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ARTICLE

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Unichiral 2-(2′-Pyrrolidinyl)-1,4-benzodioxanes: the 2*R*,2′*S* Diastereomer of the *N*-Methyl-7-hydroxy Analogue Is a Potent $\alpha4\beta2$- and $\alpha6\beta2$-Nicotinic Acetylcholine Receptor Partial Agonist

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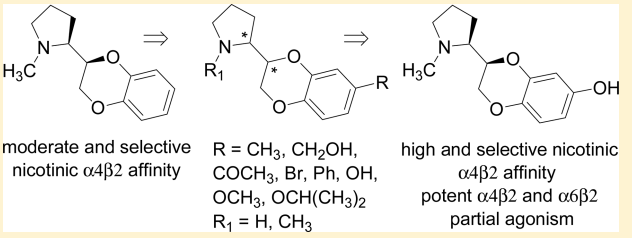
Supporting Information

ABSTRACT: A series of unichiral 7-substituted 2-(1′-methyl-2′-pyrrolidinyl)-1,4-benzodioxanes were synthesized and tested for the affinity for the $\alpha4\beta2$ and $\alpha7$ central nicotinic receptors; the 2*R*,2′*S* diastereomer of the 7-OH analogue [(*R*,*S*)-**7**], unique in the series, has a high $\alpha4\beta2$ affinity (12nM K_i). *N*-Demethylation and configuration inversion of the stereocenters greatly weaken its $\alpha4\beta2$ affinity, confirming that such a rigid molecule can be considered a new template for $\alpha4\beta2$ ligands. Docking analysis showed how (*R*,*S*)-**7** is capable of strongly and specifically interacting with the amino acidic counterpart of the $\alpha4\beta2$ receptor binding site. Further pharmacological characterization demonstrated that (*R*,*S*)-**7** also has a high affinity for the $\alpha6\beta2$ receptor, and in vitro functional tests indicated that it is a potent $\alpha4\beta2$ and $\alpha6\beta2$ partial agonist, with modest affinity and potency for the $\alpha3\beta4$ receptor. Comparison with varenicline, a well-known nicotinic partial agonist used as a smoking cessation aid, interestingly reveals similar nicotinoid profiles.



INTRODUCTION

The key role played by $\alpha4\beta2$ and/or $\alpha7$ neuronal nicotinic acetylcholine receptors (nAChRs) in a wide range of CNS functions, such as learning, memory, attention, information processing, analgesia, and nicotine addiction, makes the agonists at these receptors good candidates for the treatment of several CNS disorders, including cognitive dysfunction and neurodegenerative conditions, pain, and nicotine dependence.[1−3] Potent and selective $\alpha4\beta2$ and $\alpha7$ nAChR agonists are of great interest to drug research, and recent studies have intensively focused on the structural differences which determine the relative selectivity for these two major brain subtypes.[4,5] Representatives of the known $\alpha4\beta2$- or $\alpha7$-ligands or newly designed nicotinoids have been submitted to batteries of assays to assess the subtype affinity and activity, while SAR analysis and modeling studies have tentatively rationalized the difference in subtype activation and subtype binding modes.[4−10]

Protonated or quaternarized amine and a hydrogen bond acceptor and π-electron rich group (HBA/π) have been established as the two key elements of the nicotinic agonist pharmacophore.[11−13] According to current models of nicotinic receptors and of their binding modes, the charged moiety of the ligand would interact with the side chains of tyrosine and tryptophan residues well-conserved across nAChR subtypes, thus not providing much basis for designing subtype-selective ligands. On the other hand, interaction of HBA/π with a less conserved and more heterogeneous subpocket, involving, in the case of $\alpha4\beta2$ subtype, polar residues of $\beta2$ subunit, could be exploited to selectively promote $\alpha4\beta2$ vs $\alpha7$ affinity.[8,14] Recently, we have reported the moderate and selective $\alpha4\beta2$ affinity of one of the four stereoisomers of 2-(1-methyl-2-pyrrolidinyl)-1,4-benzodioxane (**1**), designed as rigidified analogues of prolinol phenyl ether and conceived as potential templates and scaffolds for nicotinic ligands (Chart 1).[15]

The rigidity of **1** and the significant variations in affinity depending on the methylation of nitrogen and on the configuration of the two stereocenters have allowed to well support SAR with docking analysis.[15] The results, showing the binding potential of the benzodioxane as a HBA/π group, prompted us to investigate if the introduction of substituents, in particular at its 7-position, can positively affect the $\alpha4\beta2/\alpha7$ affinity profile. Here, we report the synthesis, the pharmacological evaluation, and the SAR analysis of the unichiral 7-substituted 2-(2-pyrrolidinyl)benzodioanes **2**−**9** constructed as new $\alpha4\beta2$ nAChR ligands (Chart 2).

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CHEMISTRY

All the stereoisomers of the compounds **7** and **7a** and only two diastereomers, epimers at the dioxane stereocenter, of the compounds **2**−**6** and **8**−**9** were prepared. We applied the synthetic strategy previously reported for **1** and **1a**,[15] with the modifications made necessary by the susbstituent at the 7-position of benzodioxane. *N*-Protected (*S*)- and (*R*)-2-bromoacetylpyrrolidine were reacted with the suitable *o*,*p*-disubstituted sodium phenate, prepared according to literature,[16−18] or by original methods, having OBn or OMEM at the *ortho* position and the *para*-substituent identical to that of the target benzodioxane (Br, Ph, Ac) or easily convertible into it (CHO, OBn). The enantiomeric excess of the resultant *S* and *R* phenoxyacetyl pyrrolidines **12**−**16** was determined by chiral HPLC and found to be always more than 97%. The carbonyl reduction of the *S* isomers of **12**−**16** with sodium borohydride or lithium aluminum hydride in THF afforded the (*R*,*S*)/(*S*,*S*) diastereomeric mixture of the secondary alcohols **17**−**21** (Scheme 1). All of these mixture were resolved by chromatography except for **17** and **18**.

Scheme 2 reports the successive transformations of **17** and **18** leading to the compounds **2**−**4**. Overnight hydrogenation of (*R*,*S*)/(*S*,*S*)-**17** yielded (*R*,*S*)- and (*S*,*S*)-**22**, which were separated by chromatography, intramolecularly cyclized to *N*-Boc protected 2-pyrrolidinyl-7-methylbenzodioxanes (*S*,*S*)- and (*R*,*S*)-**23** by Mitsunobu reaction, and finally reduced to the corresponding *N*-methyl analogues (*S*,*S*)- and (*R*,*S*)-**2**. Otherwise, a short hydrogenation of (*R*,*S*)/(*S*,*S*)-**17** produced only debenzylation.

Chart 1. Design of Conformationally Restricted Prolinol Phenyl Ethers

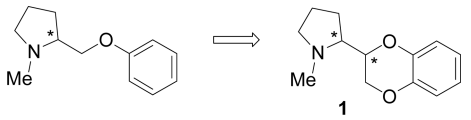
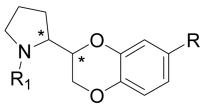


Chart 2. Target 7-Substituted 2-(2-Pyrrolidinyl)benzodioxanes



1	R = H	R_1 = CH_3	**6**	R = Ph	R_1 = CH_3
1a	R = H	R_1 = H	**7**	R = OH	R_1 = CH_3
2	R = CH_3	R_1 = CH_3	**7a**	R = OH	R_1 = H
3	R = CH_2OH	R_1 = CH_3	**8**	R = OCH_3	R_1 = CH_3
4	R = $COCH_3$	R_1 = CH_3	**9**	R = $OCH(CH_3)_2$	R_1 = CH_3
5	R = Br	R_1 = CH_3			

The resultant (*R*,*S*) and (*S*,*S*) *p*-hydroxymethylphenyl ethers **24** were tritylated at the benzylic OH-group, separated, and cyclized to 7-trityloxymethylbenzodioxanes (*S*,*S*)- and (*R*,*S*)-**26**. The final treatment with lithium aluminum hydride, followed by acidic hydrolysis, caused Boc reduction to methyl and detritylation, providing (*S*,*S*)- and (*R*,*S*)-**3**. The (*R*,*S*)/(*S*,*S*) diastereomeric mixture of the secondary alcohol **18**, treated with hydrogen, underwent debenzylation, *N*-deprotection and, after adding formaldehyde, reductive *N*-methylation. The successive separation of diastereomeric *p*-acetylphenyl ethers **27** and the Mitsunobu intramolecular cyclization gave (*S*,*S*)- and (*R*,*S*)-**4**.

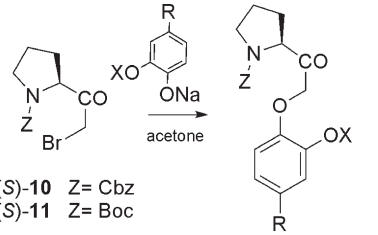
Scheme 3 shows the routes from the resolved diastereomeric alcohols **19**−**21** to the compounds **5**−**9**. The intermediates (*R*,*S*)- and (*S*,*S*)-**19** were treated with trifluoroacetic acid to remove the MEM protection and cyclized to (*S*,*S*)- and (*R*,*S*)-**5**, while (*R*,*S*)- and (*S*,*S*)-**20** were debenzylated, cyclized, and reduced to (*S*,*S*)- and (*R*,*S*)-**6**. Mesylation of the secondary hydroxyl group of the *o*,*p*-dibenzyloxyphenyl ethers (*R*,*S*)- and (*S*,*S*)-**21**, removal of both benzyl protections, and nucleophilic intramolecular displacement of mesylate afforded (*S*,*S*)- and (*R*,*S*)-**33**, the common intermediates to the (*S*,*S*) and (*R*,*S*) diastereomers of **7**, **7a**, **8**, and **9**. In fact, *N*-deprotection gave (*S*,*S*)- and (*R*,*S*)-**7a**, which were *N*-methylated to yield (*S*,*S*)- and (*R*,*S*)-**7**, while *O*-methylation and *O*-isopropylation, followed by Boc reduction, provided (*S*,*S*)- and (*R*,*S*)-**8** and (*S*,*S*)- and (*R*,*S*)-**9**, respectively.

Starting from the (*S*,*R*)/(*R*,*R*) diastereomeric mixture of **21**, obtained by reduction of (*R*)-**16**, the same synthetic strategy illustrated for (*S*,*S*)- and (*R*,*S*)-**7** and for (*S*,*S*)- and (*R*,*S*)-**7a**, gave (*R*,*R*)- and (*S*,*R*)-**7** and the corresponding normethyl analogues (*R*,*R*)- and (*S*,*R*)-**7a**.

BIOLOGY

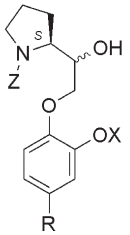
Binding Studies. The synthesized compounds were tested in vitro on rat cerebral cortex to evaluate their affinity for the central nicotinic receptors; [^{3}H]-epibatidine and [^{125}I]-αBungarotoxin were used as radioligand to label $\alpha4\beta2$ and $\alpha7$ receptors, respectively. Nicotine was included in the series for comparison. The results are listed in Table 1, together with those previously reported for the stereoisomers of **1** and **1a**.[15] The compounds with the highest $\alpha4\beta2$ affinity were (*R*,*S*)-**7** (0.012 μM K_i) and (*S*,*S*)-**7** (0.421 μM K_i), and their affinity for the $\alpha7$ subtype was respectively 35-fold (0.427 μM K_i) and 47-fold (20 μM K_i) lower than that for the $\alpha4\beta2$ subtype. Both compounds were also tested for their affinity for the human $\alpha3\beta4$ subtype: (*R*,*S*)-**7** showed 26-fold lower affinity (0.310 μM K_i) than that determined for the $\alpha4\beta2$ subtype, whereas (*S*,*S*)-**7** had very similar affinity for the $\alpha3\beta4$ (0.698 μM K_i) and $\alpha4\beta2$ subtype (0.421 μM K_i). As (*R*,*S*)-**7** had the greatest affinity and selectivity for the $\alpha4\beta2$ subtype, we also studied its affinity for the

Scheme 1. Synthesis of the *R*,*S* and *S*,*S* Stereoisomers of the Intermediates 17−21[a]



(*S*)-**12**	R = CHO	X = Bn	Z = Boc
(*S*)-**13**	$COCH_3$	Bn	Cbz
(*S*)-**14**	Br	MEM	Cbz
(*S*)-**15**	Ph	Bn	Boc
(*S*)-**16**	OBn	Bn	Boc

a



(*R*,*S*)/(*S*,*S*)-**17**	R = CH_2OH	X = Bn	Z = Boc
(*R*,*S*)/(*S*,*S*)-**18**	$COCH_3$	Bn	Cbz
(*R*,*S*)-**19**	Br	MEM	Me
(*S*,*S*)-**19**	Br	MEM	Me
(*R*,*S*)-**20**	Ph	Bn	Boc
(*S*,*S*)-**20**	Ph	Bn	Boc
(*R*,*S*)-**21**	OBn	Bn	Boc
(*S*,*S*)-**21**	OBn	Bn	Boc

[a] Reagents and conditions: (a) $NaBH_4$ or $LiAlH_4$, THF, rt.

