Brief Articles

α_1-Adrenoceptor Antagonists. 6.[1] Structural Optimization of Pyridazinone−Arylpiperazines. Study of the Influence on Affinity and Selectivity of Cyclic Substituents at the Pyridazinone Ring and Alkoxy Groups at the Arylpiperazine Moiety

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Received January 20, 2003

In continuing our search for selective α_1-adrenoceptor (AR) antagonists, we have synthesized new alkoxyarylpiperazinylalkylpyridazinone derivatives. The new compounds were tested for their affinity toward α_1- and α_2-AR and toward the 5-HT_{1A} receptor. α_1-AR affinity data are in the subnanomolar range, with **3** showing an affinity of 0.052 nM, about 5-fold higher than prazosin. None of the studied compounds was found to be α_1/α_2 selective, but **8** showed an interesting 5-HT_{1A}/α_1 affinity ratio of 119.

Introduction

In continued attempts to identify potent and selective α_1-AR antagonists, we have previously published SAR and 3D QSAR studies on arylpiperazinylalkylpyridazinone derivatives designed on the basis of a pharmacophoric model[2] suggesting the following three-dimensional structural properties for an ideal α_1-AR antagonist (Table 1). (1) A positively ionizable group, corresponding to the more basic nitrogen atom of the piperazine ring, and (2) an ortho- or meta substituted phenyl ring, both of them constituting the arylpiperazine system, satisfy three (namely, PI HY1-HY2, respectively) of the five features of the pharmacophoric hypothesis. Moreover, (3) a polar group (corresponding to the pyridazinone ring) able to provide a hydrogen bond acceptor feature filling the HBA portion of the pharmacophore is required at the edge of the molecule opposite the arylpiperazine moiety. Finally, (4) a third hydrophobic moiety, corresponding to the terminal molecular portions directly linked to the pyridazinone ring, was hypothesized to fit the HY3 feature of the model.

The pharmacophoric model also suggested[2,3] that (1) the hydrophobic region accommodating the substituted phenyl ring is able to locate substituents larger than a methoxy group, (2) both affinity and selectivity are dependent on the length of the polymethylene chain connecting the pyridazinone and the arylpiperazine moieties, being a heptyl spacer optimal for α_1 affinity, and (3) a (hetero)cyclic fragment bigger than an aromatic five-membered ring is required as the terminal molecular portion for best α_1-AR affinity.

On the basis of these suggestions, **1**, derived from our previous work in this field and bearing the terminal furoylpiperazinyl moiety similarly to the reference compound prazosin, was chosen as a hit compound to be structurally optimized for improving affinity and selectivity toward α_1-AR. With the length of the alkyl chain fixed on a seven-carbon atom sequence, the new compounds have been obtained by variation of (i) the bulkiness of the ortho substituent on the phenyl ring of the arylpiperazine moiety, (ii) the terminal molecular portion opposite the arylpiperazine moiety (i.e., the furoyl group of **1**), and (iii) substituents and substitution pattern on the central pyridazinone nucleus.

All of them were evaluated for their affinity toward α_1-AR, α_2-AR, and 5-HT_{1A} serotoninergic receptors, and SAR studies are reported here.

Chemistry

Compounds **2**−**16** were synthesized as outlined in Scheme 1. In detail, a mixture of 4,5-dichloropyridazin-3(2*H*)-one (**18**) and the appropriate 1-substituted piperazine (**19**) was refluxed for 15 h in ethanol and Et_3N to afford intermediates **20a**−**d** (method A). Next, 4-chloro-5-[4-(2-furoyl)piperazin-1-yl]pyridazin-3(2*H*)-one (**20a**), 4-chloro-5-{[4-[2-(1,4-benzodioxan)methyl]piperazin-1-yl}pyridazin-3(2*H*)-one (**20b**), 4-chloro-5-{[4-[2-(2-methoxyphenoxy)ethyl]piperazin-1-yl}pyridazin-3(2*H*)-one (**20c**), and 4-chloro-5-{[4-[2-(2-ethoxyphenoxy)ethyl]piperazin-1-yl}pyridazin-3(2*H*)-one (**20d**) were in turn transformed into intermediates **21a**−**d** by treating with 1,7-dibromoheptane in acetone and potassium carbonate

