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Synthesis and Biological Activity of New 1,4-Benzodioxan-arylpiperazine Derivatives. Further Validation of a Pharmacophore Model for α_1-Adrenoceptor Antagonists

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Abstract—A series of WB4101 (**1**)-related benzodioxanes (**2**–**17**) have been synthesized by replacing the phenoxyethyl moiety of **1** with a *N*-alkyl piperazine bearing a cyclic substituent (a substituted or unsubstituted phenyl group, a pyridine or pyridazinone ring, a furoyl moiety) at the second nitrogen atom. The binding profile of these compounds has been assessed by radioligand receptor binding assay at α_1- and α_2-adrenoceptors, in comparison to prazosin and rauwolscine, respectively. Moreover, structure–activity relationships have been derived for compounds **2**–**17** based on their fitting to a pharmacophore model for α_1-adrenoceptor antagonists recently proposed by our research group. In a parallel way, the same compounds have been used to further test the predictive power and statistical significance of the model itself. The accuracy of the results obtained also in this case revealed the robustness of the calculated pharmacophore model and led to the identification of the molecular structural moieties which are thought to contribute to the biological activity.

Introduction

The α_1-adrenergic receptors (α_1-AR) are a family of G-protein coupled seven-transmembrane helix receptors which are mainly involved in the cardiovascular and central nervous system.[1] It is now clear that α_1-AR are comprised of multiple subtypes. To date, they are classified into α_{1A}, α_{1B}, and α_{1D},[2] which possess high affinity for prazosin, and the corresponding cloned counterparts α_{1a}, α_{1b}, α_{1d},[3] respectively. In addition to these three subtypes, the existence of an additional α_1-AR (called α_{1L}) has been postulated.[4]

In a similar way, α_2-AR have been classified in four subtypes, called α_{2A}, α_{2B}, α_{2C}, α_{2D}, respectively.[2c]

Molecular cloning studies[5] have shown that the α_1- and α_2-adrenoceptors have many common features which could reflect their similar mechanisms of action. As a consequence of such similarities, synthetic compounds with affinity towards α_1-AR are expected to potentially bind to both α_1 and α_2 receptors.

In this context, many literature reports have highlighted that the arylpiperazinylalkyl moiety is a key element in defining α_1-AR antagonist activity.[6] Moreover, great attention has been paid to compounds bearing the 2-aminomethyl-1,4-benzodioxane fragment, such as **1** (Fig. 1), due to their potent and highly selective α_1-AR antagonist activity.[7]

Research efforts in the area of α_1-AR receptor antagonists have led to the discovery of some clinically useful antihypertensive drugs,[8] acting by relaxation of the vascular smooth muscle which contains high concentrations of α_1-AR. In a similar way, α_1-AR blockers have been employed in the treatment of benign prostatic hyperplasia, due to the significant improvements in symptoms and flow rates in patients with bladder outflow obstruction.[9–11]

The goal of our research project was the synthesis of new benzodioxane-arylpiperazine hybridized compounds possibly characterized by high affinity and selectivity towards α_1-AR with respect to α_2-AR. Thus, in an effort

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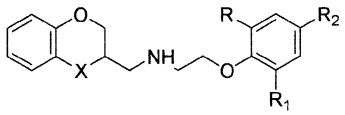


Figure 1. 1 (WB 4101): X = O, R = R_1 = OMe, R_2 = H **20** (Benoxathian): X = S, R = R_1 = OMe, R_2 = H **21**: X = O, R = R_1 = H, R_2 = OMe.

to improve the α_1-AR binding affinity, we decided to modify the lead structure of **1** to propose a new class of compounds that would allow exploration of the role played by the arylpiperazinylalkyl moiety in the activity towards α_1-AR. In the course of our studies in the field of new and potentially selective α_1-AR antagonists, we have recently synthesized several compounds (**2**–**9**, Table 1)[12] structurally related to **1** and characterized by the arylpiperazine ring attached through an ethyl or propyl spacer to the 2-aminomethyl-1,4-benzodioxane moiety. Since some of them showed an interesting activity (i.e., compounds **2** and **3** towards α_1-AR, and compound **9** toward α_2-AR, respectively), in order to better define the optimum structural properties required to interact with α-AR, new compounds (**10**–**17**, Scheme 1 and Table 1) with diverse substituents on the piperazine ring have been synthesized.