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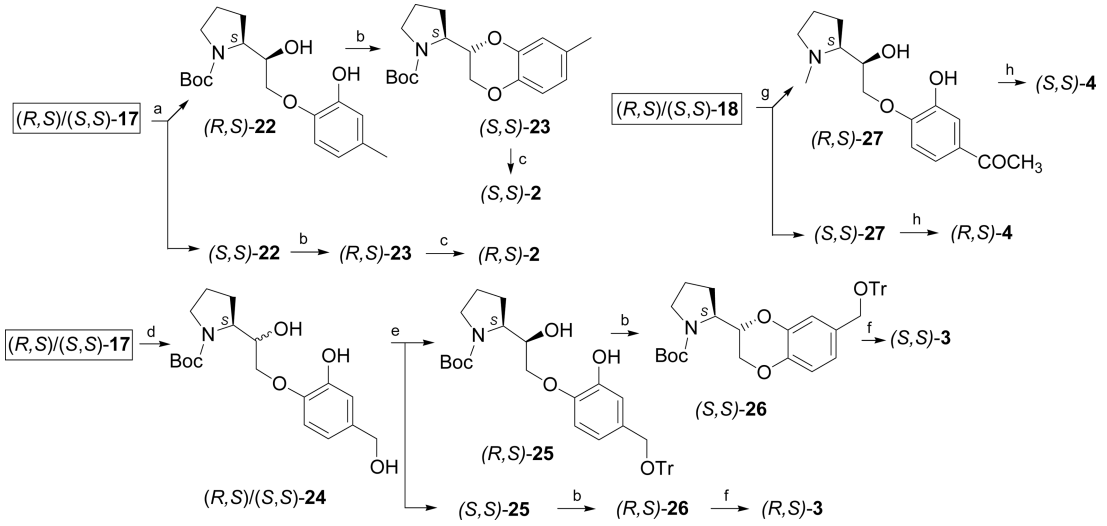
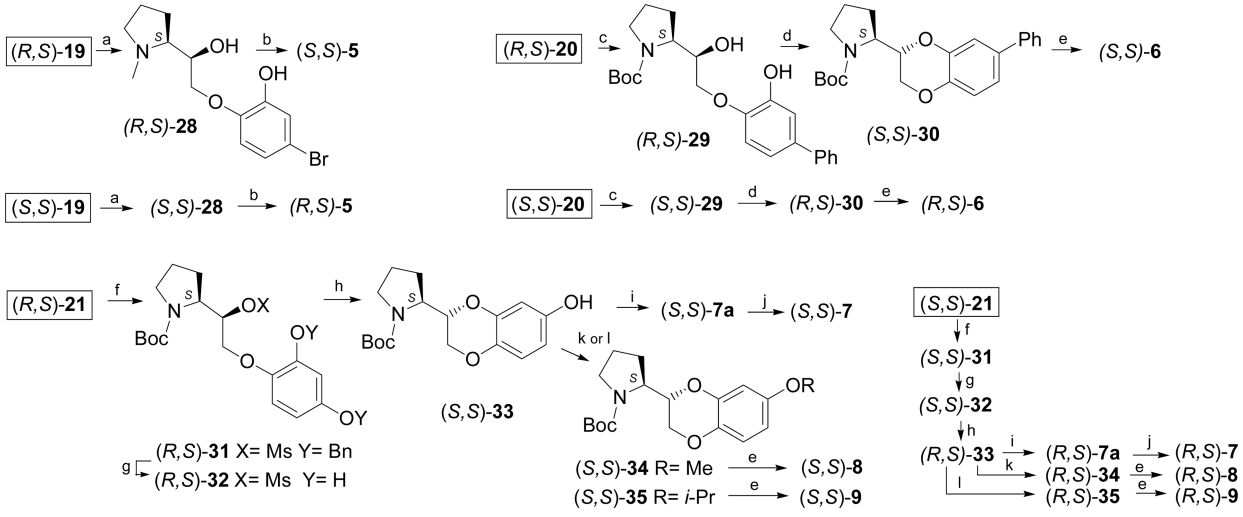
Scheme 2. Synthesis of the *R,S* and *S,S* Stereoisomers of Compounds 2−4[a]



[a] Reagents and conditions: (a) H_2, Pd/C, MeOH, rt, 24 h; (b) $(Ph)_3P$, DIAD, THF, 100 °C, 10 min, MW; (c) $LiAlH_4$, THF, reflux, 3 h; (d) H_2, Pd/C, MeOH, rt, 1 h; (e) TrCl, TEA, DME, reflux 16 h; (f) $LiAlH_4$, THF, reflux, 2 h then HCl 1M, MeOH, rt, 3 h; (g) H_2, Pd/C, CH_2O, MeOH, rt, 16 h; (h) Ph_3P, reflux, or $(CH_3)_3P$, rt, DEAD, THF, 16 h.

Scheme 3. Synthesis of the *R,S* and *S,S* Stereoisomers of Compounds 5−9[a]



[a] Reagents and conditions: (a) CF_3COOH, DCM, rt, 30 min; (b) $(CH_3)_3P$, DEAD, THF, rt, 24 h; (c) H_2, Pd/C, MeOH, rt, 48 h; (d) $(CH_3)_3P$, DEAD, THF, 100 °C, 10 min, MW; (e) $LiAlH_4$, THF, reflux, 1−2 h; (f) MsCl, TEA, DCM, rt, 5 h; (g) H_2 5 atm, Pd/C, MeOH, rt, 16 h; (h) K_2CO_3, DME reflux 6 h, (i) CF_3COOH, DCM, rt, 2 h; (j) H_2 Pd/C, CH_2O, MeOH, rt, 16 h; (k) DMC, DBU, 90 °C, 16 h; (l) NaH, *i*-propyl bromide, DME, 60 °C, 16 h.

native immunoimmobilized $\alpha6\beta2^*$ subtype, which was 0.013 μM K_i, and almost identical to that for the $\alpha4\beta2$ subtype (Figure 1).

In Vitro Functional Activity at nAChR. The affinity measurements indicate that (*R,S*)-7 has a high affinity for $\alpha4\beta2$ and $\alpha6\beta2^*$ receptors but give no indication of its possible functional activity. To study the functional response of (*R,S*)-7, we tested its ability to induce [^{3}H]DA release from striatal slices using an in vitro 96-well plate filter assay[19] and compared its functional profile with that of the full agonist nicotine.

As shown in Figure 2, both nicotine (EC_{50} 67 nM; $pEC_{50} \pm$ SE, 7.17 ± 0.04) and (*R,S*)-7 (EC_{50} 82 nM; $pEC_{50} \pm$ SE, 7.08 ± 0.07) induced [^{3}H]DA release from striatal slices in a dose-dependent manner and with similar potency, but the intrinsic agonist efficacy of (*R,S*)-7 was only 51% of that of nicotine. The $\alpha4\beta2$ and $\alpha6\beta2$ subtypes each contribute 50% of the nicotine-induced striatal [^{3}H]DA release[20] and, as the binding studies showed that (*R,S*)-7 also binds the native $\alpha6\beta2^*$ subtype, we quantified its effect on [^{3}H]DA release mediated by the $\alpha6\beta2^*$ subtype. To this end, striatal slices were incubated with 100 nM αconotoxin MII (α-MII), a specific antagonist of the $\alpha6\beta2^*$ subtype. Figure 2 shows that the presence of α-MII led to a further decrease in the ability of (*R,S*)-7 to induce [^{3}H]DA release with almost no change in the potency

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Table 1. Nicotine and Compounds 1−9: Affinity for Native Receptor Subtypes, Present in Rat Brain Membranes, Labeled by [^{3}H]-Epibatidine, and [^{125}I]-αBungarotoxin, and Docking Scores (kcal/mol) in a α4β2 Nicotinic Receptor Model[a]

	α4β2 nAChR [^{3}H]-Epi K_i (μM)	α7 nAChR [^{125}I]-αBgtx K_i (μM)	energy (kcal/mol)		α4β2 nAChR [^{3}H]-Epi K_i (μM)	α7 nAChR [^{125}I]-αBgtx K_i (μM)	energy (kcal/mol)
nicotine	0.004 (18)	0.234 (29)					
(*R*,*S*)-**1**	0.26 (32)	21 (44)	−30.21	(*R*,*S*)-**6**	35 (20)	36 (45)	−24.97
(*S*,*S*)-**1**	0.47 (30)	14.6 (54)	−28.45	(*S*,*S*)-**6**	3.1 (23)	51 (45)	−28.05
(*S*,*R*)-**1**	12.5 (17)	35 (45)		(*R*,*S*)-**7**	0.012 (13)	0.427 (34)	−40.86
(*R*,*R*)-**1**	43.8 (16)	14.8 (35)		(*S*,*S*)-**7**	0.421 (30)	20 (36)	−30.43
(*R*,*S*)-**1a**	2.1 (30)	36 (41)		(*S*,*R*)-**7**	3.5 (9)	4.5 (72)	−17.75
(*S*,*S*)-**1a**	14.4 (32)	32.2 (45)		(*R*,*R*)-**7**	3 (7)	1.3 (63)	−21.67
(*S*,*R*)-**1a**	88 (18)	14.7 (42)		(*R*,*S*)-**7a**	9 (26)	33 (34)	−27.78
(*R*,*R*)-**1a**	11.6 (17)	27 (38)		(*S*,*S*)-**7a**	1.2 (20)	53 (36)	−25.00
(*R*,*S*)-**2**	42 (19)	126 (50)	−24.01	(*S*,*R*)-**7a**	18 (24)	35 (70)	−16.83
(*S*,*S*)-**2**	5.7 (21)	64 (43)	−27.99	(*R*,*R*)-**7a**	1.3 (9)	3 (71)	−19.42
(*R*,*S*)-**3**	51 (19)	60 (79)	−24.41	(*R*,*S*)-**8**	17 (12)	32 (38)	−24.43
(*S*,*S*)-**3**	9.5 (19)	12 (41)	−27.05	(*S*,*S*)-**8**	4.2 (9)	26 (43)	−27.56
(*R*,*S*)-**4**	97 (21)	110 (51)	−25.24	(*R*,*S*)-**9**	9.3 (14)	6.1 (32)	−24.71
(*S*,*S*)-**4**	11.7 (10)	30 (50)	−28.91	(*S*,*S*)-**9**	3.2 (10)	43 (42)	−27.79
(*R*,*S*)-**5**	8.1 (10)	22 (42)	−23.21				
(*S*,*S*)-**5**	3 (10)	22 (43)	−28.91	K_d (nM)	0.036 (22)	0.3 (35)	

[a] The K_d and K_i values were derived from [^{3}H]-epibatidine and [^{125}I]-αBungarotoxin saturation and two competition binding experiments on rat brain membranes as described in ref 23. The curves were fitted using a nonlinear least-squares analysis program and the *F* test. The numbers in brackets represent the %CV. The affinities of compounds **1** and **1a** are those previously reported in ref 15.

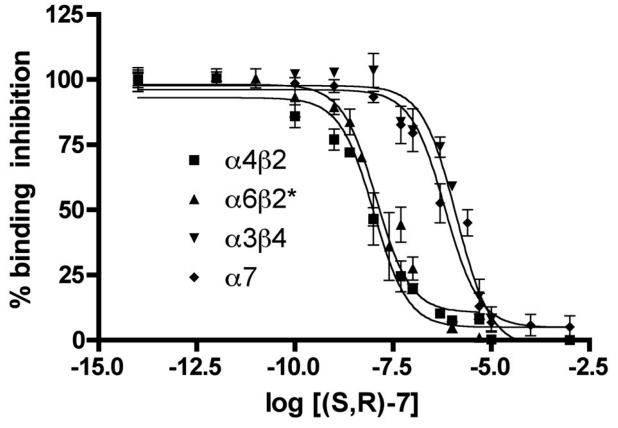

Figure 1. Inhibition of [^{3}H]-epibatidine binding to the α4β2, α6β2*, and α3β4 subtypes and of [^{125}I]-αBungarotoxin binding to the α7 subtype by increasing concentration of (*R*,*S*)-7. (*R*,*S*)-7 was preincubated with the indicated subtypes for 5 min, and then [^{3}H]-epibatidine 100 pM (for α4β2 and α6β2*) or 250 pM (α3β4) or 1 nM [^{125}I]-αBungarotoxin (α7) was added and left overnight at 4 °C. The K_i values were determined by fitting three separate experiments using LIGAND program.[14] In each experiment, each dilution of the drug was tested in triplicate. All of the values are expressed in relation to [^{3}H]-epibatidine-specific binding to α4β2, α6β2*, and α3β4 receptors and [^{125}I]-αBungarotoxin to α7 receptors (considered as 100%). The curves were generated using Prism 4.0 (GraphPad, San Diego, CA).

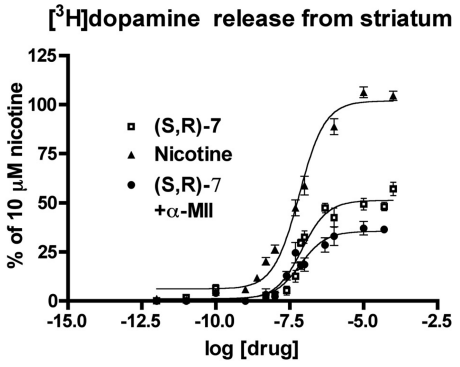

Figure 2. Concentration−response curves of agonist-induced [^{3}H]DA release from rat striatal slices. The slices were preloaded with 100 nM [^{3}H]DA and exposed to the indicated concentration of nicotine, (*R*,*S*)-7 alone, or in the presence of 100 nM αMII. Results were normalized and expressed as a percentage of 10 μM nicotine-induced release. The data points represent the mean SEM of 3−4 separate experiments performed in triplicate.

(EC_{50} of 72 nM; pEC_{50} ± SE, 7.14 ± 0.11). These results indicate that (*R*,*S*)-7 induces [^{3}H]DA release by acting as a partial agonist on both α4β2 and α6β2* subtypes. As a control, we also tested the effect of varenicline, a cytisine derivative recently introduced in the treatment of smoking cessation,[21] and, in agreement with previously published data,[22] found that it is a partial agonist with an intrinsic agonist efficacy that is 49% of that of 10 μM nicotine and an EC_{50} of 46 nM, which is very similar to that of (*R*,*S*)-7. To study the functional effect of (*R*,*S*)-7 on the α3β4 subtype, we tested its ability to induce [^{3}H]NA release, which is mediated by the α3β4 subtype in rat. We found that (*R*,*S*)-7 is able to induce [^{3}H]NA release with an EC_{50} of 17.6 μM (pEC_{50} ± SE, 4.75 ± 0.09) and an efficacy similar to that of 100 μM nicotine. In line with the finding of binding studies showing low affinity for the α3β4 subtype, this functional assay showed that (*R*,*S*)-7 is less potent on this subtype than on β2-containing receptors but similarly efficacious as nicotine, thus acting as a full agonist.

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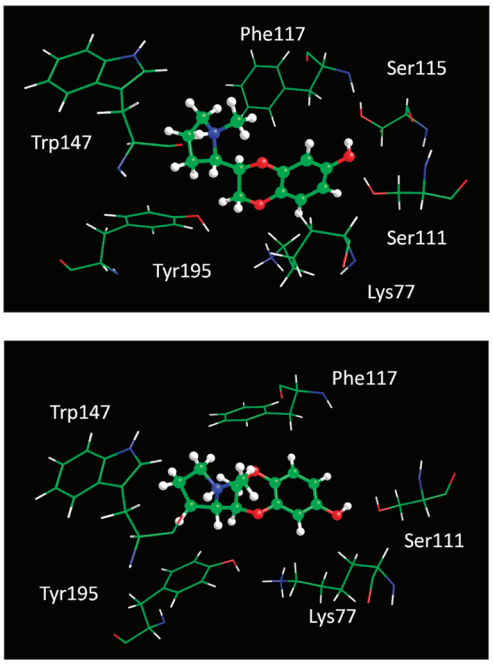

Figure 3. Main polar contacts stabilizing the putative complexes between $\alpha4\beta2$ nAChR binding site and (*R*,*S*)-7 (upper panel) and (*S*,*S*)-7 (lower panel).

DOCKING

As detailed under Computational Methods, the proposed nicotinoids were investigated by a docking analysis with the available rat $\alpha4\beta2$ model (PDB ID: 1OLE),[24] the chiral protonated tertiary nitrogen of pyrrolidine being in *S* configuration. The Figure 3 compares the best obtained complexes for (*R*,*S*)-7 and (*S*,*S*)-7. The protonated nitrogen atom of both stereoisomers stabilizes a reinforced H-bond with the carbonyl group of Trp147($\alpha4$), while the carbon atoms of the pyrrolidine ring contact some apolar residues not displayed for clarity [e.g., Val109($\beta2$), Phe117($\beta2$), Leu119($\beta2$), and Tyr195($\alpha4$)]. Similarly to what already observed for unsubstituted compounds (*R*,*S*)-**1** and (*S*,*S*)-**1**, the 7-hydroxy derivatives (*R*,*S*)-7 and (*S*,*S*)-7 differently arrange their benzodioxane ring because the pivotal H-bonds with Lys77($\beta2$) and Tyr195($\alpha4$) are elicited by O(1) in (*S*,*S*)-7 and by O(4) in (*R*,*S*)-7, thus resulting in a beneficially greater distance between mutually repulsive Lys77 and pyrrolidine nitrogen in the latter isomer. In addition, the (*R*,*S*)-7 isomer is able to insert the hydroxyl function in a very narrow subpocket where it can elicit two additional H-bonds with the side chain of Ser111($\beta2$) and Ser115($\beta2$). Conversely, the less affinitive (*S*,*S*)-7 isomer arranges the hydroxyl function in a different region quite distant from the mentioned serine residues, where it cannot stabilize significant polar interactions.

