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Design, synthesis, and α_1-adrenoceptor binding properties of new arylpiperazine derivatives bearing a flavone nucleus as the terminal heterocyclic molecular portion☆

Laura Betti,[a] Monia Floridi,[b] Gino Giannaccini,[a] Fabrizio Manetti,[c,*] Chiara Paparelli,[b] Giovannella Strappaghetti[b,*] and Maurizio Botta[c]

[a]Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy
[b]Dipartimento di Chimica e Tecnologia del Farmaco, Università di Perugia, Via del Liceo 1, 06123 Perugia, Italy
[c]Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, Via Aldo Moro, 53100 Siena, Italy

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Abstract—Following our research project aimed at obtaining new compounds with high affinity and selectivity toward α_1-adrenoceptors (AR), a new class of piperazine derivatives was designed, synthesized and biologically tested. The new compounds **1**–**13** are characterized by a flavone system linked, through an ethoxy or propoxy spacer, to a phenyl- or pyridazinone-piperazine moiety. Biological data showed an interesting profile for the phenylpiperazine subclass found to have a nanomolar affinity toward α_1-AR, and less pronounced affinity for α_2-AR and the 5-HT_{1A} serotoninergic receptor. A discussion on the structure–activity relationship (SAR) of such compounds is also reported, on the basis of the flavone substitution pattern, length and functionalization of the spacer, and disruption of the phenylpiperazine system.

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1. Introduction

Benign prostatic hyperplasia (BPH) is characterized by an excessive growth of the glandular cells and myofibroblasts of periurethral and transition zones, leading to urinary obstruction and lower urinary tract symptoms (LUTS), mainly due to the physical compression of the urethra. Although both α_1- and α_2-AR are present, the contraction of the human prostate was found to be mediated by α_1-AR, whith α_2-AR playing any significant role.[2] As a consequence, α_1-AR antagonists were successfully used in the treatment of BPH to relieve the LUTS.

In this context, in continued research efforts in the area of α_1-AR antagonists, starting from the prototype of α_1-AR antagonists bearing a benzodioxane moiety (WB 4101, Fig. 1), we have previously designed and synthesized benzodioxane–arylpiperazine hybridized compounds whose chemical structure incorporated both a 1,4-benzodioxan-2-yl nucleus and an *ortho*-substituted phenylpiperazine system bridged by an unsubstituted alkyl chain of variable length.[3] Such compounds were characterized by interesting α_1-AR antagonist activity and unrelevant selectivity between α_1- and α_2-AR. As a consequence, with the aim of improving α_1-AR affinity and selectivity, we decided to modify the original benzodioxane–arylpiperazine scaffold to propose a new class of compounds that would allow exploration of the role played by the arylpiperazine, the alkyl chain, and the terminal heterocyclic fragment on the α_1-AR activity and selectivity toward α_2-AR and 5-HT_{1A} serotoninergic receptor. For this purpose, a survey of the literature focused on α_1-AR antagonists, led to the suggestion that, while the oxygen atom at the position 1 of WB 4101 contributes to receptor binding, the oxygen atom at the position 4 could have a structural function in determining the optimal conformation of such a compound for interaction with the corresponding receptor.[4] Moreover, compounds bearing a phenyl ring at the position 3 (such as phendioxan, Fig. 1) or an aryl substituent, were characterized by a significant enhancement of α_1-AR selectivity with respect to the parent compound.[5]

Keywords: α_1-Adrenoceptor affinity; Flavone; Pharmacophore; Pyridazinone-piperazine.

* Corresponding authors. Tel.: +39-0577-234330; fax: +39-0577-234333 (F. M.); tel.: +39-075-5855136; fax: +39-075-5855161 (G. S.). E-mail: manettif@unisi.it; noemi@unipg.it

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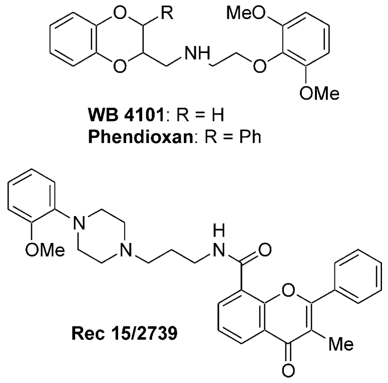


Figure 1.

On the basis of these considerations, we have planned to modify the structure of the WB 4101 benzodioxane ring according to the following guidelines. (1) Insertion of a phenyl ring at the C3 position, as found in the structure of phendioxan. (2) Replacement of the O4 oxygen atom with a carbonyl group and insertion of a double bond between C2 and C3. This transformation introduced into the molecule a structural rigidificaton allowing for the investigation of the role played by this coplanar system in determining α_1-AR affinity and avoiding, at the same time, complicating factors during the synthesis of the new compounds, such as the *cis*/*trans* relationship due to the C2–C3 disubstitution or the stereochemistry on C2.

As a result of such modifications, we have kept the resulting flavone moiety, as already found in Rec 15/2739 (Fig. 1), and inserted it, enriched with a 3-hydroxy substituent, into the new ortho alkoxyphenylpiperazinylalkyloxy flavone derivatives **1** and **2**, differing on the length of the alkyl spacer (ethyl and propyl, respectively). To further investigate the influence of such a flavone nucleus on the affinity toward the panel of receptors studied, two additional substitution patterns were considered, corresponding to a 6- and 7-hydroxyflavone fragment, leading to compounds **5**, and **3**, **4**, respectively. Compound **6** has been synthesized to assess the hypothesis that increased affinity was usually associated with bulkier substituent at the ortho position on the phenyl ring attached to the piperazine nucleus. Moreover, an additional subset of derivatives (namely, compounds **7**–**13**) has been obtained by replacement of the alkoxyphenyl moiety with a pyridazinone nucleus, with the aim of evaluating the importance of the integrity of the phenylpiperazine system for α_1-AR affinity. Finally, to evaluate the role of the spacer, in addition to variations on its length, a hydroxy group has been inserted in the central carbon atom of a propyl spacer to obtain compounds **12** and **13**.