We report here the synthesis of the new benzodioxane-arylpiperazines **10**–**17** and the α_1-, α_2-adrenoceptor blocking properties of compounds **2**–**17**. Moreover, the relationships between chemical features of these compounds and their binding affinity data have been derived on the basis of a five-feature pharmacophore model for α_1-AR antagonists previously built by our research group, and consisting of a positive ionizable, three hydrophobic, and a hydrogen bond acceptor pharmacophore features.[13]

Chemistry

The synthesis of compounds **10**–**17** is highlighted in Scheme 1. These compounds were prepared by reacting the 1-(2-chloroethyl)- or 1-(3-chloropropyl)-4-arylpiperazines **19a**–**h**, in turn synthesized following the method described by Bourdais,[14] with 2-aminomethyl-1,4-benzodioxane (**18**),[15] in dry ethanol and in the presence of sodium carbonate.

Details on the synthesis of compounds **2**–**9** have been reported elsewhere.[12]

Results and Discussion

This work is a part of a project aimed at synthesizing arylpiperazine derivatives displaying high affinity and selectivity for α_1-AR with respect to α_2-AR, to be potentially applied in the treatment of hypertension or benign prostatic hyperplasia.

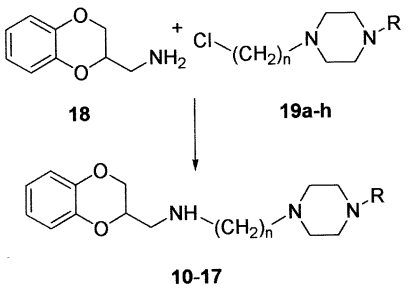
Being aware that both the α_1- and α_2-AR are heterogenic species, but taking into account that our major interest is the synthesis of selective α_1-AR antagonists (with respect to α_2-AR antagonists), the following comments on the structural features of compounds **2**–**17** are only referred to the native α_1- and α_2-adrenoceptors, and not to their relative subtypes.

By taking as a starting point an hybridized structure containing both the arylpiperazinylalkyl and the benzodioxane moieties, the role of either the aryl or the alkyl group in defining affinity for α_1- and α_2-AR was investigated.

Table 1. α_1- and α_2-Adrenergic receptors binding affinities for benzodioxane-arylpiperazine derivatives **2**–**17**

Compd	*n*	R	K_i[a] (nM)		
			α_1-AR[b]	α_2-AR	α_2/α_1
2[c]	2	*o*-Methoxyphenyl	37.0 ± 5.3 (64)	67.0 ± 9.2	1.81
3[c]	3	*o*-Methoxyphenyl	16.5 ± 4.0 (3.9)	134.0 ± 15.0	8.12
4[c]	2	*o*-Chlorophenyl	113.7 ± 15.2 (71)	221.1 ± 37.0	1.95
5[c]	3	*o*-Chlorophenyl	127.0 ± 13.9 (67)	104.9 ± 9.0	0.82
6[c]	2	Phenyl	201.3 ± 34.0 (290)	186.7 ± 35.0	0.92
7[c]	3	Phenyl	147.7 ± 20.0 (110)	380.2 ± 50.0	2.58
8[c]	2	2-Pyridinyl	415.8 ± 39.0 (710)	238.8 ± 17.0	0.57
9[c]	3	2-Pyridinyl	346.7 ± 30.0 (280)	25.6 ± 3.0	0.08
10	2	*p*-Methoxyphenyl	986.0 ± 87.0 (1040)	163.7 ± 32.0	0.16
11	3	*p*-Methoxyphenyl	718.2 ± 74.0 (790)	96.8 ± 7.0	0.13
12	2	*o*-Fluorophenyl	359.4 ± 45.0 (290)	30.0 ± 4.0	0.08
13	3	*o*-Fluorophenyl	203.0 ± 14.0 (63)	855.4 ± 79.0	4.21
14	2	2-Methyl-4-chloropyridazin-3(2*H*)-one-5-yl	269.0 ± 40.0 (100)	293.1 ± 25.0	1.09
15	3	2-Methyl-4-chloropyridazin-3(2*H*)-one-5-yl	292.5 ± 35.0 (120)	140.8 ± 15.0	0.48
16	2	2-Furoyl	177.2 ± 23.0 (120)	158.5 ± 16.0	0.89
17	3	2-Furoyl	331.0 ± 74.0 (250)	61.0 ± 5.0	0.18
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:[27] $K_i = IC_{50}/1 + (L/K_d)$ 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) and K_d of [^{3}H]-rauwolscine binding to rat cortex membranes was 4 nM (α_2).
[b]In parentheses, calculated affinity values.
[c]The synthesis of compounds **2**–**9** has been reported elsewhere.[12]



Scheme 1. Compounds: **10** and **19a**, $n=2$, R = *p*-methoxy phenyl; **11** and **19b**, $n=3$, R = *p*-methoxyphenyl; **12** and **19c**, $n=2$, R = *o*-fluorophenyl; **13** and **19d**, $n=3$, R = *o*-fluorophenyl; **14** and **19e**, $n=2$, R = 2-methyl-4-chloropyridazin-3(2*H*)-one-5-yl; **15** and **19f**, $n=3$, R = 2-methyl-4-chloropyridazin-3(2*H*)-one-5-yl; **16** and **19g**, $n=2$, R = 2-furoyl; **17** and **19h**, $n=3$, R = 2-furoyl. Reagents: ethanol, K_2CO_3, Δ.

