α_1-Adrenoceptor Antagonists. 4.[1] Pharmacophore-Based Design, Synthesis, and Biological Evaluation of New Imidazo-, Benzimidazo-, and Indoloarylpiperazine Derivatives

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As a part of a program aimed at discovering compounds endowed with α_1-adrenoceptor (AR) blocking properties, in this paper we describe the synthesis and biological characterization of the compounds designed to fully match a three-dimensional pharmacophore model for α_1-AR antagonists previously developed by our research group. Accordingly, the structure of trazodone (**1**), identified during a database search performed by using the model as a 3D query, was chosen as the starting point for this study and modified following suggestions derived from a literature survey. In particular, the triazolopyridine moiety of trazodone was replaced with different heteroaromatic rings (such as imidazole, benzimidazole, and indole), and a pyridazin-3(2*H*)-one moiety was inserted into the scaffold of the new compounds to increase the overall length of the molecules and to allow for a complete fit into all the pharmacophore features. Our aim was also to study the influence of the position of both the chloro and the methoxy groups on the piperazine phenyl ring, as well as the effect of the lengthening or shortening of the polymethylene spacer linking the phenylpiperazine moiety to the terminal heterocyclic portion. Compounds obtained by such structural optimization share a 6-(imidazol-1-yl)-, 6-(benzimidazol-1-yl)-, or 6-(indol-1-yl)pyridazin-3(2*H*)-one as a common structural feature that represents an element of novelty in the SAR of arylpiperazine compounds acting toward α_1-AR. Biological evaluation by radioligand receptor binding assays toward α_1-AR, α_2-AR, and 5-HT_{1A} serotoninergic receptors indicated compounds characterized by very good α_1-AR affinity and selectivity. Very interestingly, chemical features (such as the *o*-methoxyphenylpiperazinyl moiety and an alkyl spacer of three or four methylene units) that generally do not allow for 5-HT_{1A}/α_1 selectivity led to compounds **2c** and **6c** with a 5-HT_{1A}/α_1 ratio of 286 and 281, respectively. Finally, compounds with the best α_1-AR affinity profile (**2c**, **5f**, and **6c**) were demonstrated to be α_1-AR antagonists.

Introduction

The α_1-adrenoceptors (α_1-AR) are a family of G-protein-coupled seven-transmembrane helix receptors comprising multiple subtypes. To date, they have been characterized as α_{1A}, α_{1B}, and α_{1D} and possess high affinity for prazosin and the corresponding cloned counterparts (α_{1a}, α_{1b}, and α_{1d}, respectively). In a similar way, α_2-AR have been classified into four subtypes called α_{2A}, α_{2B}, α_{2C}, and α_{2D}.[2,3]

The fact that α_1-blockers have been employed in the treatment of benign prostatic hyperplasia (BPH) for more than 2 decades,[4,5] combined with experimental data showing BPH as the most common benign tumor in men, led in recent years to a marked increase in the search and development of new α_1-AR antagonists.[1,6−9] On the basis of these considerations and taking into account our previous experience in this field,[1] our goal was the discovery of novel compounds characterized by high affinity for α_1-AR and, possibly, selectivity toward α_1 receptors with respect to α_2-AR.

For this purpose, the starting point of this work was structure **1** (Scheme 1), identified as a hit compound by a database search based on a pharmacophore model for α_1-AR antagonists. Following suggestions taken from a literature survey on the field of α_1-AR antagonists, we decided to computationally investigate the influence of diverse structural modifications of **1** on α_1-AR affinity. Accordingly, by a theoretical approach based on the program Catalyst[10] and the above-mentioned pharmacophore model, we designed two different compounds (**2b** and **3b**; Scheme 2) characterized by a common structural element consisting of an ortho (methoxy and chloro, respectively) substituted phenylpiperazinylalkylpyridazinone moiety bearing a terminal benzimidazole group. In silico prediction of their α_1-AR binding properties (4.2 and 8.5 nM for compounds **2b** and **3b**, respectively; Table 1) led to the suggestion that these com-

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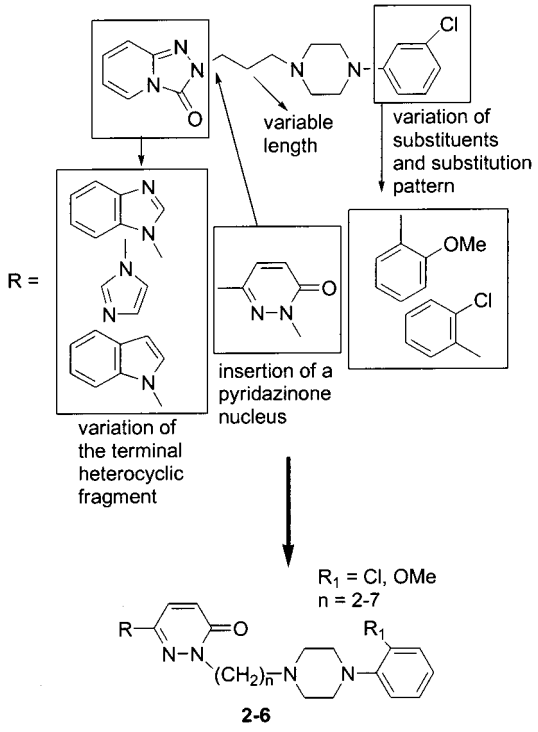
Scheme 1. Trazodone **1**. Low Fit to a Pharmacophore Model for α_1 Adrenoceptor Antagonists



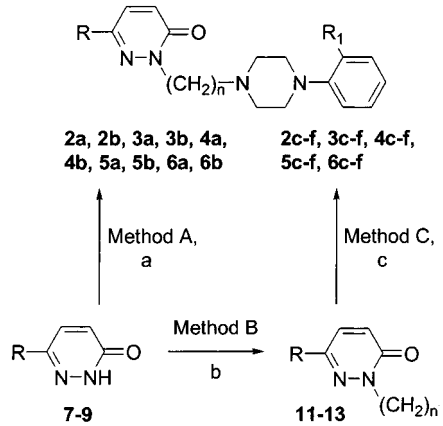
pounds could represent a very interesting synthetic target.

Here, we report the rational design and synthesis of the new compounds **2**−**6** and their biological properties evaluated by radioligand binding assays on α_1-AR, α_2-AR, and 5-HT_{1A} serotoninergic receptors. While they exhibit moderate to high affinity toward α_1-AR, with values ranging from 0.9 nM (compound **6c**) to 472.4 nM (compound **5a**), few of them retain an interesting affinity for α_2-AR (i.e., **2c**, **3c**, **3f**, **5f**, and **6c**), but none of compounds **2**−**6** showed significant potency toward 5-HT_{1A} (K_i values higher than 98 nM). Interestingly, good selectivity was found for compounds **2c** (5-HT_{1A}/α_1 ratio of 286), **6c** (5-HT_{1A}/α_1 ratio of 281), and **6f** (α_2/α_1 ratio of 278). Moreover, both the most active (**6c** with an affinity of 0.9 nM toward α_1-AR) and selective (**6c** and **6f**) compounds belong to the indole subset.

