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Synthesis, Biological Evaluation, and Docking Studies of Tetrahydrofuran- Cyclopentanone- and Cyclopentanol-Based Ligands Acting at Adrenergic α_1- and Serotonine 5-HT$_{1A}$ Receptors

Adolfo Prandi,[†] Silvia Franchini,[†] Leda Ivanova Manasieva,[†] Paola Fossa,[‡] Elena Cichero,[‡] Gabriella Marucci,[§] Michela Buccioni,[§] Antonio Cilia,[∥] Lorenza Pirona,[∥] and Livio Brasili*[,†]

[†]Dipartimento di Scienze Farmaceutiche, Università degli Studi di Modena e Reggio Emilia, Via Campi 183, 41125 Modena, Italy

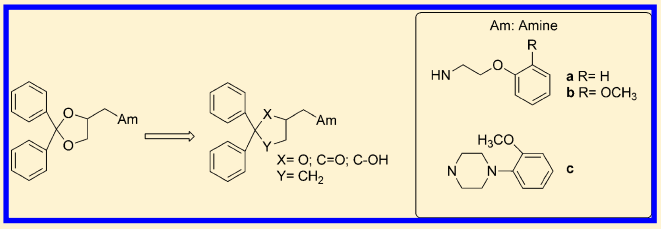
[‡]Dipartimento di Scienze Farmaceutiche, Università degli Studi di Genova, Viale Benedetto XV 3, 16132 Genova, Italy

[§]Dipartimento di Scienze Chimiche, Università degli Studi di Camerino, Via S. Agostino 1, 62032 Camerino, Italy

[∥]Divisione Ricerca e Sviluppo, Recordati SpA, Via Civitali 1, 20148 Milano, Italy

Supporting Information

ABSTRACT: A series of aralkylphenoxyethylamine and aralkylmethoxyphenylpiperazine compounds was synthesized and their in vitro pharmacological profile at both 5-HT$_{1A}$ receptors and α_1-adrenoceptor subtypes was measured by binding assay and functional studies. The results showed that the replacement of the 1,3-dioxolane ring by a tetrahydrofuran, cyclopentanone, or cyclopentanol moiety leads to an overall reduction of in vitro affinity at the α_1-adrenoceptor while both potency and efficacy were increased at the 5-HT$_{1A}$ receptor. A significant improvement of 5-HT$_{1A}$/α_1 selectivity was observed in some of the cyclopentanol derivatives synthesized (**4a** *cis*, **4c** *cis* and *trans*). Compounds **2a** and **4c** *cis* emerged as novel and interesting 5-HT$_{1A}$ receptor antagonist (pK_i = 8.70) and a 5-HT$_{1A}$ receptor partial agonist (pK_i = 9.25, pD_2 = 9.03, E_{max} = 47%, 5-HT$_{1A}$/α_{1a} = 69), respectively. Docking studies were performed at support of the biological data and to elucidate the molecular basis for 5-HT$_{1A}$ agonism/antagonism activity.



1. INTRODUCTION

Serotonin (5-HT) receptors have been studied for almost three decades and have provided researchers with many targets for drug action. To date, 14 receptor subtypes have been identified and grouped into seven subfamilies (5-HT$_1$ to 5-HT$_7$) on the basis of molecular cloning, amino acid sequence, pharmacology, and signal transduction data.

The 5-HT$_{1A}$ receptor is implicated in numerous behavioral and physiological functions including cognition, psychosis, feeding/satiety, temperature regulation, anxiety, depression, sleep, pain perception, and sexual activity and represents an attractive target for drug discovery. In particular, 5-HT$_{1A}$ agonists and partial agonists have shown to be effective in the treatment of anxiety, depression, and psychosis.[1−4] Recently, it was suggested that 5-HT$_{1A}$ agonists shown neuroprotective properties.[5] Moreover, it was observed in animal models that 5-HT$_{1A}$ receptor agonists may rival the opioids in pain relief therapy.[6] More recently, 5-HT$_{1A}$ agonists may be useful as levodopa adjuvants in the treatment of Parkinson's disease.[7,8] The potential therapeutic applications of 5-HT$_{1A}$ receptor antagonists have been evaluated, for example, in cognition disorders such as the cognitive impairment associated with Alzheimer's disease.[9] Several studies have shown that 5-HT$_{1A}$ autoreceptor antagonists can be used in coadministration with SSRIs (selective serotonin reuptake inhibitors) to potentiate the antidepressant effects.[10]

Despite the fact that 5-HT$_{1A}$ receptors differ significantly from the other 5-HT receptor subtypes, their trans-membrane amino acid sequence shows substantial similarity with the α_1-adrenoceptors (approximately 45% identity).[11] As a consequence, a number of 5-HT$_{1A}$ ligands also bind to adrenergic receptors with high affinity and poor selectivity.

Several structurally different compounds are known to bind 5-HT$_{1A}$ receptors. Among these, a large number of *N*4-substituted *N*1-arylpiperazines and phenoxyethylamines have been extensively studied.[12−15] In Chart 1, some examples are reported.

In recent years, our efforts in the search for new and selective 5-HT$_{1A}$ receptor ligands have led to the discovery of a new class of 1,3-dioxolane-based arylpiperazines[14] and 1,3-dioxolane-based phenoxyethylamine.[15] In particular, compounds **1a**, **1b**, and **1c** (Chart 2), due to their good affinity for 5-HT$_{1A}$ receptors, were considered as lead compounds for further optimization. In functional studies at the 5-HT$_{1A}$ receptors, they behaved as partial agonists with pD_2 values ranging between 7.3 and 8.8. In addition, they displayed also high

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Chart 1. Some Representative Ligands for 5-HT$_{1A}$ Receptors

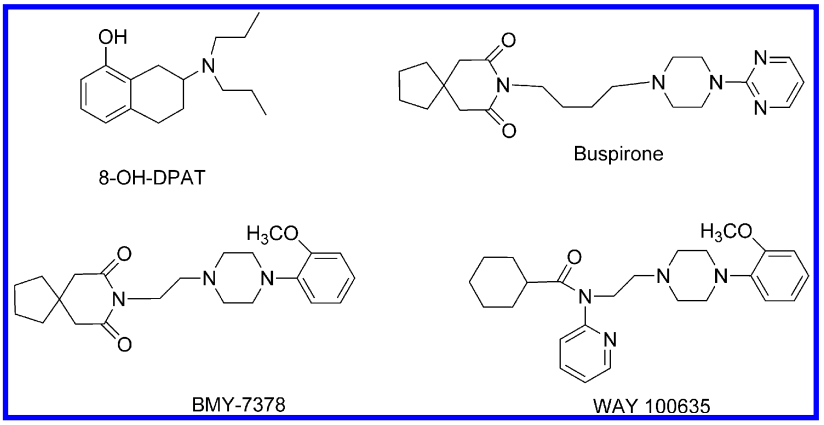
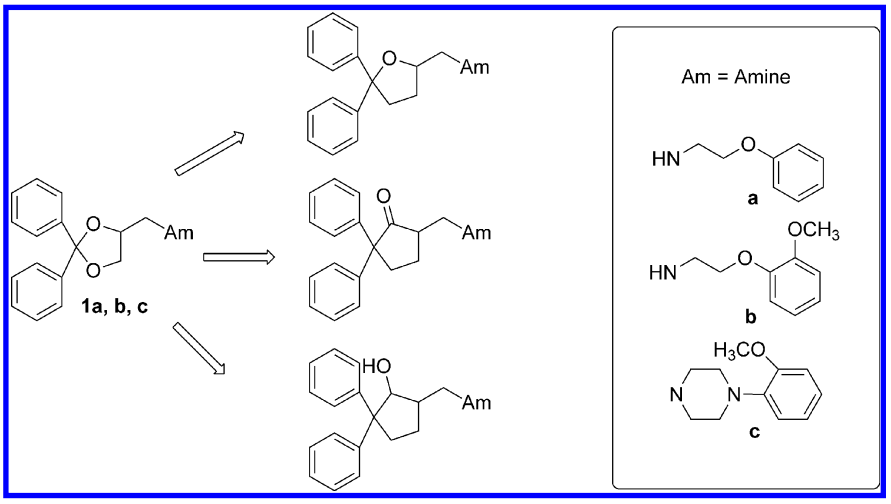
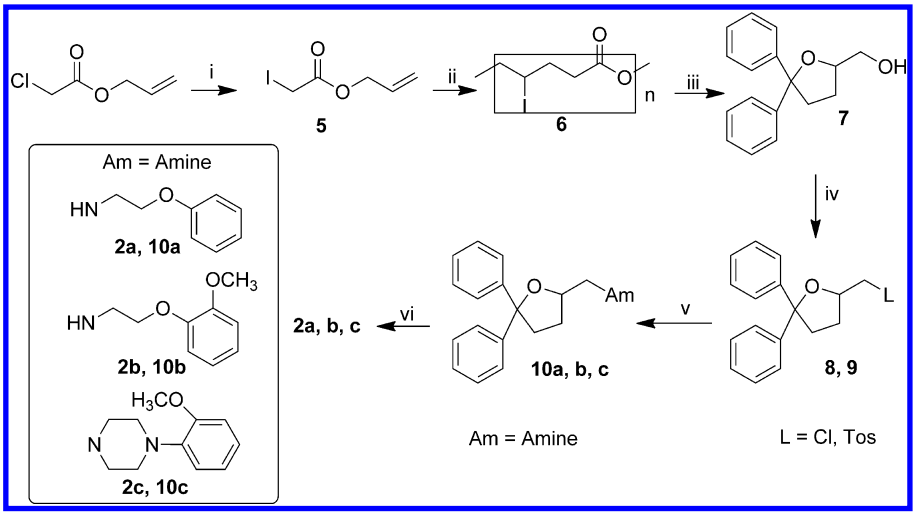


Chart 2. Structural Modifications of the 1,3-Dioxolane-Based Lead Compounds 1a, 1b, and 1c



Scheme 1. [a]



[a]Reagents and conditions: (i) KI, acetone, 3.5 h. (ii) Et_3B, CH_2Cl_2, rt, 3 h. (iii) PhMgBr 3M in Et_2O, THF, rt, 2 h. (iv) For **8**: pTsCl, C_5H_5N, CH_2Cl_2, rt, 24 h. For **9**: $SOCl_2$, C_5H_5N, PhMe, 1.5 h. (v) Appropriate amine (Am), KI (for **9**), 2-methoxyethanol, reflux, 18−24 h. (vi) $C_2H_2O_4$, Et_2O.

affinity for α_1-adrenoceptor subtypes, resulting in a poor receptor selectivity.