The synthesis and pharmacological profile on isolated rat vas deferens of compounds with an *o*-methoxyphenyl (**2**,**3**), *o*-chlorophenyl (**4**,**5**), phenyl (**6**,**7**), and 2-pyridinyl (**8**,**9**) substituent have been previously reported by some of us.[12] Moreover, with the aim of obtaining the maximum coverage in the kinds (i.e., substituents) and relative positions (i.e., substitution pattern) of the molecular chemical features, the benzodioxane-arylpiperazine class has been enlarged by synthesizing additional compounds (**10**–**17**) with a different substitution on the piperazine ring and the alkyl chain characterized by two or three methylene units.

The α_1- and α_2-adrenoceptor binding affinities of benzodioxane-arylpiperazine derivatives **2**–**17**, expressed as K_i values, have been assessed by radioligand receptor binding assay and reported in Table 1.

Considering the binding data, it is possible to observe that affinity for α_1-AR is markedly affected by replacement of the *o*-methoxyphenyl group with all other substituents, with the *p*-methoxy derivatives **10** and **11** showing the lowest affinity. In the *o*-substituted series, the chloro and fluoro derivatives are all less active than the corresponding methoxy counterparts **2** and **3**, with the chloro substituent associated with a modest enhancement in affinity with respect to the fluoro derivatives (see compounds **4** and **5** vs **12** and **13**).

To further analyze, at a quantitative level, the relationships between the structural properties of compounds **2**–**17** and their relative affinity data, we have applied a pharmacophore model for α_1-AR antagonists recently reported by our research group.[13] Starting from some arylpiperazine-pyridazinone derivatives and various other molecules collected from the literature, the model has been developed resorting to a ligand-based drug design method, with the aim of gaining an insight into the structural factors responsible for α_1 affinity.

A quantitative SAR has been derived on the basis of how well each of compounds **2**–**17** was able to fit the pharmacophore features of the model proposed for α_1-AR antagonists.

In a parallel way, the benzodioxan-arylpiperazine derivatives reported in this paper have been used as a test set to further assess the statistical significance and the predictive power of the pharmacophore model. In fact, benzodioxan-arylpiperazines **2**–**17** belong to a class of α_1-AR antagonists never included either in the training set or in the test set considered in our previous work to generate the pharmacophore model. As a consequence, the aim of the current study was the investigation of the structural features of compounds **2**–**17** responsible for α_1-AR affinity and compare them to the features previously identified with a view to contribute to the understanding of the interaction of all these compounds with the corresponding receptor (α_1-AR). In this context, we have assumed that they all bind in the same way to the receptor.

Moreover, because no experimental data on the biologically relevant conformations of the selected compounds are available, we resorted to a molecular mechanics approach (the 2D–3D sketcher of Catalyst)[16] to build the conformational models to be used in the fitting procedure to the pharmacophore.

In addition, due to the fact that the biological evaluation of all the chiral compounds was carried out using racemic mixtures, while the biological activity of chiral compounds is usually due to one of the enantiomers [as an example, (*S*)-**1** has been reported to be much more active than the corresponding *R* enantiomer], it was arbitrarily decided to model the chiral compounds with undefined chirality, thus allowing the program to choose which configuration of the asymmetric carbon atom (i.e., C2 on the benzodioxane ring) common to all compounds **2**–**17** was most appropriate.

Evaluation of how well derivatives **2**–**17** are able to fit the pharmacophore highlighted that the chemical functionalities of the model are all matched by the chemical groups of **3** (Fig. 2), the most active compound of the whole set, taken as a representative example of all benzodioxane derivatives. Particularly, the arylpiperazinyl moiety maps the region where a cluster of features, known as crucial elements to interact with the putative receptor, lies. While the ortho-substituted phenyl ring (corresponding to the aromatic feature of the De Marinis' pharmacophore model)[17] occupies both HY1 and HY2, the piperazine N4 nitrogen atom is located inside the positive ionizable feature PI, also identified by all the previously proposed pharmacophore models for α_1-AR antagonists. Among all the orientations of **3** into the pharmacophore, it was found that in most cases the remaining two features (HBA and HY3, respectively) of the model are interacting with the ligand in such a way as the secondary amine nitrogen overlaps HBA (corresponding to the polar region in the De Marinis' pharmacophore), while the phenyl ring of the benzodioxane moiety only partially matches HY3 of the pharmacophore. Affinity of **3** in this orientation within the model was predicted to be 3.9 nM versus an experimental value of 16.5 nM.