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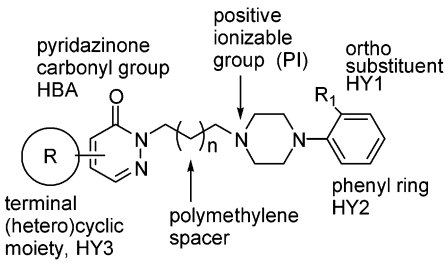
10.1021/jm0307842 CCC: $25.00
Published on Web 06/11/2003

Table 1. α_1-AR, α_2-AR, and 5-HT_{1A} Affinities for **1**−**17**



			K_i [c] (nM)		
compd[a]	R[b]	R_1	α_1-AR	α_2-AR	5-HT_{1A}
1[d]	*a*	−OMe	1.9 ± 0.1	520 ± 0.2	ND[e]
2[f]	*a*	−OEt	0.5 ± 0.02	4.0 ± 0.2	ND[e]
3[f]	*a*	−O^{i}Pr	0.052 ± 0.007	0.56 ± 0.19	0.90 ± 0.09
4	*a*	−OMe	0.37 ± 0.09	1.23 ± 0.14	0.42 ± 0.16
5	*a*	−OEt	0.23 ± 0.03	0.80 ± 0.07	0.35 ± 0.10
6	*a*	−O^{i}Pr	0.08 ± 0.01	0.66 ± 0.05	1.16 ± 0.72
7	*b*	−OMe	1.68 ± 0.50	0.85 ± 0.16	11.1 ± 5.5
8	*b*	−OEt	0.42 ± 0.01	2.16 ± 0.08	49.8 ± 16.7
9	*b*	−O^{i}Pr	0.31 ± 0.08	3.34 ± 0.22	2.47 ± 0.91
10	*b*	−OMe	0.54 ± 0.10	1.45 ± 0.21	0.40 ± 0.29
11	*b*	−OEt	0.43 ± 0.08	2.40 ± 0.42	1.02 ± 0.51
12	*b*	−O^{i}Pr	0.32 ± 0.02	13.4 ± 3.2	1.52 ± 0.80
13[f]	*d*	−OMe	0.55 ± 0.17	1.59 ± 0.15	0.82 ± 0.31
14[f]	*d*	−OEt	0.43 ± 0.08	2.04 ± 0.24	1.32 ± 0.62
15[f]	*d*	−O^{i}Pr	0.26 ± 0.04	3.21 ± 0.13	2.54 ± 1.02
16[f]	*c*	−OEt	0.58 ± 0.09	8.22 ± 0.34	ND[e]
17[d]	*c*	−OMe	1.45 ± 0.14	4.64 ± 0.49	ND[e]
P			0.24 ± 0.05		
R				4.0 ± 0.3	
D					2.0 ± 0.2

[a] Compounds **2**−**15** have been submitted to an Italian patent.[4] [b] *a*, 2-furoyl; *b*, 1,4-benzodioxan-2-yl; *c*, 2-(2-methoxyphenoxy)ethyl; *d*, 2-(2-ethoxyphenoxy)ethyl. [c] The K_i binding data were calculated as described in the Experimental Section. The K_i values are the mean ± SD of a series of separate assays, each performed in triplicate. Inhibition constants (K_i) were calculated according to the equation of Cheng and Prusoff:[5] $K_i = IC_{50}/(1 + [L]/K_d)$ where [L] is the radiolabeled ligand concentration and K_d its dissociation constant. K_d of [^{3}H]prazosin (**P**) binding to rat cortex membranes was 0.24 nM (α_1), K_d of [^{3}H]rauwolscine (**R**) binding to rat cortex membranes was 4 nM (α_2), and K_d of [^{3}H]8-OH-DPAT (**D**) binding to rat cortex membranes was 2 nM (5-HT_{1A}). [d] Compounds reported elsewhere by our research group.[2] [e] ND: not determined. [f] Compounds reported elsewhere by our research group.[1]