Rational Design

In our previous publications,[1b,c] we described the construction and validation of a three-dimensional pharmacophore model of α_1-AR antagonists sharing a phenylpiperazinylalkyl scaffold as a common structural feature and bearing a wide variety of heterocyclic moieties at the edge of the alkyl spacer. During a validation step performed by using the model as a three-dimensional query in a database search, the antidepressant drug trazodone (**1**, Scheme 1), also reported to have affinity for α_1-AR,[11,12] has been identified as a hit compound. However, trazodone shows a structure characterized by chemical features able to satisfy only partially the spatial constraints imposed by the pharmacophore model (for a representation of the pharmacophore features, see Figure 1). In particular, while the positive ionizable group (PI) and the hydrogen bond acceptor feature (HBA) of the model were perfectly fitted by the piperazine N1 atom and the carbonyl group of **1**, respectively, two peripheral hydrophobic regions were only partially matched by the *m*-chlorophenyl moiety and the condensed pyridine ring of **1**, respectively. As a consequence, the affinity of trazodone for α_1-AR has been calculated by the model to be 220 nM vs a reported experimental value of 281 nM (IC_{50} value).

Scheme 2[a]



[a] Compounds: **2a**, R = 1-benzimidazolyl, n = 2, R_1 = OMe; **2b**, R = 1-benzimidazolyl, n = 3, R_1 = OMe; **2c**, R = 1-benzimidazolyl, n = 4, R_1 = OMe; **2d**, R = 1-benzimidazolyl, n = 5, R_1 = OMe; **2e**, R = 1-benzimidazolyl, n = 6, R_1 = OMe; **2f**, R = 1-benzimidazolyl, n = 7, R_1 = OMe; **3a**, R = 1-benzimidazolyl, n = 2, R_1 = Cl; **3b**, R = 1-benzimidazolyl, n = 3, R_1 = Cl; **3c**, R = 1-benzimidazolyl, n = 4, R_1 = Cl; **3d**, R = 1-benzimidazolyl, n = 5, R_1 = Cl; **3e**, R = 1-benzimidazolyl, n = 6, R_1 = Cl; **3f**, R = 1-benzimidazolyl, n = 7, R_1 = Cl; **4a**, R = 1-imidazolyl, n = 2, R_1 = OMe; **4b**, R = 1-imidazolyl, n = 3, R_1 = OMe; **4c**, R = 1-imidazolyl, n = 4, R_1 = OMe; **4d**, R = 1-imidazolyl, n = 5, R_1 = OMe; **4e**, R = 1-imidazolyl, n = 6, R_1 = OMe; **4f**, R = 1-imidazolyl, n = 7, R_1 = OMe; **5a**, R = 1-imidazolyl, n = 2, R_1 = Cl; **5b**, R = 1-imidazolyl, n = 3, R_1 = Cl; **5c**, R = 1-imidazolyl, n = 4, R_1 = Cl; **5d**, R = 1-imidazolyl, n = 5, R_1 = Cl; **5e**, R = 1-imidazolyl, n = 6, R_1 = Cl; **5f**, R = 1-imidazolyl, n = 7, R_1 = Cl; **6a**, R = 1-indolyl, n = 2, R_1 = 1-imidazolyl, n = 7, R_1 = Cl; **6a**, R = 1-indolyl, n = 2, R_1 = OMe; **6b**, R = 1-indolyl, n = 3, R_1 = OMe; **6c**, R = 1-indolyl, n = 4, R_1 = OMe; **6d**, R = 1-indolyl, n = 5, R_1 = OMe; **6e**, R = 1-indolyl, n = 6, R_1 = OMe; **6f**, R = 1-indolyl, n = 7, R_1 = OMe; **7**, R = 1-benzimidazolyl; **8**, R = 1-imidazolyl; **9**, R = 1-indolyl; **11a**, R = 1-benzimidazolyl, n = 4; **11b**, R = 1-benzimidazolyl, n = 5; **11c**, R = 1-benzimidazolyl, n = 6; **11d**, R = 1-benzimidazolyl, n = 7; **12a**, R = 1-imidazolyl, n = 4; **12b**, R = 1-imidazolyl, n = 5; **12c**, R = 1-imidazolyl, n = 6; **12d**, R = 1-imidazolyl, n = 7; **13a**, R = 1-indolyl, n = 4; **13b**, R = 1-indolyl, n = 5; **13c**, R = 1-indolyl, n = 6; **13d**, R = 1-indolyl, n = 7. Reagents: (a) **10a**, **10b**, **10c**, or **10d**, K_2CO_3, acetone; (b) $Br(CH_2)_nBr$ (n = 4−7), K_2CO_3, acetone; (c) **14** or **15**, Na_2CO_3, isoamyl alcohol.

The pharmacophore model mentioned above also provided a summary of the structural features to be considered important for the affinity and selectivity toward α_1-AR with respect to α_2-AR. In particular, an *o*-methoxy substituent on the phenylpiperazine moiety causes high α_1-AR affinity and α_2/α_1 selectivity; the length of the polymethylene chain bridging the arylpiperazine and the terminal heterocyclic group is the key element for bringing the terminal portions of this molecule to the optimal distance for interacting with the corresponding pharmacophoric features HBA and HY3. Finally, the analysis of the fitting mode (to the pharmacophore model) of compounds bearing an extended terminal moiety (i.e., phenoxyethylpiperazinylpyridazinone) or a long alkyl chain spacer suggested the existence of an "extrasize" portion of such structures (exceeding the pharmacophore itself) corresponding to the

Table 1. The α_1- and α_2-Adrenergic and 5-HT_{1A} Serotoninergic Receptors Binding Affinities for Compounds **2**−**6**