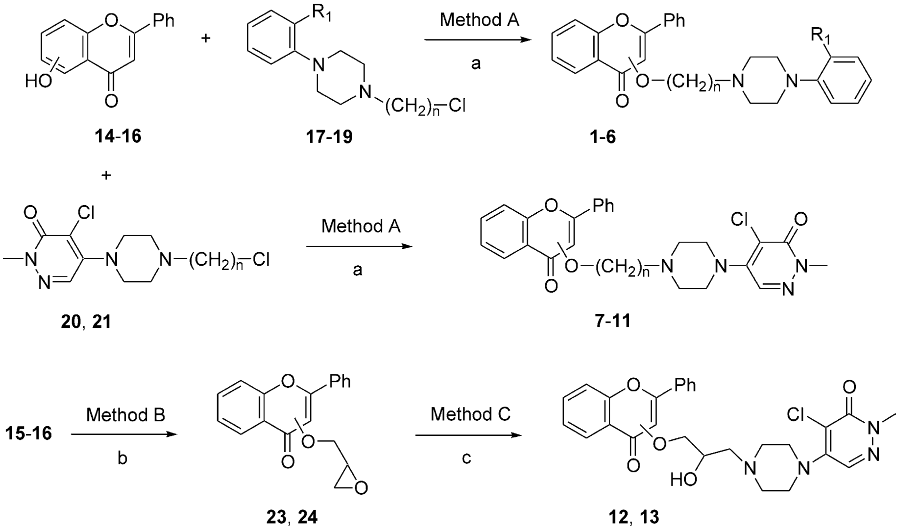
2. Chemistry

The target compounds **1**–**13** were synthesized as outlined in Scheme 1.

In detail, 3-hydroxyflavone (**14**) or 7-hydroxyflavone (**15**) in turn reacted with 1-(2-methoxyphenyl)-4-(2-chloroethyl)piperazine (**17**) or 1-(2-methoxyphenyl)-4-(3-chloropropyl)piperazine (**18**) (both of them synthesized following the method described by Bourdais)[6] in acetone in the presence of dry potassium carbonate (method A) to afford compounds **1**–**2** and **3**–**4**, respectively, in yields ranging from 30 to 45%.

The same reaction has been applied using 3-hydroxyflavone or 7-hydroxyflavone that in turn reacted with 2-methyl-4-chloro-5-[4-(2-chloroethyl)piperazin-1-yl]pyridazin-3(2*H*)-one (**20**) or 2-methyl-4-chloro-5-[4-(3-chloropropyl)piperazin-1-yl]pyridazin-3(2*H*)-one (**21**)[7] to prepare compounds **7**–**8** and **9**–**10**, respectively, in yields ranging from 40 to 60%.

In a similar way, according to method A, compounds **5**, **6**, and **11** were prepared by direct alkylation of 6-hydroxyflavone (**16**) using the arylpiperazine **17**, 1-(2-isopropyl-



Scheme 1. Compounds: **14**, 3-hydroxyflavone; **15**, 7-hydroxyflavone; **16**, 6-hydroxyflavone; **17**, $n = 2$, R_1 = OMe; **18**, $n = 3$, R_1 = OMe; **19** $n = 2$, R_1 = O/Pr; **20**, $n = 2$; **21**, $n = 3$; **23**, 7-(2,3-epoxypropoxy)flavone; **24**, 6-(2,3-epoxypropoxy)flavone. Reagents: (a) acetone, dry K_2CO_3; (b) **22**, KOH, aqueous EtOH; (c) **25**, EtOH, reflux.

oxyphenyl)-4-(2-chloroethyl)piperazine (**19**),[8] and the pyridazinone-arylpiperazine **20**, respectively.

Finally, reaction of **15** or **16** with an excess of epichlorohydrin (**22**) and potassium hydroxide in aqueous ethyl alcohol (method B) gave the 7-(2,3-epoxypropoxy)-flavone (**23**) and 6-(2,3-epoxypropoxy)flavone (**24**),[9] respectively. The opening of these epoxyde intermediates was realized using an excess of 2-methyl-4-chloro-5-(piperazin-1-yl)pyridazin-3(2*H*)-one (**25**) in refluxing ethanol (method C) to afford compounds **12** and **13**, respectively.

Chemical and physical data, as well as elemental analysis of the new compounds are reported in Tables 1 and 2.

Table 1. α_1-AR, α_2-AR and 5-HT$_{1A}$ Serotoninergic receptor binding affinities of compounds **1**–**17**

Compd	*n*	R$_1$	Sub[b]	K_i[a] (nM)		
				α_1-AR[c]	α_2-AR	5-HT$_{1A}$
1	2	OMe	3	5.9±2.6 (1.4)	1045±219	155.0±50
2	3	OMe	3	7.8±0.7 (5.4)	530±42	3.2±0.8
3	2	OMe	7	5.7±0.7 (14)	810±28	164±68
4	3	OMe	7	7.7±0.8 (2.1)	1055±148	85±2
5	2	OMe	6	7.2±0.3 (7.8)	561±48	74±36
6	2	O*i*Pr	6	2.6±0.6	6.3±0.9	91±38
7	2		3	55±12 (290)	>10,000	742±54
8	3		3	75±6	>10,000	717±24
9	2		7	235±35	>10,000	>10,000
10	3		7	205±21	>10,000	>10,000
11	2		6	581±24	6500±670	>10,000
12			7	101±23	889±50	>10,000
13			6	437±60	3264±302	>10,000
Prazosin				0.24±0.05		
Rauwolscine					4.0±0.3	

[a] The K_i binding data were calculated as described in the Experimental. 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:[15] $K_i = IC_{50}/1 + (L/K_d)$ when [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), K_d of [^{3}H]-rauwolscine binding to rat cortex membranes was 4 nM (α_2), and K_b of [^{3}H]8-OH-DPAT binding to rat cortex membranes was 2 nM (5-HT$_{1A}$).

[b] Substitution pattern on the flavone nucleus.

[c] In parentheses, predicted affinity values calculated by Catalyst, are reported.

3. Results and discussion

This work is a part of a project aimed at synthesizing arylpiperazine derivatives showing high affinity and selectivity for α_1-AR with respect to α_2-AR and 5-HT$_{1A}$ serotoninergic receptors, to be potentially applied in the treatment of benign prostatic hyperplasia or hypertension.

Being aware that both the α_1- and α_2-AR are heterogenic species, but taking into account that our major interest is the synthesis of selective α_1-AR antagonists (with respect to α_2-AR and 5-HT$_{1A}$ antagonists), the following comments on the structural features of compounds **1**–**13** are only referred to the native α_1- and α_2-adrenoceptors, and not to their relative subtypes.

