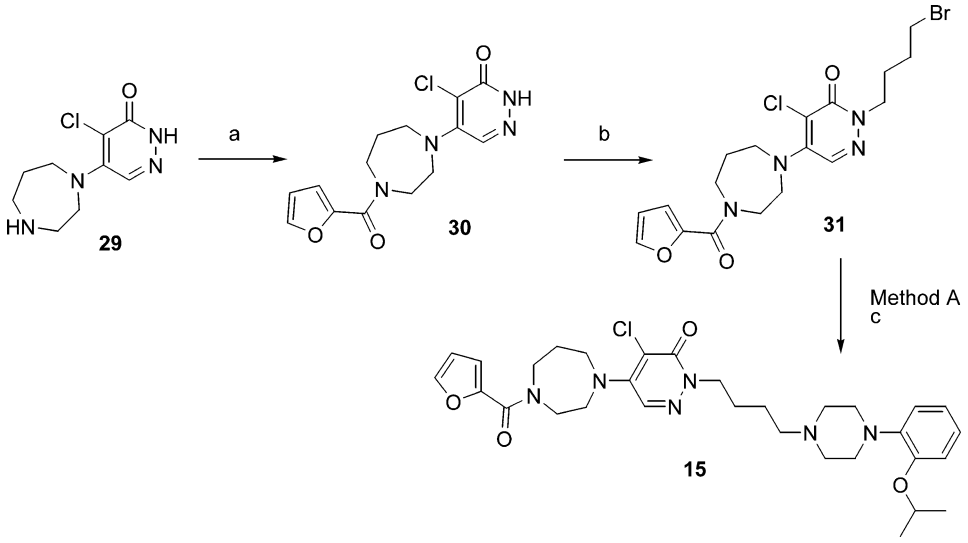
Condensation of 4-chloro-5-(1,4-diazepin-1-yl)pyridazin-3(2*H*)-one **29** with furoyl chloride in chloroform and sodium bicarbonate led to 4-chloro-5-[4-(2-furoyl)-[1,4-diazepin-1-yl]pyridazin-3(2*H*)-one (**30**), that was in turn alkylated with 1,4-dibromobutane to prepare intermediate **31** (Scheme 2). Reaction of **31** with 1-(2-isopropoxyphenyl)piperazine in acetonitrile in the presence of dry potassium carbonate gave the final compound **15**.

3. Pharmacology

The pharmacological activity profile of compounds **5**–**19** was evaluated for their affinity toward α_1-AR, α_2-AR, and 5-HT_{1A}R by determining for each compound the ability to diplace [^{3}H]prazosin, [^{3}H]rauwolscine, and [^{3}H]8-OH-DPAT, respectively, from specific binding sites on rat cerebral cortex. K_i values, reported in Table 1, were determined on the basis of three competition binding experiments in which seven drug concentra-



Scheme 1. Compounds **20**, R = 2-furoyl; **21**, R = 2-ethoxyphenyl; **22**, *n* = 4, R = 2-furoyl; **23**, *n* = 4, R = 2-ethoxyphenyl; **24**, *n* = 7, R = 2-ethoxyphenyl. Reagents and conditions: (a) 1-(2-furoyl)piperazine or 1-(2-ethoxyphenyl)piperazine, EtOH, Et_3N; (b) 1,4-dibromobutane or 1,7-dibromoheptane, acetone, K_2CO_3 or Na_2CO_3, reflux; (c) acetonitrile or acetone or isoamyl alcohol, K_2CO_3, reflux; (d) **25**, **26**, **27**, or **28**, acetone, K_2CO_3, reflux.



Scheme 2. Reagents and conditions: (a) furoyl chloride, $CHCl_3$, Na_2CO_3; (b) 1,4-dibromobutane; (c) 1-(2-isopropoxyphenyl)piperazine, acetonitrile, K_2CO_3, reflux.

tions, run in triplicate, were used. Further details are reported in Section 6.

Moreover, to determine the intrinsic activity of compounds with the best affinity profile toward α_1-AR (namely, **5** and **17**), competition studies were performed in the presence and in the absence of 1 mM GTP using the radiolabeled antagonist [^{3}H]prazosin. In Table 2, the GTP shift values of the selected compounds and the antagonist reference compound prazosin were reported.

At the α_1 receptor, the selected compounds displayed no significant GTP shift, suggesting that they elicited an antagonist profile as prazosin.