(method B). Compounds **2** and **3** were obtained from **21a** with 1-(2-ethoxyphenyl)piperazine (**22b**) and 1-(2-isopropoxyphenyl)piperazine (**22c**), respectively, in isoamyl alcohol and sodium carbonate (method C). Following method C, while **16** was prepared from **21c** by reaction with **22b**, compounds **13**, **14**, and **15** were obtained by reaction between **21d** and 1-(2-methoxyphenyl)piperazine (**22a**), **22b**, and **22c**, respectively. Finally, **7**−**9** were obtained from the reaction between the intermediate **21b** and **22a**−**c**, respectively.

When the reaction between **18** and the piperazines **19a**,**b** was performed in an apolar aprotic solvent such as dry dioxane instead of a polar protic solvent like ethyl alcohol, the usual 4-chloro-5-substituted pyridazinones **20a**,**b** were obtained together with the corresponding 4-substituted-5-chloro isomers (**23a**,**b**). Isomers were easily separated by chromatography on a silica gel column, eluting with EtOH/CH_2Cl_2. In particular, intermediates **23a**,**b** obtained in dry 1,4-dioxane and dry $KHCO_3$ (method D) starting from **19a**,**b** were in turn alkylated following method B to give the corresponding bromoalkyl derivatives **24a**,**b**. Following method C, while reaction between **24a** and **22a**−**c** afforded **4**−**6**, respectively, **10**−**12** were obtained by treating **24b** with the same arylpiperazines **22a**−**c**, respectively.

Biology

The pharmacological profile of **2**−**16** was evaluated for their affinities toward α_1-AR, α_2-AR, and 5-HT_{1A} serotoninergic receptor by determining for each compound the ability to displace [^{3}H]prazosin, [^{3}H]rauwolscine, and [^{3}H]8-OH-DPAT, respectively, from specific binding sites on rat cerebral cortex. K_i values were determined on the basis of three competition binding experiments in which seven drug concentrations, run in triplicate, were used.[2,3]

Moreover, to determine the intrinsic activity of compounds with the best affinity profile toward α_1-AR (namely, **3**, **9**, and **15**), 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.

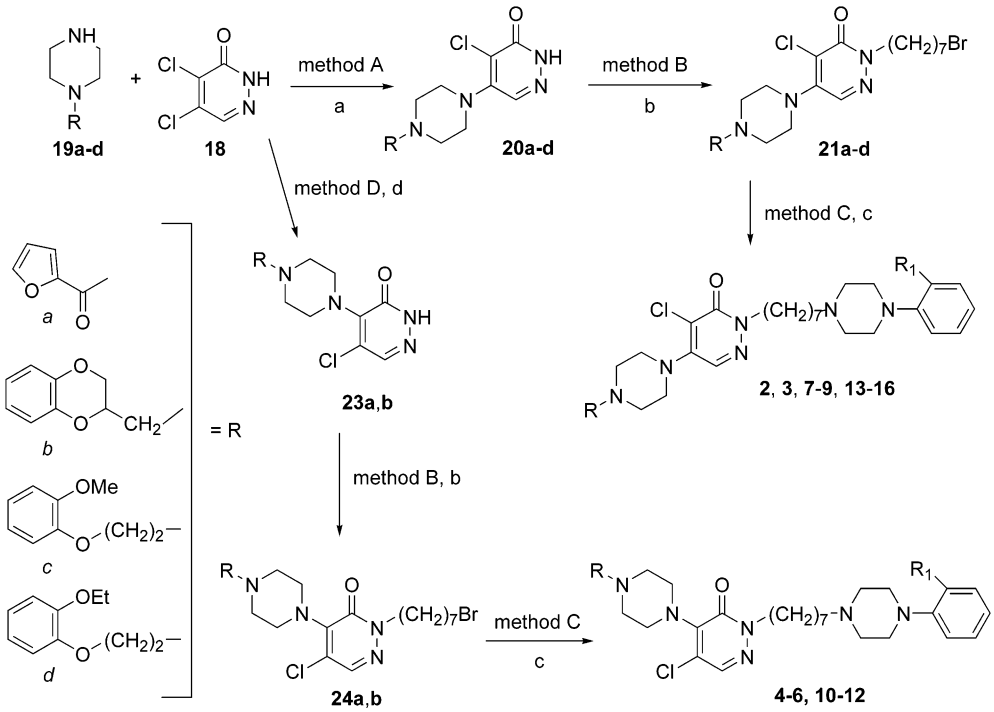
At the α_1 receptor, the selected compounds displayed no significant GTP shift, suggesting that they elicited an antagonist profile such as prazosin.