With the aim of further improving serotoninegic potency, efficacy, and especially selectivity and better understand structural requirements for 5-HT$_{1A}$ binding and activation, we aimed to generate structural variants of the lead structures, focusing our attention on the 1,3-dioxolane moiety, replacing it with other five-membered rings bearing a H-bond acceptor and

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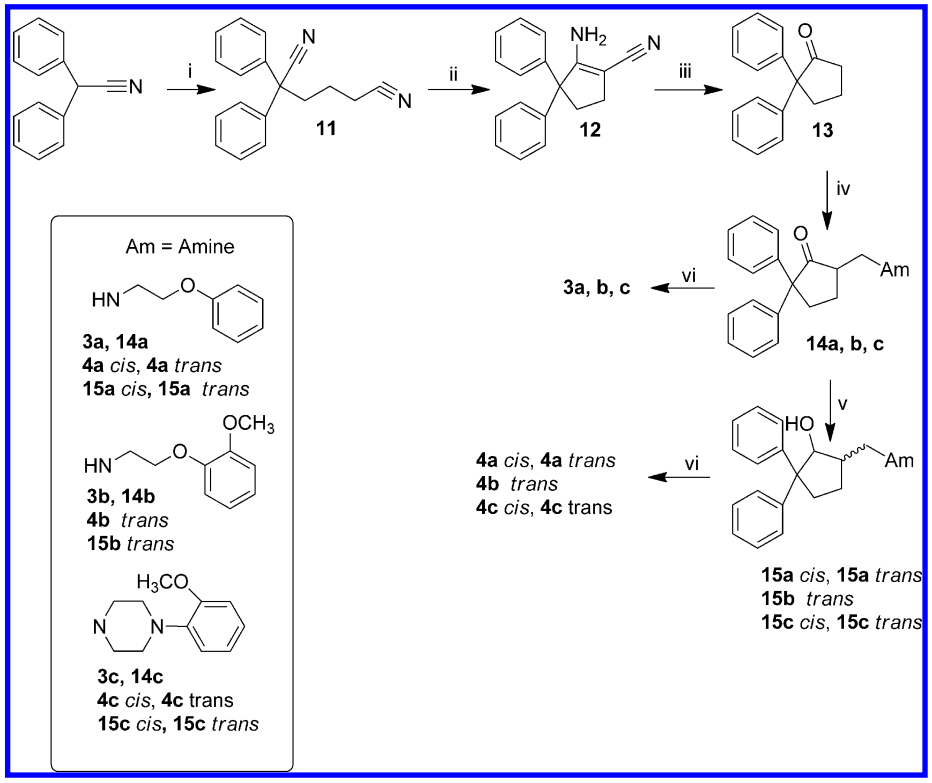
Scheme 2[a]



[a]Reagents and conditions: (i) $NaNH_2$, PhMe, reflux, 4 h; 4-chlorobutyronitrile, reflux, 12 h; (ii) $NaNH_2$, PhMe, reflux, 12 h; (iii) H_2SO_4 conc, H_2O, rt, 24 h; (iv) appropriate amine (Am·HCl), paraformaldehyde, C_2H_5OH, reflux, 25 h; (v) $NaBH_4$, C_2H_5OH, rt, 23 h; (vi) $C_2H_2O_4$, Et_2O.

donor group (cyclopentanol) or H-bond acceptor groups (tetrahydrofuran or cyclopentanone) (Chart 2). This study allowed us to discover a new series of potent and selective 5-HT_{1A} receptor agonists. Moreover it was found that some structural modification are able to direct the activity toward antagonist activity.

To rationalize the pharmacological results and support and guide the chemical exploration, in silico docking studies on compounds **2**−**4a,b,c** were performed on a theoretical 5-HT_{1A} three-dimensional model previously set up in-house[16] to simulate the interaction of the synthesized compounds with the receptor binding sites.

As reported,[16] our model was built using the X-ray coordinates of the human β2 adrenoreceptor in complex with a partial inverse agonist, carazolol (pdb code: 2RH1).[17] Notably, X-ray coordinates of the human β2 adrenoreceptor in complex with an irreversible agonist has recently become available (pdb code: 3PDS).[18]

Thus, to verify the reliability of the obtained 5-HT_{1A}/agonist and 5-HT_{1A}/antagonist models, the key interactions with the $5HT_{1A}$ agonist or antagonist were compared with those present in the human β2 adrenoreceptor 3PDS and 2RH1 complexes, respectively. The final results, being in agreement with the experimental data, allowed us to validate the model built as a useful tool for virtual screening procedures and for development of new tetrahydrofuran-, cyclopentanone-, and cyclopentanol-based derivatives, potentially active on the 5-HT_{1A} receptor.

2. RESULTS AND DISCUSSION

2.1. Chemistry. Phenoxyethylamine and [(2-methoxy)-phenoxy]ethylamine were produced by reacting chloroacetamide with the appropriate phenol and then reducing with diborane as previously described.[19] 2-Methoxyphenylpiperazine was purchased from Sigma.

Substituted tetrahydrofurans **2a,b,c** were synthesized as outlined in Scheme 1. The key intermediate **7** was obtained through the reaction of the radical oligomer of allyl iodoacetate with Grignard's reagent as previously described.[20] In brief, the reaction of allyl iodoacetate (**5**) in CH_2Cl_2 at room temperature (rt) with Et_3B (0.1 equiv)/O_2 (trace) as the initiator gave the radical oligomeric mixture **6**. The reaction of **6** with phenylmagnesium bromide in THF at rt led to the formation of the corresponding tetrahydrofuran derivative **7**, from which either the chloro derivative **8** or the tosyl derivative **9** were obtained. The intermediates **8** and **9** were then transformed into the corresponding substituted amine derivatives **10a,b** and **10c**, respectively.

Substituted cyclopentanones (**3a,b,c**) and cyclopentanols (**4a,b,c**) were prepared as outlined in Scheme 2.

2,2-Diphenylcyclopentanone **13** was synthesized by alkylation of diphenylacetonitrile with a 4-chlorobutyronitrile, followed by base-catalyzed Thorpe−Ziegler cyclization to afford the enaminonitrile **12**. Acid hydrolysis (H_2SO_4) of the enaminonitrile gave the desired ketone **13** in good yields.[21] A Mannich reaction between cyclopentanone (**13**) and the appropriate amines as hydrochloride salt and aqueous paraformaldehyde gave the desired compounds **14a,b,c**.

The cyclopentanol derivatives **15a,b,c** were obtained by simple reduction of the carbonyl group of **14a,b,c** in the presence of $NaBH_4$. The reduction of the carbonyl group to alcohol leads to the introduction of a second chiral center with the formation of a diastereomeric pair *cis*/*trans*, except for **14b**, for which only the *trans* form was obtained. The mixture of the

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two isomers was separated by flash chromatography at this stage, and their relative stereochemistry was elucidated by ^{1}H NMR. All the free amines were dissolved in diethyl ether and transformed into the corresponding oxalate salts which, after filtration, were crystallized from methanol.

2.2. Biological Activity. The pharmacological profile of the compounds synthesized **2**−**4a**,**b**,**c** and BMY-7378, 8-OH-DPAT, and WAY100635 as reference compounds, was evaluated by radioligand binding assay using [^{3}H]prazosin to label cloned human α_1-adrenoceptors, expressed in CHO cells, and [^{3}H]8-OH-DPAT to label cloned human 5-HT$_{1A}$ receptors, expressed in HeLa cells.[22] Selectivity ratios 5-HT$_{1A}$/α_1 were calculated using the highest affinity at α_1 receptors regardless the subtype.

From a preliminary analysis of the results obtained, summarized in Table 1, for the α_1-adrenoceptor subtypes, a general reduction in affinity was observed compared to the lead compounds **1a**, **1b**, and **1c**. Conversely, a general increase of in vitro potency at the 5-HT$_{1A}$ receptor was observed for the 2-methoxyphenylpiperazine derivatives, enhancing 5-HT$_{1A}$/α_{1a} receptor selectivity, with an inversion of selectivity ratio which goes from 0.01 for lead **1c** to 11 for **3c** and 69 and 56 for **4c** *cis* and **4c** *trans*, respectively.

Table 1. pK_i[a] and in Vitro Selectivity[b] Values of Compounds 1−4a,b,c and BMY-7378, 8-OH-DPAT, and WAY100635 in Human Recombinant α_1 and 5-HT$_{1A}$ Receptors

compd	p$K_i\alpha_{1a}$	p$K_i\alpha_{1b}$	p$K_i\alpha_{1d}$	pK_i5HT$_{1A}$	5HT$_{1A}$/α_1[c]
1a	7.43	7.20	7.94	8.45	3
1b	7.71	7.33	8.03	9.22	15
1c	9.58	8.17	9.09	7.64	0.01
2a	7.27	6.91	7.50	8.70	16
2b	7.57	7.40	8.20	9.08	8
2c	9.19	8.05	8.41	8.36	0.1
3a	<6	<6	6.41	7.98	37
3b	<6	<6	6.85	8.46	41
3c	7.40	6.85	6.81	8.46	11
4a *cis*	<6	<6	<6	8.03	>107
4a *trans*	<6	6.54	7.03	8.02	10
4b *trans*	6.96	7.13	8.24	9.49	18
4c *cis*	7.41	6.78	7.17	9.25	69
4c *trans*	7.35	6.61	7.17	9.10	56
BMY-7378	6.42	6.15	8.89	8.90	1
8-OH DPAT	6.82	<6	<6	8.43	40
WAY100635	7.73	7.18	8.34	9.48	14

[a] K_i values were calculated at one or two concentrations and agreed within 10%. [b] Antilog of the difference between the pK_i values for α_1-adrenoreceptors and 5-HT$_{1A}$ receptor. [c] Selectivity ratios 5HT$_{1A}$/α_1 were calculated using the highest affinity at α_1 receptors regardless the subtype.

Functional activity of the α_1-adrenoceptors was further evaluated on a variety of isolated tissues, using BMY-7378, as reference compound. Blocking activity was assessed by antagonism of (−)-noradrenaline-induced contraction of rat prostatic vas deferens (α_{1A}),[23] thoracic aorta (α_{1D}),[24] and by antagonism of (−)-phenylephrine-induced contraction of rat spleen (α_{1B}).[25] The antagonist potencies to α_1-adrenoceptor subtypes are summarized in Table 2. With regard to the α_{1A}- and α_{1D}-subtypes, with few exceptions, the trend of activities parallels the affinities found of binding studies, as the potencies are lower than those of the lead compounds **1a**, **1b**, and **1c**.

Table 2. Antagonist Potency, Expressed as pK_b ± SEM Values[a] and Selectivities[b] of 1−4a,b,c and BMY-7378 at α_1 Adrenoceptors in Isolated Prostatic Vas Deferens (α_{1A}), Spleen (α_{1B}), and Thoracic Aorta (α_{1D}) of Rats

compd	p$K_b\alpha_{1A}$	p$K_b\alpha_{1B}$	p$K_b\alpha_{1D}$	α_{1D}/α_{1A}	α_{1D}/α_{1B}	α_{1A}/α_{1B}
1a	6.16	5.86	8.37	162	323	2
1b	7.53	7.36	8.65	13	20	1.5
1c	8.24	6.69	8.14	0.8	28	35
2a	6.26	6.20	7.21	9	10	1
2b	7.24	6.92	8.09	7	15	2
2c	7.65	6.85	7.50	0.7	4	6
3a	<5	6.95	6.30	>20	0.2	>0.01
3b	6.39	6.92	6.54	1.4	0.4	0.3
3c	6.28	7.54	6.43	1.4	0.08	0.05
4a *cis*	5.23	6.26	6.88	45	4	0.09
4a *trans*	5.43	6.67	6.86	1.4	1.5	0.06
4b *trans*	6.57	7.70	8.42	71	5	0.07
4c *cis*	5.62	7.25	7.21	39	0.9	0.02
4c *trans*	6.39	7.14	6.82	3	0.5	0.2
BMY-7378	7.01	7.48	8.40	25	8	0.3

[a] pK_b values were calculated according to van Rossum[36] at one or two concentrations and agreed within 2%. [b] Antilog of the difference between the pK_b values for α_{1A}, α_{1B}, and α_{1D} adrenoreceptors.

Surprisingly, with regard to the α_{1B} subtype an enhancement in antagonistic potency is observed with respect to the lead compounds **1a**, **1b**, and **1c**. Interesting for future design of α_{1B}-antagonist is the 10-fold increase observed with compound **3a** (with respect to compound **1a**) and the 10-fold α_{1B}/α_{1A} and α_{1B}/α_{1D} selectivity in the case of compound **3c**.

The functional characterization of 5-HT$_{1A}$ receptors was also performed according to the methods of Stanton and Beer,[26] using [^{35}S]GTPγS binding, in cell membranes from HeLa cells transfected with human cloned 5-HT$_{1A}$ receptor. Stimulation of [^{35}S]GTPγS binding was expressed as the percent increase in binding above the basal value, considering that the maximal stimulation observed is with 8-OH-DPAT and was thus taken as 100%.