4. Results and discussion

Several of the new compounds were obtained keeping fixed the terminal heterocyclic moiety, while the length of the alkylen spacer, the substituents, and the substitu-

Table 1. α_1-AR, α_2-AR, and 5-HT_{1A} binding affinities for compounds **5**–**19**

Compound	K_i (nM)[a]			α_2/α_1 ratio	5-HT_{1A}/α_1 ratio
	α_1-AR	α_2-AR	5-HT_{1A}		
5	0.35 ± 0.03	71 ± 6	1.3 ± 0.45	203	3.6
6	4.8 ± 1.1	45 ± 7	11 ± 4	9.4	2.3
7	223 ± 55	65 ± 15	233 ± 35	3.4[b]	1.0
8	1819 ± 760	2793 ± 320	209 ± 40	1.5	8.7[c]
9	14 ± 2	70 ± 10	17 ± 1	5.0	1.2
10	146 ± 43	137 ± 31	137 ± 19	1.1[b]	1.1[c]
11	9461 ± 750	40%[d]	20%[d]		
12	9.4 ± 2.3	201 ± 40	618 ± 150	21	66
13	35 ± 16	341 ± 38	118 ± 35	9.7	3.4
14	29 ± 15	182 ± 37	1153 ± 225	6.3	40
15	0.93 ± 0.04	123 ± 34	2.0 ± 0.6	132	2.1
16	0.45 ±0.02	1.5 ± 0.2	5.0 ± 0.5	3.3	11
17	0.28 ± 0.02	31 ± 5.8	3.2 ± 0.4	111	11
18	0.70 ± 0.18	6.8 ± 1.0	3.9 ± 0.3	9.7	5.6
19	2.0 ± 0.6	18 ± 3	7.2 ± 2.1	9.0	3.6
Prazosin	0.24 ± 0.05				
Rauwolscine		4.0 ± 0.3			
OH-DPAT			2.0 ± 0.2		

[a] K_i binding data were calculated as described in Section 6. K_i values are means ± SD of separate series assay, each performed in triplicate. They were calculated according to the Cheng and Prusoff equation:[14] $K_i = IC_{50}/[1 + ([L]/K_d)]$, where [L] is the ligand concentration and K_d its dissociation constant. K_d of [^{3}H]prazosin binding to rat cortex membranes was 0.24 nM (α_1-AR), K_d of [^{3}H]rauwolscine binding to rat cortex membranes was 4 nM (α_2-AR), and K_d of [^{3}H]8-OH-DPAT binding to rat cortex membranes was 2 nM (5-HT_{1A}).

[b] As α_2/α_1 ratio.

[c] As α_1/5-HT_{1A} ratio.

[d] Percent inhibition at the 10 μM dose.

Table 2. Intrinsic Activity of Compounds **5** and **17** to α_1-AR

Compound	K_i toward α_1[a]		GTP shift
	−GTP (nM)	+GTP (nM)	
5	0.21 ± 0.05	0.28 ± 0.04	1.33
17	0.37 ± 0.06	0.40 ± 0.05	1.08
Prazosin	0.23 ± 0.06	0.28 ± 0.05	1.22

[a] Displacement of [^{3}H]prazosin from rat cerebral cortex membranes in the absence and in the presence of 1 nM GTP. Values are taken from three experiments, expressed as means ± SEM.

tion pattern on the arylpiperazine moiety were varied. In detail, with the alkylen chain as a four-carbon atom spacer, the alkoxy group at the phenylpiperazine moiety was increased to an ethoxy (**6**) and isopropoxy (**5**) substituent, leading to an affinity enhancement of about one and two orders of magnitude, respectively, with respect to the parent *o*-methoxy derivative **1**[3] (Chart 1) (4.8, 0.35 nM vs 33.5 nM), in agreement with previous experimental results showing that alkoxy groups bulkier

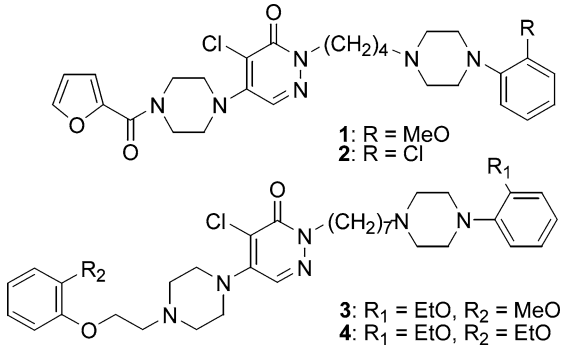


Chart 1.

than a methoxy substitutent influenced positively affinity of the higher homologues.[5] Superposition of such compounds to the pharmacophoric model for α_1-AR antagonists showed very similar orientations and accounted for the different affinity on the basis of the fact that larger alkoxy substituents were able to fill one of the features of the HY1–HY2 hydrophobic system better than a smaller group (such as MeO).