A further analysis of mapping modes of compound **3** and other benzodioxanes onto the pharmacophore showed two additional interaction pathways. In parti-

cular, **3** is able to locate itself in such a manner that the benzodioxane ring maps both HY1 and HY2, while the *o*-methoxyphenyl substituent lies into HY3 (Fig. 3). Consequently, the secondary amino nitrogen and the piperazine nitrogen bearing the propyl chain interchanged their positions, becoming the first one the PI group and the second one the HBA feature, respectively. This arrangement, derived by simply reversing the orientation of the ligand, is not as favoured, mainly because of the conformational strain associated to the piperazine ring.

A third different orientation was found characterized by a conformational rearrangement mainly involving the alkylamino spacer of the ligand. In fact, while the arylpiperazine group maintains the usual location within the pharmacophore (HY1–HY2–PI), the alkylamino chain causes the shortening of the distance between the terminal rings of the ligand, assuming a C-shaped conformation. As a consequence, the benzodioxane system undergoes a translation resulting in a perfect match of the oxygen atom at the 1-position (O1) to HBA.

The last finding is worthy of further consideration on the basis of a literature report[18] describing that, while O4 does not interact with the receptor by hydrogen bond, O1 is a crucial key in defining the role of benzodioxane ring in receptor binding. In fact, with the exception of a carbonyl group, replacement of O1 with

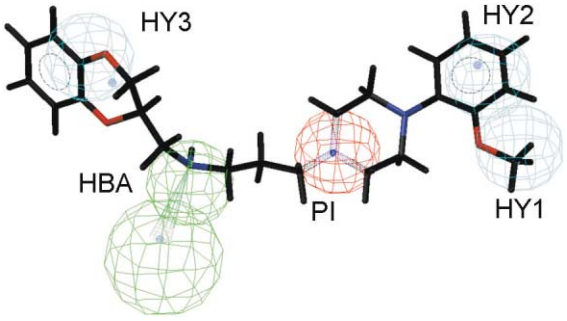


Figure 2. Compound **3**, the most active of the whole class of benzodioxane-arylpiperazine derivatives, mapped to the pharmacophore model for α_1-adrenoceptor antagonists. Pharmacophore features are color coded: cyan for hydrophobics (HY1, HY2, and HY3), green for a hydrogen bond acceptor (HBA), and red for a positive ionizable feature (PI).

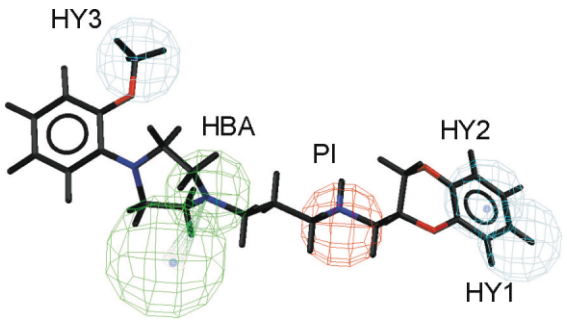


Figure 3. Alternative orientation of compound **3** within the pharmacophore model. This arrangement is not as favoured due to the strained conformation of piperazine. Pharmacophore features are color coded as in Figure 2.

different substituents usually led to derivatives less active than the parent compound. In this context, we have performed a superposition of several benzodioxane derivatives (taken from the literature) into the model. In particular, the *S* enantiomer of **1**, a potent and selective α_1-AR antagonist, lies into the pharmacophore in such a manner that O1 interacts with HBA (Fig. 4). The amine nitrogen and the *o*-methoxyphenyl substituent match PI and HY1–HY2, respectively. Spatial location of the condensed phenyl ring allows only a partial fit into HY3, not in accordance with the very high affinity of (*S*)-**1** for α_1-AR reported to be 0.16, 2.5, and 0.25 nM for α_{1A}, α_{1B}, α_{1D}, respectively.[19] The difference in the predicted *vs* actual affinity of (*S*)-**1** (predicted value 15 nM) led us to re-consider the possible size of HY3. Thus, we have increased by 0.5 Å the radius of the sphere representing this hydrophobic feature. As a result, an improved fit (from 9 to 11) and a predicted affinity value of 0.17 nM, in good agreement with the experimental data, were found. Although this finding has to be considered as a preliminary result, it suggested that a hydrophobic region larger than HY3 might lead to an improvement of the ligand affinity to the receptor. Evaluation of the influence of the HY3 size on the affinity towards α_1-AR is currently under investigation.