The results, as shown in Table 3, indicate that the majority of compounds are active in stimulating 5-HT$_{1A}$ receptors with high potency values and a relative efficacy of about 50%. Interestingly, three compounds (**2a**, **2c**, **3c**) behave as neutral antagonists with pK_i values ranging from 8.3 to 8.7. Given the well-known difficulty in discovering new antagonists of the 5-HT$_{1A}$ receptor, this result is important, as antagonistic activity does not appear to be conferred to a given compound by a distinct structural element (or elements).

*2.2.1. Phenoxyethylamine Derivatives (**2a**, **3a**, **4a** cis and trans).* The isosteric replacement of the oxygen atom at position 3 on the 1,3-dioxolane ring of the lead **1a** with a methylene group to obtain tetrahydrofuran derivative **2a** suggests that the presence of the second oxygen atom is not essential for 5-HT$_{1A}$ and α_1 adrenergic affinity. In fact, compound **2a** shows slightly higher affinity for 5-HT$_{1A}$, together with a little decrease in affinity for α_1. This results in a little improvement in 5-HT$_{1A}$/α_{1D} selectivity. Interestingly, in functional assays for 5-HT$_{1A}$ receptors, compound **2a** behaved as a neutral antagonist with high affinity (pK_i = 8.70) (Table 3).

The substitution of the ether function of **2a** with a carbonyl group to obtain cyclopentanone derivative **3a** decreased both α_1 and 5-HT$_{1A}$ binding affinity. This reduction is more

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Table 3. Agonist Potency (pD_2) and Relative Effectiveness (E_{max})[a] in the Agonist-Induced [^{35}S]GTPγS Binding Assay at Human 5-HT_{1A} Receptors

compd	pD_2	$\%E_{max}$
1a	8.8	24.4
1b	7.36	31.6
1c	8.3	16.2
2a	na[e] (pK_i = 8.70)	
2b	9.04	53.4
2c	na (pK_i = 8.36)	
3a	7.44	25.5
3b	8.37	39.4
3c	na (pK_i = 8.46)	
4a *cis*	7.81	46.5
4a *trans*	7.94	64.9
4b *trans*	8.96	64.1
4c *cis*	9.03	47.1
4c *trans*	8.74	37.3
BMY-7378	9.27	26
8-OH DPAT	7.83	100
WAY100635	na (pK_i = 9.48)	

[a]Maximal stimulation expressed as a percentage of the maximal 8-OH-DPAT response. [e]na: neutral antagonist.

significant for the adrenergic system for which a little increase in selectivity (5-HT_{1A}/α_{1D} = 37) is observed. Moreover, this modification restores the partial agonistic behavior, accompanied, however, by a potency 25-fold lower than that of the lead **1a**.

The introduction of an H-bond acceptor and donor hydroxyl group, as in the cyclopentanol derivatives **4a** *cis* and *trans*, instead of an H-bond acceptor ether or carbonyl group, present in compounds **2a** and **3a**, dramatically decreased α_1 adrenergic affinity, leaving 5-HT_{1A} binding affinity almost unchanged. In particular, compound **4a** *cis* showed a 35-fold increase in 5-HT_{1A}/α_{1D} selectivity compared to lead **1a**, and a 10-fold reduction of activity as agonist.

*2.2.2. 2-Methoxyphenoxyethylamine Derivatives (**2b**, **3b**, **4b** trans).* In the 2-methoxyphenoxyethylamine series, the above-mentioned isosteric substitutions (oxygen/methylene/carbonyl/hydroxyl) led to compounds characterized by an increased agonistic potency for 5-HT_{1A} receptors compared to the lead **1b**. In particular, the tetrahydrofuran derivative **2b** was 50-fold more potent than the lead **1b**, followed by the cyclopentanol derivative **4b** *trans* (40-fold) and the cyclopentanone derivative **3b** (10-fold). All compounds also show an increase in relative efficacy ($\%E_{max}$). With the exception of derivative **4b** *trans*, the increased agonistic potency was accompanied by a slight decrease in affinity for 5-HT_{1A} compared to lead **1b**. Only cyclopentanone derivative **3b** has a satisfactory value of 5-HT_{1A}/α_{1d} selectivity (41) due to a decrease in affinity for all three α_1 adrenoceptor subtypes. The most interesting compound of this series was the cyclopentanol derivative **4b** *trans*: the presence of an H-bond acceptor and donor group increased the affinity for $5HT_{1A}$, agonistic potency, and efficacy (pK_i = 9.49, pD_2 = 8.96, $\%E_{max}$ = 64).

By comparing cyclopentanone **3a** and cyclopentanoles **4a** *cis* and *trans* bearing a phenoxyethylamine lateral chain, with the same rings, bearing a 2-methoxyphenoxyethylamine chain, **3b** and **4b** *trans* respectively, it is possible to highlight the fact that the presence of a methoxy group improved 5-HT_{1A} affinity and agonistic potency by about 10-fold. Modeling studies confirm that the presence of a methoxy group stabilizes the interaction with the receptor's Thr121 binding pocket by H-bond formation.

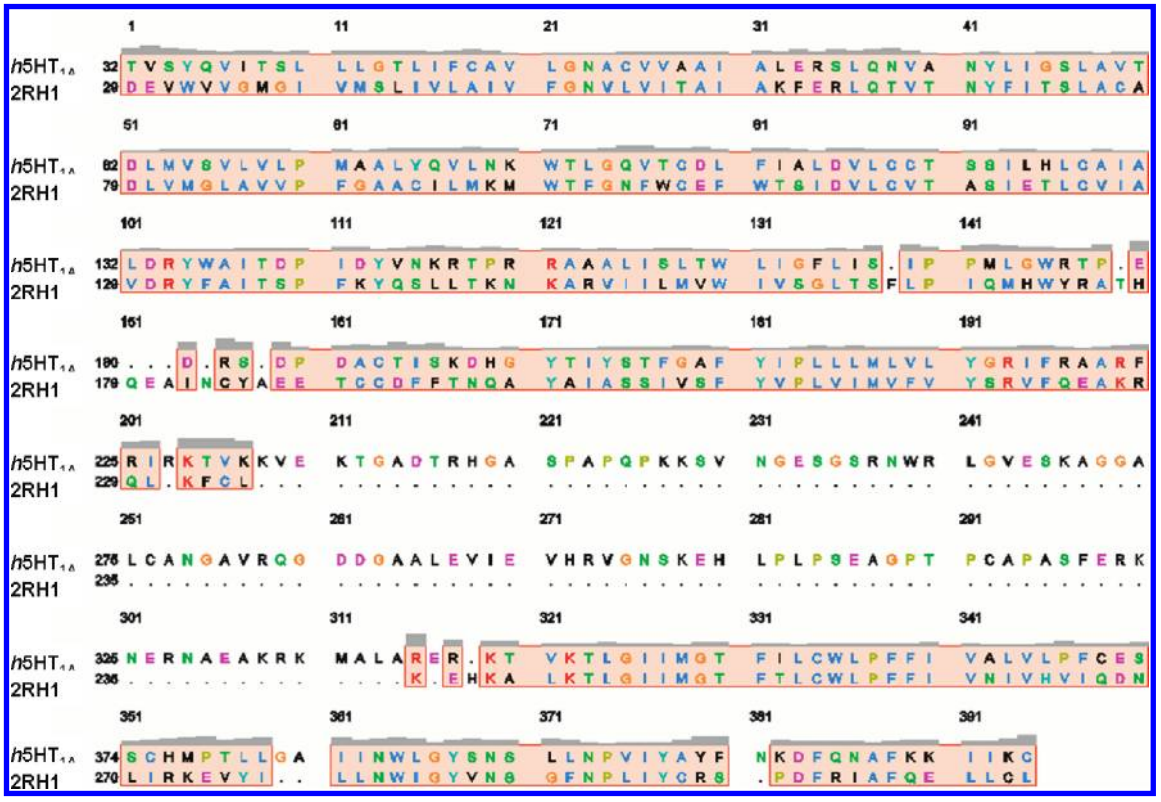

Figure 1. Sequence alignment of the $5HT_{1A}$ receptor on the basis of the human β2 adrenoreceptor (2RH1) coordinates.

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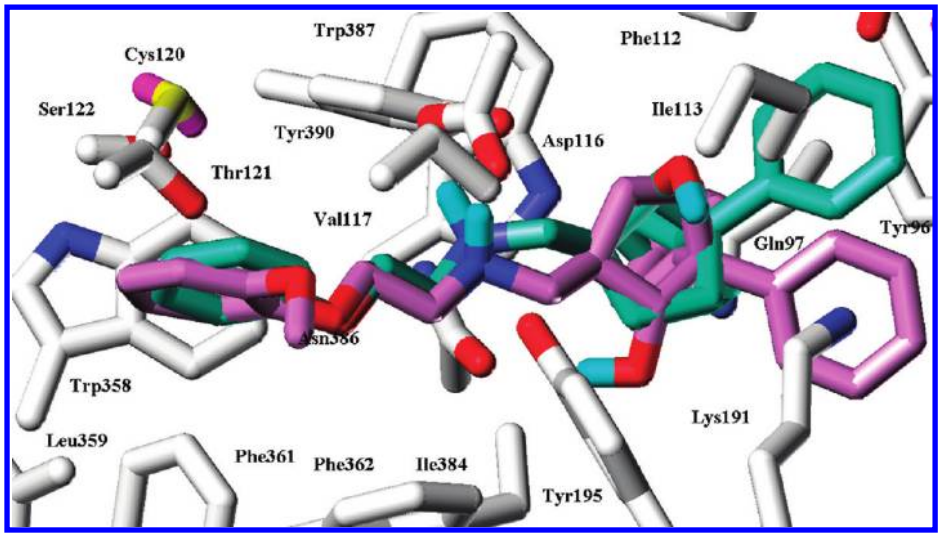

Figure 2. Selected docking pose of the $5HT_{1A}$ agonists **4b** *trans* (the *RR* isomer, depicted in violet) and **4a** *cis* (the *RS* isomer, depicted in green) into the $5HT_{1A}$ putative binding site. For simplicity, only the most important protein residues are depicted and labeled.

*2.2.3. 2-Methoxyphenylpiperazine Derivatives (**2c**, **3c**, **4c** cis and trans).* The biological results revealed that the structural modifications on the 1,3-dioxolane core influenced either 5-HT_{1A} functional activity or 5-HT_{1A}/α_{1a} selectivity.

By comparing tetrahydrofuran **2c** and cyclopentanone **3a**, it can be observed that the introduction of a ring bearing an H-bond acceptor group together with the presence of the piperazine moiety made both compounds 5-HT_{1A} antagonists with similar affinity to one another (pK_i = 8.36 and 8.46, respectively). Modeling studies confirmed that both compounds interact with receptor's Lys191 binding pocket through H-bond formation. Unfortunately, compound **2c** showed higher affinity to the α_{1a} adrenergic subtypes than to the 5-HT_{1A} serotoninergic receptor. In contrast, compound **3c** showed a switch in selectivity profile toward the 5-HT_{1A} receptor due to the dramatic decrease in α_1 adrenergic affinity.

The replacement of 1,3-dioxolane ring with an H-bond donor ring to give cyclopentanol derivatives **4c** *cis* and *trans* increased 5-HT_{1A} affinity of about 30−40-fold as well as agonist potency and efficacy. Both diastereomers binds preferentially to 5-HT_{1A} receptors showing an inverted selectivity profile than the lead **1c**. In particular, compound **4c** *trans* emerges for its good selectivity (5-HT_{1A}/α_{1a} = 69).

By examining each couple of diastereomeric pairs (**4a** *cis* and *trans* and **4c** *cis* and *trans*), it was evident that stereochemistry does not play a significant role at 5-HT_{1A} receptor. In both cases, *cis* and *trans* isomers were equiactive. Even at α_1 receptor subtypes, the two showed similar affinities to one another.