Moreover, enlargement of the piperazine ring constituting a portion of the terminal molecular fragment to a diazepino nucleus (**15**) led to an affinity less than 3-fold lower with respect to the corresponding piperazino derivative **5** (0.93 nM vs 0.35 nM), suggesting that the size of such a ring is not a crucial key in determining interactions with the receptor. This result was in agreement with the fact that the piperazino and the diazepino rings are both able to match the terminal hydrophobic feature (HY3) of the model, thus contributing in a similar way to define affinity of such derivatives.

SAR considerations reported in the literature for arylpiperazines also suggested that a chloro substituent (instead of an alkoxy group) at the *ortho* position of the phenyl ring of the arylpiperazine moiety was also suitable for affinity toward α_1-AR.[3] Moreover, although the *ortho* position is the optimum for affinity toward α_1-AR, a variation in the substitution pattern (a *meta* substituent instead of an *ortho* group) was interesting to find out whether changing the position of the substituent in the aryl moiety significantly changes the affinity. In agreement with this experimental evidence, shifting the chloro susbtituent of compound **2**[3] (Chart 1) into

the *meta* position (**9**), no significant variation in affinity was observed (19 nM vs 14 nM, respectively). In a similar way, shortening the alkylen spacer of a methylene unit led to compound **12** with an affinity value comparable to that of **9** (9 nM vs 14 nM). On the other hand, when the *m*-chloro substituent of **9** was replaced by a bulkier CF_3 group (**10**), affinity underwent a remarkable decrease of about one order of magnitude, from 14 nM to 146 nM, probably due to a bad steric effect of the larger substituent. A less pronounced reduction of affinity was also found in compounds with a propylen spacer after transformation of the *m*-Cl derivative **12** into the corresponding CF_3 analogue **13** (9 nM vs 35 nM, respectively). These results suggested that the length of the spacer and the size of the *meta* substituent at the arylpiperazine moiety contributed at the same time to define affinity values. In particular, while the *m*-CF_3 group was always disfavored with respect to the corresponding Cl substitution, the difference in affinity was much more evident in derivatives with a butylen spacer than with respect to those with a propylen spacer. This was probably due to the fact that lengthening the alkylen chain of a methylene unit could cause steric clashes involving the *m*-CF_3 group to become more marked, leading to a deep reduction of affinity.

Compound **11** was found to be inactive toward all the receptors tested, being 9461 nM its affinity value against α_1-AR, while only a 40% and 20% of specific [^{3}H]prazosin and [^{3}H]8-OH-DPAT binding was found at the test dose (10 μM) toward α_1-AR and 5-HT_{1A}R, respectively.

Finally, four of the new compounds (namely, **7**, **8**, **11**, and **14**) were appropriately designed to confirm the hypothesis that the replacement of the substituted phenylpiperazine ring (constituting the arylpiperazine moiety) with different substituents led in most cases to a marked drop in affinity toward α_1-AR. In particular, **14** retained appreciable affinity toward α_1-AR (29 nM), while compounds **7**, **8**, and **11** were characterized by unsignificant affinity toward the same receptor (223, 1819, and 9461 nM, respectively), in agreement with the experimental evidence that such a moiety plays a crucial role in defining the affinity of phenylpiperazinylalkylpyridazinone derivatives and related compounds.[1c]

Compounds **16**–**19** were designed and synthesized, according to previous findings suggesting that a butylen or heptylen chain is the optimal spacer and that an alkoxy group (at the *ortho* position on the phenyl ring of the arylpiperazine moiety) larger than a methoxy substituent led to enhanced affinity.[5] In addition, based on the observation that phenoxyethylpiperazinylpyridazinone moiety as the terminal molecular fragment was characterized by an 'extra-size' portion exceeding the pharmacophoric features,[3] such a large substituent was replaced by a smaller *o*-ethoxyphenylpiperazinylpyridazinone, to verify the hypothesis[9] that a reduced size of the terminal fragment could lead to compounds retaining good α_1-AR affinity. As a result, compound **17** was characterized by an affinity value of 0.28 nM, slightly lower than that of the corresponding derivative **3**[10] (Chart 1), bearing the extended terminal fragment (0.43 nM). Differently, compound **19** was found to be about 8-fold less active with respect to **4**[10] (Chart 1) (2.0 vs 0.26 nM). Moreover, when the length of the alkylen spacer was reduced from a heptylen to a butylen chain, affinity underwent a slight decrease for ethoxy derivatives **17** and **16** (0.28 vs 0.45 nM, respectively), while it increased from 2.0 to 0.7 nM for isopropoxy derivatives **19** and **18**, respectively.