Results and Discussion

Affinity data reported in Table 1 clearly showed that all the new compounds were characterized by a very interesting biological profile. In fact, with the exception of **7** (K_i = 1.68 nM), affinity values toward α_1-AR were all in the subnanomolar range (comparable to that of the reference compound prazosin) or lower. On the basis of both α_1-AR affinity data and structural properties of the studied compounds, some suggestions can be ruled out. (i) As expected, increasing the size of the ortho alkoxy substituent on the phenyl ring of the arylpiperazine moiety afforded compounds with enhanced affinity toward α_1-AR. In fact, without any exception, the largest isopropoxy group led to the best α_1-AR affinity profile. Very interestingly, a couple of compounds (namely, **3** and **6**) showed an affinity (0.05 and 0.08 nM, respectively) significantly higher than the reference compound prazosin. (ii) The substitution pattern on the pyridazinone ring is not important for defining affinity toward α_1-AR, affinities being comparable to each other among various subclasses of compounds (**1**−**3** versus **4**−**6**, respectively, and **7**−**9** versus **10**−**12**, respectively). (iii) Comparison between the furoyl class and the remaining series suggested that the terminal fragment was not a fundamental key in influencing α_1 affinity in compounds bearing an *o*-methoxy and *o*-ethoxy substituent (**1**,**2** versus **7**,**8** and **13**,**14**, respectively, and **4**,**5** versus **10**,**11**, respectively). On the contrary, among the isopropoxy derivatives, compounds belonging to the furoyl series showed affinity values of about 6-fold (**3** versus **9**) and 4-fold (**6** versus **12**) lower than the corresponding benzodioxane derivatives. Similarly, **3** was 5-fold more active than **15**.

Regarding the α_2 affinity values, while in the furoyl series higher affinity was associated with a larger substituent (as for α_1-AR affinity), both the benzodioxane and alkoxyphenoxyethyl derivatives showed lower affinity with bulkier substituents. Moreover, the inter-

Scheme 1[a]



[a] Compounds: **1**, R = *a*, R_1 = OMe; **2**, R = *a*, R_1 = OEt; **3**, R = *a*, R_1 = O^{i}Pr; **4**, R = *a*, R_1 = OMe; **5**, R = *a*, R_1 = OEt; **6**, R = *a*, R_1 = O^{i}Pr; **7**, R = *b*, R_1 = OMe; **8**, R = *b*, R_1 = OEt; **9**, R = *b*, R_1 = O^{i}Pr; **10**, R = *b*, R_1 = OMe; **11**, R = *b*, R_1 = OEt; **12**, R = *b*, R_1 = O^{i}Pr; **13**, R = *d*, R_1 = OMe; **14**, R = *d*, R_1 = OEt; **15**, R = *d*, R_1 = O^{i}Pr; **16**, R = *c*, R_1 = OEt; **17**, R = *c*, R_1 = OMe; **19a**, R = *a*; **19b**, R = *b*; **19c**, R = *c*; **19d**, R = *d*; **20a**, R = *a*; **20b**, R = *b*; **20c**, R = *c*; **20d**, R = *d*; **21a**, R = *a*; **21b**, R = *b*; **21c**, R = *c*; **21d**, R = *d*; **23a**, R = *a*; **23b**, R = *b*; **24a**, R = *a*; **24b**, R = *b*. Reagents: (a) EtOH, Et_3N; (b) $Br(CH_2)_7Br$, acetone, K_2CO_3; (c) **22a**, **22b**, or **22c**, isoamyl alcohol, Na_2CO_3; (d) dry 1,4-dioxane, $KHCO_3$.