The α_1- and α_2-adrenoceptor and 5-HT$_{1A}$ serotoninergic receptor binding affinities of the new flavone derivatives, expressed as K_i values, have been assessed by radioligand receptor binding assay and reported in Table 1.

Considering the binding data, it was possible to observe that α_1-AR affinity of all the phenylpiperazine derivatives was in the nanomolar range, from 2.6 nM of compound **6** through 7.8 nM of **2**. On the contrary, derivatives obtained by replacement of the *o*-methoxyphenyl moiety with a pyridazinone nucleus were characterized by a significant decrease in α_1-AR affinity data, ranging from 55 nM of 7 through 581 nM of **11**. Similarly, functionalization of the spacer linking the piperazine ring to the flavone moiety was detrimental for α_1-AR affinity (see below).

To further analyze, at a quantitative level, the relationships between the structural properties of compounds **1**–**13** and their relative affinity data, we have used a pharmacophoric model for α_1-AR antagonists recently reported by our research group.[10] Starting from some arylpiperazine–pyridazinone derivatives and various other molecules collected from the literature, the model has been developed applying a ligand-based drug design method, with the aim of gaining an insight into the structural factors responsible for α_1-AR affinity. Five features constitute the model (Fig. 2), namely, three hydrophobic regions HY, a hydrogen bond acceptor HBA, and a positively ionizable group PI.

Table 2. Elemental analysis and physical data of compounds **1**–**13**

Compd	Yield (%)	Mp	Formula	Elemental analysis data: found (%), calculated (%)		
				C	H	N
1	35	188–196	$C_{28}H_{28}N_2O_4 \cdot 2HCl \cdot H_2O$	61.49, 61.43	6.29, 5.87	5.01,5.12
2	30	190–196	$C_{29}H_{30}N_2O_4 \cdot HCl \cdot 2H_2O$	64.07, 64.15	6.07, 6.45	4.75, 5.15
3	30	232–238	$C_{28}H_{28}N_2O_4 \cdot 2HCl \cdot 0.5H_2O$	62.05, 62.47	5.69, 5.76	5.04, 5.20
4	40	228–232	$C_{29}H_{30}N_2O_4 \cdot 2HCl$	63.65, 64.10	5.98, 5.89	5.03, 5.15
5	60	137–143	$C_{28}H_{28}N_2O_4 \cdot 2HCl \cdot 1.5H_2O$	60.55, 60.44	6.06, 5.94	5.12, 5.03
6	25	199–203	$C_{30}H_{32}N_2O_4 \cdot 3HCl \cdot 1.5H_2O$	57.96, 58.03	5.88, 6.12	4.34, 4.51
7	60	244–249	$C_{26}H_{25}ClN_4O_4 \cdot HCl$	58.67, 58.99	5.17, 4.91	10.17, 10.58
8	40	239–243	$C_{27}H_{27}ClN_4O_4 \cdot 3HCl$	52.71, 52.61	5.10, 4.87	8.68, 9.09
9	60	270–278	$C_{26}H_{25}ClN_4O_4 \cdot HCl \cdot 0.5H_2O$	58.34, 58.00	4.80, 5.01	9.99, 10.41
10	40	267–273	$C_{27}H_{27}ClN_4O_4 \cdot HCl \cdot H_2O$	57.35, 57.76	5.23, 5.35	9.97, 9.98
11	40	216–218	$C_{26}H_{25}ClN_4O_5 \cdot HCl \cdot 1.5H_2O$	56.35, 56.12	5.32, 5.21	9.68, 10.07
12	45	239–242	$C_{27}H_{27}ClN_4O_5 \cdot 2HCl \cdot 0.5H_2O$	53.76, 53.61	5.20, 4.96	9.38, 9.27
13	60	191–195	$C_{27}H_{27}ClN_4O_5 \cdot HCl$	61.40, 61.03	5.69, 5.27	10.11, 10.54

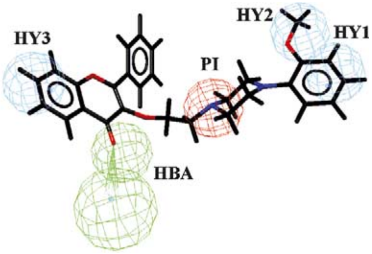


Figure 2. Compound **1** fitted into the five-feature pharmacophoric model for compounds with affinity toward α_1-AR. The HY1–HY2 hydrophobic system is matched by the phenyl ring and its *ortho* methoxy substituent, the positively ionizable group PI corresponded to the most basic nitrogen atom of the piperazine ring, the carbonyl oxygen atom was the hydrogen bond acceptor group HBA, and, finally, the condensed phenyl ring of the flavone moiety satisfied the terminal hydrophobic region HY3 of the pharmacophore. The pharmacophic features are color coded: hydrophobic HY, blue; positively ionizable PI, red; hydrogen bond acceptor HBA, green.

Moreover, because no experimental data on the biologically relevant conformations of the new compounds were available, we resorted to a molecular mechanics approach (the 2D–3D sketcher of Catalyst)[11] to build the conformational models to be used in the fitting procedure to the pharmacophore.

Analysis of the fitting mode of **1** into the pharmacophoric model, depicted in Figure 2, evidenced that the arylpiperazine moiety of such a compound interacted with the HY1–HY2–PI system. In particular, the HY2 and HY1 features are filled by the *o*-methoxy substituent and the phenyl ring, respectively, while the piperazine nitrogen atom directly linked to the alkyl spacer, corresponded to the positive ionizable group (PI) of the pharmacophore. The terminal flavone fragment was able to satisfy, with its carbonyl group and the condensed phenyl ring, respectively, both the hydrogen bond accceptor feature (HBA) and the additional hydrophobic region HY3 of the pharmacophoric model. In such an orientation with respect to the features of the model, compound **1** was predicted to have an affinity value of 1.4 nM toward α_1-AR, in good agreement with the experimental observation (5.9 nM). Lengthening of the alkyl spacer connecting the arylpiperazine moiety to the flavone fragment, from an ethyl to a propyl chain only produced a slight variation of α_1-AR affinity. In fact, affinity of compound **2**, bearing a three carbon atom linker, was found to be 7.8 nM, comparable to that of the corresponding ethyl derivative **1** (5.9 nM). As expected, the orientation of both **1** and **2** into the pharmacophoric model was very similar, with the arylpiperazine system and the flavonic condensed phenyl ring of both compounds lying in the same regions of space. The most relevant difference in orientation was that the 2-phenylpyrane nucleus of **2** underwent a translation mainly due to a conformational rearrangement of the elongated spacer. The affinity of **2** was predicted to be 5.4 nM versus an experimental value of 7.8 nM.