2.3. Modeling Studies. The $5HT_{1A}$ model previously built by us[16] has been used for docking simulations of all the compound isomers. As shown in Figure 1, the protein model was derived by the alignment of the $5HT_{1A}$ sequence on the X-ray coordinates of human β2 adrenoreceptor (2RH1). The reliability of the alignment was verified by the high value of the pairwise percentage residue identity (PPRI = 36%).

Accordingly, a consistent number of 5-HT_{1A} residues resulted to be conserved between the two GPCRs: (i) Val37, Val51, Gly53, Asn54, Val57, Ala60, Ile61, and Ala62 residues in TM1, (ii) Val70, Asn72, Tyr73, Ile75, Ser77, Leu78, Ala79, Asp82, Leu83, Val89, Pro91, and Ala94 in TM2, (iii) Cys109, Asp116, Val117, Leu118, Cys119, Thr121, Ser123, Ile124, Leu127, Cys128, Ile130, Ala131, Asp133, Arg134, Tyr135 (the DRY motif; 133−135 residues), Ala137, Ile138, and Thr139 in TM3, (iv) the Ala153, Ile157, Trp161, Leu166, and Ser168 residues in TM4, (v) Tyr195, Ile197, Ser199, Phe204, Tyr205, Phe207, Leu208, Met211, Thr215, Arg217, Phe219, and Ala222 in TM5, (vi) Glu340, Lys342, Lys345, Thr346, Leu347, Gly348, Ile349, Ile350, Met351, Gly352, Thr353, Phe354, Leu356, Cys357, Trp358, Leu359, Pro360 (the CWXP motif; 357−360 residues), Phe361, Phe362, Ile363, Val364, and Val367 in TM6, and (vii) Asn386, Trp387, Gly389, Tyr390, Asn392, Ser393, Asn396, Pro397, Ile399, and Tyr400 (the NPXIY sequence; 396−400 residues) in TM7.

The 5-HT_{1A} putative binding site, determined as described in the Experimental Section, was used for docking simulations of all the data set compounds.

2.3.1. Cyclopentanol Derivative Binding Mode. According to our calculations, compounds bearing a flexible (**4b** *trans* and **4a** *cis*) or a rigid (**4c** *cis*) linker located between the diphenylsubstituted cyclopentanol core and the phenyl ring share the same binding mode. More specifically, the *cis* isomer **4c** *cis* and the *trans* isomer **4c** *trans* displayed docking poses comparable with those of the *trans* and *cis* ones, respectively, **4b** *trans* (or **4a** *cis*).

As shown in Figure 2, the *trans* isomer **4b** *trans* (depicted in violet, the *RR* isomer was revealed to be the most probable) showed H-bond interactions between the linker amino group hydrogen atom and the key residue Asp116, the cyclopentanoyl hydroxyl moiety and the Asn386 side chain carbonyl group, and also between the 2-methoxyphenyl ring and the Thr121 side chain. In addition, the nitrogen atom located in the linker could be involved in H-bond contacts with the Tyr390 hydroxyl group. van der Waals contacts are displayed between: (i) the two phenyl ring linked to the cyclopentanol core and Tyr96, Val98, Ile113, and Tyr195, (ii) the cyclopentanol core and Val117, Tyr195, and Trp387, (iii) the 2-methoxyphenyl and Cys120, Ala203, Trp358, Phe361, Phe362, and Tyr390. The two phenyl ring and the 2-methoxyphenyl moiety were also involved in $\pi-\pi$ stacking with Trp358, Phe361, Phe362, and Tyr390, respectively. One of two phenyl rings linked to the cyclopentanol core was also engaged in cation−π interactions with the ε-amino group of Lys191.

The *cis* isomer **4a** *cis* (depicted in green, the *RS* isomer was shown to be the most probable) shows H-bond interactions

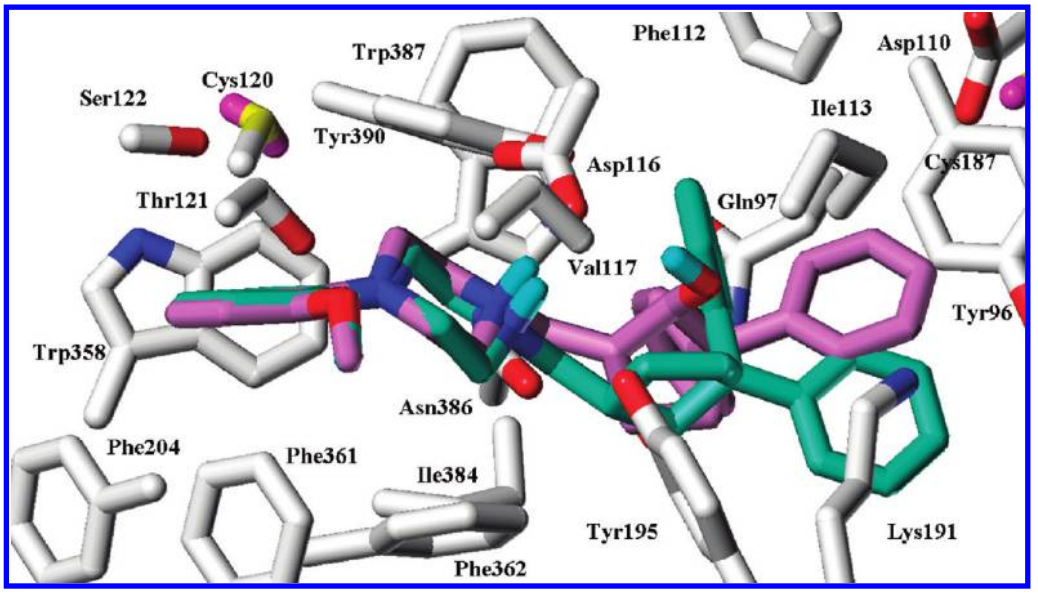

Figure 3. Selected docking pose of **4c** *cis* (the *SR* isomer, depicted in green) and **4c** *trans* (the *RR* isomer, depicted in violet) isomers into the $5HT_{1A}$ putative binding site. For simplicity, only the most important protein residues are depicted and labeled.

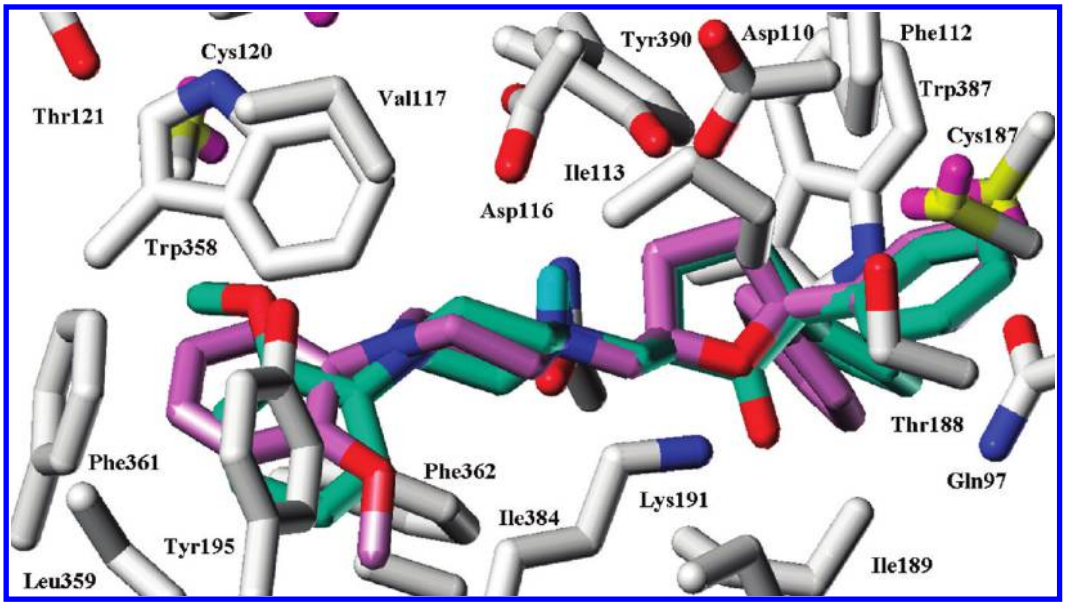

Figure 4. Selected docking pose of the $5HT_{1A}$ antagonists **2c** (the *R* isomer, depicted in violet) and **3c** (the *R* isomer, depicted in green) into the $5HT_{1A}$ putative binding site. For simplicity, only the most important protein residues are depicted and labeled.

between the linker amino group hydrogen atom and the cyclopentanoyl hydroxyl moiety and the Asp116 side chain. Thus, no H-bond contacts with the Asn386 side chain carbonyl group could be detected. As observed for the corresponding *trans* isomer, the nitrogen atom on the linker could be involved in H-bond contacts with the Tyr390 hydroxyl group, while van der Waals contacts were displayed between: (i) the two phenyl ring linked to the cyclopentanol core and Tyr96, Val98, Ile113, Ile189, and Tyr195, (ii) the cyclopentanol core and Val117, Tyr195, and Trp387, (iii) the phenoxy ring and Cys120, Ala203, Trp358, Phe361, and Tyr390. The two phenyl ring and the phenoxy moiety are also involved in $\pi-\pi$ stacking with Tyr96, Tyr195 and with Trp358, Phe361, and Tyr390, respectively.

The *cis* isomer **4c** *cis* (see Figure 3, depicted in green; the *SR* isomer was shown to be the most probable) and the *trans* isomer **4c** *trans* (see Figure 2, depicted in violet; the *RR* isomer was shown to be the most probable) displayed the same hydrophilic and hydrophobic interactions described above for the *trans* isomer **4b** *trans* and the *cis* isomer **4a** *cis*, respectively, except for the establishment of H-bond interactions with Tyr390. This hypothesis seems reasonable because the piperazine nitrogen atom is protonated and, thus, does not display the same interactions observed for the **4a** *cis* nitrogen atom. Furthermore, the compound **4c** *trans* hydroxyl moiety was engaged in H-bond contacts with the residue Asn386 carbonyl side chain.

Because all these compounds display $5\text{-}HT_{1A}$ agonistic behavior, our calculations highlight a selection of residues as being involved in the agonistic binding

2.3.2. Tetrahydrofuran Derivative Binding Mode. As shown in Figure 4 (for simplicity, only the compound **2c** docking pose

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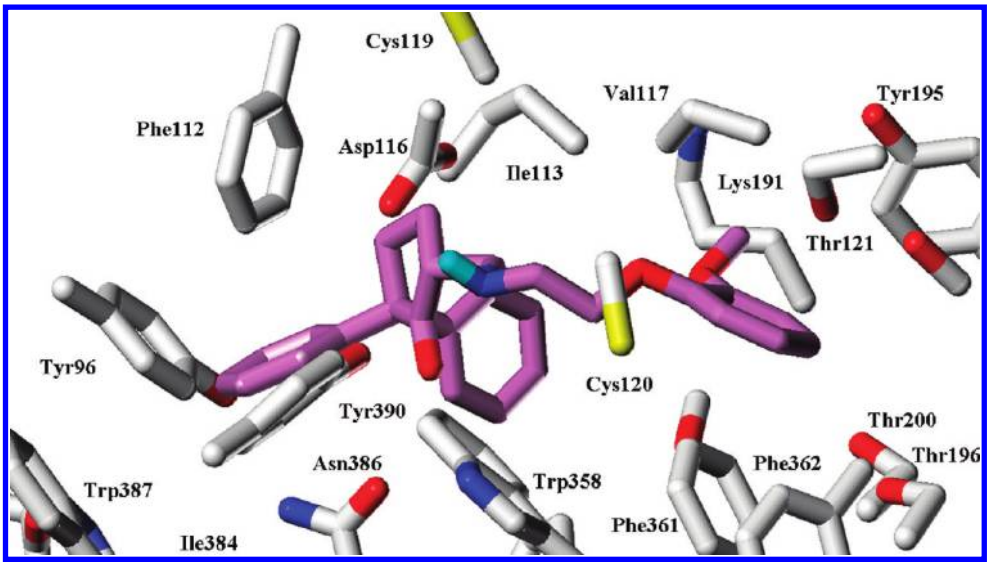

Figure 5. Selected docking pose of **3b** *R* isomer (depicted in violet) into the $5HT_{1A}$ putative binding site. For simplicity, only the most important protein residues are depicted and labeled.

has been selected for depiction, colored in violet), compounds **2a** and **2c** (the *R* isomers were shown to be the most probable) display the following common H-bond interactions between: (i) the tetrahydrofuran oxygen atom and the Lys191 ε-amino group and (ii) the linker amino group hydrogen atom (or protonated piperazine nitrogen atom) and the key residue Asp116. van der Waals contacts were displayed between the two phenyl rings linked to the tetrahydrofuran core and Ala93, Tyr96, Ile113, Thr188, Ile189, Lys191, and between the phenoxy (or 2-methoxyphenyl) moiety and Val117, Cys120, and Tyr195. The two phenyl rings and the phenoxy (or 2-methoxyphenyl) group were also involved in $\pi-\pi$ stacking with Tyr96 and with Tyr195, respectively. In addition, one of the two phenyl rings linked to the tetrahydrofuran core was also engaged in cation$-\pi$ interactions with the ε-amino group of Lys191.