Table 2. Intrinsic Activity of Compounds **3**, **9**, and **15** toward α_1-AR

	K_i toward α_1[a] (nM)		
compd	−GTP	+GTP	GTP shift
3	0.047 ± 0.003	0.081 ± 0.005	1.7
9	0.23 ± 0.03	0.33 ± 0.05	1.4
15	0.28 ± 0.04	0.22 ± 0.02	0.8
prazosin	0.26 ± 0.05	0.32 ± 0.06	1.2

[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 the mean ± SEM.

esting selectivity of **1** (α_2/α_1 = 274) was lost mainly because of an improvement of the α_2 affinity profile, and none of the studied compounds showed appreciable selectivity, 42 being the highest α_2/α_1 ratio found for **12**.

In summary, bulky alkoxy groups at the phenylpiperazine moiety in conjunction with larger (with respect to the furoyl moiety) or conformationally flexible terminal fragments are required for selectivity toward α_1-AR.

As far as 5-HT_{1A} affinity was concerned, **8** was found with an appreciable α_1-AR selectivity (5-HT_{1A}/α_1 ratio of 119), suggesting that the new pyridazinone−arylpiperazines can be good templates for the development of novel α_1 selective ligands with respect to the 5-HT_{1A} receptor.

Conclusions

On the basis of suggestions derived from our previous work in the field of α_1-AR antagonists, a number of novel arylpiperazine−pyridazinone-containing compounds were designed, synthesized, and evaluated for their biological properties. As a result, each of them was found to have a high affinity for α_1-AR comparable to or higher (up to 4- to 5-fold higher) than the affinity of the reference compound prazosin. Moreover, **8**, belonging to the benzodioxane class and retaining an α_1-AR affinity in the subnanomolar range (0.42 nM), was characterized by a marked decrease in 5-HT_{1A} affinity, thus leading to an interesting 5-HT_{1A}/α_1 selectivity of about 120. As a consequence, this compound has been chosen as a lead to pursue α_1 selectivity with respect to serotoninergic 5-HT_{1A} affinity.

Experimental Section

Chemistry. Melting points were determined using 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) are relative to that for tetramethylsilane used as an internal reference standard. Elemental analyses are within ±0.4% of theorical values. Precoated Kiesegel 60 F_{254} plates (Merck) were used for TLC. The corresponding hydrochloride derivatives were prepared by bubbling dry HCl into the dry solution of the compound.

Synthesis. Specific examples presented below illustrate the general synthetic methods A−D.

Method A Example. 4-Chloro-5-{4-[2-(2-ethoxyphenoxy)-ethyl]piperazin-1-yl}pyridazin-3(2*H*)-one (20d). A mixture of 4,5-dichloropyridazin-3(2*H*)-one **18** (4.45 g, 27 mmol) and 1-[2-(2-ethoxyphenoxy)ethyl]piperazine **19d** (7.30 g, 30 mmol) in dry ethanol and Et_3N was refluxed under stirring for 15 h. The mixture was evaporated under reduced pressure and purified by chromatography on a silica gel column, eluting with a EtOH/CH_2Cl_2 mixture (6:94) to give a 50% yield of a white solid: mp 140−143 °C. ^{1}H NMR ($CDCl_3$) δ: 1.42 (t, 3H, J = 6

Hz, CH_3), 2.80 (m, 4H, H-pip), 2.95 (m, 2H, CH_2), 3.45−3.50 (m, 4H, H-pip), 4.10 (quart 2H, CH_2−CH_3), 4.25 (t, 2H, $J = 6$ Hz, CH_2), 6.80−6.95 (m, 4H, H-arom), 7.68 (s, 1H, H6-pyrid), 11.82 (s, 1H, NH-pyrid).