While most of the new compounds showed low (2793 nM) to moderate (45 nM) affinity toward α_2-AR, the remaining compounds **16**–**19** were characterized by interesting biological data (from 1.5 to 31 nM). Moreover, **17** also showed an appreciable α_1 selectivity (α_2/α_1 ratio of 111), similar to **15** and **5** with α_2/α_1 ratio of 132 and 203, respectively.

As expected on the basis of the fact that an *o*-alkoxyphenylpiperazinyl group as the arylpiperazine moiety[11] and/or a propylen or butylen spacer represented optimum chemical features for inducing high affinity for both α_1-AR and 5-HT_{1A}R,[12] affinity values of the new compounds toward 5-HT_{1A}R followed the same trend already found for affinity toward α_1-AR, with a few exceptions. In fact, the ethoxy derivative **6** showed an affinity of 11 nM, lower than that of the corresponding isopropoxy analogue **5** whose affinity of 1.3 nM was comparable with that of compound **15** (2.0 nM), bearing the same *ortho* substituent at the phenylpiperazine moiety and a diazepino ring on the terminal molecular fragment, instead of a piperazino nucleus. Similarly, affinity of compounds **9** and **10** (17 and 137 nM, respectively) was comparable to that found toward α_1-AR (14 and 146 nM, respectively). On the contrary, affinity of compounds **12**–**14** (618, 118, and 1153 nM, respectively) was found to be significantly lower with respect to affinity toward α_1-AR (9.4, 35, and 29 nM, respectively). Moreover, compounds **16**–**19** showed affinity value toward the 5-HT_{1A}R in the nanomolar range (5.0, 3.2, 3.9, and 7.2 nM, respectively). As a consequence of the statement that affinity values toward α_1-AR and 5-HT_{1A}R of compounds belonging to this structural class are generally comparable, no appreciable selectivity was found for the new compounds among the two receptors. The highest 5-HT_{1A}R/α_1-AR ratio values are 66 and 40, associated to compounds **12** and **14**, respectively.

Finally, in addition to both an *o*-alkoxyphenylpiperazinyl group and a spacer of a given length, a furoylpiperazinylpyridazinone and an *o*-ethoxyphenylpiperazinylpyridazinone moiety could be considered as terminal fragments well tolerated for ligand-5-HT_{1A}R interaction, on the basis of the nanomolar affinity found for compounds **5**, **6**, **9**, **15**, and **16**–**19**, respectively.

5. Conclusions

Among the new arylpiperazine–pyridazinone derivatives, compounds **5**, **15**, and **17** emerge because of their subnanomolar α_1-AR affinity (0.35, 0.93, and 0.28 nM, respectively) and significant selectivity over α_2-AR (203, 132, and 111, respectively). Such derivatives have

been selected for further studies in order to investigate their affinity toward the α_1-AR subtypes.

6. Experimental

6.1. Chemistry

Starting materials were purchased from Aldrich-Italia (Milan). Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC 200 MHz instrument in the solvent indicated below. Chemical shift values (parts per million, ppm) are relative to tetramethylsilane used as an internal reference standard. Elemental analyses are within ±0.4% of theoretical values. Precoated Kieselgel 60 F_{254} plates (Merck) were used for TLC. The corresponding hydrochlorides were prepared by bubbling dry HCl into the dry solution of the compound.

6.2. Syntheses

Specific examples presented below illustrate general synthetic methods A–B.

6.3. 4-Chloro-5-[4-(2-ethoxyphenyl)piperazin-1-yl)pyridazin-3(2*H*)-one (21)

A mixture of 1-(2-ethoxyphenyl)piperazine hydrochloride (2.60 g, 11.0 mmol), 4,5-dichloro-pyridazin-3(2*H*)-one (1.80 g, 11.0 mmol) in ethanol and Et_3N was stirred under reflux for 20 h. The mixture was evaporated under reduced pressure and the compound was purified by crystallization with EtOH to give 60% yield of a white solid: mp 190–191 °C.