Substitution of the oxygen atom at 4-position of benzodioxane moiety led to benoxathian (**20**, Fig. 1) with a slightly reduced activity (0.2, 4.0, and 0.4 nM for α_{1A}, α_{1B}, α_{1D}, respectively)[19] with respect to (*S*)-**1**. Since both O4 of (*S*)-**1** and S4 of **20** lie in an 'empty' region of space (where no pharmacophore features are present), the program well predicts (0.59 nM) the affinity of such a thioderivative. In a similar way, the model also accounts for the marked drop in affinity found for the *p*-methoxy derivative (**21**, Fig. 1)[20] of (*S*)-**1**, the calculated affinity value being 340 nM. The main reason for this decreased activity was the complete inability of **21** to match one of the HY1–HY2 features of the model, according to the trend found for diverse *p*-substituted phenyl derivatives.

Finally, with the purpose of evaluating the ability of the pharmacophore model to discriminate between enantiomeric pairs, the difference between predicted and experimental values has been calculated for *S* and *R* enantiomers of some compounds used in this study. As

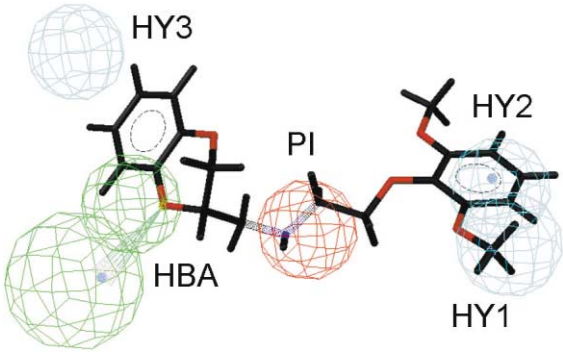


Figure 4. The *S* enantiomer of WB4101 (**1**) superposed to the pharmacophore model. The oxygen atom at the 1-position of the benzodioxane ring corresponds to the HBA feature of the model. Pharmacophore features are color coded as in Figure 2.

an example, the predicted affinity value for (*R*)-**1** was 4.0 nM versus an experimentally determined affinity of 39.8 nM.[20] While no substantial differences in orientation of (*S*)-**1** and (*R*)-**1** with respect to the pharmacophore features was found, the enhanced predicted value for affinity of (*S*)-**1** toward α_1-AR was due to a slightly more productive interaction with HBA.

In a similar way, *S* enantiomers of the benzodioxane-arylpiperazine derivatives have been selected by the program as the optimum structures to interact with the model, although predicted values for *S* enantiomers are in all cases almost identical to the affinity values calculated for *R* enantiomers.

Among the three orientations of **3** into the pharmacophore, the program underscores the first one to best rationalize the effect deriving from the substitution on the phenyl ring. The substitution pattern on the phenyl ring of the arylpiperazine moiety is a crucial element to be accounted for with the purpose to rationalize the relationships between structural properties and affinities of compounds **2**–**17**. Pharmacological tests highlighted that the methoxy group at the ortho position is the substituent associated with the highest α_1-AR antagonist activity within the whole set of benzodioxanes under study. Moreover, the presence of a chloride, fluoride, or hydrogen atom instead of the methoxy group led in all cases to compounds about one order of magnitude (or more) less active than **3** (Table 1). The effect of replacement of the *o*-methoxy group with a chlorine atom was difficult to rationalize on the basis of previous work pointing out that both the two substituents are able to interact, equally weighted by the program, with the same hydrophobic portion of the pharmacophore model.[13] Decreased activity associated with **4** and **5** with respect to **2** and **3** can be justified taking into account that, during estimation of affinity values based on the orientation of the ligand, the program runs with the aim of finding the highest number of contacts between pharmacophore and ligand.[21] As a consequence of forcing the benzodioxane ring into HY3, the ortho substituent at the opposite terminal portion of the molecule, with the exception of the methoxy group, is only able to partially map one of the HY1–HY2 features. In summary, the pharmacophore model accounts for this trend in the biological data with the ability of each of the above substituents to fit the HY2 feature. Particularly, while the *o*-methoxyphenyl moiety possesses optimum structural features to perfectly map the HY1–HY2 system of the model, the *o*-chloro- and *o*-fluorophenyl groups are only able to partially match into HY1–HY2, with a consequent reduction in the predicted activity (i.e., 67 and 63 nM are the calculated affinity values for compounds **5** and **13**, respectively). In a similar way, derivatives where the aromatic group is unsubstituted (**6** and **7**) or corresponding to a pyridine ring (**8** and **9**), are unable to map one of the HY1-HY2 features, with consequent decreased activity (i.e., calculated values for compounds **7** and **9** are 110 and 280 nM, respectively). Based on these considerations, we were unable to justify by means of the pharmacophore model the slight improvement in α_1-AR affinity of the unsubstituted phenyl compounds versus the corresponding *o*-fluoro derivatives. For this purpose, it is important to note that Catalyst is unable to take into account electrostatic effects possibly involved in defining the activity of these phenyl and pyridine derivatives.