The sequence alignment, as computed for rat $\alpha4$, $\alpha3$, $\alpha6$, $\alpha7$, $\beta2$, and $\beta4$ subunits and reported in Figure S1 (Supporting Information), shows that the main residues interacting with the charged pyrrolidine and the benzodioxane oxygen atom (i.e., Trp147 and Tyr195) belong to highly conserved regions, while Ser111 and Ser115 belong to a very variable region. In particular, none of the aligned sequences conserves serine residues corresponding to Ser111 and Ser115 of $\beta2$ subunit apart from $\beta4$, which shows a conserved serine residue corresponding to Ser115 (i.e., Ser119 in $\beta4$ subunit). This suggests that the first residues can contribute to ligand potency only, while the $\beta2$ serine residues can concur to ligand selectivity, confirming that, as a rule, selectivity can be increased by reinforcing the contacts with the less conserved regions of $\beta2$ subunit.

The narrowness of the subpocket harboring the hydroxyl function prevents a suitable pose of the other selected substituents. As an example, Figure S2 (Supporting Information) reports the best complex obtained for (*R*,*S*)-**4**. Although the carbonyl of the acetyl substituent of (*R*,*S*)-**4** seems to conserve a weak contact with Ser111, the methyl of the same substituent clashes against the side chains of the mentioned serine residues, thus inducing a detrimental distortion of the entire binding site and preventing the key polar contacts between ligand and receptor as described for (*R*,*S*)-7.

Figure S2 shows what happens when the benzodioxane configuration of (*R*,*S*)-**4** is inverted. All the contacts described for (*S*,*S*)-7 are conserved by (*S*,*S*)-**4**, while the acetyl substituent is inserted into a less stringent region, which roughly corresponds to the entrance of the binding cavity. Here, the acetyl does not elicit significant interactions and at least conserves a weak H-bond with Ser111.

Taken together, docking results confirm that the subpocket lined by the two serine residues can be contacted only by very small and polar 7-substituents able to both accept and donate H-bonds and born by benzodioxane in *R* configuration. Conversely, the adjacent region, in which the 7-substituents of the *S*,*S* isomers are harbored, is surely wider and more tolerant but unable to stabilize strong interactions with the ligand.

Docking results on the other two stereoisomers of the hydroxyl derivative, (*S*,*R*)-7 and (*R*,*R*)-7, as well as on the four stereoisomers of nor-methyl **7a**, emphasize the critical role of an optimal arrangement of protonated pyrrolidine to elicit the required H-bond with Trp147($\alpha4$). Indeed, (*S*,*R*)-7 and (*R*,*R*)-7 unfavorably contact Trp147($\alpha4$) with the *N*-methyl group, while a repulsive interaction of one of the two nitrogen protons of **7a** stereoisomers with Lys77 cannot be avoided. Such a detrimental approach, exacerbated by the rigidity of these molecules, is shown also by the *N*-methyl derivatives, when the protonated nitrogen is in *R* configuration, but it is bypassed thanks to the possibility of inverting the nitrogen chirality.

Docking scores, as computed by an electrostatic interaction energy with distance-dependent dielectric constant and compiled in Table 1, are in line with the above observation: (a) (*R*,*S*)-7 shows the largely best score, (b) (*R*,*S*)-**1** has an intermediate score, (c) the *R*,*S* isomers of the other 7-substituted derivatives display energy scores markedly worse than unsubstituted (*R*,*S*)-**1**, (d) all the 7-substituted *S*,*S* isomers, irrespective of the substituent, report very similar scores, which are in turn similar to that of unsubstituted (*S*,*S*)-**1**, (e) the score differences between the four isomers of **7** are remarkable, and (f) the docking energies of all the **7a** are scarce independently on the configurations. Finally, docking energies address the importance of protonation induced chirality because the two most affinitive (*R*,*S*)-7 and (*S*,*S*)-7 show quite poor scores (−21.87 and −18.45 kcal/mol, respectively) when considered with the charged nitrogen in *R* configuration.

DISCUSSION

The $\alpha4\beta2$ affinity and selectivity of (*R*,*S*)-**1** assimilate benzodioxane to other bicycles, which show analogous nicotinic profiles when bearing 2-pyrrolidinyl, such as quinoline,[6] furopyridine,[25] and

chroman.[26] In the case of 1,4-benzodioxan-2-yl group, the presence of the stereocenter and the interaction potential of both oxygen atoms as HBAs are additional features, whose combined influence has to be considered. For the unsubstituted N-methylpyrrolidinyl-benzodioxane **1**, we had previously argumented that the presence of the two dioxane oxigens seems to obscure the role of dioxane chirality. Indeed, the inversion of dioxane C(2) of (*R*,*S*)-**1**, the most $\alpha4\beta2$ affinitive stereoisomer of **1**, having *R* configuration at the dioxane stereocenter and *S* at the pyrrolidine C(2), slightly affects the $\alpha4\beta2$ affinity (cf. 0.26 μM K_i of (*R*,*S*)-**1** and 0.47 μM K_i of (*S*,*S*)-**1**), while inversion of pyrrolidine configuration results in a 170-fold decrease (cf. 0.26 μM K_i of (*R*,*S*)-**1** and 43.8 μM K_i of (*R*,*R*)-**1**). Consistently with these data, docking analysis had shown different, but ever productive accommodations for the dioxane ring in both the configurations thanks to the capability of interacting with the same amino acids through O(4) or, in alternative, through O(1), whereas pyrrolidine ring can be correctly arranged in the binding pocket only when its C(2) has *S* configuration.[15]

These results naturally proposed pyrrolidinylbenzodioxane for the same investigations on the effect of substituents at the aromatic ring on the nicotinic affinity already made for the phenyl and pyridyl ethers of prolinol, which are well-known nicotinoids.[27−29] Rigidity of the whole molecule, on the one hand, and chirality of the HBA/π group and presence of two potential HBAs in it, on the other hand, made such an investigation particularly attractive. The same substituent would differently affect affinity in dependence on these factors, increasing the chance of selecting substituted derivatives which optimally fit into the receptor binding site. Moreover, any ameliorating substitution would be highly informative for the binding site structure and pharmacophore modeling. However, consideration that four stereoisomers of a differently substituted 2-(2-phenoxy-1-hydroxyethyl)pyrrolidyne had to be synthesized for each selected substituent or substitution pattern forced us to limit the study to the diastereomeric pairs with *S* configuration at the pyrrolidine stereocenter and to monosubstitution, in particular at the benzodioxane C(7). The former choice was suggested by the sensibly higher $\alpha4\beta2$ affinities of such diastereoisomers in the case of the unsubstituted lead **1**. The latter one was inspired by the SAR studies on the phenyl and pyridyl ethers of prolinol, indicating, on the basis of wide and systematic investigations, the substitutions at the *meta*-position to the methyleneoxy linker as the most productive ones.[28,29] Our docking analyses[15] on (*R*,*S*)-**1** had previously showed the proximity of benzodioxane C(7), corresponding to the above *meta*-carbon of pyridyl and phenyl ethers, to the hydroxyls of two serine residues (Ser111 and Ser 115), belonging, moreover, to $\beta2$ subunit, which affords the minus side of the binding site. The presence of these two functions indicated OH, a both HBA and HBD group, as the first-choice substituent, although, quite surprisingly, the reported long series of phenyl- and pyridine-modified analogues of phenyl and pyridyl ethers of prolinol had never enclosed any hydroxyphenyl or hydroxypyridyl ether. The increase of $\alpha4\beta2$ affinity from the 0.26 μM K_i value of unsubstituted (*R*,*S*)-**1** to 12 nM for the 7-OH substituted analogue (*R*,*S*)-**7** was consistent with the working hypothesis. The choice of the successive substituents, namely hydroxymethyl, methyloxy, isopropyloxy, acetyl, and methyl, was mainly aimed at verifying the importance of the postulated interactions between the benzodioxane OH and the two serine OH. Suppression of both HBA and HBD properties of the substituent (CH_3) as well as of only its HBD character ($COCH_3$, OCH_3, and $OCH(CH_3)_2$) with no or modest increase of its volume resulted in dramatically lowered affinities (see 10−100 μM K_i of (*R*,*S*)-**2**, (*R*,*S*)-**4**, (*R*,*S*)-**8**, and (*R*,*S*)-**9**). Also the simple homologation to CH_2OH was highly deleterious (see 51 μM K_i of (*R*,*S*)-**3**). After that, two substituents substantially different from OH, namely bromo and phenyl, were considered, but these substitutions were pejorative as well. Halo substitution, which is reported to enhance the affinity of prolinol phenyl-ethers[29] and not to affect that of prolinol 3-pyridyl ethers,[28] when accomplished in 3 and in 5, respectively, resulted in a 30-fold weaker affinity of the bromobenzodioxane derivative (cf. 8.1 μM K_i of (*R*,*S*)-**5** and 0.26 μM K_i of (*R*,*S*)-**1**). The 7-phenyl analogue (*R*,*S*)-**6** had 130-fold lower affinity compared to (*R*,*S*)-**1**.

Interestingly, the inversion of the configuration of the benzodioxane stereocenter produced opposite effects on the 7-hydroxy analogue compared to all the other 7-substituted analogues. Compound (*S*,*S*)-**7** possesses 35-fold weaker affinity than (*R*,*S*)-**7** and behaves as OH had no influence on the interaction, because its moderate submicromolar $\alpha4\beta2$ affinity is equal to that of unsubstituted (*S*,*S*)-**1**. On the contrary, all the *S*,*S* stereoisomers of the other substituted analogues show modest 1−10 μM $\alpha4\beta2$ affinities, which are however from 3- to 11-fold higher than the corresponding *R*,*S* stereoisomers. Furthermore, the substitutions at C(7) seem to be better tolerated than in the case of the *R*,*S* stereoisomers because their $\alpha4\beta2$ affinities are not so far from that of unsubstituted (*S*,*S*)-**1** as those of the *R*,*S* stereoisomers from (*R*,*S*)-**1**. Docking analysis well supports these results, showing how the substituent at C(7) of benzodioxane can be profitably settled in a narrow subpocket of the binding site only if it is small and capable of strongly and specifically interacting with the amino acidic counterpart and if the bicycle has *R* configuration at C(2). This is the case, unique in the *R*,*S* series, of the OH of (*R*,*S*)-**7** interacting with the two $\beta2$ serine residues. When, on the contrary, benzodioxane is in *S* configuration, different substituents can be accepted. However, the absence of tight interactions between them and the counterpart makes their contribution negligible (OH) or slightly negative (the other substituents), attenuating the diastereopreference. This latter is always for the *S*,*S* isomer, with the obvious exception of **7** due to the mentioned interactions proper only to the hydroxyl of its *R*,*S* isomer.

These observations prompted us to closely examine compound **7**. We decided to characterize all its four stereoisomers and, in addition, the four stereoisomers of its nor-methyl analogue **7a**.

In line with compounds **1** and **1a**, the modest $\alpha4\beta2$ affinities of (*S*,*R*)-**7** and (*R*,*R*)-**7** and of all the four stereoisomers of **7a** confirm that the ligand recognition is conditioned by the *S* configuration of the pyrrolidine stereocenter and by the presence of only one proton at positively charged nitrogen. These results, which can be satisfactorily rationalized with the aid of docking analysis, as discussed in detail in the previous paragraph, stress those obtained for compounds **1** and **1a**. Comparison of the $\alpha4\beta2$ affinity ratios makes evident that the 7-OH substitution significantly enhances the eudismic index of the *R*,*S*/*S*,*R* pair (cf. 2.46 of **7** with 1.68 of **1**), the epimeric eudismic index of the *R*,*S*/*S*,*S* and *R*,*S*/*R*,*R* pairs (cf. 1.55 and 2.40 of **7** with 0.26 and 2.23 of **1**, respectively) and the *N*-methyl/*N*-desmethyl affinity ratio (cf. 750 of (*R*,*S*)-**7**/(*R*,*S*)-**7a** with 8 of (*R*,*S*)-**1**/(*R*,*S*)-**1a**). These unidirectional increases unambiguously indicate that the introduction of the 7-OH substituent produces a tighter ligand−receptor interaction with more stringent steric demand.

All the tested pyrrolidinyl benzodioxanes have poor $\alpha7$ affinity; none of them reaches submicromolar values of K_i except for (*R*,*S*)-**7**, which however maintains a significant $\alpha4\beta2$ versus

$\alpha7$ selectivity, similar to that of nicotine and to those of (*S*,*S*)-**7**, (*S*,*S*)-**7a**, (*R*,*S*)-**1**, and (*S*,*S*)-**1**, namely the compounds with the highest $\alpha4\beta2$ affinities after (*R*,*S*)-**7**. The other compounds display both $\alpha4\beta2$ and $\alpha7$ modest affinities. These data indicate pyrrolidinyl benzodioxanes, unsubstituted or 7-OH substituted, as new $\alpha4\beta2$ selective nicotinoids. The presence of Ser113 in the $\alpha7$ subunit, although not aligned to Ser111 and Ser115 of the $\beta2$ subunit (Figure S1, Supporting Information), might explain the enhancement of $\alpha7$ affinity from 21 μM K_i of unsubstituted (*R*,*S*)-**1** to 0.427 μM K_i of 7-OH substituted (*R*,*S*)-**7** and, although the $\alpha3\beta4$ affinity of (*R*,*S*)-**1** is unavailable as a reference value, also the submicromolar $\alpha3\beta4$ affinity of (*R*,*S*)-**7** might be advantaged by interactions of benzodioxane OH with conserved Ser119, corresponding to Ser115 of $\beta2$ subunit (Figure S1, Supporting Information).

The further investigations on the neuropharmacological profile of (*R*,*S*)-**7** show that this compound has high affinity for native $\beta2$-containing receptors, both $\alpha4\beta2$ and $\alpha6\beta2$, and that this affinity is much higher not only than that for $\alpha7$ subtype but also than that for $\alpha3\beta4$ subtype. The $\alpha7$ receptor is localized in the central and peripheral nervous systems, where it is involved in many important physiological functions, whereas the $\alpha3\beta4^*$ subtype is predominant in the autonomic ganglia and adrenal medulla and its distribution in the CNS is very limited. In autonomic ganglia, $\alpha3\beta4^*$ receptors are located at presynaptic terminals, where they facilitate spontaneous and evoked Ach release, and at postsynaptic terminals, where they mediate ganglionic fast synaptic transmission and control the function of organs such as heart, intestine, and bladder. The low affinity of (*R*,*S*)-**7** is a promising characteristic, as it suggests that the compound will not have undesirable side effects due the $\alpha3\beta4$ receptors in the peripheral nervous system.