Notably, both compounds display $5\text{-}HT_{1A}$ antagonistic behavior, lacking H-bond interactions with residues Asn386 and Tyr390 (as previously described for the $5\text{-}HT_{1A}$ agonists **4c** *cis*, **4c** *trans*, and **4a** *cis*). In contrast, the introduction of the flexible linker aminoethyl group (between the tetrahydrofuran core and the phenoxy moiety) and of a methoxy group at the phenoxy ring *ortho* position, makes compound **2b** a $5\text{-}HT_{1A}$ agonist. Accordingly, it seemed to display H-bond interactions between the methoxy group and the linker nitrogen atom with residues Thr121 and Asn386, respectively.

2.3.3. Cyclopentanone Derivative Binding Mode. Compound **3c** (the *R* isomer was shown to be the most probable) displays a binding mode comparable with that of compound **2c**, the *R* isomer, and shows $5\text{-}HT_{1A}$ antagonistic behavior (see Figure 5, the compound **3c** selected docking pose is depicted in green). In brief, compound **3c** displayed H-bond interactions between the linker piperazine protonated nitrogen atom and the key residue Asp116 and between the cyclopentanone carbonyl group and the Lys191 ε-amino group. van der Waals contacts are displayed between the two phenyl ring linked to the cyclopentanone core and Ala93, Tyr96, Ile113, Thr188, Ile189, and Lys191 and between the 2-methoxyphenyl moiety and Val117, Cys120, and Tyr195. The two phenyl rings and the 2-methoxyphenyl group were also involved in $\pi-\pi$ stacking with Tyr96 and Tyr195, respectively. As shown in Figure 5 (for simplicity only the binding mode selected by us for compound **3b** is depicted), compounds **3a** and **3b** (the *R* isomers were shown to be the most probable) display the following common H-bond interactions between: (i) the cyclopentanone oxygen atom and the Tyr390 and (ii) the linker amino group hydrogen atom and the Asp116 side chain. The compound **3b** methoxy group is involved in H-bond interaction with Thr121, while van der Waals contacts were displayed between the two phenyl ring linked to the cyclopentanone core and Ala93, Tyr96, Phe112, Ile113, Trp358, and Tyr390 and between the phenoxy (or 2-methoxyphenyl) moiety and Val117, Cys120, Tyr195, Phe204, and Phe362. The two phenyl rings and the phenoxy (or 2-methoxyphenyl) group were also involved in $\pi-\pi$ stacking with Tyr96, Phe112, Trp358, and Tyr390 and with Tyr195, Phe204, and Phe362, respectively.

Lead compounds **1a** and **1b** (the *R* isomers were shown to be the most probable), bearing a flexible linker located between the dioxolane core and the phenoxy moiety display a binding mode comparable with that of the agonist **2b**. Thus, H-bond interactions were detected between: (i) the amino group hydrogen atom and the key residue Asp116 and (ii) the nitrogen atom and Asn386. Moreover, one of the two oxygen atoms on the dioxolane core and the methoxy group (for **1b**) were involved in H-bonds with Tyr390 and Thr121, respectively.

As far as the lead compound **1c** is concerned (the *R* isomer was shown to be the most probable), the piperazine protonated nitrogen and one of the two oxygen atoms located on the dioxolane core were engaged in H-bond contacts with the key residues Asp116 and Tyr390, respectively. Accordingly, all three of these compounds were characterized by $5\text{-}HT_{1A}$ agonistic activity.

In keeping with site-directed mutagenesis data and with the computational results available in the literature,[27] all these ligands display a salt bridge between the piperazine protonated nitrogen and the Asp116 side chain. As reported in Table 4, H-bond contacts with Thr121, Asn386, Tyr390, or Lys191 seemed to determine the agonist or neutral antagonist behavior of the compounds.

On the basis of these results, the presence of an H-bond donor group on the diphenyl-disubstituted ring and the

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Table 4. The Most Important $5HT_{1A}$ Residues Which Are Involved in the Agonist or Antagonist Binding Mode Are Listed[a]

	$5HT_{1A}$ agonist	$5HT_{1A}$ antagonist
H-bond interactions	**Asp116**, **Asn386**, Tyr390, Thr121	**Asp116**, Lys191
$\pi-\pi$ stacking	Tyr96, **Tyr195**, **Phe204**, **Trp358**, **Phe361**, **Phe362**, **Trp387**, **Tyr390**	Tyr96, **Tyr195**
van der Waals contacts	Val98, Ile113, Ile189, **Val117**, Cys120, Ala203	Ala93, Ile113, **Val117**, Ile189, Cys120

[a]The residues which are conserved between the $5HT_{1A}$ and the β2-adrenoreceptor are reported in bold. In the two columns, the $5HT_{1A}$ residues which correspond to the key aminoacids involved in the 3PDS, and in the 2RH1 H-bond contacts, respectively, are underlined.

introduction of an alkyl amino group at the ligand linker portion makes these compounds 5-HT_{1A} agonists (by enhancing the hydrophilic contacts with residues Asp116 and/or Asn386, and Tyr390). Moreover, the presence of a methoxy group on the phenoxy (or phenyl) ring (linked to the diphenyl-disubstituted core) could improve the agonistic potency (through an H-bond with Thr121). The reliability of these data was supported by the higher pD_2 values of **4b**-*trans* isomer (pD_2 = 8.96) and **3b**-*R* isomer (pD_2 = 8.37) in comparison with those of **4a**-*trans* isomer (pD_2 = 7.94) and **3a**-*R* isomer (pD_2 = 7.44). Accordingly, all the cyclopentanol derivatives discussed here showed 5-HT_{1A} agonistic activity.

For 5-HT_{1A} agonist activity, the introduction of a diphenyl-disubstituted ring bearing H-bond acceptor groups (together with the H-bond acceptor and donor atom at the ligand linker portion) could also be possible by enhancing H-bond contacts with Tyr390. In agreement with these results, compound **2b** (pD_2 = 9.04; both the linker nitrogen atom and the tetrahydrofuran oxigen atom were involved in H-bond with Tyr390) showed higher potency compared to compound **3b** (pD_2 = 8.37; only the cyclopentanone oxygen atom was engaged in H-bond contacts with Tyr390).

In contrast, the introduction of a diphenyl-disubstituted ring bearing H-bond acceptor groups *in tandem* with the presence of the piperazine moiety at the ligand linker portion made these compounds 5-HT_{1A} antagonists (see compound **3c** and *R* isomer of **2c** docking poses previously discussed). Notably, these compounds interact with Lys191, while no H-bond with Tyr390 or Asn396 were detected.

To verify the reliability of the obtained 5-HT_{1A}/agonist and 5-HT_{1A}/antagonist models, we have compared the results obtained by means of our homology and docking studies, with the experimental data concerning the template GPCR β2 adrenoreceptor, available in complex with an agonist (pdb code: 3PDS) and with an antagonist compound (pdb code: 2RH1).

Both the β2-adrenoreceptor agonist and antagonist displayed H-bond contacts with residues Asp113 and Asn312. On the other hand, only the agonist ligand is engaged in additional H-bonds with Ser203 and Ser207, while the antagonist interacts by $\pi-\pi$ stacking with Phe193, being projected toward Ser203, Ser207, and Thr195.

Notably, the key residues Asp113 and Asn312 were conserved between the β2-adrenoreceptor and the $5HT_{1A}$ protein, with respect to the corresponding Asp116 and Asn386 in the 5-HT_{1A} sequence. Furthermore, the β2-adrenoreceptor Phe193, which was engaged in contacts with the antagonist compound, corresponds to the 5-HT_{1A} Lys191. Interestingly, according to our calculations the 5-HT_{1A} antagonist behavior seemed to be determined by the formation of H-bonds with Lys191. Furthermore, the β2-adrenoreceptor Phe193, which was engaged in contacts with the antagonist compound, corresponds to the 5-HT_{1A} Ile189, which also belong to the 5-HT_{1A} putative binding site.

The final results, being quite in agreement with the experimental data concerning the β2-adrenoreceptor complexes, can be considered reasonable and then allow us to validate the model built as a useful tool for virtual screening procedures and for the development of new tetrahydrofuran-, cyclopentanone-, and cyclopentanol-based derivatives, potentially active on the 5-HT_{1A} receptor.

3. CONCLUSION

In conclusion, starting from the 1,3-dioxolane-based compounds, we have discovered a new series of 5-HT_{1A} receptor ligands. Compounds **2a** and **4c** *cis* emerged as promising 5-HT_{1A} receptor ligands, the former showing 5-HT_{1A} antagonistic behavior and the latter being one of the most potent and selective agonists. On the light of the potential application of both agonists and antagonists, it appears worthwhile to further investigate this new class of compounds. More extensive structure−activity relationship studies are being performed and will be reported in due course.

4. EXPERIMENTAL SECTION

4.1. Chemistry. The structural characterization was done by NMR and elemental analyses techniques (CHN, elemental analyzer model 1106, Carlo Erba Instruments). All the assayed compounds displayed a purity ≥96%, determined by Elemental Analyses. Melting points were determined on a Büchi 510 capillary melting points apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker DPX 200 Avance, working at 200.13 MHz and at a temperature of 300 K; chemical shifts are reported as δ (ppm) relative to tetramethylsilane (s = singlet, brs = broad singlet, d = doublet, dd = double doublet, t = triplet, pseudot = pseudotriplet, td = triple doublet, qd = double quadruplet, m = multiplet). Silica gel TLC plates (Merck, Kiesgel 60, F_{254}) were used to monitor the progression of the reactions. Chromatographic separations were performed on silica gel columns (Kieselgel 60, 0.040−0.063 mm, Merck) by flash chromatography.

*4.1.1. Prop-2-en-1-yl 2-iodoacetate (Allyliodoacetate) (**5**).* A mixture of 9.4 g (69.86 mmol) of allylchloroacetate and 12.76 g of KI (76.8 mmol) in 60 mL of acetone was vigorously stirred at rt for 3.5 h. Then the reaction mixture was diluted with water and poured into a separating funnel; the organic layer was collected, dried over anhydrous Na_2SO_4, and filtered. Solvent and unreacted allylchloroacetate were removed at 30 °C, under reduced pressure (30−70 mbar), to give 12.5 g (79%) of the title compound. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.73 (s, 2H), 4.67 (m, 2H), 5.36 (m, 2H), 5.95 (m, 1H).