The distance between the arylpiperazine and benzodioxane moieties is influenced by the length of the alkylamino chain acting as a spacer between the two rings of compounds **2**–**17**. Both the intrinsically high conformational flexibility of the Csp^3–Csp^3 polymethylene sequence and the length of the whole chain are crucial parameters influencing the goodness-of-fit of each compound to the chemical features of the pharmacophore model. In fact, while the PI, HY1, and HY2 features are mapped by all ligands, HBA and HY3 can be mapped only depending on the length of the polymethylene chain. Particularly, compounds characterized by $n=2$ are estimated to be weakly active, due to difficult in mapping the HY3 feature. As an example, affinity of compound **2** has been predicted 64 nM versus an experimental value of 37 nM, while the corresponding propyl derivative **3** has been predicted to have an affinity of 3.9 nM versus an experimental value of 16.5 nM). This finding is in agreement with the experimental data of phenyl derivatives. In fact, with the exception of **4** and **5**, among compounds bearing the phenylpiperazine moiety, the ethyl spacer-bearing derivatives are all less active than the corresponding propyl analogues. Alternative orientations of compounds bearing the ethylene chain are characterized by an enhanced mapping to HY3, with the piperazinyl ring undergoing a deep conformational rearrangement leading to a very strained structure of the ligand.

The lengthening of the ethyl chain to a propyl spacer led to several compounds characterized by a higher conformational flexibility. This structural variation is responsible for the enhanced fit to the pharmacophore with a consequent improvement of the calculated affinity values. On the contrary, in the pyridazinone and furoyl series, compounds bearing an ethyl spacer (**14** and **16**) where slightly more active than the corresponding propyl derivatives (**15** and **17**). Both pyridazinone and furoyl derivatives showed the same orientation within the model, with the benzodioxane moiety matching both HY1–HY2. In addition, compounds **14** and **15** are characterized by a PI group corresponding to the piperazine nitrogen bearing the alkyl chain, while the methyl group of the pyridazinone ring was the HY3 feature. In this orientation, both compounds were unable to fit HBA, while alternative mappings, characterized by the carbonyl moiety located into HBA and the methyl group in a very partial fit to HY3, led to predicted affinity values of 100 and 120 nM for **14** and **15**, respectively. On the other hand, the amino nitrogen of compounds **16** and **17** was the PI feature, while the piperazine nitrogen bearing the alkyl chain and the furane ring of **17** matched HBA and HY3, respectively. A different situation was found for **16** where a higher affinity was predicted as the consequence of fitting between the carbonyl group and HBA.

The preferential orientation of the pyridazinone and furoyl moieties of compounds **14**, **15** and **16**, **17** into the pharmacophore was similar to that previously found for pyridazinone derivatives used to build the pharmacophore itself[17] and for prazosin, respectively. Moreover, the ethyl spacer represents the optimum structural requirement (with respect to the propyl chain) to allow for a fit into both PI and HY3, according to experimental data showing for compounds **14** and **16** a slightly increased affinity with respect to **15** and **17**, respectively.

Finally, in accordance with experimental data, *p*-methoxy substituted compounds **6** and **7** have been predicted to have the highest activity values within the whole set, in agreement with some recent findings reporting para substituents as unfavourable to ligand-receptor binding.[6b,22] As an example, the predicted affinity of **11** was 790 nM, versus an experimental value of 718 nM.