^{1}H NMR ($CDCl_3$) δ: 1.47 (t, 3H, CH_2-CH_3); 3.22–3.25 (m, 4H, H-pip.); 3.63–3.65 (m, 4H, H-pip.); 4.10 (quart., 2H, CH_2–CH_3); 6.84–6.94 (m, 4H, H-arom); 7.61 (s, 1H, H-pyrid.); 11.42 (s, 1H, NH).

6.4. 2-(4-Bromobutyl)-4-chloro-5-[4-(2-ethoxyphenyl)piperazin-1-yl)pyridazin-3(2*H*)-one (23)

A mixture of **21** (0.40 g, 1.30 mmol), 1,4-dibromo-butane (0.43 g, 2.00 mmol), and dry potassium carbonate (0.28 g, 2.00 mmol) in 20 mL of acetone was stirred under reflux for 6 h. After evaporation under reduced pressure, the compound was purified by chromatography on a silica gel column, eluting with a EtOH/CH_2Cl_2 mixture (2:98) to give 60% yield of a yellow dense oil.

^{1}H NMR ($CDCl_3$) δ: 1.46 (t, 3H, CH_2–CH_3); 1.88–1.96 (m, 4H, $2CH_2$); 3.19–3.24 (m, 4H, H-pip.); 3.43 (t, 2H, CH_2); 3.54–3.59 (m, 4H, H-pip.); 4.02–4.20 (m, 4H, CH_2–CH_3, CH_2); 6.84–7.00 (m, 4H, H-arom); 7.76 (s, 1H, H-pyrid.).

6.5. 2-(7-Bromoheptyl)-4-chloro-5-[4-(2-ethoxyphenyl)piperazin-1-yl)pyridazin-3(2*H*)-one (24)

A mixture of **21** (0.50 g, 1.50 mmol), 1,7-dibromo-heptane (0.58 g, 2.25 mmol), and dry potassium carbonate (0.31 g, 2.25 mmol) in 20 mL of acetone was stirred under reflux for 7 h. After evaporation under reduced pressure, the compound was purified by chromatography on a silica gel column, eluting with a EtOH/CH_2Cl_2 mixture (2:98) to give 50% yield of a yellow oil.

^{1}H NMR ($CDCl_3$) δ: 1.23–1.58 (m, 9H, CH_2–CH_3, $3CH_2$); 1.74–1.84 (m, 4H, $2CH_2$); 3.20–3.35 (m, 4H, H-pip.); 3.41 (t, 2H, CH_2); 3.55–3.60 (m, 4H, H-pip.); 3.99–4.17 (m, 4H, CH_2–CH_3; CH_2); 6.84–7.00 (m, 4H, H-arom); 7.76 (s, 1H, H-pyrid.).

6.6. 2-(4-Bromobutyl)-phthalazin-1(2*H*)-one (25)

A mixture of phthalazin-1(2*H*)-one (0.80 g, 5.50 mmol), 1,4-dibromobutane (1.80 g, 8.20 mmol), and dry potassium carbonate (1.13 g, 8.20 mmol) in 30 mL of acetone was stirred under reflux for 24 h. The mixture was evaporated under reduced pressure and the compound was purified by chromatography on a silica gel column, eluting with CH_2Cl_2 to give 50% yield of an oil.

^{1}H NMR ($CDCl_3$) δ: 1.83–1.97 (m, 4H, $2CH_2$); 3.38 (t, 2H, J = 7 Hz, CH_2); 4.20 (t, 2H, J = 7 Hz, CH_2); 7.59–7.73 (m, 3H, H-arom); 8.06 (s, 1H, H-phthal.); 8.29–8.31 (m, 1H, H-arom).

6.7. 1-(3-Bromopropyl)-4-(3-chlorophenyl)piperazine (26)

Using the method reported by Bourdais,[8] a mixture of 1-(3-chlorophenyl)piperazine hydrochloride (1.50 g, 9.10 mmol), 1,3-dibromo-propane (2.80 g, 13.0 mmol), and dry potassium carbonate (1.90 g, 14.0 mmol) in 20 mL of dry dimethylformamide was stirred for 24 h at room temperature (rt). The compound was purified by chromatography on a silica gel column, eluting with ethyl acetate to give 25% yield of a yellow dense oil.