*4.1.2. (5,5-Diphenyloxolan-2-yl)methanol (**7**).* Triethylborane (1.22 mL, 1.22 mmol, 1 M solution in hexane) was added, dropwise, to allyl iodoacetate **5** (2.3 g, 10.22 mmol) in dry CH_2Cl_2 (20 mL), and the mixture was stirred at rt for 3 h. TLC monitoring showed that **5** was totally consumed. ^{1}H NMR monitoring indicated that no vinylic proton signals could be observed. The resulting solution, which contained the oligomeric mixture **6**, was then directly used in the following deoligomerization reaction. The oligomeric mixture **6** was concentrated in vacuo, and the residue was dissolved in 50 mL of dry THF. Phenylmagnesiumbromide (13 mL, 40.9 mmol, 3 M solution in Et_2O) was added, dropwise, to the solution at rt over 2 h. The reaction mixture was further stirred at rt overnight. Hydrochloric acid (2N) was added until the pH was slightly acidic. The resulting mixture was then extracted with ether (30 mL, 3×), and the combined organic phase

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dried over anhydrous Na_2SO_4. After solvent removal, the crude exctract was purified by flash chromatography on silica gel (20/80 cyclohexane/ethyl acetate) to give 0.65 g (25%) of the title compound as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.93 (m, 2H), 2.63 (m, 2H), 3.60 (dd, J = 5.7, 11.5, 1H), 3.78 (dd, J = 3.3, 11.5, 1H), 4.34 (m, 1H), 7.15−7.55 (m, 10H). ^{1}H NMR (200 MHz, DMSO): δ 1.59−1.94 (m, 2H), 2.38 (m, 1H), 2.65 (m, 1H), 3.30−3.59 (m, 2H), 4.05 (qd, J = 5.6, 7.4, 1H), 4.68 (t, J = 5.6, 1H), 7.05−7.38 (m, 6H), 7.38−7.50 (m, 4H).

*4.1.3. 5-(Chloromethyl)-2,2-diphenyloxolane (**8**).* To a solution of **7** (0.64 g, 2.53 mmol) in 15 mL of pyridine/toluene (1:2) was added dropwise, at 0 °C, $SOCl_2$ (0.24 mL, 3.3 mmol) in 2 mL of toluene. The reaction was left at rt for 1 h and then refluxed for 15 min. After solvent removal, the crude, dissolved in CH_2Cl_2, was washed with a saturated solution of $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered, and evaporated. The crude extract was purified by flash chromatography on silica gel (50/50 cyclohexane/ethyl acetate) to give 0.34 g (50%) of the title compound. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.93 (m, 1H), 2.13 (m, 1H), 2.54 (m, 1H), 2.70 (m, 1H), 3.54 (dd, J = 6.7, 10.8, 1H), 3.66−3.74 (dd, J = 3.70, 10.8, 1H), 4.39 (m, 1H), 7.13−7.38 (m, 6H), 7.38−7.52 (m, 4H). ^{1}H NMR (200 MHz, DMSO): δ 1.77 (m, 1H), 1.95 (m, 1H), 2.46 (m, 1H), 2.74 (m, 1H), 3.71 (m, 2H), 4.28 (qd, J = 5.44, 7.56, 1H), 7.10−7.37 (m, 6H), 7.37−7.52 (m, 4H).

*4.1.4. (5,5-Diphenyloxolan-2-yl)methyl 4-methylbenzene-1-sulfonate (**9**).* To a solution of **7** (0.75 g, 2.95 mmol) in 1.5 mL of pyridine (8.85 mmol) and 10 mL of dry CH_2Cl_2, *p*TsCl (0.84 g, 4.42 mmol) was added portionwise, at 0 °C, over 30 min. The reaction was stirred, at rt, for 24 h. After solvent removal, the crude, dissolved in $CHCl_3$, was washed with a saturated solution of $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered, and evaporated. The crude exctract was purified by flash chromatography on silica gel (80/20 cyclohexane/ethyl acetate) to give 0.75 g (62%) of the title compound. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.83 (m, 1H), 2.02 (m, 1H), 2.33−2.53 (m, 4H), 2.62 (m, 1H), 4.09 (m, 2H), 4.34 (m, 1H), 7.10−7.45 (m, 12H), 7.70−7.85 (m, 2H)

*4.1.5. General Procedure for the Synthesis of Amines **10a,b,c**.* To a solution of chloromethyl derivate **8** in 5 mL of 2-methoxy-ethanol or tosyl derivative **9** in 5 mL of DMF, a large excess (3−4 equiv) of the appropriate ammine (Am) and a catalytic ammount of KI (in case of **10a** and **10b**) were added. The reaction was heated at reflux for 18−24 h. After cooling at rt, the solvent was removed under reduced pressure. The crude, dissolved in $CHCl_3$, was washed with a solution of 5% NaOH (3×) and brine (1×) and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the desired amine as oil.

*4.1.6. [(5,5-Diphenyloxolan-2-yl)methyl](2-phenoxyethyl)amine (**10a**) and Oxalate Salt (**2a**).* The crude extract was purified by flash chromatography on silica gel (40/60 cyclohexane/ethyl acetate) to give 0.15 g (0.40 mmol, 57%) of the title compound. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.75 (m, 1H), 2.02 (bs, 1H), 2.09 (m, 1H), 2.61 (m, 2H), 2.83 (dd, J = 8.4, 12.1, 1H), 2.99 (dd, J = 3.7, 12.1, 1H), 3.20 (m, 2H), 4.16 (pseudot, J = 5.2, 2H), 4.44 (m, 1H), 6.82−7.05 (m, 3H), 7.10−7.37 (m, 8H), 7.37−7.50 (m, 4H). The free amine was transformed into the corresponding oxalate salt which was crystallized from methanol to give compound **2a**: mp 217−218 °C. ^{1}H NMR (200 MHz, DMSO): δ 1.70 (m, 1H), 1.97 (m, 1H), 2.52 (m, 1H), 2.74 (m, 1H), 3.10 (dd, J = 8.4, 12.6, 1H), 3.22 (dd, J = 3.5, 12.6, 1H), 3.44 (m, 2H), 4.28 (pseudot, J = 5.2, 2H), 4.17−4.41 (m, 1H), 6.91−7.01 (m, 3H), 7.13−7.37 (m, 8H), 7.47−7.51 (m, 4H); $C_{27}H_{29}NO_6$ Anal. (C, H, N).

*4.1.7. [(5,5-Diphenyloxolan-2-yl)methyl][2-(2-methoxyphenoxy)-ethyl]amine (**10b**) and Oxalate Salt (**2b**).* The crude extract was purified by flash chromatography on silica gel (50/50 cycloexane/ethyl acetate → 20/80 cycloexane/ethyl acetate) to give 0.093 g (0.23 mmol, 42%) of the title compound. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.61 (bs, 1H), 1.70 (m, 1H), 2.05 (m, 1H), 2.61 (m, 2H), 2.85 (d, J = 6.0, 2H), 3.13 (m, 2H), 3.83 (s, 3H), 4.17 (pseudot, J = 5.5, 2H), 4.38 (m, 1H), 6.81−7.05 (m, 4H), 7.09−7.36 (m, 6H), 7.38−7.51 (m, 4H). The free amine was transformed into the corresponding oxalate salt which was crystallized from methanol to give compound **2b**; mp 146−150 °C. ^{1}H NMR (200 MHz, DMSO): δ 1.69 (m, 1H), 2.00 (m, 1H), 2.50 (m, 1H), 2.73 (m, 1H), 3.21 (m, 2H), 3.41 (m, 2H), 3.70 (s, 3H), 4.22 (pseudot, J = 5.6, 2H), 4.30 (m, 2H), 6.72−7.09 (m, 4H), 7.09−7.41 (m, 6H), 7.41−7.58 (m, 4H); $C_{28}H_{31}NO_7$ Anal. (C, H, N).

*4.1.8. 1-[(5,5-Diphenyloxolan-2-yl)methyl]-4-(2-methoxyphenyl)-piperazine (**10c**) and Oxalate Salt (**2c**).* The crude extract was purified by flash chromatography on silica gel (75/25 cyclohexane/ethyl acetate) to give 0.15 g (0.35 mmol, 95%) of the title compound. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.76 (m, 1H), 2.09 (m, 1H), 2.47−2.87 (m, 6H,), 2.95 (m, 2H), 3.14 (m, 4H), 3.89 (s, 3H), 4.43 (m, 1H), 6.81−7.08 (m, 4H.), 7.12−7.39 (m, 6H.), 7.39−7.52 (m, 4H). The free amine was transformed into the corresponding oxalate salt which was crystallized from methanol to give compound **2c**; mp 195−198 °C. ^{1}H NMR (200 MHz, DMSO): δ 1.67 (m, 1H), 2.00 (m, 1H), 2.40−2.80 (m, 2H), 3.00−3.50 (m, 11H), 3.79 (s, 3H), 4.36 (m, 1H); 6.88−7.02 (m, 4H), 7.09−7.39 (m, 6H), 7.40−7.56 (m, 4H); $C_{30}H_{34}N_2O_6$ Anal. (C, H, N).

*4.1.9. 2,2-Diphenylhexanedinitrile (**11**).* To a solution of diphenylacetonitrile (36,8 g, 190 mmol) in 220 mL of toluene, sodium amide (7,8 g, 199 mmol) was slowly added. The reaction mixture was refluxed for 4 h. After the mixture was cooled, 4-chlorobutyronitrile (20 g, 195 mmol) dissolved in 40 mL of toluene, was added, dropwise, over 30 min. The reaction mixture was then refluxed for 12 h. After cooling at rt, the organic layer was washed with water (3×) and brine (1×) and dried over anhydrous Na_2SO_4. After solvent removal, the crude extract was purified by flash chromatography on silica gel (85/15 cyclohexane/ethyl acetate) to give 18.1 g (69 mmol, 36%) of the title compound. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.83 (m, 2H, H4), 2.43 (t, J = 6.8, 2H), 2.58 (m, 2H), 7.26−7.42 (m, 10H).

*4.1.10. 2-Amine-3,3-diphenyl-cyclopent-1-enecarbonitrile (**12**).* To a solution of sodium amide (2,6 g, 67 mmol) in 60 mL of toluene, the dinitrile **11** (17,5 g, 67 mmol) was added, portionwise, at rt. The reaction mixture was refluxed for 12 h. After cooling at rt, the organic layer was washed with water (3×) and brine (1×) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give 15 g (86%) of the title compound, which was used in the next step without further purification. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.48 (m, 2H), 2.64 (m, 2H), 4.25−4.51 (bs, 2H), 7.21−7.41 (m, 10H).

*4.1.11. 2,2-Diphenyl-cyclopentanone (**13**).* To a solution of 36 mL of H_2SO_4 conc in 65 mL of water, the enaminonitrile **12** (7 g, 27 mmol) was added, portionwise, at rt. The reaction mixture was refluxed for 24 h. After cooling to rt, the mixture was poured into 500 mL of ice and extracted with $CHCl_3$ (×3). The combined organic layers were washed with a saturated solution of $NaHCO_3$ (2×) and brine (1×) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give 5 g (78%) of the title compound as a white solid (mp 80 °C). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.98 (m, 2H), 2.47 (t, J = 7.5, 2H), 2.74 (t, J = 6.6, 2H), 7.26 (m, 10H).

*4.1.12. General Procedure for the Synthesis of Amines (Mannich Bases) **14a,b,c**.* To a solution of 2,2-diphenylcyclopentanone **13** (0.5−1.0 g) in 8 mL of ethanol, an excess (3−4 equiv) of the appropriated amine (Am) and paraformaldehyde (1.5 equiv) were added. The reactione mixture was refluxed for 1 h, and then an additional 1.5 equiv of paraformaldehyde was added. The mixture was refluxed for further 12 h. After cooling to rt, the solvent was removed under reduced pressure. The crude, dissolved in CH_2Cl_2, was washed with a solution of 5% NaOH (3×) and brine (1×) and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the desired amine as oil.