The results of these preliminary in vitro studies show that (*R*,*S*)-**7**, compared with nicotine, has similar potency and significantly less intrinsic activity on [^{3}H]DA release and that this is due to reduced intrinsic activity at both the $\alpha4\beta2$ and $\alpha6\beta2^*$ subtypes. Given the importance of DA release in the mesolimbic dopaminergic system in determining nicotine dependence, a partial agonist of $\alpha4\beta2$ and $\alpha6\beta2$ subtypes may be useful in the treatment of nicotine addiction. The pharmacological profile of (*R*,*S*)-**7** is very similar to that of varenicline, a currently marketed smoking cessation aid,[20] in terms of subtype specificity. (*R*,*S*)-**7** has a lower affinity for $\beta2$-containing receptors than varenicline, but it resulted in having the same intrinsic partial agonist activity and the same potency as varenicline when tested for [^{3}H]DA release.[22,30] The difference in the binding affinity of varenicline and (*R*,*S*)-**7** and their very similar functional potency may reflect a difference in their ability to desensitize the $\beta2$-containing receptors measured by binding studies.[22,30] Like varenicline, (*R*,*S*)-**7** may facilitate the extinction of behaviors associated with nicotine dependence by partially stimulating $\beta2$-containing receptors in the absence of nicotine while preventing nicotine from triggering a full response.

CONCLUSIONS

Introduction of OH substituent to C(7) of (2*R*,2′*S*)-2-(1′-methyl-2′-pyrrolidinyl)-1,4-benzodioxane increases $\alpha4\beta2$ nicotinic affinity from 0.26 to 0.012 μM K_i, maintaining the good $\alpha4\beta2$ vs $\alpha7$ selectivity of the parent unsubstituted compound. Other substituents at this position seem to be not tolerated and *R* and *S* configuration of the two stereocenters are strictly required for optimal interaction as well as *N* methylation. The rigidity of the pyrrolidinylbenzodioxane scaffold allows these results to be satisfactorily rationalized by docking in a $\alpha4\beta2$ receptor model. Furthermore, the high affinity of (2*R*,2′*S*)-7-hydroxy-2-(1′-methyl-2′-pyrrolidinyl)-1,4-benzodioxane also for the $\alpha6\beta2^*$ nicotinic subtype suggests that the 7-hydroxy-benzodioxane substructure is a HBA/π group capable of selectively promoting the interaction with nicotinic $\beta2$-containing receptors. The potent $\alpha4\beta2$ and $\alpha6\beta2$ partial agonism, together with the modest affinity and activity at the $\alpha3\beta4^*$ subtype, are worthy features of the title compound, which substantiate such a hypothesis.

EXPERIMENTAL SECTION

Chemistry. 1H NMR spectra were recorded operating at 300 MHz. Chemical shifts are given in parts per million relative to residual solvent ($CHCl_3$ or DMSO) as internal standard. Optical rotations were determined by a Jasco P-1010 polarimeter. HPLC analyses were performed on a Kromasyl Amycoat column using Hewlett-Packard 1050 instrument. Thermal analyses were performed on 2−5 mg samples in closed pans at 5 °C/min using DSC 2010 TA INSTRUMENTS. Elemental analyses (CHN) are within ±0.40% of theoretical values. Purifications were performed using KP-Sil 32−63 μm 60 Å cartridges and Merck silica gel (particle size 40−63 μm). The results of elemental analyses indicated that the purity of all tested compounds was higher than 95%. 2-Benzyloxyphenates *p*-substituted with CHO and OBn were prepared according to refs 17 and 18 and transformed into sodium salts by treatment of their solution in methanol with 1 M aqueous NaOH. The preparations of the sodium salts of 2-benzyloxyphenols *p*-substituted with Ph and $COCH_3$ are reported in the Supporting Information as well as the preparation of the sodium 2-MEM-4-bromophenate from 2-hydroxy-4-bromoacetophenone, in turn obtained from 3-bromophenol according to ref 16.

In each described preparation, the moles of reagents are given for one mole of substrate.

General Procedure for Synthesis of Compounds 12−16. The *S* and *R* isomers of target compounds **12**, **15**, and **16** were synthesized combining *N*-Boc protected (*S*)- and (*R*)-2-bromoacetylpyrrolidine with sodium 2-benzyloxyphenate *p*-substituted with CHO (**12**), Ph (**15**) and OBn (**16**). The *S* and *R* isomers of target compounds **13** and **14** were synthesized by combining *N*-Cbz protected (*S*)- and (*R*)-2-bromoacetylpyrrolidine with sodium 2-benzyloxy-4-acetylphenate (**13**) and 2-(2-methoxyethoxymethoxy)-4-bromophenate (**14**). All the reactions were carried out in acetone at room temperature using exceeding bromoketone (1.1 mol) with the exception of **15** (0.8 mol of bromoketone in DMF).

*(S)-N-Boc-2-(o-benzyloxy-p-formylphenoxyacetyl)pyrrolidine [(S)-**12**].* Obtained as an amorphous solid in 95% yield after chromatography on silica gel (cyclohexane/EtOAc 1:1): $[\alpha]_D^{25} = -35.1$ (*c* 1, $CHCl_3$), 99.35% ee (*n*-hexane/ethanol 8:2). ^{1}H NMR ($CDCl_3$) δ 9.85 (s, 0.33H), 9.82 (s, 0.67H), 7.52−7.27 (m, 7H), 6.98 (d, 0.66H, J = 8.25 Hz), 6.90 (d, 0.34H, J = 8.25 Hz), 5.18 (s, 2H), 4.98 (s, 1.35H), 4.81 (s, 0.65H), 4.60−4.55 (m, 1H), 3.38−3.56 (m, 2H), 2.18−1.77 (m, 4H), 1.47 (s, 5.4H), 1.41 (s, 3.6H).

*(S)-N-Cbz-2-(o-benzyloxy-p-acetylphenoxyacetyl)pyrrolidine [(S)-**13**].* Obtained as a white solid in 82% yield after chromatography on silica gel (cyclohexane/EtOAc 1:1): mp 91.8 °C; $[\alpha]_D^{25} = -21.5$ (*c* 1, $CHCl_3$); 97.51% ee (*n*-hexane/ethanol 8:2). ^{1}H NMR ($CDCl_3$) δ 7.61−7.52 (m, 2H), 7.47−7.26 (m, 10H), 6.86 (d, 0.6H, J = 8.53 Hz), 6.54 (d, 0.4H, J = 8.26 Hz), 5.18−5.01 (m, 4H), 4.90 (s, 1.25H), 4.71−4.62 (m, 1.75H), 3.59−3.49 (m, 2H), 2.54 (s, 1.87H), 2.52 (s, 1.13H), 2.17−2.11 (m, 1H), 1.97−1.77 (m, 3H).

*(S)-N-Cbz-2-(o-methoxyethoxymethoxy-p-bromophenoxyacetyl)-pyrrolidine [(S)-**14**].* Obtained as an amorphous solid in 80% yield after

chromatography on silica gel (cyclohexane/EtOAc 1:1): $[\alpha]_D^{25} = -17.7$ (*c* 1, MeOH); 98.62% ee (*n*-hexane/ethanol 8:2). ^{1}H NMR (DMSO 100 °C) δ: 7.40−7.20 (m, 6H), 7.11−7.01 (m, 1.5H), 6.82−6.70 (m, 1.5H), 5.19 (s, 2H), 5.08 (s, 2H), 4.82 (s, 2H), 4.68−4.60 (m, 1H), 3.80−3.71 (m, 2H), 3.52−3.40 (m, 4H), 3.22 (s, 3H), 2.25−2.09 (m, 1H), 1.96−1.76 (m, 3H).

*(S)-N-Boc-2-(o-benzyloxy-p-phenylphenoxyacetyl)pyrrolidine [(S)-**15**].* Obtained as an amorphous solid in 58% yield after chromatography on silica gel (toluene/EtOAc 8:2): $[\alpha]_D^{25} = -25.3$ (*c* 1, $CHCl_3$); 97.67% ee (*n*-hexane/ethanol 8:2). ^{1}H NMR ($CDCl_3$) δ 7.54−7.28 (m, 10H), 7.20−7.11 (m, 2H), 6.97−6.93 (m, 1H), 5.19 (s, 2H), 4.89 (s, 1H), 4.76 (s, 1H), 4.68−4.60 (m, 1H), 3.52−3.40 (m, 2H), 2.20−2.09 (m, 1H), 1.96−1.76 (m, 3H), 1.47 (s, 4.5 H), 1.40 (s, 4.5 H).

*(S)-N-Boc-2-(o,p-dibenzyloxyphenoxyacetyl)pyrrolidine [(S)-**16**].* Obtained as an oil in 67% yield after chromatography on silica gel (tolune/EtOAc 95:5): $[\alpha]_D^{25} = -12.7$ (*c* 1, MeOH); 99.42% ee (*n*-hexane/*i*-propyl alcohol 8:2). ^{1}H NMR ($CDCl_3$) δ 7.28−7.43 (m, 10H), 6.85 (m, 1H), 6.64 (m, 1H), 6.46 (m, 1H), 4.76 (s, 2H), 5.06 (s, 1H), 4.98 (s, 1H), 4.76 (s, 1H), 4.65 (s, 1H), 4.55−4.60 (m, 1H), 3.37−3.50 (m, 2H), 2.04−2.17 (m, 1H), 1.70−1.92 (m, 3H), 1.45 (s, 4.5H), 1.36 (s, 4.5H).

*(R)-N-Boc-2-(o-benzyloxy-p-formylphenoxyacetyl)pyrrolidine [(R)-**12**].* $[\alpha]_D^{25} = +35.4$ (*c* 1, $CHCl_3$); 97.52% ee.

*(R)-N-Cbz-2-(o-benzyloxy-p-aceylphenoxyacetyl)pyrrolidine [(R)-**13**].* $[\alpha]_D^{25} = +20.1$ (*c* 1, $CHCl_3$); 97.42% ee.

*(R)-N-Cbz-2-(o-methoxyethoxymethoxy-p-bromophenoxyacetyl)pyrrolidine [(R)-**14**].* $[\alpha]_D^{25} = +18.2$ (*c* 1, MeOH); 99.35% ee.

*(R)-N-Boc-2-(o-benzyloxy-p-phenylphenoxyacetyl)pyrrolidine [(R)-**15**].* $[\alpha]_D^{25} = +25.2$ (*c* 1, $CHCl_3$); 98.01% ee.

*(R)-N-Boc-2-(o,p-dibenzyloxyphenoxyacetyl)pyrrolidine [(R)-**16**].* $[\alpha]_D^{25} = +12.7$ (*c* 1, MeOH); 99.64% ee.

General Procedure for Synthesis of Compounds 17−21. The target compounds were obtained by treatment of the *S* isomers of **12**−**16** and of (*R*)-**16** with 2 mol of $NaBH_4$ (**17**, **18**, and **21**), 3 mol of $NaBH_4$ (**20**), or 2 mol of $LiAlH_4$ (**19**) in THF. The reactions were carried out at temperatures between −10 and −20 °C except for **21** (room temperature) for 0.5−3 h. The diastereomeric mixture of **17** and **18** were not resolved.

*(1R,2′S)- and (1S,2′S)-1-(1′-Boc-2′-pyrrolidinyl)-2-(o-benzyloxy-p-hydroxymethylphenoxy)ethanol [(R,S)/(S,S)-**17**].* Obtained as a crude mixture of diastereoisomers in 85% yield. ^{1}H NMR ($CDCl_3$) δ 7.46−7.29 (m, 5H), 6.99−6.87 (m, 3H), 5.09 (s, 1H), 5.07 (s, 1H), 5.01 (bs, 1H), 4.59 (s, 1H), 4.57 (s, 1H), 4.18−3.91 (m, 4H), 3.50−3.34 (m, 1H), 3.32−3.23 (m, 1H), 2.18−1.70 (m, 4H), 1.47 (s, 4.5H), 1.45 (s, 4.5H).

*(1R,2′S)- and (1S,2′S)-1-(1′-Cbz-2′-pyrrolidinyl)-2-(o-benzyloxy-p-acetylphenoxy)ethanol [(R,S)/(S,S)-**18**].* Obtained as a diastereomeric mixture in 88% yield after removal of the *p*-hydroxyethyl analogue by chromatography on silica gel (cyclohexane/EtOAc 1:1). ^{1}H NMR ($CDCl_3$) δ 7.59−7.55 (m, 2H), 7.46−7.26 (m, 10H), 6.96−6.88 (m, 1H), 5.20−5.10 (m, 4H), 4.73 (bs, 1H), 4.27−3.85 (m, 3H), 3.64−3.30 (m, 2H), 2.54 (s, 3H, CH_3), 2.24−2.17 (m, 1H), 2.15−1.82 (m, 2H), 1.76−1.69 (m, 1H).

*(1R,2′S)-1-(1′-Methyl-2′-pyrrolidinyl)-2-(o-methoxyethoxymethoxy-p-bromophenoxy)ethanol [(R,S)-**19**].* Obtained as an oil in 35% yield after chromatography on silica gel (DCM/MeOH/30%NH_3 95:5:2.5): $[\alpha]_D^{25} = -21.2$ (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.28 (d, 1H, *J* = 2.2 Hz), 7.06 (dd, 1H, *J* = 2.2 and 8.53 Hz), 6.79 (d, 1H, *J* = 8.53 Hz), 5.25 (s, 2H), 3.99−3.90 (m, 2H), 3.86−3.83 (m, 2H), 3.81−3.77 (m, 1H), 3.58−3.55 (m, 2H), 3.38 (s, 3H), 3.11−3.07 (m, 1H), 2.95−2.82 (bs, 1H), 2.72−2.68 (m, 1H), 2.45 (s, 3H), 2.39−2.33 (m, 1H), 1.98−1.68 (m, 4H).

*(1S,2′S)-1-(1′-Methyl-2′-pyrrolidinyl)-2-(o-methoxyethoxymethoxy-p-bromophenoxy)ethanol [(S,S)-**19**].* Obtained as an oil in 29% yield after chromatography on silica gel (DCM/MeOH/30%NH_3 95:5:2.5): $[\alpha]_D^{25} = -19.1$ (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.30 (d, *J* = 2.20 Hz, 1H), 7.06 (dd, 1H, *J* = 2.20 and 8.53 Hz), 6.80 (d, 1H, *J* = 8.53 Hz), 5.26 (s, 2H), 4.16−4.11 (m, 1H), 4.04−3.98 (m, 1H), 3.92−3.84 (m, 3H), 3.57−3.54 (m, 2H), 3.38 (s, 3H), 3.13−3.07 (m, 1H), 3.02−2.82 (bs, 1H), 2.46−2.38 (m, 1H), 2.36 (s, 3H,), 2.32−2.23 (m, 1H), 1.87−1.77 (m, 4H).