Transformation of the 3-hydroxyflavone moiety of **1** into the corresponding 7-hydroxy derivative, as in compounds **3** and **4**, did not influence the α_1-AR affinity in a significative way. Accordingly, they showed an affinity of 5.7 and 7.7 nM, respectively. Regarding the orientation of **3** with respect to the pharmacophore, the HBA and PI features are filled by the flavone carbonyl group and the most basic nitrogen atom of the piperazine ring, respectively, similarly to compound **1**. On the contrary, the phenyl ring at the 2-position of the flavone system corresponded to the HY3 feature,[12] and the HY1-HY2 system, also in this case, was filled by the *o*-methoxyphenyl moiety, but in the opposite orientation with respect to that found in **1**. That is, the methoxy substituent was located inside the HY1 sphere and the phenyl ring into the HY2 sphere. Very similar considerations can be made for compound **4**, where the elongation of the central chain led only to a partial spatial rearrangement of the piperazine ring. Predicted affinity values for **3** and **4** were 14 and 2.1 nM.

Compound **5**, bearing a 6-hydroxyflavone system, was found to have an affinity of 7.2 nM. Such a compound interacts with the pharmacophore in a similar way in respect to the 7-hydroxyflavone derivative **3**, with a small variation in the location of the condensed phenyl ring. Predicted affinity of **5** was 7.8 nM versus an experimental value of 7.2 nM.

An enhancement of affinity was found for compound **6** (2.6 nM), probably due to the increment of bulkiness of the *ortho* substituent on the phenyl ring bound to the piperazine nucleus. Superposition of this compound into the pharmacophore showed that the isopropyloxy group is able to better fit the HY1–HY2 hydrophobic system, in agreement with our previous results.[1,13]

Moreover, replacement of the *o*-methoxyphenyl moiety with a pyridazinone nucleus produced a marked decrease in affinity toward α_1-AR. In particular, 3-hydroxyflavone derivatives **7** and **8** showed values of about one order of magnitude higher with respect to **1** and **2**. Analogously, the 7-hydroxyflavone derivatives **9** and **10** showed affinity values 40- and 27-fold higher with respect to **3** and **4**. Finally, the 6-hydroxyflavone derivative **11** showed an affinity 80-fold higher than the corresponding methoxyphenyl counterpart **5**.

Superposition of pyridazinone derivatives into the pharmacophore accounted for their decreased affinity. In fact, affinity of compound **7**, taken as a representative example of the whole pyridazinone subset, was predicted to be 290 nM, as a consequence of the partial fit of such a compound into the pharmacophoric features. Particularly, while HBA, PI and HY2 were matched by the carbonyl group, the most basic nitrogen atom of the piperazine ring, and the methyl group on the pyridazinone nucleus, the HY3 feature was only partially mapped by the condensed phenyl ring of the flavone moiety. Unexpectedly, the remaining feature, HY1, was completely omitted, even if the structure of the [5-(4-chloropyridazinon-3(2*H*)yl)]piperazin-1-yl moiety is very similar to that of the *o*-chlorophenylpiperazine portion, that was demonstrated to be able to fit both the HY1 and HY2 features of the pharmacophore. In other words, the pyridazinone nucleus and the chlorine atom were expected to fill the HY1 and the HY2 features of the pharmacophore, similarly to an *o*-chlorophenyl moiety. Such an orientation was impossible to be found because the pyridazinone nucleus, due to the presence of its carbonyl group and the N1 nitrogen atom (both of them hydrogen bond acceptors), was not recognized by the program as a hydrophobic element, and consequently not forced to occupy neither HY1 nor HY2. As a consequence, the lack of mapping HY1 can be rationalized considering that during the fitting operation aimed at finding a chemical group able to profitably interact with the HY1–HY2, the program chose the methyl group of the pyridazinone (with respect to the chlorine atom) for matching HY2. During the same step of calculations, the pyridazinone–piperazine system underwent a conformational rearrangement (with respect to an *o*-chlorophenylpiperazine moiety) leading to completely omit HY1.

Finally, functionalization of the spacer between the piperazine and flavone moieties was found to have a slight influence on affinity. In fact, compound **12** bearing a hydroxy group at the central carbon atom of the propyl spacer, was characterized by an affinity of 101 nM, about 2-fold lower than the corresponding unsubstituted derivative **10**. Affinity of **12** was predicted by the pharmacophoric model to be 360 nM, in agreement with the experimentally determined value. Alignment of **12** into the pharmacophore showed that the hydroxy group was the hydrogen bond acceptor group instead of the flavone carbonyl moiety, while the 2-phenyl ring at the flavone nucleus corresponded to the HY3 feature of the pharmacophoric model.

Regarding α_2-AR affinity, it can be noticed that compounds **1**–**5** were characterized by values of about two orders of magnitude (or more) higher than the corresponding affinity values toward α_1-AR. As a consequence, they assumed an interesting selectivity for α_1-AR toward α_2-AR, being, as an example, 177 the α_2/α_1 affinity ratio for compound **1**. An exception to this trend was represented by **6** characterized by an α_2-AR affinity value (6.3 nM) about 90-fold lower than the corresponding *o*-methoxy derivative **5** (561 nM), leading to the lack of any α_2/α_1 selectivity. On the other hand, among the pyridazinone subset, only compounds **7** and **8** retained some selectivity (180 and 133, respectively) for α_1-AR toward α_2-AR, mainly due to the fact that such compounds showed any affinity toward α_2-AR.

The 5-HT$_{1A}$ affinity of these new flavone derivatives was worthy of further consideration. In fact, differently from both the α_1-AR and α_2-AR affinity that was in principle not dependent from the length of the spacer and from the substitution pattern on the flavone system, the last structural elements were suggested to influence affinity toward the serotoninergic receptor. Compounds with an ethyl spacer showed affinity lower than the corresponding propyl derivatives (**1** versus **2**, and **3** versus **4**). Moreover, variation of the flavone substitution pattern evidenced that both the 3-hydroxy and 6-hydroxyflavone derivatives were better recognized by the 5-HT$_{1A}$ receptor with respect to the corresponding 7-hydroxy derivatives. As an example, **2** possessed an affinity of 3.2 nM, that is 26-fold lower with respect to **4** (85 nM). Similarly to results obtained for α_2-AR, the pyridazinone derivatives showed any significant affinity toward 5-HT$_{1A}$.