*4.1.13. 5-{[(2-Phenoxyethyl)amino]methyl}-2,2-diphenylcyclopentan-1-one (**14a**) and Oxalate Salt (**3a**).* The crude extract was purified by flash chromatography on silica gel (80/20 cyclohexane/ethyl acetate) to give 0.91 g (50%) of the title compound. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.71 (bs, 1H), 1.76 (m, 1H), 2.22 (m, 1H), 2.48−3.15 (m, 5H), 2.98 (t, J = 5.1, 2H), 4.03 (pseudot, J = 5.1, 2H), 6.86−7.02 (m, 3H), 7.15−7.35 (m, 12H). The free amine was transformed into the corresponding oxalate salt which was crystallized from methanol to give compound **3a**; mp 142−145 °C. ^{1}H NMR (200

MHz, DMSO): δ 1.62 (m, 1H), 2.24 (m, 1H), 2.62 (m, 1H), 2.83−2.97 (m, 5H), 3.19−3.29 (m, 3H), 4.16 (pseudot, J = 5.0, 2H), 6.93−7.00 (m, 3H), 7.13−7.38 (m, 12H); $C_{28}H_{29}NO_6$ Anal. (C, H, N).

*4.1.14. 5-({[2-(2-Methoxyphenoxy)ethyl]amino}methyl)-2,2-diphenylcyclopentan-1-one (**14b**) and Oxalate Salt (**3b**).* The crude extract was purified by flash chromatography on silica gel (20/80 cyclohexane/ethyl acetate) to give 0.75 g (36%) of the title compound. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.78 (m, 1H), 1.84 (bs, 1H), 2.23 (m, 1H), 2.40−3.15 (m, 5H), 3.0 (m, 2H), 3.84 (s, 3H), 4.09 (pseudot, J = 5.6, 2H), 6.83−6.99 (m, 4H), 7.14−7.34 (m, 10H). The free amine was transformed into the corresponding oxalate salt which was crystallized from methanol to give compound **3b**; mp 143−145 °C. ^{1}H NMR (200 MHz, DMSO): δ 1.63 (m, 1H), 2.25 (m, 1H), 2.58 (m, 1H), 2.84−3.05 (m, 5H), 3.16−3.43 (m, 3H), 3.71 (s, 3H), 4.17 (pseudot, J = 5.0, 2H), 6.78−7.08 (m, 4H), 7.09−7.47 (m, 10H); $C_{29}H_{31}NO_7$ Anal. (C, H, N).

*4.1.15. 5-{[4-(2-Methoxyphenyl)piperazin-1-yl]methyl}-2,2-diphenylcyclopentan-1-one (**14c**) and Oxalate Salt (**3c**).* The crude extract was purified by flash chromatography on silica gel (80/20 cyclohexane/ethyl acetate) to give 0.31 g (35%) of the title compound. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.88 (m, 1H), 2.30 (m, 1H), 2.44 (dd, J = 9.1, 12.4 1H), 2.52−2.80 (m, 6H, H3a), 2.88 (m, 1H), 2.91 (dd, J = 3.6, 12.4, 1H), 3.04 (pseudot, J = 4.6, 4H), 3.86 (s, 3H), 6.84−7.05 (m, 4H), 7.18−7.36 (m, 10H). The free amine was transformed into the corresponding oxalate salt which was crystallized from methanol to give compound **3c**; mp 131−139 °C. ^{1}H NMR (200 MHz, DMSO): δ 1.67 (m, 1H), 2,27 (m, 1H), 2.58 (m, 1H), 2.62−3.25 (m, 14H), 3.77 (s, 3H), 6.87−6.98 (m, 4H), 7.12−7.38 (m, 10H); $C_{31}H_{34}N_2O_6$ Anal. (C, H, N).

*4.1.16. General Procedure for the Synthesis of Amines **15a** cis and trans, **15b** trans, and **15c** cis and trans.* To a solution of the appropriated ketone (**14a** or **14b** or **14c**) dissolved in the minimum of ethanol, was added, dropwise, at 0 °C, a suspension of $NaBH_4$ (1.5 equiv) in ethanol. The reaction mixture was allowed to stand 2−3 h at rt and then was cooled to 0 °C and quenched with 0.1 N HCl. After evaporation of the ethanol, the residue was basified to pH = 12 with a saturated solution of $NaHCO_3$ and extracted with $CHCl_3$ (3×). Evaporation of the solvent gave the desired cyclopentanol as oil. Purification and diastereomeric separations were accomplished by flash chromatography on silica gel.

*4.1.17. cis-5-{[(2-Phenoxyethyl)amino]methyl}-2,2-diphenylcyclopentan-1-ol (**15a** cis) and Oxalate Salt (**4a** cis).* Flash chromatography: 90/10 ethyl acetate/methanol. 0,15 g (26%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.66 (m, 1H), 1.86 (m, 1H), 1.93 (m, 1H), 2.05 (bs, 1H), 2.38 (m, 1H), 2.61 (dd, J = 8.2, 11.4, 1H), 2.70 (m, 2H), 2.91 (dd, J = 4.4, 11.4, 1H) 2.98 (m, 2H), 4.05 (pseudo t, J = 5.1, 2H), 5.02 (d, J = 4.7, 1H), 6.87−6.99 (m, 3H), 7.03−7.47 (m, 12H). The free amine was transformed into the corresponding oxalate salt which was crystallized from methanol to give compound **4a** *cis*; mp 200−203 °C. ^{1}H NMR (200 MHz, DMSO): δ 1.53 (m, 1H), 1.84 (m, 1H), 2.22 (m, 1H), 2.40 (m, 2H), 2.79 (m, 2H), 2.97 (dd, J = 6.8, 12.3, 1H), 3.18 (dd, J = 7.5, 12.3, 1H), 3.32 (pseudot, J = 5.2, 2H), 4.20 (pseudot, J = 5.2, 2H), 4.92 (d, J = 3.5, 1H), 6.89−7.49 (m, 15H); $C_{28}H_{31}NO_6$ Anal. (C, H, N)

*4.1.18. trans-5-{[(2-Phenoxyethyl)amino]methyl}-2,2-diphenylcyclopentan-1-ol (**15a** trans) and Oxalate Salt (**4a** trans).* Flash chromatography: 90/10 ethyl acetate/methanol, 0.24 g (42%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.38 (m, 1H), 1.80- 2.18 (m, 3H), 2.22 (bs, 1H), 2.68- 2.90 (m, 3H), 2.98 (pseudot, J = 4.7, 2H), 3.09 (dd, J = 4.3, 11.4, 1H), 4.05 (pseudot, J = 4.7, 2H), 4.44 (d, J = 9.5, 1H), 6.74−6.99 (m, 3H), 7.04−7.47 (m, 12H). The free amine was transformed into the corresponding oxalate salt which was crystallized from methanol to give compound **4a** *trans*; mp 189−194 °C. ^{1}H NMR (200 MHz, DMSO): δ 1.49 (m, 1H), 1.82- 2.23 (m, 3H), 2.40 (m, 2H), 2.64 (m, 2H), 3.02 (dd, J = 8.3, 12.0, 1H), 3.19 (dd, J = 4.5, 12.0, 1H), 3.27 (pseudot, J = 5.1 2H), 4.19 (pseudot, J = 5.1, 2H), 4.33 (d, J = 9.3, 1H), 6.88−7.03 (m, 3H), 7.04−7.38 (m, 12H); $C_{28}H_{31}NO_6$, Anal. (C, H, N).

*4.1.19. trans-5-({[2-(2-Methoxyphenoxy)ethyl]amino}methyl)-2,2-diphenylcyclopentan-1-ol (**15b** trans) and Oxalate Salt (**4b** trans).* Flash chromatography: 90/10 ethyl acetate/methanol, 0.15 g (28%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.38 (m, 1H), 1.90 (td, J = 9.7, 4.6, 1H), 2.00−2.17 (m, 2H), 2.18 (bs, 1H), 2.69−2.81 (m, 3H), 3.02 (m, 2H), 3.10 (dd, J = 4.4, 8.8, 1H), 3.78 (s, 3H), 4.08 (pseudot, J = 5.0, 2H), 4.45 (d, J = 9.7, 1H) 6.83−6.98 (m, 4H) 7.09−7.45 (m, 10H). The free amine was transformed into the corresponding oxalate salt which was crystallized from methanol to give compound **4b** *trans*; mp 182−186 °C. ^{1}H NMR (200 MHz, DMSO): δ 1.50 (m, 1H), 1.80−2.20 (m, 3H), 2.66 (m, 1H), 2.95 (m, 3H), 3.07 (dd, J = 7.6, 12.2, 1H), 3.20 (dd, J = 4.3, 12.2, 1H), 3.27 (pseudot, J = 5.2, 2H), 3.75 (s, 3H), 4.15 (pseudot, J = 5.2, 2H), 4.33 (d, J = 9.5, 1H), 6.79−7.06 (m, 4H), 7.06−7.39 (m, 10H); $C_{29}H_{33}NO_7$ Anal. (C, H, N)

*4.1.20. cis-5-{[4-(2-Methoxyphenyl)piperazin-1-yl]methyl}-2,2-diphenylcyclopentan-1-ol (**15c** cis) and Oxalate salt (**4c** cis).* Flash chromatography: 90/10 cyclohexane/ethyl acetate, 0.10 g (5%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.48 (m, 1H), 1.89 (m, 1H), 2.25 (m, 1H), 2.30−2.95(m, 9H), 3.13 (m, 4H), 3.82 (s, 3H), 5.08 (d, J = 4.4, 1H), 6.80−7.1 (m, 4H), 7.10−7.50 (m, 10H). The free amine was transformed into the corresponding oxalate salt which was crystallized from methanol to give compound **4c** *cis*; mp 194−200 °C. ^{1}H NMR (200 MHz, DMSO): δ 1.59 (m, 1H), 1.89 (m, 1H), 2.22 (m, 1H), 2.42 (m, 1H), 2.78 (m, 1H), 2.85−3.30 (m, 12H), 3.77 (s, 3H), 4.91 (d, J = 4.2, 1H), 6.85−7.50 (m, 14H); $C_{31}H_{36}N_2O_6$ Anal. (C, H, N)

*4.1.21. trans-5-{[4-(2-Methoxyphenyl)piperazin-1-yl]methyl}-2,2-diphenylcyclopentan-1-ol (**15c** trans) and Oxalate Salt (**4c** trans).* Flash chromatography: 90/10 cyclohexane/ethyl acetate, 0.16 g (8%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.33 (m, 1H), 1.95−2.20 (m, 3H), 2.50−2.90 (m, 8H), 3.08 (m, 4H), 3.85 (s, 3H), 4.43 (d, J = 8.9, 1H), 6.83−7.04 (m, 4H), 7.15−7.37 (m, 8H), 7.43−7.47 (m, 2H). The free amine was transformed into the corresponding oxalate salt which was crystallized from methanol to give compound **4c** *trans*; mp 206−211 °C with dec. ^{1}H NMR (200 MHz, DMSO): δ 1.49 (m, 1H), 1.85−2.20 (m, 3H), 2.65 (m, 1H), 2.80−3.20 (m, 12H), 3.88 (s, 3H), 4.25 (d, J = 9.3, 1H), 6.85−7.00 (m, 4H), 7.10−7.40 (m, 10H); $C_{31}H_{36}N_2O_6$ Anal. (C, H, N)

4.2. Modeling Studies. All the compounds isomers were built, parametrized (Gasteiger−Huckel method), and energy minimized within MOE using MMFF94 forcefield.[28,29]

Both the protonated and in the unprotonated forms were evaluated for each structure.