*(1R,2′S)-1-(1′-Boc-2′-pyrrolidinyl)-2-(o-benzyloxy-p-phenylphenoxy) ethanol [(R,S)-**20**].* Obtained as an oil in 39% yield after chromatography on silica gel (toluene/EtOAc 8:2): $[\alpha]_D^{25} = -27.3$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.52−7.28 (m, 10H), 7.26−7.17 (m, 2H), 7.03 (d, 1H, *J* = 8.26 Hz), 5.17 (s, 2H), 5.02 (bs, 1H), 4.16−4.02 (m, 3H), 4.00−3.82 (m, 1H), 3.40−3.20 (bs, 1H), 3.22−3.19 (bs, 1H), 2.04−1.69 (m, 4H), 1.48 (s, 9H).

*(1S,2′S)-1-(1′-Boc-2′-pyrrolidinyl)-2-(o-benzyloxy-p-phenylphenoxy) ethanol [(S,S)-**20**].* Obtained as an oil in 50% yield after chromatography on silica gel (toluene/EtOAc 8:2): $[\alpha]_D^{25} = -43.3$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.57−7.29 (m, 10H), 7.18−7.14 (m, 2H), 7.01 (d, 1H, *J* = 7.98 Hz), 5.15 (s, 2H), 4.56 (bs, 1H), 4.20−3.90 (m, 4H), 3.48−3.37 (m, 1H), 3.30−3.19 (m, 1H), 2.20−2.12 (m, 1H), 2.05−1.62 (m, 3H), 1.46 (s, 9H).

*(1R,2′S)-1-(1′-Boc-2′-pyrrolidinyl)-2-(o,p-dibenzyloxyphenoxy)ethanol [(R,S)-**21**].* Obtained as an oil in 49% yield after chromatography on silica gel (toluene/EtOAc 85:15): $[\alpha]_D^{25} = -35.8$ (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.24−7.42 (m, 10H), 6.86 (d, 1H, *J* = 8.81 Hz), 6.63 (d, 1H, *J* = 2.75 Hz), 6.49 (dd, 1H, *J* = 8.81 and 2.75 Hz), 4.99 (s, 2H), 5.06 (s, 2H), 4.08−3.90 (m, 4H), 3.47−3.39 (m, 1H), 3.27−3.18 (m, 1H), 2.17−2.08 (m, 1H), 1.93−1.65 (m, 3H,), 1.44 (s, 9H).

*(1S,2′S)-1-(1′-Boc-2′-pyrrolidinyl)-2-(o,p-dibenzyloxyphenoxy)ethanol [(S,S)-**21**].* Obtained as an oil in 27% yield after chromatography on silica gel (toluene/EtOAc 85:15): $[\alpha]_D^{25} = -17.4$ (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.27−7.45 (m, 10H), 6.90 (d, 1H, *J* = 8.80 Hz), 6.64 (d, 1H, *J* = 2.75 Hz), 6.50 (dd, 1H, *J* = 8.80 and 2.75 Hz), 5.07 (s, 2H), 5.00 (s, 2H), 3.88−4.08 (m, 4H), 3.42−3.50 (m, 1H), 3.25−3.32 (m, 1H), 1.80−2.03 (m, 3H), 1.65−1.77 (m, 1H), 1.46 (s, 9H).

*(1R,2′R)-1-(1′-Boc-2′-pyrrolidinyl)-2-(o,p-dibenzyloxyphenoxy)ethanol [(R,R)-**21**].* Obtained as an oil in 31% yield after chromatography on silica gel (toluene/EtOAc 85:15): $[\alpha]_D^{25} = +14.8$ (*c* 1, MeOH). ^{1}H NMR identical to (*S*,*S*)-**21**.

*(1S,2′R)-1-(1′-Boc-2′-pyrrolidinyl)-2-(o,p-dibenzyloxyphenoxy)ethanol [(R,S)-**21**].* Obtained as an oil in 49% yield after chromatography on silica gel (toluene/EtOAc 85:15): $[\alpha]_D^{25} = +36.2$ (*c* 1, MeOH). ^{1}H NMR identical to (*S*,*R*)-**21**.

(1*R*,2′*S*)-1-(1′-Boc-2′-pyrrolidinyl)-2-(*o*-hydroxy-*p*-methylphenoxy)ethanol [(*R*,*S*)-22]. Obtained as an oil in 35% yield by hydrogenation (H_2 1 atm, 10% Pd(C)) of (*R*,*S*)/(*S*,*S*)-**17** in methanol for 24 h and chromatography on silica gel (toluene/EtOAc 8:2): $[\alpha]_D^{25} = -76.0$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.88 (bs, 1H), 6.79 (d, 1H, *J* = 7.90 Hz), 6.75 (s, 1H), 6.57 (d, 1H, *J* = 7.90 Hz), 6.27 (bs, 1H), 4.07−4.00 (m, 2H,), 3.96−3.90 (m, 1H), 3.82−3.76 (m, 1H), 3.60−3.44 (m, 1H,), 3.38−3.25 (m, 1H), 2.25 (s, 3H), 2.12−2.01 (m, 1H), 1.88−1.70 (m, 3H), 1.48 (s, 9H).

(2*S*,2′*S*)-2-(1′-Boc-2′-pyrrolidinyl)-7-methyl-1,4-benzodioxane [(*S*,*S*)-23]. Obtained as an oil in 60% yield by treatment of (*R*,*S*)-**22** with PPh_3 (1.2 mol) and DIAD (1.2 mol) in THF under microwave irradiation at 100 °C and 300 W for 10 min and chromatography on silica gel (toluene/EtOAc 8:2): $[\alpha]_D^{25} = -134.9$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.76 (d, 1H, *J* = 8.25 Hz,), 6.69 (s, 1H), 6.62 (d, 1H, *J* = 8.25 Hz), 4.28 (dd, 1H, *J* = 1.93 and 11.28 Hz), 4.12−3.90 (m, 3H), 3.49−3.36 (m, 2H), 2.24 (s, 3H), 2.21−2.14 (m, 1H), 2.02−1.80 (m, 3H), 1.48 (s, 9H).

(2*S*,2′*S*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-methyl-1,4-benzodioxane [(*S*,*S*)-2]. Obtained as an oil in 83% yield by treatment of (*S*,*S*)-**23** with $LiAlH_4$ (2 mol) in boiling THF for 3 h: $[\alpha]_D^{25} = -106.6$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.77−6.74 (m, 2H), 6.62 (dd, 1H, *J* = 1.65 Hz and 8,25 Hz), 4.25 (dd, 1H, *J* = 1.93 and 11.00 Hz), 4.09 (ddd,

1H, J = 1.93 Hz, 4.40 Hz e 7.98 Hz), 3.98 (dd, 1H, J = 7.98 Hz, e 11.00 Hz), 3.16−3.08 (m, 1H), 2.53−2.46 (m, 1H), 2.43 (s, 3H), 2.30−2.22 (m, 1H), 2.24 (s, 3H), 1.93−1.74 (m, 4H). Anal. ($C_{14}H_{19}NO_2$) C, H, N.

(1*S*,2′*S*)-1-(1′-Boc-2′-pyrrolidinyl)-2-(*o*-hydroxy-*p*-methylphenoxy)ethanol [(*S*,*S*)-22]. Obtained as an oil in 29% yield besides (*R*,*S*)-**22** and separated from (*R*,*S*)-**22** by chromatography on silica gel (toluene/EtOAc 8:2): $[\alpha]_D^{25}$ = −13.0 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.59 (bs, 1H), 6.77−6.74 (m, 2H), 6.58 (d, 1H, J = 7.97 Hz,), 5.12 (bs, 1H), 4.15−3.98 (m, 3H), 3.90−3.78 (m, 1H), 3.58−3.40 (m, 1H), 3.37−3.20 (m, 1H), 2.25 (s, 3H), 2.10−1.75 (m, 4H), 1.46 (s, 9H).

(2*R*,2′*S*)-2-(1′-Boc-2′-pyrrolidinyl)-7-methyl-1,4-benzodioxane [(*R*,*S*)-23]. Obtained as an oil in 60% yield from (*S*,*S*)-**22** in the same manner as (*S*,*S*)-**23** from (*R*,*S*)-**22**: $[\alpha]_D^{25}$ = −45.4 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.69−6.48 (m, 3H), 4.32−4.03 (m, 3H), 3.85−3.75 (m, 1H), 3.52−3.28 (m, 2H), 2.16 (s, 3H), 2.03−1.73 (m, 4H), 1.40 (s, 9H).

(2*R*,2′*S*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-methyl-1,4-benzodioxane [(*R*,*S*)-2]. Obtained as an oil in 80% yield from (*R*,*S*)-**23** in the same manner as (*S*,*S*)-**2** from (*S*,*S*)-**23**: $[\alpha]_D^{25}$ = −5.5 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.74 (d, 1H, J = 8.20 Hz), 6.70 (d, 1H, J = 1.47 Hz), 6.62 (dd, 1H, J = 1.47 Hz and 8,20 Hz), 4.26 (dd, 1H, J = 2.05 and 11.14 Hz), 4.11 (dt, 1H, J = 2.05 and 7.33 Hz), 3.96 (dd, 1H, J = 7.33 and 11.14 Hz), 3.13−3.07 (m, 1H), 2.56−2.66 (m, 1H), 2.49 (s, 3H), 2.32−2.26 (m, 1H), 2.24 (s, 3H), 1.97−1.65 (m, 4H). Anal. ($C_{14}H_{19}NO_2$) C, H, N.

(1*R*,2′*S*)- and (1*S*,2′*S*)-1-(1′-Boc-2′-pyrrolidinyl)-2-(*o*-hydroxy-*p*-hydroxymethylphenoxy)ethanol [(*R*,*S*)/(*S*,*S*)-24]. Obtained as an oil in 35% yield by hydrogenolysis (H_2 1 atm, 10% Pd(C)) of (*R*,*S*)/(*S*,*S*)-**17** in methanol for 1 h and chromatography on silica gel (cyclohexane/EtOAc 1:1). ^{1}H NMR ($CDCl_3$) δ 6.92−6.76 (m, 3H), 4.57 (s, 2H), 4.15−3.93 (m, 3H), 3.89−3.83 (m, 1H), 3.55−3.34 (m, 1H), 3.32−3.26 (m, 1H), 2.09−1.69 (m, 4H), 1.48 (s, 4.5H), 1.46 (s, 4.5H).

(1*R*,2′*S*)-1-(1′-Boc-2′-pyrrolidinyl)-2-(*o*-hydroxy-*p*-trityloxymethylphenoxy)ethanol [(*R*,*S*)-25]. Obtained as an amorphous solid in 36% yield by treatment of (*R*,*S*)/(*S*,*S*)-**24** with trityl chloride (1.2 mol) and exceeding TEA in boiling DME overnight and chromatography on silica gel (cyclohexane/EtOAc 8:2): $[\alpha]_D^{25}$ = −23.4 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.84 (bs, 1H), 7.53−7.50 (m, 6H), 7.33−7.17 (m, 9H), 7.03 (d, 1H, J = 1.65 Hz), 6.88 (d, 1H, J = 8.25 Hz), 6.77 (dd, 1H, J = 8.25 and 1.65 Hz), 6.19 (bs, 1H), 4.12−4.06 (m, 4H), 3.98 (dd, 1H, J = 10.46 and 5.23 Hz), 3.87−3.78 (m, 1H), 3.56−3.51 (m, 1H), 3.35−3.27 (m, 1H), 2.36−2.02 (m, 1H), 1.93−1.66 (m, 3H), 1.65 (s, 3H), 1.49 (s, 6H).

(2*S*,2′*S*)-2-(1′-Boc-2′-pyrrolidinyl)-7-trityloxy-1,4-benzodioxane [(*S*,*S*)-26]. Obtained as an amorphous solid in 37% yield by treatment of (*R*,*S*)-**25** with PPh_3 (2.2 mol) and DIAD (2.2 mol) in THF under microwave irradiation at 100 °C and 300 W for 10 min and chromatography on silica gel (cyclohexane/EtOAc 9:1): $[\alpha]_D^{25}$ = −91.7 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.61−7.45 (m, 6H), 7.38−7.00 (m, 9H), 6.98−6.91 (m, 1H), 6.86−6.79 (m, 2H), 4.31 (dd, 1H, J = 11.27 and 1.92 Hz), 4.11−3.93 (m, 4H), 3.53−3.39 (m, 3H), 2.24−2.19 (m, 1H), 2.08−1.87 (m, 3H), 1.48 (s, 9H).

(2*S*,2′*S*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-hydroxymethyl-1,4-benzodioxane [(*S*,*S*)-3]. Obtained as an oil in 93% yield by treatment of (*S*,*S*)-**26** with $LiAlH_4$ (3 mol) in boiling THF for 2 h and by detritylation of the resultant crude reduction product with 1 M aqueous HCl (7 mol) in methanol for 3 h at room temperature: $[\alpha]_D^{25}$ = −91.7 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.95 (d, 1H, J = 1.90 Hz), 6.86−6.79 (m, 2H), 4.54 (s, 2H), 4.25 (dd, 1H, J = 11.00 and 1.79 Hz), 4.08 (ddd, 1H, J = 7.98 Hz, 4.40 and 1.79 Hz), 3.98 (dd, 1H, J = 11.00 and 7.98 Hz), 3.10 (ddd, 1H, J = 8.80 Hz, 4.12 and 2.47 Hz), 2.50 (m, 1H), 2.41 (s, 3H), 2.25 (pq, 1H, J = 8.80), 2.08 (bs, 1H), 1.92−1.73 (m, 4H). Anal. ($C_{14}H_{19}NO_3$) C, H, N.

(1*S*,2′*S*)-1-(1′-Boc-2′-pyrrolidinyl)-2-(*o*-hydroxy-*p*-trityloxymethylphenoxy)ethanol [(*S*,*S*)-25]. Obtained as an amorphous solid in 22% yield besides (*R*,*S*)-**25** and separated from (*R*,*S*)-**25** by chromatography on silica gel (cyclohexane/EtOAc 8:2): $[\alpha]_D^{25}$ = −6.5 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.53−7.49 (m, 6H), 7.33−7.17 (m, 9H), 7.03 (d, 1H, J = 1.74 Hz), 6.86 (d, 1H, J = 8.25 Hz), 6.79 (dd, 1H, J = 8.25 and 1.74 Hz), 5.01 (bs, 1H), 4.13−4.06 (m, 5H), 3.92−3.86 (m, 1H,), 3.58−3.46 (m, 1H), 3.32−3.26 (m, 1H), 2.36−1.79 (m, 4H), 1.75 (s, 2H), 1.47 (s, 7H).

(2*R*,2′*S*)-2-(1′-Boc-2′-pyrrolidinyl)-7-trityloxy-1,4-benzodioxane [(*R*,*S*)-26]. Obtained as an amorphous solid in 76% yield by treatment of (*S*,*S*)-**25** with PPh_3 (2.2 mol) and DIAD (2.2 mol) in THF under microwave irradiation at 100 °C and 300 W for 10 min and chromatography on silica gel (cyclohexane/EtOAc 8:2): $[\alpha]_D^{25}$ = −27.4 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.52−7.48 (m, 6H), 7.34−7.21 (m, 9H), 6.95−6.85 (m, 1H), 6.83−6.79 (m, 2H), 4.37−4.23 (m, 3H), 4.04 (s, 2H), 3.95−3.92 (m, 1H), 3.56−3.33 (m, 2H), 2.20−2.03 (m, 3H), 1.95−1.83 (m, 1H), 1.46 (s, 9H).