From the above considerations, a summary of the interaction pathways between benzodioxane derivatives and the pharmacophore model for α_1-AR antagonists can be drawn as follows. (i) Phenyl or pyridine derivatives show, as a preferential orientation, the arylpiperazine moiety located within the HY1–HY2–HBA system, in agreement with other arylpiperazines used to build the model itself. On the contrary, furoyl- and pyridazinonepiperazine undergo a reversion of the orientation, having the benzodioxane moiety located within HY1–HY2. As a consequence, the basic, positive ionizable nitrogen, accessible to the receptor and easily protonable at physiological pH, can be alternately represented by the piperazine N4 nitrogen or the secondary amine nitrogen. (ii) The *o*-methoxy substituted phenyl ring of compounds **2** and **3** is associated with the best activity toward α_1-AR. A marked decrease in activity was found in the *o*-chloro, *o*-fluoro, unsubstituted or pyridine derivatives. As expected, the *p*-methoxy substituent led to a dramatic drop in affinity. (iii) A polar group corresponding to the carbonyl moiety of **14**–**17** and the secondary amino nitrogen in the remaining compounds, is the preferential hydrogen bond acceptor substituent. (iv) The polymethylene chain linking the arylpiperazine to the benzodioxane ring is an important structural feature in determining affinity of compounds **2**–**17**. In fact, as demonstrated by the pharmacophore fitting studies reported here, the alkyl moiety determines the distance at which both the benzodioxane and arylpiperazine rings are located, and, accordingly, the goodness-of-fit to the pockets defined by the HY1–HY2–PI and HBA–HY3 features, respectively. These results are in good agreement with the generally accepted statement that an α_1-AR antagonist interacts with the corresponding receptor by a three-pocket binding pathway: the most important structural element for blocking α_1-AR is a protonated nitrogen interacting with the carboxylate of an aspartic residue. The remaining molecular portions of the ligand fit two binding pockets almost symmetrically located with respect to the aspartate. (v) Finally, an additional pharmacophore element (HY3) accommodates (portions of) the benzodioxane, furoyl, and pyridazinone moieties of compounds **2**–**17**.

Conclusions

Several compounds belonging to a hybridized class of benzodioxane-arylpiperazine derivatives have been prepared by variation of the alkyl spacer linking the terminal benzodioxane and arylpiperazine rings and by modification of the aryl moiety bound to the piperazine. All these compounds have been tested for their α_1- and α_2-AR antagonist properties.

Some structural features have been demonstrated to markedly affect affinity for both α_1- and α_2-AR. In particular, the *o*-methoxyphenyl group bound to the piperazine ring led to the best α_1- affinity profile. In fact, derivatives **2** and **3** showed an interesting affinity towards α_1-AR. On the contrary, the remaining compounds, characterized by a different substitution on the piperazine ring, are all less active than the *o*-methoxyphenyl derivatives, with *p*-methoxyphenyl compounds showing the highest values in affinity (i.e., they are the less active compounds).

The alkyl spacer is also important in defining affinity, the propyl spacer being the optimum linker between the terminal heterocycles.

Benzodioxane-arylpiperazine derivatives **2**–**17** were also used to further test the predictive power of a pharmacophore model for α_1-AR antagonists. In particular, the model was able to predict accurately the receptor affinity of such compounds not used in the construction of the model itself. Moreover, this model justifies the importance of the main pharmacophore groups (particularly, the *o*-methoxyphenyl moiety and the basic nitrogen atom) as well as of their relative distance. In fact, while the substitution pattern on the phenyl ring considerably affect the affinity properties (with the *o*-methoxy substituent associated with the best activity), spatial considerations are particularly important for the phenyl ring on the piperazine moiety and the basic nitrogen atom, since their spatial locations are critical for the biological activity, as generally accepted for α_1-AR antagonists.

Considering that the model was able to rationalize the major structure–activity relationships for these compounds, and its statistical significance has been previously highlighted, we can conclude that this pharmacophore model may represent a powerful tool for subsequent three dimensional quantitative structure–activity relationships (3D-QSAR) studies on the antagonists of α_1-adrenoceptors.

Experimental

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. Chemical shift values (ppm) are relative to tetramethylsilane used as an internal standard. Elemental analyses are

within ±0.4% of theoretical values. Precoated Kiesegel 60 F_{254} plates (Merck) were used for TLC.

Method for the preparation of compounds 10–17. An example. *N*-{2-[4-(4-Methoxyphenyl)piperazin-1-yl]ethyl}-2-aminomethyl-1,4-benzodioxane (10). A mixture of 1-(2-chloroethyl)-4-(4-methoxyphenyl)piperazine (**19a**) (0.25 g, 0.001 mol), 2-aminomethyl-1,4-benzodioxane (**18**) (0.49 g, 0.003 mol) and sodium carbonate (0.10 g, 0.001 mol) in dry ethanol was refluxed under stirring for 6 h. The mixture was filtered and the organic phase was evaporated under reduced pressure. The residue was purified by chromatography on a silica gel column eluting with $EtOH/CH_2Cl_2$ (1:9) to give **1** (55%) as an oil; ^1H NMR ($CDCl_3$) δ 1.90 (s, 1H, NH), 2.45–2.60 (m, 6H, 4H-pip, CH_2), 2.70–2.90 (m, 4H, $2CH_2$), 3.00–3.10 (m, 4H, H-pip), 3.70 (s, 3H, OCH_3), 3.90–4.00 (m, 1H, H-benzodiox), 4.20–4.30 (m, 2H, H-benzodiox), 6.70–6.90 (m, 8H, H-arom). The corresponding hydrochloride had mp 140–145 °C. Anal. calcd for $C_{22}H_{29}N_3O_3 \cdot 3HCl$: C, 53.62; H, 6.50; N, 8.50. Found: C, 53.20; H, 6.90; N, 8.15.