^{1}H NMR ($CDCl_3$) δ: 1.97–2.08 (m, 2H, CH_2); 2.49–2.59 (m, 6H, CH_2, 4H-pip.); 3.48(t, 2H, J = 7 Hz, CH_2); 3.79–3.85 (m, 4H, H-pip.); 6.73–6.85 (m, 3H, H-arom); 7.15 (m, 1H, H-arom).

6.8. 4-Chloro-5-[4-(2-furoyl)-[1,4]diazepan-1-yl]pyridazin-3(2*H*)-one (30)

A mixture of 4,5-dichloro-pyridazin-3(2*H*)-one (2.00 g, 12.0 mmol), homopiperazine (1.80 g, 18.0 mmol) in ethanol and Et_3N was stirred under reflux for 5 h. The mixture was evaporated under reduced pressure and the compound was purified by crystallization with methanol to give a 50% yield of homopiperazine derivate **29** as a yellow solid: mp 167–170 °C

Subsequently a mixture of **29** (1.00 g, 4.40 mmol), furoyl chloride (0.58 g, 4.46 mmol), and sodium bicarbonate (0.56 g, 6.60 mmol) in 40 mL of chloroform was stirred for 20 h, rt. After evaporation under reduced pressure, **30** was purified by chromatography on a silica gel column, eluting with a EtOH/CH_2Cl_2 mixture (6:94) to give 55% yield of a white solid: mp 184–186 °C.

^{1}H NMR ($CDCl_3$) δ: 2.16–2.18 (m, 2H, CH_2-homopip.); 3.63–3.90 (m, 8H, H-homopip.); 6.42–6.45 (m, 1H, H-fur); 7.07–7.09 (m, 1H, H-fur); 7.46–7.48 (m, 1H, H-fur); 7.68 (s, 1H, H-pyrid.); 12.20 (s, 1H, NH).

6.9. 2-(4-Bromobutyl)-4-chloro-5-[4-(2-furoyl)-[1,4]diazepan-1-yl]pyridazin-3(2*H*)-one (31)

A mixture of 4-chloro-5-[4-(2-furoyl)-[1,4]diazepan-1-yl]pyridazin-3(2*H*)-one (**30**) (0.28 g, 0.87 mmol), 1,4-dibromo-butane (0.28 g, 1.3 mmol) and dry potassium carbonate (0.18 g, 1.3 mmol) in acetone was stirred under reflux for 10 h. After evaporation under reduced pressure, the compound was purified by chromatography on a silica gel column, eluting with a EtOH/CH_2Cl_2 mixture (4:96) to give 60% yield of a yellow oil.

^{1}H NMR ($CDCl_3$) δ: 1.83–1.95 (m, 4H, $2CH_2$); 2.14–2.16 (m, 2H, CH_2-homopip.); 3.65–4.15 (m, 10H, 8H-homopip., CH_2); 4.45 (m, 2H, CH_2); 6.47–6.49 (m, 1H, H-fur); 7.07–7.09 (m, 1H, H-fur); 7.46–7.48 (m, 1H, H-fur); 7.68 (s, 1H, H-pyrid.).

6.10. Method A Example. 4-Chloro-5-[4-(2-furoyl)piperazin-1-yl]-2-{4-[4-(2-isopropoxyphenyl)piperazin-1-yl]butyl}pyridazin-3(2*H*)-one (5)

A mixture of 2-(4-bromobutyl)-4-chloro-5-[4-(2-furoyl)piperazin-1-yl]pyridazin- 3(2*H*)-one (**22**)[3] (0.48 g, 1.08 mmol), 1-(2-isopropoxyphenyl)piperazine[6] (0.24 g, 1.08 mmol) in 30 mL of acetonitrile in the presence of dry potassium carbonate (0.18 g, 1.30 mmol) was stirred under reflux for 2 h. After evaporation under reduced pressure, the residue was purified by chromatography on a silica gel column, eluting with a EtOH/CH_2Cl_2 mixture (6:94) to give 60% yield of a dense oil.