Successively docking studies were performed, using the 5-HT_{1A} model previously built by us.[12] Briefly, the protein model was derived by the alignment of the 5-HT_{1A} (P08908) fast sequence on the X-ray coordinates of human β2 adrenoreceptor (2RH1), on the basis of the Blosum62 matrix (MOE software). The gap regions between the template and the target protein sequences, concerning 5-HT_{1A} loop domain, were built by means of the MOE loop library.

The putative 5-HT_{1A} receptor binding site was determined starting from the fact that, for the ligand activity, formation of a polar interaction between the ligand and Asp116 is necessary.[30−32] Thus, the MOE tool SiteFinder was employed to identify the proper protein pocket, including Asp116 and those residues highlighted by the rhodopsin-based homology modeling studies developed by Nowak.[27]

Each isomer was docked into the 5-HT_{1A} putative ligand binding site, by means of the Surflex docking module implemented in Sybyl-X1.0. Then, for all the compounds, the best docking geometries (selected on the basis of the SurFlex scoring functions) were refined by ligand−receptor complex energy minimization (CHARMM27) by means of the MOE software. To verify the reliability of the derived docking poses, the obtained 5-HT_{1A}/*cis* conformer and 5-HT_{1A}/*trans* conformer complexes were further investigated by docking calculations (10 run), using MOE-Dock (Genetic algorithm; applied on the poses already located into the putative 5-HT_{1A} receptor). The conformers showing lower energy scoring functions and rmsd values (respect to the starting poses) were selected as the most stable and allowed us to identify the most probable enantiomers with 5-HT_{1A}. At this step, for all compounds bearing a tertiary amino group (piperazine derivatives), the protonated conformers showed to be the most probable (by means of lower values of energy scoring functions). This result may be explained by an H-bond interaction between the protonated

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piperazine nitrogen atom and Asp116. This interaction, able to stabilize the 5-HT_{1A}/ligand complex, was not highlighted by the corresponding unprotonated ligand.

On the contrary, for those compounds bearing a secondary amino group (the ones with the flexible linker), the interaction with the protein resulted to be more favored in their unprotonated form (lower energy scoring functions). In fact, this latest form could display an additional H-bond with Thr390, plus the one with Asp116. The corresponding protonated form displayed only a single H-bond with Asp116.

4.3. Biological Assays/Pharmacology. *4.3.1. Radioligand Binding Assay at Human Recombinant 5-HT_{1A} Receptors and α_1 Adrenoceptor Subtypes.* A human cell line (HeLa) stably transfected with genomic clone G-21 coding for the human 5-HT_{1A} serotoninergic receptor was used. Cells were grown as monolayers in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and gentamycin (100 μg/mL) under 5% CO_2 at 37 °C. Cells were detached from the growth flask at 95% confluence by a cell scraper and were lysed in ice-cold Tris (5 mM) and EDTA buffer (5 mM, pH 7.4). Homogenates were centrifuged for 20 min at 40000*g*, and pellets were resuspended in a small volume of ice-cold Tris/EDTA buffer (above) and immediately frozen and stored at 70 °C until use. On the day of experiment, cell membranes were resuspended in binding buffer (50 mM Tris, 2.5 mM $MgCl_2$, and 10 mM pargiline, pH 7.4). Membranes were incubated in a final volume of 0.32 mL for 30 min at 30 °C with 1 nM [^{3}H]8-OH-DPAT, in the absence or presence of various concentrations of the competing drugs (1pM to 1 μM); each experimental condition was performed in triplicate. Nonspecific binding was determined in the presence of 10 μM 5-HT.[22]

Binding to recombinant human α_1 adrenoceptor subtypes was performed in membranes from Chinese hamster ovary (CHO) cells transfected by electroporation with DNA expressing the gene encoding each α_1 adrenoceptor subtype. Cloning and stable expression of the human α_1 adrenoceptor genes were performed as described.[33] CHO cell membranes were incubated in 50 mM Tris (pH 7.4) with 0.2 nM [^{3}H]prazosin, in a final volume of 0.32 mL for 30 min at 25 °C, in the absence or presence of competing drugs (1 pM to 1 μM). Nonspecific binding was determined in the presence of 10 μM phentolamine. The incubation was stopped by addition of ice-cold Tris buffer and rapid filtration through Unifilter B filters (Perkin-Elmer) using a Filtermate cell harvester (Packard), and the radioactivity retained on the filters was determined by (TopCount, Perkin-Elmer) liquid scintillation counting at 90% efficiency.

4.3.2. [^{35}S]GTPγS Binding Assay. The effects of the various compounds tested on [^{35}S]GTPγS binding in HeLa cells expressing the recombinant human 5-HT_{1A} receptor were evaluated according to the method of Stanton and Beer[26] with minor modifications. Stimulation experiments: Cell membranes were resuspended in buffer containing 20 mM HEPES, 3 mM $MgSO_4$, and 120 mM NaCl (pH 7.4). The membranes were incubated with 30 μM GDP, and various concentrations (from 0.1 nM to 10 μM) of test drugs or 8-OH-DPAT (reference curve) for 20 min at 30 °C in a final volume of 0.5 mL. Samples were transferred to ice, [^{35}S]GTPγS (200 pM) was added, and samples were incubated for another 30 min at 30 °C.

4.3.3. Functional Antagonism in Isolated Tissues. Male Wistar rats (275−300 g) were killed by cervical dislocation, and the organs required were isolated, freed from adhering connective tissues and set up rapidly under a suitable resting tension in 20 mL organ baths containing physiological salt solution kept at 37 °C and aerated with 5% CO_2−95% O_2 at pH 7.4. Concentration−response curves were constructed by cumulative addition of agonist. The concentration of agonist in the organ bath was increased approximately 3-fold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady. Contractions were recorded by means of a force displacement transducer connected to the Mac Lab system PowerLab/800. In addition, parallel experiments in which tissues did not receive any antagonist were run in order to check any variation in sensitivity.

All animal testing was carried out according to European Communities Council Directive of 24 November 1986 (86/609/EEC).

4.3.3.1. Vas Deferens Prostatic Portion. This tissue was used to assess the antagonism toward α_{1A}-adrenoceptors.[23] Prostatic portions of 2 cm length were mounted under 0.5 g tension at 37 °C in Tyrode solution of the following composition (mM): NaCl, 130; KCl, 1; $CaCl_2$, 1.8, $MgCl_2$, 0.89; NaH_2PO_4, 0.42; $NaHCO_3$, 25; glucose, 5.6. Cocaine hydrochloride (0.1 μM) was added to the Tyrode to prevent the neuronal uptake of (−)-noradrenaline. The preparations were equilibrated for 60 min with washing every 15 min. After the equilibration period, tissues were primed twice by addition of 10 μM noradrenaline. After another washing and equilibration period of 60 min, a noradrenaline concentration−response curve was constructed (basal response). The antagonist was allowed to equilibrate for 30 min before constructing a new concentration−response curve to the agonist. (−)-Noradrenaline solutions contained 0.05% $Na_2S_2O_5$ to prevent oxidation.

4.3.3.2. Spleen. This tissue was used to assess the antagonism toward α_{1B}-adrenoceptors.[25] The spleen was removed and bisected longitudinally into two strips, which were suspended in tissue baths containing Krebs solution of the following composition (mM): NaCl, 120; KCl, 4.7; $CaCl_2$, 2.5, $MgSO_4$, 1.5; KH_2PO_4, 1.2; $NaHCO_3$, 20; glucose, 11; K_2EDTA, 0.01. Propranolol hydrochloride (4 μM) was added to block β-adrenoceptors. The spleen strips were placed under 1 g resting tension and equilibrated for 2 h. The cumulative concentration−response curves to phenylephrine were measured isometrically and obtained at 30 min intervals, the first one being discarded and the second taken as a control. The antagonist was allowed to equilibrate for 30 min before constructing a new concentration−response curve to the agonist.

4.3.3.3. Aorta. This tissue was used to assess the antagonism toward α_{1D}-adrenoceptors.[24] Thoracic aorta was cleaned from extraneous connective tissues and placed in Krebs solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 1.9, $MgSO_4$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11.7; K_2EDTA, 0.01. Cocaine hydrochloride (0.1 μM) and propranolol hydrochloride (4 μM) were added to prevent the neuronal uptake of (−)-noradrenaline and to block β-adrenoceptors, respectively. Two helicoidal strips (15 mm × 3 mm) were cut from each aorta beginning from the end most proximal to the heart. The endothelium was removed by rubbing with filter paper: the absence of acetylcholine (100 μM)-induced relaxation to preparations contracted with (−)-noradrenaline (1 μM) was taken as an indicator that the vessel was denuded successfully. Vascular strips were then tied with surgical thread and suspended in a jacketed tissue bath containing Tyrode solution. Strip contractions were measured isometrically. After at least a 2 h equilibration period under an optimal tension of 1 g, cumulative (−)-noradrenaline concentration−response curves were recorded at 1 h intervals, the first two being discarded and the third one taken as control. The antagonist was allowed to equilibrate with the tissue for 30 min before the generation of the fourth cumulative concentration−response curve to (−)-noradrenaline. (−)-Noradrenaline solutions contained 0.05% K_2EDTA in 0.9% NaCl to prevent oxidation.

4.3.4. Data Analysis. Binding data were analyzed using the nonlinear curve-fitting program Allfit.[34] None of the pseudo-Hill coefficients (n^H) were significantly different from unity ($p > 0.05$). Equilibrium dissociation constants (K_i) were derived from the Cheng−Prusoff equation[35] $K_i = IC_{50}/(L/K_d)$, where L and K_d are the concentration and the equilibrium dissociation constant of the radioligand. pK_i values are the mean of 2−3 separate experiments performed in duplicate. Stimulation of [^{35}S]GTPγS binding induced by the compounds tested was expressed as the percent increase in binding above basal value, with the maximal stimulation observed with 8-OH-DPAT taken as 100%. The concentration−response curves of the agonistic activity were analyzed by Allfit as reported above. The maximum percentage of stimulation of [^{35}S]GTPγS binding (E_{max}) achieved for each drug, and the concentration required to obtain 50% of E_{max} (pD_2 = −log$_{10}$ [EC_{50}]), were evaluated. In functional studies, responses were expressed as a percentage of the maximal contraction

observed in the agonist concentration−response curves, taken as a control, which were analyzed by pharmacological computer programs. pK_b values were calculated according to van Rossum[36] at one or two antagonist concentrations. Each concentration was tested at least four times.

■ ASSOCIATED CONTENT

Supporting Information

Elemental analysis for compounds **1**−**6**, **7c**, **7t**, **8t**, **9c** (+2H_2O), and **9t**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone, +39 (0)59 205 5139. Fax: +39 (0)59 205 5131. E-mail: livio.brasili@unimore.it.

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■ ABBREVIATIONS USED

α_1, alpha$_1$ adrenergic receptor; 5-HT, 5-hydroxytryptamine (serotonin); 5-Ht$_{1A}$, serotonin$_{1A}$ receptor; K_i, inhibition constant; Kb, antagonist affinity constant; E_{max}, maximal response; pD_2, negative logarithm of half-maximun effective concentration (EC_{50}); THF, tetrahydrofuran; BMY-7378, 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]-decane-79-dione; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetraline; WAY100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamide; [^{35}S]GTPγS, guanosine-5′-*O*-(γthio)triphosphate; NMR, nuclear magnetic resonance; TLC, thin layer chromatography; DMSO, dimethylsulfoxide; DMF, dimethylformamide; Et_2O, diethylether; $C_2H_2O_4$, oxalic acid; pTsCl, *p*-toluensulfonyl chloride; HeLa, human cell line; CHO, Chinese hamster ovary; GDP, guanosine-5′-diphosphate; GPCR, G protein-coupled receptor

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