(2*R*,2′*S*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-hydroxymethyl-1,4-benzodioxane [(*R*,*S*)-3]. Obtained as an oil in 90% yield by treatment of (*R*,*S*)-**26** with $LiAlH_4$ (3 mol) in boiling THF for 2 h and by detritylation of the resultant crude reduction product with 1 M aqueous HCl (7 mol) in methanol for 3 h at room temperature: $[\alpha]_D^{25}$ = +2.5 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.91−6.90 (m, 1H), 6.84−6.79 (m, 2H), 4.56 (s, 2H), 4.27 (dd, 1H, J = 10.97 and 2.19 Hz), 4.09 (dt, 1H, J = 7.31 and 2.19 Hz), 3.96 (dd, 1H, J = 10.97 and 7.31 Hz), 3.12−3.06 (m, 1H), 2.64−2.57 (m, 1H), 2.48 (s, 3H), 2.32−2.23 (pq, 1H), 2.00−1.62 (m, 4H). Anal. ($C_{14}H_{19}NO_3$) C, H, N.

(1*R*,2′*S*)-1-(1′-Methyl-2′-pyrrolidinyl)-2-(*o*-hydroxy-*p*-acetylphenoxy)ethanol [(*R*,*S*)-27]. Obtained as an amorphous solid in 70% yield by hydrogenation (H_2 1 atm, 10% Pd(C)) of (*R*,*S*)/(*S*,*S*)-**18** in methanol for 2 h and, after adding an excess of formalin, for additional 16 h and subsequent chromatography on silica gel (DCM/MeOH/TEA 95:5:2): $[\alpha]_D^{25}$ = −3.9 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.51 (d, 1H, J = 1.93 Hz), 7.45 (dd, 1H, J = 8.39 and 1.93 Hz), 6.91 (d, 1H, J = 8.39 Hz), 4.68 (bs, 2H), 4.11 (dd, 1H, J = 9.91 and 4.13 Hz), 4.03 (dd, 1H, J = 9.91 and 5.78 Hz), 3.92−3.89 (m, 1H), 3.19−3.14 (m, 1H), 2.80−2.77 (m, 1H), 2.53 (s, 3H), 2.52 (s, 3H), 2.50−2.43 (m, 1H), 2.15−1.95 (m, 1H), 1.88−1.67 (m, 3H).

(2*S*,2′*S*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-acetyl-1,4-benzodioxane [(*S*,*S*)-4]. Obtained as an oil in 60% yield by treatment of (*R*,*S*)-**27** with PMe_3 (2 mol) and DEAD (1.1 mol) in THF at room temperature for 16 h and chromatography on silica gel (DCM/MeOH/30% NH_3 98:2:0.25): $[\alpha]_D^{25}$ = +17.7 (*c* 0.8, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.57 (d, 1H, J = 1.92 Hz), 7.48 (dd, 1H, J = 8.53 and 1.92 Hz), 6.90 (d, 1H, J = 8.53 Hz), 4.34 (d, 1H, J = 9.35 Hz), 4.12−4.02 (m, 2H), 3.15−3.09 (m, 1H), 2.56−2.50 (m, 1H), 2.51 (s, 3H), 2.43 (s, 3H), 2.32−2.23 (m, 1H), 1.94−1.75 (m, 4H). Anal. ($C_{15}H_{19}NO_3$) C, H, N.

(1*S*,2′*S*)-1-(1′-methyl-2′-pyrrolidinyl)-2-(*o*-hydroxy-*p*-acetylphenoxy)ethanol [(*S*,*S*)-27]. Obtained as an amorphous solid in 27% yield besides (*R*,*S*)-**27** and separated from (*R*,*S*)-**27** by chromatography on silica gel (DCM/MeOH/TEA 95:5:2): $[\alpha]_D^{25}$ = −13.0 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.51 (d, 1H, J = 3.30 Hz), 7.46 (dd, 1H, J = 8.52 and 3.30 Hz), 6.85 (d, 1H, J = 8.52 Hz), 5.66 (bs, 2H), 4.28−4.23 (m, 1H), 4.07 (dd, 1H, J = 9.63 and 3.58 Hz), 3.96 (dd, 1H, J = 9.63 and 7.98 Hz), 3.22−3.16 (m, 1H), 2.57−2.40 (m, 1H), 2.52 (s, 3H), 2.45 (s, 3H), 2.40−2.30 (m, 1H), 1.99−1.90 (m, 1H), 1.86−1.73 (m, 3H).

(2*R*,2′*S*)- 2-(1′-Methyl-2′-pyrrolidinyl)-7-acetyl-1,4-benzodioxane [(*R*,*S*)-4]. Obtained as an oil in 52% yield by treatment of (*S*,*S*)-**27** with PPh_3 (1.1 mol) and DEAD (1.1 mol) in boiling THF for 16 h and chromatography on silica gel (DCM/MeOH/30% NH_3 98:2:0.25): $[\alpha]_D^{25}$ = −118.4 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.51 (d, 1H, J = 1.93 Hz), 7.48 (dd, 1H, J = 8.25 and 1.93 Hz), 6.89 (d, 1H, J = 8.25 Hz), 4.36 (dd, 1H, J = 11.28 and 2.20 Hz), 4.14 (dt, 1H, J = 8.10 and 2.20 Hz), 4.02 (dd, 1H, J = 11.28 and 8.10 Hz), 3.14−3.07 (m, 1H), 2.68−2.61 (m, 1H),

2.52 (s, 3H), 2.50 (s, 3H), 2.34−2.26 (pq, 1H), 1.99−1.90 (m, 1H), 1.82−1.65 (m, 3H). Anal. ($C_{15}H_{19}NO_3$) C, H, N.

(1*R*,2′*S*)-1-(1′-methyl-2′-pyrrolidinyl)-2-(*o*-hydroxy-*p*-bromophenoxy)ethanol [(*R*,*S*)-28]. Obtained as an oil in quantitative yield by treatment of (*R*,*S*)-**19** with CF_3COOH (4 mol) in DCM at room temperature for 30 min: $[\alpha]_D^{25} = -15.4$ (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.05 (d, 1H, J=2.20 Hz), 6.85 (dd, 1H, *J* = 2.20 and 8.53 Hz), 6.77 (d, 1H, *J* = 8.53 Hz), 4.76 (bs, 2H), 4.03−3.91 (m, 2H), 3.85−3.80 (pq, 1H), 3.17−3.12 (m, 1H), 2.77−2.71 (m, 1H), 2.48 (s, 3H), 2.45−2.39 (m, 1H), 2.04−1.68 (m, 4H).

(2*S*,2′*S*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-bromo-1,4-benzodioxane [(*S*,*S*)-5]. Obtained as an oil in 69% yield by treatment of (*R*,*S*)-**28** with PMe_3 (1.5 mol) and DEAD (1.1 mol) in THF at room temperature for 24 h and chromatography on silica gel (EtOAc/TEA 98:2): $[\alpha]_D^{25} = -133.7$ (*c* 0.5, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.08 (d, 1H, *J* = 2.20 Hz), 6.91 (dd, 1H, *J* = 2.2 and 8.53 Hz), 6.72 (d, 1H, *J* = 8.53 Hz), 4.27 (dd, 1H *J* = 1.93 and 11.00 Hz), 4.06 (ddd, 1H, *J* = 1.93 Hz, 4.40 and 7.98 Hz), 3.97 (dd, 1H, *J* = 7.98 and 11.00 Hz), 3.12−3.06 (m, 1H), 2.50−2.46 (m, 1H), 2.41(s, 3H), 2.30−2.24 (m, 1H), 1.88−1.75 (m, 4H). Anal. ($C_{13}H_{16}BrNO_2$) C, H, N.

(1*S*,2′*S*)-1-(1′-Methyl-2′-pyrrolidinyl)-2-(*o*-hydroxy-*p*-bromophenoxy)ethanol [(*S*,*S*)-28]. Obtained as an oil in quantitative yield by treatment of (*S*,*S*)-**19** with CF_3COOH (4 mol) in DCM at room temperature for 30 min: $[\alpha]_D^{25} = -5.8$ (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.06 (d, 1H, *J* = 2.2 Hz), 6.87 (dd, 1H, *J* = 2.2 and 8.53 Hz), 6.73 (d, 1H, *J* = 8.53 Hz), 6.30 (bs, 2H), 4.13−4.08 (m, 1H), 4.05−4.00 (m, 1H), 3.84−3.78 (m, 1H), 3.15−3.11 (m, 1H), 2.40 (s, 3H), 2.38−2.28 (m, 2H), 1.86−1.76 (m, 4H).

(2*R*,2′*S*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-bromo-1,4-benzodioxane [(*R*,*S*)-5]. Obtained as an oil in 69% yield from (*S*,*S*)-**28** as described for (*S*,*S*)-**5**: $[\alpha]_D^{25} = -2.8$ (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.03 (d, 1H, *J* = 2.20 Hz), 6.91 (dd, 1H, *J* = 2.20 and 8.52 Hz), 6.74 (d, 1H, *J* = 8.52 Hz), 4.31 (dd, 1H, *J* = 2.20 and 11.28 Hz), 4.11 (dt, 1H, *J* = 2.20 and 7.15 Hz), 3.96 (dd, 1H, *J* = 7.15 and 11.28 Hz), 3.12−3.06 (m, 1H), 2.65−2.57 (m, 1H), 2.47 (s, 3H), 2.33−2.24 (pq, 1H), 1.97−1.72 (m, 4H). Anal. ($C_{13}H_{16}BrNO_2$) C, H, N.

(1*R*,2′*S*)-1-(1′-Boc-2′-pyrrolidinyl)-2-(*o*-hydroxy-*p*-phenylphenoxy)ethanol [(*R*,*S*)-29]. Obtained as an oil in 85% yield by hydrogenolysis (H_2 1 atm, 10% Pd(C)) of (*R*,*S*)-**20** in methanol for 48 h: $[\alpha]_D^{25} = -34.1$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.92 (bs, 1H), 7.55 (d, 2H, *J* = 7.15 Hz), 7.40 (t, 2H, *J* = 7.15 Hz), 7.31 (d, 1H, *J* = 7.15 Hz), 7.20 (d, 1H, *J* = 1.93 Hz), 7.02 (dd, 1H, *J* = 1.93 and 8.26 Hz), 6.98 (d, 1H, *J* = 8.26 Hz), 6.23 (bs, 1H), 4.14−4.07 (m, 1H), 4.06−3.99 (m, 1H), 3.90−3.80 (m, 1H), 3.61−3.49 (m, 1H), 3.38−3.27 (m, 1H), 2.13−2.05 (m, 1H), 2.00−1.65 (m, 3H,), 1.50 (s, 9H).

(2*S*,2′*S*)-2-(1′-Boc-2′-pyrrolidinyl)-7-phenyl-1,4-benzodioxane [(*S*,*S*)-30]. Obtained as an oil in 60% yield by treatment of (*R*,*S*)-**29** with PMe_3 (2.2 mol) and DEAD (2.2 mol) in THF under microwave irradiation at 100 °C and 300 W for 10 min and chromatography on silica gel (cyclohexane/EtOAc 7:3): $[\alpha]_D^{25} = -126.4$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.54 (d, 2H, *J* = 7.7 Hz), 7.40 (t, 2H, *J* = 7.7 Hz), 7.34−7.22 (m, 1H), 7.19−7.05 (m, 2H), 6.94 (d, 1H, *J* = 8.25 Hz), 4.35 (d, 1H, *J* = 11.27 Hz), 4.25−3.90 (m, 3H), 3.62−3.35 (m, 2H), 2.36−2.20 (m, 1H), 2.10−1.80 (m, 3H), 1.43 (s, 4.5H), 1.42 (s, 4.5H).

(2*S*,2′*S*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-phenyl-1,4-benzodioxane [(*S*,*S*)-6]. Obtained as an oil in 70% yield by treatment of (*S*,*S*)-**30** with $LiAlH_4$ (2 mol) in boiling THF for 1 h: $[\alpha]_D^{25} = -83.4$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.55 (d, 2H, *J* = 7.15 Hz), 7.41 (t, 2H, *J* = 7.15 Hz), 7.31 (d, 1H, *J* = 7.15 Hz), 7.24 (d, 1H, *J* = 2.20 Hz), 7.10 (dd, 1H, *J* = 8.52 and 2.20 Hz), 6.95 (d, 1H, *J* = 8.52 Hz), 4.31 (dd, 1H, *J* = 1.92 and 11.27 Hz), 4.17 (ddd, 1H, *J* = 1.92 Hz, 4.40 and 8.00 Hz), 4.06 (dd, 1H, *J* = 8.00 and 11.27 Hz), 3.18−3.13 (m, 1H), 2.58−2.52 (m, 1H), 2.46 (s, 3H), 2.33−2.25 (m, 1H), 2.03−1.77 (m, 4H). Anal. ($C_{19}H_{21}NO_2$) C, H, N.

(1*S*,2′*S*)-1-(1′-Boc-2′-pyrrolidinyl)-2-(*o*-hydroxy-*p*-phenylphenoxy)ethanol [(*S*,*S*)-29]. Obtained as an oil in 93% yield from (*S*,*S*)-**20** as described for (*R*,*S*)-**29**: $[\alpha]_D^{25} = -7.8$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.55 (d, 2H, *J* = 7.15 Hz), 7.40 (t, 2H, *J* = 7.15 Hz), 7.34−7.20 (m, 2H), 7.08−7.01 (m, 1H), 6.98−6.93 (m, 1H), 5.08 (bs, 1H), 4.20−4.06 (m, 3H), 4.00−3.90 (m, 1H), 3.61−3.42 (m, 1H), 3.33−3.21 (m, 1H), 2.08−1.68 (m, 4H), 1.47 (s, 9H).

(2*R*,2′*S*)-2-(1′-Boc-2′-pyrrolidinyl)-7-phenyl-1,4-benzodioxane [(*R*,*S*)-30]. Obtained as an oil in 72% from (*S*,*S*)-**29** as described for (*S*,*S*)-**30**: $[\alpha]_D^{25} = -44.1$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.53 (d, 2H, *J* = 7.43 Hz), 7.40 (t, 2H, *J* = 7.43 Hz), 7.32−7.21 (m, 1H), 7.20−7.15 (m, 1H), 7.14−7.02 (m, 1H), 6.93 (d, 1H, *J* = 8.25 Hz), 4.50−4.18 (m, 3H), 4.06−3.90 (m, 1H), 3.65−3.39 (m, 2H), 2.20−2.00 (m, 3H), 1.98−1.86 (m, 1H), 1.47 (s, 4.5H), 1.57 (s, 4.5H).