On the basis of the results described above, some considerations can be ruled out as follows. (1) The integrity of the phenylpiperazine system is a crucial key in determining affinity toward all the receptors considered. In fact, compounds bearing a pyridazinone-piperazine scaffold were characterized by low affinity toward α_1-AR, with respect to the corresponding *o*-alkoxyphenylpiperazine derivatives. Moreover, they showed any significant affinity for both α_2-AR and 5-HT$_{1A}$ serotoninergic receptors. This could be a consequence of the different hydrophilic/lipophilic character of this portion of the molecule, as also suggested by our previous studies in this field[3] and by literature reports showing that simple heterocyclic rings, such as pyridine or pyrimidine, usually led to a (marked) drop in affinity toward α_1-AR.[14] (2) Increasing the bulkiness of the alkoxy group at the *ortho* position on the phenyl ring attached to the piperazine nucleus led to an enhancement of affinity toward both α_1- and α_2-AR, in agreement with what was previously demonstrated by our research group.[1,13] In particular, while α_1-AR affinity was increased of less than 3-fold as a consequence of the replacement of the methoxy group of **5** with the bulkier isopropoxy substituent of **6**, the corresponding α_2-AR affinity underwent an enhancement of about 90-fold (561 nM of **5** versus 6.3 nM of **6**). On the contrary with respect to this trend, compound **5** retained a better 5-HT$_{1A}$ affinity (74 nM) in comparison to the corresponding isopropoxy derivative **6** (91 nM). (3) The different extension of the ethyl or propyl chain constituting the spacer between the arylpiperazine system and the terminal heterocyclic fragment, exerted a weak influence in determining affinity of the new compounds toward α_1-AR. In fact, although the ethyl spacer was associated with the best affinity in congeneric series of compounds (with the exception of **9** and **10**), differences in affinity between the ethyl and propyl derivatives were very small. On the contrary, an opposite trend was found for 5-HT$_{1A}$ affinity data, being propyl derivatives more active than the corresponding ethyl congeners, and being more pronounced the differences in affinity. (4) The substitution pattern of the flavone moiety was in principle unable to influence affinity, while the introduction of a 7-hydroxyflavone moiety linked to a propyl spacer caused a decrease of more than one order of magnitude in 5-HT$_{1A}$ affinity (**2** versus **4**). In particular, comparison of compounds bearing the same linker but different terminal heterocyclic portions, evidenced as the presence of a 3-hydroxy, 6-hydroxy, or 7-hydroxyflavone was unrelevant for α_1-AR affinity. In fact, compounds **1** and **3** showed affinity values in the range between 5.7 and 5.9 nM, similarly to compounds **2** and **4**, whose affinity values spanned from 7.7 to 7.8 nM. A different trend in α_1-AR affinity was found for compounds with a pyridazinone nucleus instead of the *o*-methoxyphenyl moiety. Among the subset with an ethyl spacer, the 3-hydroxyflavone derivative **7** showed an α_1-AR affinity of 55 nM, the best value between all the new pyridazinone derivatives.

Moreover, such a compound was also characterized by the best selectivity between α_1-AR and α_2-AR, being 180 the α_2/α_1 affinity ratio. Transformation of the 3-hydroxyflavone moiety into the corresponding 7- or 6-hydroxy derivative, led to compounds **9** and **11** with decreased affinity values (235 and 581 nM, respectively). A similar trend was found for compounds bearing a propyl spacer. In fact, a drop in affinity was observed for **8** and **10**, characterized by values of 75 and 205 nM, respectively. Regarding 5-HT_{1A} affinity, it can be noticed that the introduction of a 7-hydroxyflavone moiety as the terminal molecular fragment was detrimental for affinity, being compounds **3** and **4** characterized by the highest values (164 and 85 nM, respectively) with respect to the other members of the phenylpiperazine family bearing a flavone nucleus with different substitution pattern (compare **3** versus **1**, **5**, and **4** versus **2**).

4. Conclusions

On the basis of our previous work and a literature survey in the field of compounds having affinity toward α_1-AR, we have designed and synthesized new piperazine derivatives, most of them endowed with α_1-AR affinity in the nanomolar range. A SAR analysis led to the suggestion that the arylpiperazine moiety was crucial for affinity, with the *o*-isopropoxy derivative better than the corresponding *o*-methoxy compound. Its replacement with a pyridazinone-piperazine system led to a marked drop in affinity. Similarly, functionalization of the alkyl bridge connecting the arylpiperazine to the terminal heterocyclic fragment was detrimental for affinity, while variation of its length from an ethoxy to a propoxy chain produced only a slight decrease in affinity. Moreover, the substitution pattern on the flavone ring was not able to influence affinity toward both the α_1- and α_2-AR. Finally, considering that (1) replacement of the *o*-methoxy substituent with a *o*-isopropoxy group led to an enhancement of about one order of magnitude in α_2-AR affinity, with consequent lacking of any α_1-AR over α_2-AR selectivity, and (2) replacement of the whole aryl moiety with a pyridazinone nucleus gave interesting α_2/α_1 affinity ratio (as an example, 180 for compound **7**) mainly due to a deep decrease in α_2-AR affinity, the synthesis of different classes of compounds is ongoing in our laboratories with the aim of further investigate the role played by the substituent linked to the piperazine ring in defining both the affinity and selectivity toward α_1-AR and α_2-AR. Moreover, considering the fact that 5-HT_{1A} affinity was shown to be dependent on the length of the spacer, in an attempt to find compounds with higher selectivity toward this serotoninergic receptor, several structural modification of the spacer have been planned and results will be reported in due time.

5. Experimental

5.1. Chemistry

Melting points were determined using a Electrothermal 9100 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 hydrochloride derivatives were prepared by bubbling dry HCl into the dry solution of the compound.