				K_i[b] (nM)		
compd	*n*	R[a]	R_1	α_1-AR[c]	α_2-AR	5-HT_{1A}[d]
2a	2	A	$-OCH_3$	33.0 ± 5.0 (45)	350 ± 50.2	541 ± 5.7
2b	3	A	$-OCH_3$	6.5 ± 0.5 (4.2)	158 ± 15.3	382 ± 30.5
2c	4	A	$-OCH_3$	1.1 ± 0.1 (1.3)	16.0 ± 1.9	315 ± 27.2
2d	5	A	$-OCH_3$	3.1 ± 0.2 (1)	30.5 ± 4.5	127 ± 22.3
2e	6	A	$-OCH_3$	6.4 ± 0.6 (6)	113 ± 23.2	106 ± 9.5
2f	7	A	$-OCH_3$	1.7 ± 0.1 (5.4)	89.8 ± 7.3	nd
3a	2	A	−Cl	70.4 ± 5.3 (100)	265 ± 30.5	1010 ± 205
3b	3	A	−Cl	15.2 ± 1.7 (8.5)	44.7 ± 5.0	461 ± 35.4
3c	4	A	−Cl	1.3 ± 0.1 (1.2)	5.8 ± 0.6	181 ± 30.7
3d	5	A	−Cl	4.5 ± 0.3 (2.6)	127 ± 22.6	351 ± 31.8
3e	6	A	−Cl	15.1 ± 1.8 (22)	31.5 ± 4.5	617 ± 45.5
3f	7	A	−Cl	1.0 ± 0.1 (4.3)	10.3 ± 1.7	182 ± 29.5
4a	2	B	$-OCH_3$	237 ± 30.4 (250)	1492 ± 350	nd
4b	3	B	$-OCH_3$	115 ± 22.4 (190)	673 ± 55.5	463 ± 32.5
4c	4	B	$-OCH_3$	47.5 ± 6.3 (18)	393 ± 31.8	nd
4d	5	B	$-OCH_3$	37.5 ± 5.3 (38)	427 ± 35.7	223 ± 25.0
4e	6	B	$-OCH_3$	52.5 ± 5.3 (46)	131 ± 22.5	nd
4f	7	B	$-OCH_3$	6.0 ± 0.7 (0.52)	76.5 ± 6.5	98.7 ± 7.9
5a	2	B	Cl	472 ± 35.7 (160)	2072 ± 950	nd
5b	3	B	Cl	48.3 ± 6.3 (28)	436 ± 36.7	617 ± 57.4
5c	4	B	Cl	24.6 ± 3.8 (25)	65.2 ± 9.2	nd
5d	5	B	Cl	23.3 ± 2.5 (12)	134 ± 20.3	323 ± 32.2
5e	6	B	Cl	29.7 ± 4.5 (15)	138 ± 25.4	nd
5f	7	B	Cl	4.6 ± 0.3 (9.7)	24.5 ± 3.8	123 ± 23.2
6a	2	C	$-OCH_3$	22.7 ± 2.1 (23)	1098 ± 174	310 ± 32.3
6b	3	C	$-OCH_3$	3.2 ± 1.4 (1.7)	170 ± 29.1	103 ± 9.4
6c	4	C	$-OCH_3$	0.9 ± 0.1 (0.2)	20.0 ± 4.9	253 ± 24.5
6d	5	C	$-OCH_3$	6.8 ± 3.2 (18)	95.6 ± 7.9	141 ± 23.7
6e	6	C	$-OCH_3$	2.8 ± 1.9 (0.61)	104 ± 9.4	514 ± 36.7
6f	7	C	$-OCH_3$	1.1 ± 0.4 (0.66)	306 ± 32.2	181 ± 27.2
P				0.24 ± 0.05		
R					4.0 ± 0.3	
D						2.0 ± 0.2

[a] A: 1-benzimidazolyl. B: 1-imidazolyl. C: 1-indolyl. [b] The K_i binding data were calculated as described in the Experimental Section. The K_i values are means ± SD of series separate assays, each performed in triplicate. Inhibition constants (K_i) were calculated according to the equation of Cheng and Prusoff:[23] $K_i = IC_{50}/[1 + (L/K_d)]$ where [L] is the ligand concentration and K_d its dissociation constant. K_d of [^{3}H]prazosin (**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}). [c] In parentheses, predicted affinity values (in nM) are calculated by Catalyst based on the pharmacophore model for α_1-AR antagonists. [d] nd: not determined.

methoxyphenoxyethyl moiety or to the piperazine ring directly bound to the pyridazinone nucleus.[1b]

In addition to suggestions derived from the pharmacophore model, a literature survey has been performed on arylpiperazine derivatives acting toward α_1-AR. As a summary, a concise SAR can be reported as follows. Replacement of the triazolopyridine nucleus of **1** with simplified heterocycles, such as 1- and 2-benzotriazole bearing a variety of small substituents (i.e., methyl, nitro, chloro), led to α_1-AR antagonists whose affinity is not dependent on either the benzotriazole isomer tested or the substituent and substitution pattern of the heterocycle.[13] Moreover, reduction in the number of nitrogen atoms in the heteroaromatic ring system afforded compounds with enhanced affinity. Particularly, unsubstituted 1-benzimidazole and 1-indole derivatives were found to be the most active compounds within these series.[14] Finally, simple chemical modifications of the piperazine ring of **1** cause a loss of affinity toward α_1-AR.[12]

On the other hand, the classical isosteric substitution of the carbonyls of a hydantoin moiety (corresponding to the terminal heterocyclic substituent) by methylene

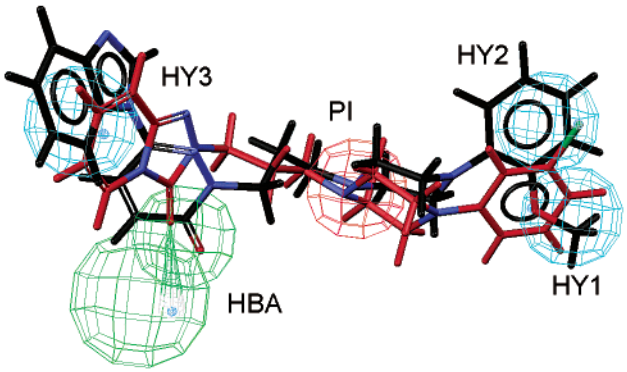


Figure 1. Compounds **1** (trazodone, red) and **2b** (black) mapped to the pharmacophore model. It is important to note that while trazodone only partially matches the HY1−HY2 and HY3 features, compound **2b** shows an enhanced fit to the pharmacophore hypothesis. Features are color-coded: cyan for hydrophobic regions (HY1−HY3), green for a hydrogen bond acceptor (HBA), and red for a positive ionizable feature (PI).

groups negatively influenced the electronic interactions for α_1 receptor binding.[15] These data are in agreement with the requirements of the pharmacophore model indicating the presence of a HBA group in the region of space occupied by the above-mentioned heteroring.