(2*R*,2′*S*)- 2-(1′-Methyl-2′-pyrrolidinyl)-7-phenyl-1,4-benzodioxane [(*R*,*S*)-6]. Obtained as an oil in 80% yield from (*R*,*S*)-**30** as described for (*S*,*S*)-**6**: $[\alpha]_D^{25} = -29.4$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.53 (d, 2H, *J* = 7.15 Hz), 7.40 (t, 2H, *J* = 7.15 Hz), 7.30 (d, 1H, *J* = 7.15 Hz), 7.15 (d, 1H, *J* = 2.20 Hz), 7.08 (dd, 1H, *J* = 8.53 and 2.20 Hz), 6.92 (d, 1H, *J* = 8.53 Hz), 4.33 (dd, 1H, *J* = 2.20 and 11.27 Hz), 4.19 (dt, 1H, *J* = 2.20 and 7.42 Hz), 4.06 (dd, 1H, *J* = 7.42 and 11.27 Hz), 3.15−3.09 (m, 1H), 2.71−2.64 (m, 1H), 2.52 (s, 3H, CH_3), 2.39−2.23 (m, 1H), 2.20−1.92 (m, 1H), 1.84−1.64 (m, 3H). Anal. ($C_{19}H_{21}NO_2$) C, H, N.

(1*R*,2′*S*)-1-(1′-Boc-2′-pyrrolidinyl)-2-(*o*,*p*-dibenzyloxyphenoxy)ethyl mesylate [(*R*,*S*)-31]. Obtained as a white solid in 55% yield from (*R*,*S*)-**21** by treatment with equimolar mesyl chloride and trimethylamine in DCM at room temperature for 5 h and subsequent chromatography on silica gel (cyclohexane/EtOAc 7:3): mp 97.3 °C; $[\alpha]_D^{25} = -61.4$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.30−7.50 (m, 10H), 6.77 (d, 1H, *J* = 8.81 Hz), 6.66 (d, 1H, *J* = 2.75 Hz), 6.47 (dd, 1H, *J* = 8.81 and 2.75 Hz), 5.10−5.30 (m, 1H), 4.91−5.00 (m, 4H), 3.99−4.11 (m, 3H), 3.36−3.50 (m, 2H), 2.82 (bs, 3H), 1.78−2.10 (m, 4H), 1.47 (s, 7H), 1.42 (s, 2H).

(1*R*,2′*S*)-1-(1′-Boc-2′-pyrrolidinyl)-2-(*o*,*p*-dihydroxyphenoxy)ethyl mesylate [(*R*,*S*)-32]. Obtained as an oil in 87% yield by hydrogenolysis (H_2 5 atm, 10% Pd(C)) of (*R*,*S*)-**31** in methanol for 16 h: $[\alpha]_D^{25} = -49.4$ (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 6.59 (m, 1H), 6.49 (d, 1H, *J* = 2.75 Hz), 6.27 (m, 1H), 5.34 (m, 1H), 4.15−3.90 (m, 4H), 3.46−3.28 (m, 3H), 3.05 (s, 3H), 1.84−2.04 (m, 4H), 1.48 (s, 9H).

(2*S*,2′*S*)-2-(1′-Boc-2′-pyrrolidinyl)-7-hydroxy-1,4-benzodioxane [(*S*,*S*)-33]. Obtained as a white solid in 77% yield by treatment of (*R*,*S*)-**32** with equimolar K_2CO_3 in boiling DME for 6 h and subsequent chromatography on silica gel (cyclohexane/EtOAc 6:4): mp 166.5 °C; $[\alpha]_D^{25} = -174.4$ (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 6.71 (d, 1H, *J* = 8.53 Hz), 6.40 (d, 1H, *J* = 2.75 Hz), 6.33 (d, 1H, *J* = 8.53 Hz), 5.10−4.90 (bs, 1H), 4.24 (dd, 1H, *J* = 11.55 and 2.20 Hz), 4.20−3.85 (m, 3H), 3.30−3.55 (m, 2H), 2.30−2.15 (m, 1H), 2.05−1.85 (m, 3H), 1.47 (s, 9H).

(2*S*,2′*S*)-2-(2′-Pyrrolidinyl)-7-hydroxy-1,4-benzodioxane [(*S*,*S*)-7a]. Obtained as a white solid in 48% yield by treatment of (*S*,*S*)-**33** with CF_3COOH in DCM at room temperature for 2 h and successive crystallization from diisopropyl ether: mp 205.5 °C; $[\alpha]_D^{25} = -76.4$ (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6) δ 8.89 (s, 1H), 6.58−6.61 (d, 1H, *J* = 8.25 Hz), 6.20 (d, 1H, *J* = 3.05 Hz), 6.16 (m, 1H), 4.21 (dd, 1H, *J* = 11.28 and 1.93 Hz), 3.83 (dd, 1H, *J* = 11.28 and 7.15 Hz), 3.70 (t, 1H, *J* = 7.15 Hz), 3.04−3.08 (m, 1H), 2.71−2.82 (m, 2H), 2.53 (bs, 1H), 1.83−1.56 (m, 4H). Anal. ($C_{12}H_{15}NO_3$) C, H, N.

(2*S*,2′*S*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-hydroxy-1,4-benzodioxane [(*S*,*S*)-7]. Obtained as a white solid in 96% yield by treatment of (*S*,*S*)-7**a** with H_2 (1 atm)/10% Pd(C) in methanol in the presence of formalin for 16 h: mp 161.3 °C; $[\alpha]_D^{25} = -95.0$ (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 8.91 (s, 1H), 6.61 (d, 1H, *J* = 8.52 Hz), 6.21 (m, 1H), 6.16 (d, 1H, *J* = 2.75 Hz), 4.20 (dd, 1H, *J* = 11.28 and 1.92 Hz), 3.92−3.97 (ddd, 1H, *J* = 7.70 Hz, 4.67 and 1.92 Hz),

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3.80−3.87 (dd, 1H, J = 11.28 and 7.71 Hz), 2.90−2.96 (m, 1H), 2.52 (m, 1H), 2.29 (s, 3H), 2.11−2.18 (m, 1H), 1.58−1.85 (m, 4H). Anal. ($C_{13}H_{17}NO_3$) C, H, N.

(1*S*,2′*S*)-1-(1′-Boc-2′-pyrrolidinyl)-2-(*o*,*p*-Dibenzyloxyphenoxy)ethyl mesylate [(*S*,*S*)-31]. Obtained as an amorphous solid in 75% yield from (*S*,*S*)-**21** as described for (*R*,*S*)-**31**; $[\alpha]_D^{25}$ = −14.5 (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.32−7.43 (m, 10H), 6.77 (d, 1H, J = 8.81 Hz), 6.67 (d, 1H, J = 2.75 Hz), 6.47 (dd, 1H, J = 8.81 and 2.75 Hz), 5.25−4.90 (m, 5H), 4.01−4.25 (m, 3H), 3.30−3.60 (m, 2H), 2.85 (s, 3H), 1.95−2.20 (m, 2H) , 1.90−1.75 (m, 2H), 1.54 (s, 2H), 1.46 (s, 7H).

(1*S*,2′*S*)-1-(1′-Boc-2′-pyrrolidinyl)-2-(*o*,*p*-dihydroxyphenoxy)ethyl mesylate [(*S*,*S*)-32]. Obtained as an oil in 82% yield from (*S*,*S*)-**31** as described for (*R*,*S*)-**32**; $[\alpha]_D^{25}$ = −29.5 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.64 (d, 1H, J = 8.53 Hz), 6.47 (s, 1H), 6.27 (d, 1H, J = 8.53 Hz), 5.32 (bs, 1H), 5.11 (m, 1H), 4.08−4.26 (m, 3H), 3.91−3.99 (pt, 1H, J = 10.18 Hz), 3.60−3.42 (m, 2H), 3.40−3.25 (m, 2H), 3.09 (s, 3H), 1.92−2.01 (m, 4H), 1.48 (s, 9H).

(2*R*,2′*S*)-2-(1′-Boc-2′-pyrrolidinyl)-7-hydroxy-1,4-benzodioxane [(*R*,*S*)-33]. Obtained as a white solid in 79% yield from (*S*,*S*)-**32** as described for (*S*,*S*)-**33**; mp 164.5 °C; $[\alpha]_D^{25}$ = −47.0 (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 6.70 (d, 1H, J = 8.53 Hz), 6.39 (d, 1H, J = 2.75 Hz), 6.30 (dd, 1H, J = 8.53 and 2.75 Hz), 4.21−4.30 (m, 4H), 3.82−3.89 (m, 1H), 3.45−3.31 (m, 2H), 2.04−1.98 (m, 3H), 1.95−1.85 (m, 1H), 1.45 (s, 9H).

(2*R*,2′*S*)-2-(2′-Pyrrolidinyl)-7-hydroxy-1,4-benzodioxane [(*R*,*S*)-7a]. Obtained as a white solid in 30% yield from (*R*,*S*)-**33** as described for (*S*,*S*)-**7a**; mp 177.3 °C; $[\alpha]_D^{25}$ = +30.2 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 6.73 (d, 1H, J = 3.02 Hz), 6.67 (d, 1H, J = 8.80 Hz), 6.30 (dd, 1H, J = 8.80 and 3.03 Hz), 4.16−4.21 (dd, 1H, J = 11.00 and 1.92 Hz), 3.98−4.04 (dt, 1H, J = 8.53 and 1.65 Hz), 3.81−3.87 (dd, 1H, J = 11.00 and 8.53 Hz), 3.20−3.28 (m, 1H), 3.09−3.17 (m, 1H), 2.94−3.03 (m, 1H), 2.00−1.78 (m, 3H), 1.65−1.53 (m, 1H). Anal. ($C_{12}H_{15}NO_3$) C, H, N.

(2*R*,2′*S*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-hydroxy-1,4-benzodioxane [(*R*,*S*)-7]. Obtained as a white solid in 85% yield from (*R*,*S*)-**7a** as described for (*S*,*S*)-**7**; mp 143.7 °C; $[\alpha]_D^{25}$ = −7.9 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 8.91 (s, 1H), 6.60 (d, J = 8.25 Hz), 6.20−6.22 (m, 1H), 6.18 (d, 1H, J = 2.76 Hz), 4.12−4.17 (dd, 1H, J = 11.28 and 2.20 Hz), 4.02−4.07 (dt, 1H, J = 7.42 and 2.20 Hz), 3.83−3.89 (dd, 1H, J = 11.28 and 7.43 Hz), 2.90−2.95 (m, 1H), 2.50−2.56 (m, 1H), 2.34 (s, 3H), 2.10−2.15 (m, 1H), 1.84−1.75 (m, 1H), 1−70−1.57 (m, 3H). Anal. ($C_{13}H_{17}NO_3$) C, H, N.

(1*R*,2′*R*)-1-(1′-Boc-2′-pyrrolidinyl)-2-(*o*,*p*-dibenzyloxyphenoxy)ethyl Mesylate [(*R*,*R*)-31]. Obtained as an amorphous solid in 97% yield from (*R*,*R*)-**21** as described for (*R*,*S*)-**31**; $[\alpha]_D^{25}$ = +14.1 (*c* 1, MeOH). ^{1}H NMR identical to (*S*,*S*)-**31**.

(1*R*,2′*R*)-1-(1′-Boc-2′-pyrrolidinyl)-2-(*o*,*p*-dihydroxyphenoxy)ethyl Mesylate [(*R*,*R*)-32]. Obtained as an oil in 86% yield from (*R*,*R*)-**31** as described for (*S*,*R*)-**32**; $[\alpha]_D^{25}$ = +29.3 (*c* 1, $CHCl_3$). ^{1}H NMR identical to (*S*,*S*)-**32**.

(2*S*,2′*R*)-2-(1′-Boc-2′-pyrrolidinyl)-7-hydroxy-1,4-benzodioxane [(*S*,*R*)-33]. Obtained as a white solid in 85% yield from (*R*,*R*)-**32** as described for (*S*,*S*)-**33**; mp 164.7 °C; $[\alpha]_D^{25}$ = +43.9 (*c* 1, MeOH). ^{1}H NMR identical to (*R*,*S*)-**33**.

(2*S*,2′*R*)-2-(2′-Pyrrolidinyl)-7-hydroxy-1,4-benzodioxane [(*S*,*R*)-7a]. Obtained as a white solid in 88% yield from (*S*,*R*)-**33** as described for (*S*,*S*)-**7a**; mp 166.7 °C; $[\alpha]_D^{25}$ = −32.9 (*c* 1, $CHCl_3$). ^{1}H NMR identical to (*R*,*S*)-**7a**. Anal. ($C_{12}H_{15}NO_3$) C, H, N.

(2*S*,2′*R*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-hydroxy-1,4-benzodioxane [(*S*,*R*)-7]. Obtained as a white solid in 91% yield from (*S*,*R*)-**7a** as described for (*S*,*S*)-**7**; mp 145.5 °C; $[\alpha]_D^{25}$ = +8.7 (*c* 1, $CHCl_3$). ^{1}H NMR identical to (*R*,*S*)-**7**. Anal. ($C_{13}H_{17}NO_3$) C, H, N.

(1*S*,2′*R*)-1-(1′-Boc-2′-pyrrolidinyl)-2-(*o*,*p*-dibenzyloxyphenoxy)ethyl Mesylate [(*S*,*R*)-31]. Obtained as an amorphous solid in 80% yield from (*S*,*R*)-**21** as described for (*R*,*S*)-**31**; $[\alpha]_D^{25}$ = +58.7 (*c* 1, $CHCl_3$). ^{1}H NMR identical to (*R*,*S*)-**31**.

(1*S*,2′*R*)-1-(1′-Boc-2′-pyrrolidinyl)-2-(*o*,*p*-dihydroxyphenoxy)ethyl Mesylate [(*S*,*R*)-32]. Obtained as an oil in 82% yield from (*S*,*R*)-**31** as described for (*R*,*S*)-**32**; $[\alpha]_D^{25}$ = +50.3 (*c* 1, MeOH). ^{1}H NMR identical to (*R*,*S*)-**32**.

(2*R*,2′*R*)-2-(1′-Boc-2′-pyrrolidinyl)-7-hydroxy-1,4-benzodioxane [(*R*,*R*)-33]. Obtained as a white solid in 83% yield from (*S*,*R*)-**32** as described for (*S*,*S*)-**33**; mp 165.4 °C; $[\alpha]_D^{25}$ = +179.1 (*c* 1, MeOH). ^{1}H NMR identical to (*S*,*S*)-**33**.

(2*R*,2′*R*)-2-(2′-Pyrrolidinyl)-7-hydroxy-1,4-benzodioxane [(*R*,*R*)-7a]. Obtained as a white solid in 70% yield from (*R*,*R*)-**33** as described for (*S*,*S*)-**7a**; mp 204.1 °C; $[\alpha]_D^{25}$ = +76.8 (*c* 1, MeOH). ^{1}H NMR identical to (*S*,*S*)-**7a**. Anal. ($C_{12}H_{15}NO_3$) C, H, N.

(2*R*,2′*R*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-hydroxy-1,4-benzodioxane [(*R*,*R*)-7]. Obtained as a white solid in 75% yield from (*R*,*R*)-**7a** as described for (*S*,*S*)-**7**; mp 160.9 °C; $[\alpha]_D^{25}$ = +101.1 (*c* 1, $CHCl_3$). ^{1}H NMR identical to (*S*,*S*)-**7**. Anal. ($C_{13}H_{17}NO_3$) C, H, N.