5.2. Synthesis. Method A examples

5.2.1. 3-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethoxy-flavone (1). A mixture of 3-hydroxyflavone **14** (0.40 g, 1.7 mmol), 1-(2-methoxyphenyl)-4-(2-chloroethyl)piperazine **17** (0.47 g, 3.4 mmol), and dry K_2CO_3 in 20 mL of acetone, was refluxed under stirring for 8 h. After filtration, the solvent was removed under reduced pressure and the residue was solubilized with CH_2Cl_2. The organic phase was extracted with aqueous NaOH 2%. The organic phase was dried with sodium sulphate and evaporated under reduced pressure. The residue was purified by chromatography on a silica gel column eluting with EtOH:CH_2Cl_2 (4:96) to give a 35% yield of a dense oil: ^{1}H NMR ($CDCl_3$) δ: 2.67–2.80 (m, 6H, 4H-pip., CH_2); 3.03–3.10 (m, 4H, H-pip.); 3.84 (s, 3H, OCH_3); 4.24 (t, 2H, $J=6$ Hz, CH_2); 6.86–6.94 (m, 4H, H-arom.); 7.39–7.66 (m, 6H, H-flav.), 8.13–8.23 (m, 3H, H-flav.). For the corresponding hydrochloride: mp 188–196 °C. Anal. ($C_{28}H_{28}N_2O_4 \cdot 2HCl \cdot H_2O$) C, H, N.

Compounds **2–11** were synthesized following method A.

5.2.2. 3-{3-[4-(2-Methoxyphenyl)]piperazin-1-yl]propoxy-flavone (2). A mixture of **14** (0.44 g, 1.8 mmol), 1-(2-methoxyphenyl)-4-(3-chloropropyl)piperazine **18** (0.50 g, 1.8 mmol), and dry K_2CO_3 (0.51 g, 3.6 mmol) in 25 mL of acetone, was refluxed for 45 h. The residue was purified by chromatography on a silica gel column eluting with EtOH:CH_2Cl_2 (5:95), to give a 30% yield of a dense oil: ^{1}H NMR ($CDCl_3$) δ: 1.90–1.98 (m, 2H, CH_2); 2.49–2.61 (m, 6H 4H-pip., CH_2); 3.00–3.10 (m, 4H, H-pip.); 3.84 (s, 3H, OCH_3); 4.11 (t, 2H, $J=6$ Hz, CH_2); 6.81–6.94 (m, 4H, H-arom.); 7.35–7.70 (m, 6H, H-flav.); 8.05–8.10 (m, 2H, H-flav.); 8.27 (d, 1H, H-flav.). For the corresponding hydrochloride: mp 190–196 °C. Anal. ($C_{29}H_{30}N_2O_4 \cdot HCl \cdot 2H_2O$) C, H, N.

5.2.3. 7-{2-[4-(2-Methoxyphenyl)]piperazin-1-yl]ethoxy}-flavone (3). It was prepared from 7-hydroxyflavone **15** (0.30 g, 1.3 mmol), **17** (0.30 g, 1.3 mmol), and dry K_2CO_3 (0.35 g, 2.6 mmol) in 20 mL of acetone at reflux for 24 h. The residue was purified by chromatography on a silica gel column eluting with EtOH:CH_2Cl_2 (4:96), to give a 30% yield of a white solid: mp 130–135 °C: ^{1}H NMR ($CDCl_3$) δ: 2.75–2.91 (m, 4H, H-pip.); 2.97 (t, 2H, $J=6$ Hz, CH_2); 3.03–3.20 (m, 4H, H-pip.); 3.85 (s, 3H, OCH_3); 4.27 (t, 2H, $J=6$ Hz, CH_2); 6.75 (s, 1H, H-flav.); 6.87–7.02 (m, 6H, H-arom.); 7.49–7.53 (m, 3H, H-flav.); 7.87–7.91 (m, 2H, H-flav.); 8.12 (d,1H, H-flav.). For the corresponding hydrochloride: mp 232–238 °C. Anal. ($C_{28}H_{28}N_2O_4 \cdot 2HCl \cdot 0.5H_2O$) C, H, N.

5.2.4. 7-{3-[4-(2-Methoxyphenyl)]piperazin-1-yl]propoxy} flavone (4). It was prepared from **15** (0.23 g, 0.7 mmol), **18** (0.26 g, 0.7 mmol), and dry K_2CO_3 (0.27 g, 1.9 mmol) in 20 mL of acetone at reflux for 45 h. The residue was purified by chromatography on a silica gel column eluting with EtOH:CH_2Cl_2 (5:95), to give a 40% yield of a white solid: mp 140–145 °C. ^{1}H NMR ($CDCl_3$) δ: 2.03–2.17 (m, 2H, CH_2); 2.62–2.73 (m, 6H, 4H-pip., CH_2); 3.10–3.14 (m, 4H, H-pip.); 3.85 (s, 3H, OCH_3); 4.21 (t, 2H, $J=6$ Hz, CH_2); 6.76–7.05 (m, 7H, 4H-arom., 3H-flav.); 7.48–7.52 (m, 3H, H-flav.); 7.88–7.93 (m, 2H, H-flav.); 8.12 (d, 1H, H-flav.). For the corresponding hydrochloride: mp 228–232 °C. Anal. ($C_{29}H_{30}N_2O_4{\cdot}2HCl$) C, H, N.

5.2.5. 6-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethoxy}-flavone (5). It was prepared from 6-hydroxyflavone **16** (0.17 g, 0.7 mmol), 17 (0.19 g, 1.3 mmol), and potassium *t*-butoxide (0.08 g, 0.7 mmol) in 15 mL of acetone at reflux for 24 h. The residue was purified by chromatography on a silica gel column eluting with EtOH:CH_2Cl_2 (1:99), to give a 60% yield of a yellow solid: mp 120–125 °C. ^{1}H NMR ($CDCl_3$) δ: 2.75–3.00 (m, 6H, 4H-pip., CH_2); 3.15–3.25 (m, 4H, H-pip.); 3.85 (s, 3H, OCH_3); 4.22 (t, $J=6$ Hz, CH_2); 5.50 (dd, 1H, H-flav.); 6.80–7.24 (m, 6H, 4H-arom., 2H-flav.); 7.40–7.60 (m, 6H, H-flav.). For the corresponding hydrochloride: mp 137–143 °C. Anal. ($C_{28}H_{28}N_2O_4{\cdot}2HCl{\cdot}1.5H_2O$) C, H, N.