Moreover, it has been well established that the length of the polymethylene spacer that separates an *o*-methoxyphenylpiperazinyl portion from a terminal pyridazin-3(2*H*)-one moiety is of great importance to the α_1 affinity of some long-chain arylpiperazines. As a general trend, elongation of the alkyl linker from two to four (and more) carbon atoms remarkably enhances the affinity of such derivatives.[1]

In light of these considerations, we have planned to synthesize new compounds based on the phenylpiperazinylalkyl molecular scaffold, appropriately modified to better meet, in comparison to trazodone, the three-dimensional structural requirements imposed by the pharmacophore model for α_1-AR antagonists. Accordingly, in an effort to improve the goodness of fit to the pharmacophore model and, possibly, the α_1-AR binding affinity, we decided to optimize the structural features of the hit compound **1** (Scheme 1). In particular, this paper describes the synthesis and biological properties of a novel series of compounds in which the phenylpiperazine moiety has been kept fixed while the terminal heterocyclic nucleus, the alkyl spacer, and the substituents on the phenyl ring bound to the piperazine N4 have been modified in several ways. In detail, the following chemical modifications were considered: (i) the terminal heterocyclic moiety was replaced by an unsubstituted 1-benzimidazole and 1-indole, according to literature reports,[13,14] or 1-imidazole ring; (ii) the substituent on the phenyl ring linked to the piperazine nucleus was an *o*-chloro or *o*-methoxy group; (iii) as suggested by the partial fit of **1** into the pharmacophore model, the overall length of the molecule was increased by inserting a pyridazinone ring between the alkyl chain and the terminal heterocyclic fragment, thus providing hydrogen bond accepting groups (such as the carbonyl moiety of the pyridazinone ring) as required by the pharmacophore model itself; (iv) the length of the alkyl chain acting as a spacer was increased through methylene group insertion up to a seven-carbon atom chain.

The effect of substitution at the ortho, meta, and para positions of the phenyl group bound to the piperazine ring has been previously discussed in many literature reports.[1b,16,17] In particular, the occupation of the ortho position usually leads to increased affinity that the pharmacophore model accounted for as a consequence of a good fit by the substituent into the HY2 feature. Substituents at the meta position are unable to significantly affect the affinity, in agreement with the pharmacophore suggestion that the *o*-substituent could partially occupy the hydrophobic region HY2. In contrast, substituents at the para position decrease the affinity toward α_1-AR. We may interpret these observations by assuming that if the substituent distance from the N1 piperazine nitrogen (the positive ionizable group of the pharmacophore model) is increased (substituent at para position), its hindrance reduces the overall activity; that is, the decrease in activity seems to depend on steric hindrance at the para position rather than on other factors.[17] A similar hypothesis, but in the opposite sense, may be assumed for the ortho and meta positions. In this case, the structure could assume a shape and size that would better accommodate the substituted phenyl ring into the HY1−HY2 pharmacophoric features, with the ortho derivatives usually more active than the corresponding meta analogues. Therefore, in this paper we only report *o*-methoxy and *o*-chloro substitutions at the phenyl ring.

Suggestions derived from ligand-based drug design studies (i.e., three-dimensional structural properties required for a compound to display α_1-AR affinity and identification of **1** as a compound that partially satisfies the constraints imposed by the pharmacophore model) combined with reported SARs on α_1-AR blockers led us to design molecules **2b** and **3b**. These structures would allow a better fit with respect to **1** into the whole pharmacophore model and, in particular, into the terminal hydrophobic features (HY1−HY2 and HY3, respectively, see Figure 1) by their *o*-methoxyphenyl (or *o*-chlorophenyl) and benzimidazole substituents, respectively. That is, the new molecules should be characterized by the essential structural elements identified by our previous work in this field, losing the extrasize portion unable to interact with any pharmacophore feature.[1b]

To evaluate the agreement between the pharmacophore model and the newly designed structures as well as to estimate the biological properties of such molecules based on their structures, prior to synthesis, we have submitted both **2b** and **3b** to a computational procedure aimed at calculating the goodness of their fit to the pharmacophore model and predicting their α_1-AR affinity. In particular, Catalyst has been applied to generate a population of conformers for each of the studied compounds. Subsequently, the Compare/Fit routine of the program has been used to choose, among all the conformations found, the structure able to allow the best orientation within the pharmacophore model, that is, the optimal fit of the molecular chemical moieties into each of the pharmacophore features. As a result, Figure 1 shows the superposition of **2b**, taken as a representative example of the studied compounds, and the five-feature pharmacophore (characterized by a positive ionizable group in red, PI; three hydrophobic regions in blue, HY1, HY2, and HY3; and a hydrogen bond acceptor feature in green, HBA). In detail, while both HY1 and HY2 are mapped by the *o*-methoxyphenyl moiety of **2b**, the N1 atom of the piperazine ring corresponds to the PI feature of the model. Moreover, the condensed phenyl ring of the benzimidazole moiety is mainly responsible for the superposition to HY3, and finally, the pyridazinone carbonyl group fulfills HBA.

The conformation of **2b** chosen by the program in the above-described orientation with respect to the pharmacophore elements gave a calculated (predicted) affinity value of 4.2 nM. Similarly, the affinity of **3b** toward α_1-AR was predicted by Catalyst to be 8.5 nM. These calculations suggested that **2b** and **3b** could represent very interesting synthetic targets.

As a consequence of these theoretical investigations, we planned to synthesize compounds **2b**, **3b**, and **2c**, the last with the purpose of taking into account the effect of the polymethylene length on the adrenoceptor affinity. Biological tests performed on compounds **2b** and **3b** confirmed the computational predictions of their α_1-AR affinity.

Finally, good affinity of such compounds combined with the aim of evaluating the influence of the above-reported structural modifications on trazodone molecule provided us with additional impetus in the synthesis of a large class of new compounds, possibly characterized by enhanced α_1-AR affinity. In particular, three subsets of molecules bearing a benzimidazole (**2**, **3**), imidazole (**4**, **5**), and indole (**6**) group as the terminal heterocyclic fragment, with variation in the spacer length and different substituents and substitution pattern on the phenyl ring attached to the piperazine nucleus, were synthesized. Replacement of the benzimidazole ring with an indole and imidazole moiety was planned to investigate the influence of electronic and steric factors, respectively, on the binding properties at α_1-AR.

Chemistry

The target compounds **2**−**6**, listed in Table 1, were synthesized as outlined in Scheme 2. In particular, alkylation of 6-(benzimidazol-1-yl)pyridazin-3(2*H*)-one (**7**), 6-(imidazol-1-yl)pyridazin-3(2*H*)-one (**8**), and 6-(indol-1-yl)pyridazin-3(2*H*)-one (**9**), prepared according to the procedure reported by Steiner and Sircar,[18] with 4-(2-methoxyphenyl)-1-(2-chloroethyl)piperazine (**10a**)[19] or 4-(2-chlorophenyl)-1-(2-chloroethyl)piperazine (**10b**)[19] in acetone in the presence of potassium carbonate (method A) afforded compounds **2a**, **4a**, **6a**, **3a**, and **5a**, respectively, in yields ranging from 40% to 60%. The same reaction has been applied to 4-(2-methoxyphenyl)-1-(2-chloropropyl)piperazine (**10c**)[19] and 4-(2-chlorophenyl)-1-(2-chloropropyl)piperazine (**10d**)[19] to prepare compounds **2b**, **4b**, **6b**, **3b**, and **5b**, respectively.