^{1}H NMR ($CDCl_3$) δ: 1.30 (d, 6H, 2 CH_3); 1.59–1.61 (m, 2H, CH_2); 1.81–1.83 (m, 2H CH_2); 2.42–2.43 (m, 2H, CH_2); 2.62–2.65 (m, 4H, H-pip.); 3.10–3.20 (m, 4H, H-pip.); 3.40–3.45 (m, 4H, H-pip.); 3.96–3.98 (m, 4H, H-pip.); 4.19 (t, 2H, J = 7 Hz, CH_2); 4.58 (sept., 1H, *CH*–$(CH_3)_2$); 6.45 (d.d., J = 1.7 and 3.4 Hz, 1H, H-fur); 6.84–6.89 (m, 4H, H-arom); 7.06 (d.d., J = 0.8 and 3.4 Hz, 1H, H-fur); 7.49–7.50 (m, 1H, H-fur); 7.60 (s, 1H, H-pyrid.). For the corresponding hydrochloride: mp 90–93 °C Anal. ($C_{30}H_{39}N_6O_4Cl{\cdot}2HCl{\cdot}3H_2O$) C, H, N.

6.11. Method B Example. 2-{4-[5-Chloro-4-[4-(2-furoyl)piperazin-1-yl]-6-oxopyridazin-1(6*H*)-yl]butyl}phthalazin-1(2*H*)-one (11)

A mixture of 2-(4-bromobutyl)phthalazin-1(2*H*)-one (**25**) (0.34 g, 1.20 mmol), 4-chloro-5-[4-(2-furoyl)piperazin-1-yl]pyridazin-3(2*H*)-one (**20**) (0.30 g, 0.970 mmol) in 20 mL of acetone in the presence of dry potassium carbonate (0.16 g, 1.20 mmol) was refluxed under stirring for 11 h. After evaporation under reduced pressure, the residue was purified by chromatography on a silica gel column eluting with a EtOH/CH_2Cl_2 mixture (4:96) to give 70% yield of a white solid: mp 57–60 °C.

^{1}H NMR ($CDCl_3$) δ: 1.84–1.88 (m, 4H, $2CH_2$); 3.40–3.43 (m, 4H, H-pip.); 3.94–3.96 (m, 4H, H-pip.); 4.19–4.25 (m, 4H, $2CH_2$); 6.48–6.50 (d.d.,1H, J = 1.7 and 3.4 Hz, H-fur); 7.04–7.24 (d.d., J = 0.8 and 3.4 Hz, 1H, H-fur); 7.48–7.77 (m, 5H, 3H-arom, 1H-fur, 1H-pyrid.); 8.12 (s, 1H, H-pyrid.); 8.36 (d, 1H, H-arom).

6.12. Biological evaluation

5-HT_{1A} receptor binding: Rat cerebral cortex was homogenized in 10 volumes of ice-cold 50 mM Tris–HCl buffer at pH 7.4 in an ultraturrax homogenizer. The homogenate was centrifuged at 48,000*g* for 15 min at 4 °C. The pellet was suspended in 35 volumes of 50 mM Tris–HCl buffer, incubated at 37 °C for 10 min to remove endogenous 5-HT, and centrifuged at 48,000*g* for 15 min at 4 °C. The resulting pellet was frozen at − 80 °C until the time of assay. The pellet was suspended in 20 volumes of ice-cold 50 mM Tris–HCl buffer at pH 7.4, and the 5-HT_{1A}R binding assay was performed in triplicate by incubating at 37 °C for 15 min in 1 mL of buffer containing aliquots of the membrane fraction (0.2–0.3 mg of protein) and 1 nM [^{3}H]8-OH-DPAT in the absence or presence of unlabeled 0 μM 8-OH-DPAT. The binding reaction was concluded by filtration through Whatman GF/C glass fiber filters under reduced pressure. Filtrates were washed twice with 5 mL aliquots of ice-cold buffer and placed in scintillation vials. The level of specific binding was obtained by subtracting the level of non-specific binding from the total level of binding and was approximated to be 85–90% of the total level of binding.

α_1-receptor binding: Rat cerebral cortex was homogenized in 20 volumes of ice-cold 50 mM Tris–HCl buffer at pH 7.7 containing 5 mM EDTA (buffer T_1) in an ultraturrax homogenizer. The homogenate was centrifuged at 48,000*g* for 15 min at 4 °C. The pellet was suspended in 20 volumes of ice-cold buffer T_1. It was then homogenized and centrifuged at 48,000*g* for 15 min at 4 °C. The resulting pellet (P_2) was frozen at − 80 °C until the time of assay.