5.2.6. 6-{2-[4-(2-Isopropoxyphenyl)piperazin-1-yl]ethoxy} flavone (6). A mixture of **16** (0.20 g, 0.8 mmol), 1-(2-isopropoxyphenyl)-4-(2-chloroethyl)piperazine **19** (0.17 g, 0.8 mmol), and K_2CO_3 (0.17 g, 0.34 mmol) in 15 mL of acetone, was refluxed for 5 h. The residue was purified by chromatography on a silica gel column eluting with EtOH:CH_2Cl_2 (1:99), to give a 25% yield of a dense oil: ^{1}H NMR ($CDCl_3$) δ: 1.34 (d, 6H, $2CH_3$); 2.77–2.92 (m, 6H, 4H-pip., CH_2); 3.06–3.15 (m, 4H, H-pip.); 4.16 (t, $J=6$ Hz, CH_2); 4.50 (q, 1H, OHCH); 5.46 (dd, 1H, H-flav.); 6.84–6.96 (m, 5H, 4H-arom., 1H-flav.); 7.13–7.20 (m, 1H, H-flav.); 7.37–7.50 (m, 6H, H-flav.). For the corresponding hydrochloride: mp 199–203 °C. Anal. ($C_{30}H_{32}N_2O_4{\cdot}3HCl{\cdot}1.5H_2O$) C, H, N.

5.2.7. 3-{2-{4-[5-(2-Methyl-4-chloro)]pyridazinon-3(2*H*)-yl}-piperazin-1-yl}ethoxyflavone (7). A mixture of 2-methyl-4-chloro-5-{[4-(2-chloroethyl)]piperazin-1-yl} pyridazin-3(2*H*)-one **20** (0.20 g, 0.34 mmol), **14** (0.16g, 0.34 mmol), and dry K_2CO_3 (0.24 g, 1.7 mmol) in 10 mL of acetone was refluxed for 45 h. The residue was purified by chromatography on a silica gel column eluting with EtOH:CH_2Cl_2 (3:97), to give a 60% yield of a dense oil: ^{1}H NMR ($CDCl_3$) δ: 2.65–2.75 (m, 4H, H-pip.); 2.80 (t, 2H, $J=6$ Hz, CH_2); 3.30–3.40 (m, 4H, H-pip.); 3.75 (s, 3H, CH_3-pyrid.); 4.25 (t, 2H, $J=6$ Hz, CH_2); 7.40–7.60 (m, 6H, 1H-pyrid., 5H-flav.); 7.70–7.77 (m, 1H, H-flav.); 8.10–8.20 (m, 2H, H-flav.), 8.26–8.28 (dd, 1H, H-flav.). For the corresponding hydrochloride: mp 244–249 °C. Anal. ($C_{26}H_{25}ClN_4O_4{\cdot}HCl$) C, H, N.

5.2.8. 3-{3-{4-[5-(2-Methyl-4-chloro)]pyridazinon-3(2*H*)-yl}piperazin-1-yl}propoxyflavone (8). It was prepared from of 2-methyl-4-chloro-5-{[4-(3-chloropropyl)]piperazin-1-yl}pyridazin-3(2*H*)-one **21** (0.37 g, 1.5 mmol), **14** (0.36 g, 1.2 mmol), and dry K_2CO_3 (0.32 g, 2.4 mmol) in 15 mL of acetone at reflux for 45 h. The residue was purified by chromatography on a silica gel column eluting with EtOH:CH_2Cl_2 (6:94) to give a 40% yield of a dense oil: ^{1}H NMR ($CDCl_3$) δ: 1.85–1.92 (m, 2H, CH_2); 2.44–2.51 (m, 6H, 4H-pip., CH_2); 3.31–3.38 (m, 4H, H-pip.); 3.75 (s, 3H, CH_3-pyrid.); 4.10 (t, 2H, $J=6$ Hz, CH_2); 7.26–7.52 (m, 6H, 5H-flav., 1H-pyrid.); 7.54–7.69 (m, 1H, H-flav.); 8.04–8.10 (m, 2H, H-flav.); 8.26 (d, 1H, H-flav.). For the corresponding hydrochloride: mp 239–243 °C. Anal. ($C_{27}H_{27}ClN_4O_4{\cdot}3HCl$) C, H, N.

5.2.9. 7-{2-[4-[5-(2-Methyl-4-chloro)]pyridazinon-3(2*H*)-yl}piperazin-1-yl}ethoxyflavone (9). It was prepared from **15** (0.24 g, 1.0 mmol), **20** (0.30 g, 1.3 mmol), and dry K_2CO_3 (0.36 g, 1.3 mmol) in 10 mL of acetone at reflux for 45 h. The residue was purified by chromatography on a silica gel column eluting with EtOH:CH_2Cl_2 (4:96), to give a 60% yield of a white solid: mp 169–174 °C. ^{1}H NMR ($CDCl_3$) δ: 2.72–2.79 (m, 4H, 4H-pip.); 2.95 (t, 2H, CH_2); 3.37–3.46 (m, 4H, H-pip); 3.73 (s, 3H, CH_3 pyrid.); 4.26 (t, 2H, CH_2); 6.77 (s, 1H, H-flav.); 6.98–7.03 (m, 2H, H-flav.); 7.50–7.58 (m, 4H, 1H-pyrid., 3H-flav.); 7.87–7.92 (m, 2H, H-flav.); 8.13 (d, 1H, H-flav.). For the corresponding hydrochloride: mp 270–278 °C. Anal. ($C_{26}H_{25}ClN_4O_4{\cdot}HCl{\cdot}0.5H_2O$) C, H, N.

5.2.10. 7-{3-[4-[5-(2-Methyl-4-chloro)]pyridazinon-3(2*H*)-yl}piperazin-1-yl}propoxyflavone (10). A mixture of **15** (0.24 g, 1.0 mmol), **21** (0.31 g, 1.3 mmol), and dry K_2CO_3 (0.36 g, 2.0 mmol) in 15 mL of acetone, was refluxed for 45 h. The residue was purified by chromatography on a silica gel column eluting with EtOH:CH_2Cl_2 (5:95), to give a 40% yield of a white solid: mp 184–189 °C. ^{1}H NMR ($CDCl_3$) δ: 2.00–2.10 (m, 2H, CH_2), 2.60–2.70 (m, 6H, 4H-pip., CH_2); 3.40–3.50 (m, 4H, H-pip.); 3.77 (s, CH_3 pyrid.); 4.17 (t, 2H, $J=6$ Hz, CH_2); 6.76 (s, 1H, H-flav.); 6.96–7.00 (m, 2H, H-flav.); 7.50–7.57 (m, 4H, 3H-flav., 1H-pyrid.); 7.88–7.93 (m, 2H, H-flav.); 8.13 (d, 1H, H-flav.). For the corresponding hydrochloride: mp 267–273 °C. Anal. ($C_{27}H_{27}ClN_4O_4{\cdot}HCl{\cdot}H_2O$) C, H, N.