Starting from **7**, **8**, and **9**, α,ω-dibromoalkanes having four to seven methylene groups were employed to prepare intermediates **11a**−**d**, **12a**−**d**, and **13a**−**d** (K_2CO_3/acetone, method B, Table 2), which in turn were converted to final compounds **2c**−**f**, **4c**−**f**, and **6c**−**f** by reaction with 1-(2-methoxyphenyl)piperazine **14** (Na_2CO_3/isoamyl alcohol, method C) and to compounds **3c**−**f** and **5c**−**f** by reaction with 1-(2-chlorophenyl)piperazine **15** (Na_2CO_3/isoamyl alcohol, method C).

Table 2. Chemical and Physical Data of the Intermediates **11**−**13**

compd	R	*n*	formula	mp (°C)	yield (%)	EtOH/CH_2Cl_2[a]
11a	1-benzimidazolyl	4	$C_{15}H_{15}ClN_4O$	147−150	45	5:95
11b	1-benzimidazolyl	5	$C_{16}H_{17}BrN_4O$	153−155	50	4:96
11c	1-benzimidazolyl	6	$C_{17}H_{19}ClN_4O$	149−152	30	10:90
11d	1-benzimidazolyl	7	$C_{18}H_{21}BrN_4O$	130−135	40	5:95
12a	1-imidazolyl	4	$C_{11}H_{13}ClN_4O$	oil	35	9:91
12b	1-imidazolyl	5	$C_{12}H_{15}BrN_4O$	oil	40	5:95
12c	1-imidazolyl	6	$C_{13}H_{17}ClN_4O$	oil	40	7:93
12d	1-imidazolyl	7	$C_{14}H_{19}BrN_4O$	oil	40	6:94
13a	1-indolyl	4	$C_{16}H_{16}ClN_3O$	oil	50	6:94
13b	1-indolyl	5	$C_{17}H_{18}BrlN_3O$	110−115	45	3:97
13c	1-indolyl	6	$C_{18}H_{20}ClN_3O$	95−100	50	4:96
13d	1-indolyl	7	$C_{19}H_{22}BrN_3O$	oil	50	3:97

[a] Eluent used for purification by chromatography on silica gel column.

Table 3. Chemical and Physical Data of the New Compounds

compd	formula	mp, °C	yield, %
2a	$C_{24}H_{26}N_6O_2$	140−155[a]	50
2b	$C_{25}H_{28}N_6O_2$	175−180[b]	35
2c	$C_{26}H_{30}N_6O_2$	165−170[a]	40
2d	$C_{27}H_{32}N_6O_2$	175−180[c]	30
2e	$C_{28}H_{34}N_6O_2$	163−165[b]	60
2f	$C_{29}H_{36}N_6O_2$	130−135[d]	50
3a	$C_{23}H_{23}ClN_6O$	185−190[e]	60
3b	$C_{24}H_{25}ClN_6O$	200−205[e]	30
3c	$C_{25}H_{27}ClN_6O$	150−155[b]	65
3d	$C_{26}H_{29}ClN_6O$	195−200[b]	50
3e	$C_{27}H_{31}ClN_6O$	145−150[f]	40
3f	$C_{28}H_{33}ClN_6O$	190−195[d]	60
4a	$C_{20}H_{24}N_6O_2$	130−135[g]	40
4b	$C_{21}H_{26}N_6O_2$	180−185[d]	30
4c	$C_{22}H_{28}N_6O_2$	110−115[b]	50
4d	$C_{23}H_{30}N_6O_2$	168−171[g]	30
4e	$C_{24}H_{32}N_6O_2$	120−122[c]	50
4f	$C_{25}H_{34}N_6O_2$	142−145[d]	30
5a	$C_{19}H_{21}ClN_6O$	55−60[g]	50
5b	$C_{20}H_{23}ClN_6O$	175−180[d]	40
5c	$C_{21}H_{25}ClN_6O$	110−115[h]	25
5d	$C_{22}H_{27}ClN_6O$	228−230[f]	35
5e	$C_{23}H_{29}ClN_6O$	120−122[a]	50
5f	$C_{24}H_{31}ClN_6O$	50−55[g]	20
6a	$C_{25}H_{27}N_5O_2$	190−195[f]	55
6b	$C_{26}H_{29}N_5O_2$	180−185[e]	60
6c	$C_{27}H_{31}N_5O_2$	135−140[g]	60
6d	$C_{28}H_{33}N_5O_2$	140−145[e]	40
6e	$C_{29}H_{35}N_5O_2$	125−130[f]	55
6f	$C_{30}H_{37}N_5O_2$	110−115[i]	55

[a] As dihydrochloride dihydrate. [b] As trihydrochloride monohydrate. [c] As trihydrochloride dihydrate. [d] As trihydrochloride. [e] As dihydrochloride. [f] As dihydrochloride monohydrate. [g] As tetrahydrochloride. [h] As dihydrochloride trihydrate. [i] As hydrochloride monohydrate.

The chemical and physical data of the new compounds are reported in Table 3.

Biology

The pharmacological profile of compounds **2**−**6** 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.

Moreover, to determine the intrinsic activity of **2c**, **5f**, and **6c** (found to be the benzimidazole, imidazole, and indole derivatives, respectively, with the best affinity profile toward α_1-AR), competition studies were performed in the presence and in the absence of 1 mM GTP using the radiolabeled antagonist [^{3}H]prazosin. The GTP shift is an in vitro parameter often indicative of intrinsic activity, representing the ratio of the compound affinity constant in the presence of GTP and the compound affinity constant in the absence of GTP. GTP modulates the affinity of agonist compounds. whereas it does not affect the affinity for an antagonist compound. Accordingly, while a GTP shift value greater than 1 is indicative of an agonist profile, a GTP shift near 1 is indicative of an antagonist profile. In Table 4, the GTP shift values of the selected compounds and antagonist reference compounds were reported.

Table 4. Intrinsic Activity of Compounds **2c**, **5f**, and **6c** to α_1-AR Expressed as GTP Shift

	$K_i\alpha_1$[a]		
compd	−GTP (nM)	+GTP (nM)	GTP shift
2c	1.2 ± 0.1	0.97 ± 0.05	0.8
5f	4.9 ± 0.3	8.33 ± 0.5	1.7
6c	1.0 ± 0.4	1.6 ± 0.2	1.6
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 means ± SEM.

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

Results and Discussion

Table 1 reports the α_1-AR, α_2-AR, and 5-HT_{1A} receptor binding affinity, expressed as K_i values, of the new compounds **2**−**6**.