Method B Example. 2-(7-Bromoheptyl)-4-chloro-5-{4-[2-(2-ethoxyphenoxy)ethyl]piperazin-1-yl}pyridazin-3(2*H*)-one (21d). A mixture of **20d** (7.60 g, 20 mmol), 1,7-dibromoheptane (7.75 g, 30 mmol), and dry potassium carbonate (4.15 g, 30 mmol) in 70 mL of acetone was refluxed under stirring for 22 h. The mixture was evaporated under reduced pressure and purified by chromatography on a silica gel column, eluting with a EtOH/CH_2Cl_2 mixture (3:97) to give 30% yield of dense oil. ^{1}H NMR ($CDCl_3$) δ: 1.25−1.45 (m, 9H, $3CH_2$, CH_3), 1.76−1.85 (m, 4H, $2CH_2$), 2.75−2.80 (m, 4H, H-pip), 2.89−2.93 (m, 2H, CH_2), 3.35−3.45 (m, 6H, CH_2, 4H-pip), 4.00−4.20 (m, 6H, $3CH_2$), 6.88−6.95 (m, 4H, H-arom), 7.59 (s, 1H, H6-pyrid).

Method C Example. 2-{7-[4-(2-Methoxyphenyl)piperazin-1-yl]heptyl}-4-chloro-5-{4-[2-(1,4-benzodioxanyl)methyl]piperazin-1-yl}pyridazin-3(2*H*)-one (7). This compound was obtained from **21b** and 1-(2-methoxyphenyl)-piperazine **22a** following the same synthetic procedure applied for **2**. The mixture was refluxed under stirring for 6 h, and the compound was purified by chromatography on a silica gel column, eluting with a EtOH/CH_2Cl_2 mixture (5:95) to give a 50% yield of a dense oil. ^{1}H NMR ($CDCl_3$) δ: 1.25−1.47 (m, 6H, $3CH_2$), 1.76−1.79 (m, 2H, CH_2), 2.30−2.42 (m, 2H, CH_2), 2.58−2.78 (m, 10H, 8H-pip, CH_2), 3.09−3.15 (m, 4H, H-pip), 3.37−3.41 (m, 4H, H-pip), 3.84 (s, 3H, OCH_3), 3.95−4.11 (m, 5H, CH_2-benzodioxan-, CH-benzodioxan-, CH_2), 4.15−4.33 (m, 2H, CH_2-benzodioxan-,), 6.80−6.90 (m, 8H, H-arom), 7.58 (s, 1H, H6-pyrid). For the corresponding hydrochloride: mp 62−68 °C. Anal. ($C_{35}H_{47}ClN_6O_4$·3HCl) C, H, N.

Method D Example. 4-{4-[(1,4-Benzodioxanyl)-2-methyl]piperazin-1-yl}-5-chloropyridazin-3(2*H*)-one (23b). A mixture of 1-[2-(1,4-benzodioxan)methyl]piperazine **19b** (0.989 g, 4.23 mmol), **18** (0.70 g, 4.23 mmol), and dry $KHCO_3$ (0.42 g, 4 mmol) in 50 mL of dry 1,4-dioxane was refluxed under stirring for 24 h. The mixture was filtered, and the filtrate was evaporaed under reduced pressure. In particular, the 4-substituted isomer **23b** was eluted with EtOH/ CH_2Cl_2 (3:97) to give 62% yield of a solid product: mp 136−139 °C. ^{1}H NMR ($CDCl_3$) δ: 2.60−2.79 (m, 6H, 4H-pip, CH_2), 3.54−3.59 (m, 4H, H-pip), 3.96−4.05 (m, 1H, CH-benzodioxan-), 4.28−4.42 (m, 2H, CH_2-benzodioxan-), 6.75−6.90 (m, 4H, H-arom), 7.60 (s, 1H, H6-pyrid), 11.29 (s, 1H, NH-pyrid).

The corresponding 5-substituted isomer **20b** was eluted with EtOH/CH_2Cl_2 (5:95) to give 35% yield of a solid product.

Acknowledgment. Financial support provided by the Italian MIUR ("Progettazione e Sintesi di Agenti Neuroprotettivi") is gratefully acknowledged. M.B. thanks the Merck Research Laboratories for the 2002 Academic Development Program Chemistry Award.

Supporting Information Available: Details of the synthesis and spectral data of some representative compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0307842