5.2.11. 6-{2-[4-[5-(2-Methyl-4-chloro)]pyridazinon-3(2*H*)-yl}piperazin-1-yl}ethoxy]flavone (11). It was prepared from **16** (0.20 g, 0.84 mmol), **20** (0.31 g, 1.3 mmol), and KOH (0.06 g, 1.0 mmol) in 15 mL of ethanol at reflux for 35 h. The residue was purified by chromatography on a silica gel column eluting with EtOH:CH_2Cl_2 (1:99) and crystallized with ethyl acetate to give a 40% yield of a white solid: mp 138–140 °C. ^{1}H NMR ($CDCl_3$) δ: 2.69–2.88 (m, 6H, 4H-pip., CH_2); 3.39–3.44 (m, 4H, H-pip.); 3.75 (s, 3H, CH_3-pyrid.); 4.12 (t, 2H, CH_2); 5.42 (dd, 1H, H-flav.); 6.98 (d, 1H, H-flav.); 7.10–7.17 (m, 1H, H-flav.); 7.35–7.57 (m, 7H, 6H-flav., 1H-pyrid.). For the corresponding hydrochloride: mp 216–218 °C. Anal. ($C_{26}H_{25}ClN_4O_4{\cdot}HCl{\cdot}1.5H_2O$), C, H, N.

5.3. Method C Examples

5.3.1. 7-{3-{4-[5-(2-Methyl-4-chloro)]pyridazinon-3(2*H*)-yl} piperazin-1-yl}-2-hydroxypropoxyflavone (12). A mixture of 7-(oxyran-2-yl-methoxy)flavone **23** (0.28 g, 0.95

mmol) and 2-methyl-4-chloro-5-[4-(piperazin-1-yl)]pyridazin-3(2*H*)-one **25** (0.34 g, 1.5 mmol) in 20 mL of dry ethyl alcohol, was refluxed for 20 h. The solvent was evaporated under reduced pressure and the residue was crystallized with CH_2Cl_2:EtOH yielding a 45% of a solid: mp 190–195 °C. ^{1}H NMR ($CDCl_3$) δ: 2.66–2.90 (m, 6H, 4H-pip., CH_2); 3.35–3.46 (m, 4H, H-pip., OH); 3.76 (s, 3H, CH_3-pyrid.); 4.14–4.26 (3H, OCH_2CH); 6.76 (s, 1H, H-flav.); 6.95–7.05 (m, 2H, H-flav.); 7.50–7.58 (m, 4H, 1H-pyrid., 3H-flav.); 7.87–7.91 (m, 2H, H-flav.); 8.13 (dd, 1H,H-flav.). For the corresponding hydrochloride: mp 239–242 °C. Anal. ($C_{27}H_{27}ClN_4O_5{\cdot}2HCl{\cdot}0.5H_2O$) C, H, N.

5.3.2. 6-{3-{4-[5-(2-Methyl-4-chloro)]pyridazinon-3(2*H*)-yl}piperazin-1-yl}-2-hydroxypropoxyflavone (13). A mixture of 6-(oxyran-2-yl-methoxy)-flavone **24** (0.24 g, 0.8 mmol) and **25** (0.30 g, 1.5 mmol) in 20 mL of dry ethyl alcohol, was refluxed for 18 h. The solvent was evaporated under reduced pressure and the residue was crystallized with EtOH, yielding a 60% of a solid: mp 181–186 °C. ^{1}H NMR ($CDCl_3$) δ: 2.56–2.89 (m, 6H, CH_2, 4H-pip.); 3.40–3.45 (m, 4H, H-pip.); 3.77 (s, 3H, CH_3-pyrid.); 3.96–4.15 (m, 3H, OCH_2CH); 5.43 (dd, 1H, H-flav.); 7.18 (dd, 1H, H-flav.); 7.35-7.46 (m, 6H, H-flav.); 7.58 (s, 1H, H-pyrid.). For the corresponding hydrochloride: mp 191–195 °C. Anal. ($C_{27}H_{27}ClN_4O_5{\cdot}HCl$) C, H, N.

5.4. Biology

5.4.1. Radioligand binding assays. The pharmacological profile of all compounds was evaluated for their affinities for α_1 and α_2-adrenoreceptors, as well as for 5-HT$_{1A}$ serotoninergic receptor by determining for each compound its ability to displace ^{3}[H]prazosin, ^{3}[H]rauwolscine, or [^{3}H]8-OH-DPAT from specific binding sites on rat cerebral cortex.

The concentration of 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 log-probit analysis with seven concentrations of the displacer, each performed in triplicate. Inhibition constants (K_i) were calculated according the equation:[15] $K_i = IC_{50}/1 + ([L]/K_d)$ where [L] is the ligand concentration and K_d its dissociation constant. K_d of [^{3}H]prazosin binding to 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$_{1A}$).

Further details have been reported elsewhere.[10,16]

5.4.2. Computational methods. All calculations and graphic manipulations were performed on a Silicon Graphics Octane R10K workstation by means of the Catalyst 4.8 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 derives from the CHARMm force field, opportunely modified and corrected.

Conformational diversity was emphasized by selection of the conformers that fell within a 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 to each mapping mode satisfying the constraints imposed by the location of the pharmacophore features.

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

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12. Regarding the 3-hydroxyflavone derivatives, the phenyl ring at the position 2 of the flavone nucleus is not directly involved in the interactions between the new compounds and the pharmacophoric model. In fact, the fitting between the flavone carbonyl group and the hydrogen bond acceptor feature of the model, forced the 2-phenyl ring to lie in a region of space surrounded by PI, HBA, and HY3, where any feature of the pharmacophore itself is located. Different orientations were found characterized by the flavone moiety that underwent a rotation of about 180 degrees, leading the endocyclic oxygen atom to correspond to the hydrogen bond acceptor group of the molecule. However, also in this case, the 2-phenyl ring was located in an empty region of space close to the HBA feature, without any interaction with the pharmacophore moieties. On the other hand, this phenyl ring is important in determining conformations of each compound belonging to the 3-hydroxyflavone subset. In fact, due to the close proximity between the phenyl ring and the alkoxy spacer (bound to the vicinal carbon atoms C2 and C3 of the flavone ring, respectively), both of them influenced each other in their conformation, also leading to a decrease in their conformational flexibility.
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