We were interested in testing if the molecular modification of **1** would result in new adrenoceptor antagonists endowed with improved potency and selectivity toward α_1-AR. The initial attempt at altering the template structure of **1** resulted in derivatives **2** characterized by the *o*-methoxyphenylpiperazinyl moiety bridged to a benzimidazolylpyridazinone scaffold through a methylene spacer with a length variable from two to seven carbon atoms.

The experimentally determined affinity of compound **2b** (6.5 nM) was in good agreement with the value calculated on the basis of the pharmacophore model for α_1-AR antagonists (4.2 nM). In addition, the lengthening of the alkyl chain by a methylene unit up to seven carbon atoms led to enhanced affinity. In particular, compounds **2c** and **2f** were found to be the most active derivatives of this subset toward α_1-AR. Not surprisingly, the best affinity was associated with compound

2c bearing a four-carbon atom linker that has been previously demonstrated to be the optimal spacer for α_1-AR antagonism in structurally related phenylpiperazinylalkyl derivatives.[1,20] In contrast, compound **2a** with $n = 2$ has been found to be weakly active (33.0 nM), in agreement with computational results showing its inability to have a good mapping to the HBA feature of the pharmacophore model, mainly because of the reduced distance between the piperazine and pyridazinone rings. Substitution of the *o*-methoxy group with a chlorine atom had no substantial influence on α_1-AR affinity. In fact, compounds **3** showed K_i values slightly higher with respect to their methoxy analogues, with the exception of compound **3f** whose affinity (1.0 nM) was enhanced in comparison to **2f** (1.7 nM). Among the remaining compounds within these subsets, the most relevant differences in affinity corresponded to about 2-fold decreased values in chloro derivatives with respect to methoxy-substituted compounds (33.0 nM of **2a** vs 70.4 nM of **3a**, 6.5 nM of **2b** vs 15.2 nM of **3b**, 6.4 nM of **2e** vs 15.1 nM of **3e**). As far as α_2-AR affinity is concerned, chloro substitution led to compounds more active than the corresponding methoxy analogues, with the exception of **3d** showing an α_2-AR affinity lower than compound **2d** (127.0 vs 30.5 nM, respectively). Finally, among all the benzimidazoles, while **3c** was the most active compound toward α_2-AR, the highest selectivity was found with compound **2f** (the α_2/α_1 ratio being 52.9).

With the aim of evaluating the influence of the terminal heterocyclic moiety directly linked to the pyridazinone ring on the affinity and selectivity toward α_1-AR, compounds **4** and **5**, bearing an imidazole instead of a benzimidazole ring, were also synthesized. A SAR analysis of compounds **4** revealed a decrease in α_1-AR affinity of at least 1 order of magnitude in comparison to the corresponding benzimidazole derivatives **2**. Compound **4f** represented the only exception to this trend, its affinity value (6.0 nM) being only 3.5-fold higher than compound **2f** (1.7 nM). Analogous considerations could be made about compounds **5** whose affinity decreased with respect to **3**. While compound **5f** retained an interesting α_1-AR affinity of 4.6 nM, **5a** showed the highest K_i value (471.4 nM) and the remaining compounds **5b**−**e** possessed affinity values ranging from 23.3 nM (**5d**) to 48.3 nM (**5b**).

It is interesting to note that while the substitution of the *o*-methoxy group in the benzimidazole derivatives **2** with a chlorine atom led to a decrease in affinity (compounds **3**), the opposite trend was shown by transformation of compounds **4** to compounds **5**. In fact, with the exception of **5a**, all the *o*-chloro-substituted imidazole derivatives were more active than the corresponding methoxy counterparts.

Moreover, compounds **4** and **5** were characterized by a substantially reduced α_2-AR affinity with respect to their benzimidazole analogues, with the exception of compound **4f**, which is slightly more active (76.5 nM) than compound **2f** (89.8 nM). None of the imidazole derivatives showed a α_2/α_1 selectivity value higher than 12.7 (associated with compound **4f**).

The last step of this project was the synthesis of compounds **6** designed with the purpose of evaluating the influence of the electronic properties of the terminal heteroring on the affinity toward adrenoceptors. Compounds **6** are *o*-methoxyphenylpiperazinylalkylpyridazinones bearing an indole moiety instead of the benzimidazole or imidazole ring considered above. Variation of the number of nitrogen atoms in the heteroring led to compounds characterized by very interesting properties in terms of both affinity and selectivity toward α_1-AR. In fact, while compound **6a** with an ethylene spacer showed the highest K_i value of this subset (22.7 nM), the remaining compounds had affinity values from 0.9 nM (**6c**) to 6.8 nM (**6d**). It is interesting to note that compounds with four- and seven-carbon-atom spacers proved to be the most active, in agreement with our findings regarding benzimidazole derivatives **2** and **3**. Moreover, **6f** was the most selective compound toward α_1-AR, showing the highest α_2/α_1 ratio (278). In contrast, none of the indole derivatives were interesting for their α_2-AR blocking properties, with the exception of **6c**, which displayed an affinity of 20.0 nM.

To summarize, it can be seen from the binding data that benzimidazoles **2** and **3** and indole derivatives **6** demonstrated moderate to high affinity for α_1-AR. Reduction of the size of the terminal heterocycle led to the imidazole compounds **4** and **5** that showed decreased affinity, with the exceptions of **4f** and **5f**, which possess a seven-methylene spacer.

Moreover, when a benzimidazole group was the terminal heterocyclic ring, the *o*-methoxy substituent on the phenyl ring attached to the piperazine N4 represented the optimal substituent with respect to a chlorine atom. In contrast, the opposite trend was found when the imidazole moiety is bound to the pyridazinone ring. In fact, when the benzimidazole group was simplified to an imidazole ring in order to examine the influence of the heterocyclic moiety on the α_1-AR affinity, the *o*-chloro derivatives showed an enhanced affinity with respect to the corresponding *o*-methoxy analogues. However, compounds **4** and **5** were all characterized by a markedly decreased affinity in comparison to the corresponding benzimidazoles **2** and **3**. Finally, the number of heterocyclic nitrogen atoms was varied leading to the indole derivatives **6**, all of which are characterized by a very interesting α_1-AR affinity. In particular, **6f**, one of the most active compounds within the whole set (with an affinity toward α_1-AR of 1.1 nM), was also found to be highly selective toward α_1-AR, showing an α_2/α_1 ratio of 278.

In addition, all the new compounds exhibited higher affinity toward α_1-AR than toward α_2-AR, but they had poor α_2/α_1 selectivity. The only exceptions to this general trend were compounds **2f**, **6b**, and **6f**, showing a α_2/α_1 ratio of 53, 53, and 278, respectively. Interestingly, the substitution of the benzimidazole group (**2f**) with an indole ring (**6b**, **6f**) was the sole difference between them.