Pellet P_2 was suspended in 20 volumes of ice-cold 50 mM Tris–HCl buffer at pH 7.7 (T_2 buffer), and the α_1 binding assay was performed in triplicate by incubating at 25 °C for 60 min in 1 mL T_2 buffer containing aliquots of the membrane fraction (0.2–0.3 mg of protein) and 0.1 nM [^{3}H]prazosin in the absence or presence of unlabeled 1 μM prazosin. The binding reaction was terminated by filtering through Whatman GF/C glass fiber filters under reduced pressure and washing twice with 5 mL of ice-cold Tris buffer. The filtrates were placed in scintillation vials, and 4 mL of Ultima Gold MN Cocktail-Packard solvent scintillation fluid was added. The radioactivity was assessed with a Packard 1600 TR scintillation counter. The level of specific binding was obtained by subtracting the level of non-specific binding from the total level of binding and was approximated to be 85–90% of the total level of binding.

α_2-receptor binding: Cerebral cortex was dissected from rat brain, and the tissue was homogenized in 20 volumes

of ice-cold 50 mM Tris–HCl buffer at pH 7.7 containing 5 mM EDTA, as reported above (buffer T_1). The homogenate was centrifuged at 48,000*g* for 15 min at 4 °C. The resulting pellet was diluted in 20 volumes of 50 mM Tris–HCl buffer at pH 7.7 and used in the binding assay.

The binding assay was performed in triplicate, by incubating aliquots of the membrane fraction (0.2–0.3 mg of protein) in Tris–HCl buffer at pH 7.7 with approximately 2 nM [^{3}H]rauwolscine in a final volume of 1 mL. Incubation was carried out at 25 °C for 60 min. Non-specific binding was defined in the presence of 10 μM rauwolscine. The binding reaction was concluded by filtration through Whatman GF/C glass fiber filters under reduced pressure. Filters were washed four times with 5 mL aliquots of ice-cold buffer and placed in scintillation vials. The level of specific binding was obtained by subtracting the level of non-specific binding from the total level of binding and approximated to be 85–90% of the total level of binding. The receptor-bound radioactivity was assessed as described above.

Compounds were dissolved in buffer or DMSO (2% buffer concentration) and added to the assay mixture. A blank experiment was carried out to determine the effect of the solvent on binding. Protein estimation was based on a reported method,[13] after solubilization with 0.75 N sodium hydroxide, using bovine serum albumin as a standard.

The concentration of tested compound that produces 50% inhibition of specific [^{3}H]prazosin or [^{3}H]rauwolscine, or [^{3}H]8-OH-DPAT binding (IC_{50}) was determined by log-probit analysis with seven concentrations of the displacer, each performed in triplicate. Inhibition constants (K_i) were calculated according to the Cheng–Prusoff equation.[14] K_d of [^{3}H]prazosin binding to cortex membranes was 0.24 nM (α_1), K_d for [^{3}H]rauwolscine binding to cortex membranes was 4 nM (α_2), and K_d of [^{3}H]8-OH-DPAT binding to cortex membranes was 2 nM (5-HT_{1A}R).

6.12.1. Molecular modeling details. All calculations and graphic manipulations were performed on an SGI Origin300 server and an Octane 12K workstation by means of the Catalyst (version 4.9) software package.[15] All the compounds used in this study were built using the 2D-3D sketcher of Catalyst. A representative family of conformations was generated for each molecule using the poling algorithm and the 'best quality conformational analysis' method. Conformational diversity was emphasized by selection of the conformers that fell within 20 kcal/mol above the lowest energy conformation found. The Compare/Fit command has been used to superpose the studied compounds into the pharmacophoric model for α_1-AR antagonists.

Acknowledgments

M.B. thanks the Merk Research Laboratories (2004 Academic Development Program Chemistry Award). F.M. wishes to thank the Divisione di Chimica Farmaceutica della Società Chimica Italiana and Farmindustria for the 'Premio Farmindustria 2004' award.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.12.009.

References and notes

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2. In the text, we use the following notation. The term 'arylpiperazine moiety' is in general referred to the molecular portion constituted by a substituted phenylpiperazine moiety directly linked to the polymethylene spacer. As an example, the *o*-isopropoxyphenylpiperazine group of compound **18** is termed as the 'arylpiperazine moiety,' while its *o*-ethoxyphenylpiperazine substituent bound to the pyridazinone nucleus constitutes the so-called 'terminal heterocyclic moiety.' Such a notation derives from the superposition mode of the new compounds to the pharmacophoric model for α_1-AR antagonists, showing their phenylpiperazine group (bound to the spacer) onto the HY1–HY2 system of the model, while the piperazine ring linked to the pyridazinone moiety lies within a region of space between HBA and HY3 (see Fig. 1).
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