Method for the preparation of compounds 19a–h. An example. 1-(2-Chloroethyl)4-(4-methoxyphenyl)piperazine (19a). This compound was prepared using the method of Bourdais.[14] A mixture of 1-(4-methoxyphenyl)piperazine (2 g, 0.01 mol), 1-bromo-2-chloroethane (1.79 g, 0.012 mol) and dry potassium carbonate (1.72 g, 0.012 mol) in DMF (10 mL) was stirred for 24 h at 25 °C. The mixture was diluted with water and extracted with CH_2Cl_2. The organic phase was dried with sodium sulfate and evaporated under reduced pressure. The residue was purified by chromatography on a silica gel column eluting with ethyl acetate to afford the title compound as an oil. Yield: 50%. ^1H NMR ($CDCl_3$) δ 2.60–2.70 (m, 4H, H-pip), 2.80 (t, $J = 7$ Hz, 2H, CH_2), 3.10–3.20 (m, 4H, H-pip), 3.65 (t, $J = 7$ Hz, 2H, CH_2), 3.80 (s, 3H, OCH_3), 6.80–7.00 (m, 4H, H-arom).

Biology

α_1-Receptor binding. Rat cerebral cortex was homogenized in 20 volumes ice-cold 50 mM Tris–HCl buffer at pH 7.7 containing 5 mM EDTA (buffer T_1) in an ultra-turrax homogenizer. The homogenate was centrifuged at 48,000*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 48,000*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 α_1 binding assay was performed in triplicate by incubating at 25 °C for 60 min in 1 mL T_2 buffer containing aliquots of the membrane fraction (0.2–0.3 mg protein) and 0.1 nM [^{3}H]-prazosin in the absence or presence of unlabelled 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 ice-cold Tris–buffer. The filters were placed in scintillation vials and 4 mL Ultima Gold MN Cocktail-Packard solvent scintillation fluid was added. The radioactivity was counted with an Packard 1600 TR scintillation counter. Specific binding was obtained by subtracting nonspecific binding from 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 48,000*g* for 15 min at 4 °C. The resulting pellet was diluted in 20 volumes of 50 mM Tris–HCl buffer at pH 7.7 and used in the binding assay.

Binding assay was perfomed in triplicate, by incubating aliquots of the membrane fraction (0.2–0.3 mg protein) in Tris–HCl buffer at pH 7.7 with approximately 2 nM [^{3}H]-rauwolscine in a final volume of 1 mL. Incubation was carried out at 25 °C for 60 min. Non-specific binding was defined in the presence of 10 μM rauwolscine. The binding reaction was concluded by filtration through Whatman GF/C glass fiber filters under reduced pressure. Filters were washed four times with 5 mL aliquots of ice-cold buffer and placed in scintillation vials. Specific binding was obtained by subtracting non specific 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 tested compound that produces 50% inhibition of specific [^{3}H]-prazosin or [^{3}H]-rauwolscine 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:[27] $K_i = IC_{50}/1 + ([L]/K_d)$ 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) and K_d of [^{3}H]-rauwolscine binding to cortex membranes was 4.0 nM (α_2).

Computational methods

All calculations and graphic manipulations were performed on a Silicon Graphics O2 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.[23] The parameter set employed to perform all the conformational calculations

derives from the CHARMm force field,[24] opportunely modified and corrected.[25]

The best quality conformational analysis approach has been selected because it provides the best possible conformational coverage within Catalyst.

Accordingly to some literature reports, conformational behavior of the arylpiperazine moiety shows the heteroring characterized by a N1–N4 di-equatorial chair. The structures of the conformers generated for compounds **14**–**17** showed a quasi-coplanar orientation of the piperazine ring with respect to the pyridazinone or furoyl moiety directly linked to it, in agreement with previous findings on piperazine derivatives.[22b] In addition, twisted or even orthogonal conformations of the piperazine C2–C3–C5–C6 system with respect to the phenyl ring of the arylpiperazinyl moiety of the ligands **2**–**13**, demonstrated to be highly profitable for α_1-AR antagonism, were also found.[6a]

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

For each Compare/Fit operation, the program 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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