5-HT$_{1A}$ Receptor Binding. 5-HT$_{1A}$ affinity data reported in Table 1 show that among compounds **2** lengthening of the polymethylene spacer led to enhanced affinity. Similarly, compounds **3a** (ethyl spacer) and **3f** (heptyl spacer) show the highest and lowest affinity values, respectively.

The 5-HT$_{1A}/\alpha_1$ ratios found for compounds **2c** (286) and **3c** (139) are worthy of further consideration. In fact, it is well-known that the *o*-methoxyphenylpiperazinyl

moiety[21] and/or a spacer length of three or four methylene units represents optimum chemical features for inducing high affinity for both α_1-adrenergic and 5-HT_{1A} serotoninergic receptors[20] (i.e., compound NAN-190 possesses a 5-HT_{1A}/α_1 selectivity of about 1).[16] On the basis of this consideration, compounds **2c**, **3c**, and **6c**, sharing a phenylpiperazinylbutyl moiety as a common structural feature, gave good and unexpected 5-HT_{1A}/α_1 selectivity.

Moreover, it has been reported that replacement of an *o*-chloro group with a methoxy substituent led to a marked improvement of 5-HT_{1A}/α_1 selectivity due to a complete loss in 5-HT_{1A} affinity.[22] In contrast, compound **3c** showed comparable α_1 affinity and improved 5-HT_{1A} affinity with respect to the corresponding methoxy counterpart **2c**, with a consequent decrease in 5-HT_{1A}/α_1 selectivity.

In summary, some of the new compounds presented in this work show an interesting and unexpected selectivity between the α_1-AR and the 5-HT_{1A} receptor. These results suggest that the new pyridazinone−arylpiperazines can be considered good templates for the development of novel α_1 selective ligands with respect to the 5-HT_{1A} receptor. The importance of such compounds is remarkable considering that the development of α_1-AR high affinity and selective antagonists with such chemical features is quite difficult. In fact, *o*-methoxy and *o*-chlorophenylpiperazinylalkyl derivatives with appreciable affinity for α_1-AR are also usually good ligands for the 5-HT_{1A} serotonergic receptor.

Conclusions

A new class of potent α_1-AR antagonists has been prepared on the basis of the suggestions derived from both a database search (performed by means of a three-dimensional pharmacophore model) and an exhaustive literature survey. These new compounds share a benzimidazolylpyridazinone, an indolylpyridazinone, or an imidazolylpyridazinone, which represents an element of novelty in the SAR of arylpiperazine derivatives acting toward α_1-AR.

A structure−affinity relationship analysis suggested the identification of some structural features very important for the affinity and selectivity of the new compounds for α_1-AR with respect to α_2-AR. In particular, (i) the presence of a methoxy group at the ortho position of the phenylpiperazine moiety led to the best α_1 affinity−selectivity profile. Although the best affinity was associated with compound **6c** (0.9 nM), three additional *o*-methoxy derivatives (i.e., **2f**, **6b**, and **6f**) also exhibited high affinity (1.7, 3.2, and 1.1 nM, respectively) and selectivity (53, 53, and 278 being the α_2/α_1 ratio, respectively). These findings led us to confirm the conclusion previously reported by us[1] and other research groups[16,20] that the ortho position could play a crucial role in the improvement of the α_1-AR antagonist properties in terms of both affinity and selectivity. Thus, we are currently investigating different alkoxy substituents at the ortho position of the phenyl ring bound to the piperazine N4 atom. (ii) The alkyl spacer bridging the phenylpiperazine moiety to the terminal heterocyclic nucleus also showed a great influence on the affinity toward adrenoceptors. As a general trend, an ethylene chain was always associated with compounds showing a weak affinity for α_1-AR, α_2-AR, and 5-HT_{1A} receptors. The lengthening of the spacer by a methylene unit to three or four carbon atoms afforded compounds with an increased affinity toward α_1- and α_2-AR. In particular, **2c**, **3c**, and **6c** were the most active compounds (in terms of α_1- and α_2-AR affinity) within the corresponding subset, with **6c** also showing the lowest K_i toward α_1-AR (0.9 nM). They showed, together with **6e** and **6f**, an interesting 5-HT_{1A}/α_1 selectivity profile. As a general trend, further elongation of the spacer to five and six carbon atoms led to a slight decrease in α_1- and α_2-AR affinity. In contrast, all but one compound bearing a seven-methylene chain as a spacer were characterized by an enhanced affinity toward both adrenergic receptors with respect to the homologues with a shorter chain. Compound **6f** was an exception because its α_2-AR affinity was measured to be about 3-fold lower with respect to **6e** (1.1 versus 2.8 nM, respectively). As a consequence, the selectivity of such a derivative was the best found among all the studied compounds (α_2/α_1 ratio of 278). These experimental results suggested that the long alkyl spacer, mainly based on its conformational flexibility, could assume a size and shape that influence the affinity (and selectivity) of compounds to α_1 and α_2 adrenoceptors. (iii) SAR considerations also led to the hypothesis that a heterocyclic terminal fragment bigger than an aromatic five-membered ring is required for best activity. In fact, compounds bearing a benzimidazole or an indole group are all characterized by higher affinity with respect to the corresponding imidazole derivatives, suggesting that the size of the terminal heteroring is able to affect the biological properties of such compounds. Finally, (iv) the number of nitrogen atoms on the heteroring is an additional element leading to a variation in affinity with indole derivatives comprising compounds **6c** (α_1 affinity of 0.9 nM, 5-HT_{1A}/α_1 selectivity of 281) and **6f** (α_1 affinity of 1.1 nM, α_2/α_1 selectivity of 278) characterized by a very interesting biological profile.

Regarding the 5-HT_{1A}/α_1 affinity profile, compounds **2c** and **6c** (bearing an *o*-methoxyphenylpiperazinyl moiety and a butyl spacer usually associated with low or null selectivity) showed an unexpected 5-HT_{1A}/α_1 ratio of about 280. This result suggested the benzimidazolyl- and indolylpyridazinone as new chemical features for designing ligands with improved selectivity toward α_1-AR with respect to 5-HT_{1A}.

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 for million) are relative to 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 hydrochlorides were prepared by bubbling dry HCl into the dry solution of the compound.

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

Method A Example. 2-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl}-6-(imidazol-1-yl)pyridazin-3(2*H*)-one (4a). A mixture of 6-(imidazol-1-yl)pyridazin-3(2*H*)-one (**8**) (0.32 g, 2.0 mmol) and 4-(2-methoxyphenyl)-1-(2-chloroethyl)piperazine

10a (0.6 g, 2.4 mmol) in 20 mL of acetone in the presence of dry potassium carbonate was refluxed for 24 h. The filtered residue was evaporated under reduced pressure and purified by chromatography on a silica gel column, eluting with an $EtOH/CH_2Cl_2$ mixture (14:86) to give a 40% yield of a dense oil: ^1H NMR ($CDCl_3$) δ 2.55−2.65 (m, 4H, H-pip), 2.75 (t, J = 7.0 Hz, 2H, CH_2), 2.80−3.00 (m, 4H, H-pip), 3.80 (s, 3H, OCH_3), 4.25 (t, J = 7.0 Hz, 2H, CH_2), 6.80−7.00 (m, 4H, H-arom), 7.15 (m, 1H, H-imid), 7.25 (d, J = 9.9 Hz, 1H, H-pyrid), 7.80 (t, J = 0.9 Hz, 1H, H-imid), 8.05 (d, J = 9.9 Hz, 1H, H-pyrid), 8.40 (t, J = 0.9 Hz, 1H, H-imid). For the corresponding hydrochloride: mp 130−135 °C. Anal. ($C_{20}H_{24}N_6O_2 \cdot 4HCl$) C, H, N.

Method B Example. 2-(4-Chlorobutyl)-6-(imidazol-1-yl)pyridazin-3(2*H*)-one (12a). A mixture of 6-(imidazol-1-yl)pyridazin-3(2*H*)-one **8** (0.49 g, 3 mmol) with 1,4-dibromochlorobutane (0.62 g, 3.6 mmol) and potassium carbonate (0.5 g, 3.6 mmol) in 25 mL of acetone was refluxed under stirring for 20 h. The filtered residue was evaporated under reduced pressure and purified by chromatography on a silica gel column, eluting with an $EtOH/CH_2Cl_2$ mixture (9:91) to give a 30% yield of a dense oil: ^1H NMR ($CDCl_3$) δ 1.80−2.10 (m, 4H, $2CH_2$), 3.55 (t, J = 7.0 Hz, 2H, CH_2), 4.20 (t, J = 7.0 Hz, 2H, CH_2), 7.10 (d, J = 9.9 Hz, 1H, H-pyrid), 7.20 (m, 1H, H-imid), 7.40 (m, 1H, 1H-imid), 7.50 (d, J = 9.9 Hz, 1H, 1H-pyrid), 8.10 (m, 1H, H-imid).

Method C Example. 2-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}-6-(imidazol-1-yl)pyridazin-3(2*H*)-one (4c). A mixture of 2-(4-bromobutyl)-6-(imidazol-1-yl)pyridazin-3(2*H*)-one (**12a**) (0.76 g, 3 mmol) with 1-(2-methoxyphenyl)piperazine **14** (0.58 g, 3 mmol) in isoamyl alcohol (20 mL) in the presence of potassium carbonate (0.38 g, 3.6 mmol) was refluxed for 24 h. The filtered residue was evaporated under reduced pressure and purified by chromatography on a silica gel column, eluting with an $EtOH/CH_2Cl_2$ mixture (15:85) to give a 50% yield of a dense oil: ^1H NMR ($CDCl_3$) δ 1.60−1.80 (m, 2H, CH_2), 1.90 (quint, J = 7.0 Hz, 2H, CH_2), 2.60 (t, J = 7.0 Hz, 2H, CH_2), 2.70−2.80 (m, 4H, H-pip), 3.10−3.20 (m, 4H, H-pip), 3.90 (s, 3H, OCH_3), 4.20 (t, J = 7.0 Hz, 2H, CH_2), 6.88−7.05 (m, 4H, H-arom), 7.15 (d, J = 9.9 Hz, 1H, H-pyrid), 7.20 (m, 1H, H-imid), 7.40−7.50 (m, 2H, 1H-pyrid, 1H-imid), 8.10 (m, 1H, H-imid). For the corresponding hydrochloride: mp 110−115 °C. Anal. ($C_{22}H_{28}N_6O_2 \cdot 3HCl \cdot H_2O$) C, H, N.

Biology. 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 48000*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 48000*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$_{1A}$ 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 nonspecific 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 48000*g* for 15 min at 4 °C. The pellet (P_1) was suspended in 20 volumes of ice-cold buffer T_1. It was then homogenized and centrifuged at 48000*g* for 15 min at 4 °C. The resulting pellet (P_2) was frozen at −80 °C until the time of assay.

The 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 of 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 suction 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 counted with a Packard 1600 TR scintillation counter. Specific binding was obtained by subtracting nonspecific binding from the total binding and was approximated to 85−90% of the total 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 48000*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.

Binding assay was perfomed 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. Nonspecific 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. Filtrates were washed four times with 5 mL aliquots of ice-cold buffer and placed in scintillation vials. Specific binding was obtained by subtracting nonspecific binding from total binding and approximated to 85−90% of total binding. The receptor-bound radioactivity was measured 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,[26] after solubilization with 0.75 N sodium hydroxide, using bovine serum albumin as the standard.

The concentration of the tested compound that produces 50% inhibition of specific [^{3}H]prazosin, [^{3}H]rauwolscine, or [^{3}H]8-OH-DPAT binding (IC_{50}) was determined by a log-probit analysis with seven concentrations of the displacer, each performed in triplicate. Inhibition constants (K_i) were calculated according to the equation[23]

$$K_i = \frac{IC_{50}}{1 + [L]/K_d}$$

where [L] is the ligand concentration and K_d its dissociation constant. K_d of [^{3}H]prazosin binding to cortex membranes was 0.24 nM (α_1), K_d of [^{3}H]rauwolscine binding to cortex membranes was 4.0 nM (α_2), and K_d of [^{3}H]8-OH-DPAT binding to cortex membranes was 2 nM (5-HT$_{1A}$).

Computational Methods. All calculations and graphic manipulations were performed on a Silicon Graphics O2 R5200 workstation by means of the Catalyst 4.6 software package.

All the compounds used in this study were built using the 2D−3D sketcher of the program. A representative family of conformations were generated for each molecule using the poling algorithm and the "best quality conformational analysis" method. The parameter set employed to perform all the conformational calculations is derived from the CHARMm force field, opportunely modified and corrected.

Conformational diversity was emphasized by selection of the conformers that fell within 20 kcal/mol range above the lowest energy conformation found.

The Compare/Fit command within Catalyst has been used to predict affinity values of the studied compounds. Particularly, the Best Fit option has been selected, which manipulates the conformers of each compound to find, when possible, different mapping modes of the ligand within the model. As a consequence, a value of the biological activity will be associated

with each mapping mode satisfying the constraints imposed by the location of the pharmacophore features.

For each Compare/Fit operation, the program provides a measure (indicated as a fit value) of how closely the pharmacophore features correspond to the molecular groups of the ligand.

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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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