(2*S*,2′*S*)-2-(1′-Boc-2′-pyrrolidinyl)-7-methoxy-1,4-benzodioxane [(*S*,*S*)-34]. Obtained as an oil in 95% yield by treatment of (*S*,*S*)-**33** with DBU (2 mol) in dimethylcarbonate at 90 °C for 16 h and successive chromatography on silica gel (cyclohexane/EtOAc 7:3); $[\alpha]_D^{25}$ = −142.2 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.78 (d, 1H, J = 8.94 Hz), 6.44 (d, 1H, J = 2.75 Hz), 6.40 (dd, 1H, J = 8.94 and 2.75 Hz), 4.26 (dd, 1H, J = 11.28 and 2.20 Hz), 4.18−4.05 (m, 1H), 4.00−3.88 (m, 2H), 3.73 (s, 3H), 3.51−3.73 (m, 2H), 2.23−2.18 (m, 1H), 2.04−1.90 (m, 3H), 1.47 (s, 9H).

(2*S*,2′*S*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-methoxy-1,4-benzodioxane [(*S*,*S*)-8]. Obtained as an oil in 85% yield by treatment of (*S*,*S*)-**34** with $LiAlH_4$ (4 mol) in boiling THF for 2 h; $[\alpha]_D^{25}$ = −104.5 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.77 (d, 1H, J = 8.80 Hz), 6.55 (d, 1H, J = 2.89 Hz), 6.40 (dd, 1H, J = 8.80 and 2.89 Hz), 4.22 (dd, 1H, J = 11.28 and 2.20 Hz), 4.13 (m, 1H), 3.95 (dd, 1H, J = 11.28 and 7.98 Hz), 3.72 (s, 3H), 3.15 (m, 1H), 2.55−2.45 (m, 1H), 2.43 (s, 3H), 2.30−2.25 (m, 1H), 1.93−1.74 (m, 3H). Anal. ($C_{14}H_{19}NO_3$) C, H, N.

(2*R*,2′*S*)-2-(1′-Boc-2′-pyrrolidinyl)-7-methoxy-1,4-benzodioxane [(*R*,*S*)-34]. Obtained as an oil in 96% yield from (*R*,*S*)-**33** as described for (*S*,*S*)-**34**; $[\alpha]_D^{25}$ = −44.9 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.77 (d, 1H, J = 8.80 Hz), 6.44−6.38 (m, 2H), 4.28−4.18 (m, 3H), 3.97−3.80 (m, 1H), 3.73 (s, 3H), 3.54−3.32 (m, 2H), 2.04−1.86 (m, 4H), 1.44 (s, 9H).

(2*R*,2′*S*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-methoxy-1,4-benzodioxane [(*R*,*S*)-8]. Obtained as an oil in 91% yield from (*R*,*S*)-**34** as described for (*S*,*S*)-**8**; $[\alpha]_D^{25}$ = −15.7 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.76 (d, 1H, J = 8.80 Hz), 6.46 (d, 1H, J = 3.02 Hz), 6.40 (dd, 1H, J = 8.80 and 3.02 Hz), 4.25 (dd, 1H, J = 11.28 and 2.20 Hz), 4.13 (dt, 1H, J = 7.15 and 2.20 Hz), 3.94 (dd, 1H, J = 11.28 and 7.15 Hz), 3.73 (s, 3H), 3.12−3.07 (m, 1H), 2.67−2.59 (m, 1H), 2.49 (s, 3H), 2.28 (pq, 1H), 1.98−1.89 (m, 1H), 1.81−1.66 (m, 3H). Anal. ($C_{14}H_{19}NO_3$) C, H, N.

(2*S*,2′*S*)-2-(1′-Boc-2′-pyrrolidinyl)-7-isopropoxy-1,4-benzodioxane [(*S*,*S*)-35]. Obtained as an oil in 84% yield by treatment of (*S*,*S*)-**33** with exceeding 2-bromopropane and NaH (1 mol) in DME at 60 °C for 16 h and subsequent chromatography on silica gel (cyclohexane/EtOAc 7:3); $[\alpha]_D^{25}$ = −133.2 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.75 (d, 1H, J = 8.80 Hz), 6.44 (d, 1H, J = 2.75 Hz), 6.39 (dd, 1H, J = 8.80 and 2.75 Hz), 4.38 (sept, 1H, J = 6.18 Hz), 4.25 (dd, 1H, J = 11.28 and 2.20 Hz), 4.20−3.85 (m, 3H), 3.55−3.32 (m, 2H), 2.25−2.10 (m, 1H,), 2.02−1.85 (m, 3H,), 1.47 (s, 9H), 1.29 (d, 6H, J = 6.18 Hz).

(2*S*,2′*S*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-isopropoxy-1,4-benzodioxane [(*S*,*S*)-9]. Obtained as an oil in 78% yield by treatment of (*S*,*S*)-**35** with $LiAlH_4$ (6 mol) in boiling THF for 2 h; $[\alpha]_D^{25}$ = −88.9 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.75 (d, 1H, J = 8.80 Hz), 6.54 (d, 1H, J = 2.88 Hz), 6.38 (dd, 1H, J = 8.80 and 2.88 Hz), 4.37 (sept, 1H, J = 6.05 Hz), 4.22 (dd, 1H, J = 11.14 and 2.20 Hz), 4.13 (ddd, 1H, J = 7.98 Hz, 3.85 and 2.20 Hz), 3.94 (dd, 1H, J = 11.14 and 7.98 Hz), 3.16−3.09 (m, 1H),

2.54−2.48 (m, 1H), 2.43 (s, 3H), 2.30−2.21 (m, 1H), 1.93−1.73 (m, 4H), 1.28 (d, 6H, J = 6.05 Hz). Anal. ($C_{16}H_{23}NO_3$) C, H, N.

(2*R*,2′*S*)-2-(1′-Boc-2′-pyrrolidinyl)-7-isopropoxy-1,4-benzodioxane [(*R*,*S*)-35]. Obtained as an oil in 66% yield from (*R*,*S*)-**33** as described for (*S*,*S*)-**35**; $[\alpha]_D^{25}$ = −38.9 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.74 (d, 1H, J = 8.52 Hz), 6.43 (d, 1H, J = 2.75 Hz), 6.38 (dd, 1H, J = 8.52 and 2.75 Hz), 4.38 (sept, 1H, J = 6.18 Hz), 4.27−4.21 (m, 3H), 3.90−3.80 (m, 1H), 3.60−3.30 (m, 2H), 2.17−1.88 (m, 4H), 1.45 (s, 5H), 1.42 (s, 4H), 1.29 (d, 6H, J = 6.18 Hz).

(2*R*,2′*S*)-2-(1′-Methyl-2′-pyrrolidinyl)-7-isopropoxy-1,4-benzodioxane [(*R*,*S*)-9]. Obtained as an oil in 72% yield from (*R*,*S*)-**35** as described for (*S*,*S*)-**9**; $[\alpha]_D^{25}$ = −2.7 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.73 (d, 1H, J = 8.80 Hz), 6.46 (d, 1H, J = 2.75 Hz), 6.39 (dd, 1H, J = 8.80 and 2.75 Hz), 4.38 (sept, 1H, J = 6.05 Hz), 4.24 (dd, 1H, J = 11.14 and 2.20 Hz), 4.12 (dt, 1H, J = 7.16 and 2.2 Hz), 3.94 (dd, 1H, J = 11.14 and 7.16 Hz), 3.13−3.07 (m, 1H), 2.66−2.59 (m, 1H), 2.49 (s, 3H), 2.28 (pq, 1H), 1.98−1.88 (m, 1H), 1.81−1.63 (m, 3H), 1.28 (d, 6H, J = 6.05 Hz). Anal. ($C_{16}H_{23}NO_3$) C, H, N.

Binding to Nicotinic Receptor Subtypes. Frozen cortex and striatum specimens taken from adult male Sprague−Dawley rats (Charles River, Calco Italy) were homogenized using a Potter homogenizer in an excess of buffer A (50 mM Tris-HCl, pH 7, 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ 2.5 mM $CaCl_2$, and 2 mM phenylmethylsulfonyl fluoride), centrifuged (60 min at 30000*g*), and rinsed twice. The homogenates were resuspended in the same buffer containing 20 μg/mL of the protease inhibitors leupeptin, bestatin, pepstatin A, and aprotinin.

[^{3}H]-epibatidine and [^{125}I]]-αBungarotoxin binding to $\alpha4\beta2$ and $\alpha7$ cortical membrane-subtypes were performed as previously described.[23] Briefly, for [^{3}H]-epibatidine, binding cortical membranes were first preincubated with 1 μM α-bungarotoxin in order to prevent the binding of [^{3}H]-epibatidine to the $\alpha7$ receptors and then with (±)-[^{3}H]-epibatidine (specific activity of 56−60 Ci/mmol, purchased from Perkin-Elmer, Boston MA). In the [^{3}H]-epibatidine saturation experiments, aliquots of cortical membranes were incubated overnight at 4 °C with concentrations of [^{3}H]-epibatidine ranging between 0.005 and 1 nM diluted in buffer A. Nonspecific binding was determined in parallel by means of incubation in the presence of 100 nM unlabeled epibatidine. At the end of the incubation, the samples were filtered on GFC filters presoaked in polyethylenimine through a harvester apparatus, and the filters were counted in a β counter. The inhibition of radioligand binding was obtained by preincubating increasing doses (10 pM to 10 mM) of the test compounds for 30 min at rt, followed by overnight incubation with a final concentration of 0.1 nM [^{3}H]-epibatidine and then incubated overnight at 4 °C.

The [^{125}I]-α-Bungarotoxin (specific activity 150 Ci/mmol, purchased from Perkin-Elmer, Boston MA) saturation binding was performed by incubating cortical membranes overnight with 0.1−10 nM concentrations of [^{125}I]-α-bungarotoxin at rt. Nonspecific binding was determined in parallel by means of incubation in the presence of 1 μM unlabeled α-bungarotoxin. After incubation, the samples were filtered as described above and the bound radioactivity directly counted in a γ counter. The inhibition of [^{125}I]-α-Bungarotoxin binding by the test compounds was measured by preincubating cortical membranes with increasing concentrations (10 pM to 10 mM) of the drug to be tested for 30 min at rt, followed by overnight incubation with a final concentration of 1 nM [^{125}I]-α-bungarotoxin at rt.

$\alpha6\beta2$ Subtype Immunoimmobilization.* The $\alpha6\beta2$* (*indicates that other subunits may be present in addition to the $\alpha6$ and $\beta2$ subunits) subtype was isolated from striatal membranes by solubilizing the membranes with 2% Triton-100 and immobilizing [^{3}H]-epibatidine labeled receptors using anti-$\alpha6$ subunit specific antibodies (Abs). Briefly, 10 μg/mL Abs were bound to microwells (Maxi-Sorp; Nunc, Roskilde, Denmark) by means of overnight incubation at 4 °C. After washing to remove the unbound Abs, rat striatal extracts were added to the wells, and after overnight incubation at 4 °C, the wells were washed and the binding experiments were performed as previously described.[20]

Heterologously Expressed $\alpha3\beta4$ Receptors. HEK 293 cells were grown in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 1% L-glutamine, 100 units/mL penicillin G, and 100 μg of streptomycin in a humidified atmosphere containing 10% CO_2. The cDNAs encoding $\alpha3$ and $\beta4$ were transfected into the HEK 293 cells at 30% confluency. The cell transfections were carried out in 100 mm Petri dishes using 30 μL of JetPEI (Polypus, France) (1 mg/mL, pH 7.2) and 10 μg of cDNAs. After 48 h transfection, the cells were collected, washed with PBS by centrifugation, and used for binding analysis.

[^{3}H]-epibatidine was bound to $\alpha6\beta2$* immunoimmobilised receptors or HEK 293 $\alpha3\beta4$ receptors by means of overnight incubation at 4 °C at concentrations ranging from 0.005 to 1 nM in a buffer containing 50 mM Tris-HCl, pH 7, 150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, and 2 mg/mL BSA in the presence (in the case of the $\alpha6\beta2$ subtype) or absence (in the case of $\alpha3\beta4$ subtype) of 0.05% Tween 20. Specific ligand binding was defined as total binding minus the binding in the presence of 100 nM cold epibatidine.

The inhibition of [^{3}H]-epibatidine binding induced by (*R*,*S*)-7 and (*S*,*S*)-7 was measured by incubating increasing concentrations for 5 min followed by overnight incubation with 0.1 nM[^{3}H]-epibatidine (in the case of the $\alpha6\beta2$* subtype) or 0.25 nM (in the case of the $\alpha3\beta4$ subtype). After incubation, the wells containing the $\alpha6\beta2$* subtype or the membranes of HEK cells transfected with $\alpha3\beta4$ subtype were washed seven times with ice-cold PBS, and [^{3}H]-epibatidine binding was determined by means of liquid scintillation counting in a β counter.

Neurotransmitter Release. [^{3}H]dopamine (DA) from striatal slices and [^{3}H] noradrenaline (NA) from hippocampal slices were measured using 96-well assays as previously described.[19] For each experiment, two rats were killed by cervical dislocation, their brain was rapidly removed, and the hippocampi and striata were dissected and transferred to ice-cold Krebs buffer (KB: 118 mM NaCl, 2.4 mM KCl, 2.4 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4 \cdot 7H_2O$, 25 mM $NaHCO_3$, 10 mM D-glucose, and 1 mM ascorbic acid), gassed with 95% O_2/ 5% CO_2 for at least 1 h at 37 °C, and the pH was adjusted to 7.4. The tissue was chopped three times (two 60° rotations) using a McIlwain tissue chopper to give slices of 150 μm. After two washes with warm KB, hippocampal and striatal slices were respectively incubated for 30 min with 150 nM [^{3}H]NA and 100 nM [^{3}H]DA, in 5 mL of KB supplemented with 10 μM pargyline to prevent the [^{3}H]catecholamine degradation that occurs at 37 °C. To remove the excess tritium, four washes with KB containing 10 μM pargyline and 0.5 μM nomifensine were performed to prevent [^{3}H]catecholamine reuptake. The slices were then loaded onto 96-well filter plates (Millipore Corporation, Milan, Italy) and incubated for 5 min with buffer in the presence or absence of antagonists or peptides under studies. Then, the buffer was removed by filtration (to obtain the basal value) and collected in a 96-well Optiplate (PerkinElmer). Buffer (100 μL) containing an agonist or an antagonist was then added to each well (in each experiment, buffer stimulation was used to determine the fractional release of [^{3}H]DA or [^{3}H]NA evoked by the buffer alone). After 5 min at 37 °C, the buffer was collected by filtration in a 96-well Optiplate to determine the fraction of [^{3}H]catecholamine released. Optiphase Supermix (Perkin-Elmer) (200 μL per well) was added to Optiplate, and the radioactivity in each well was counted for 2 min using a Microbeta 3 counter (Wallac 1450 Microbeta Trilux; PerkinElmer) with a counting efficiency of 30%. To determine the amount of tritium remaining in the slices, 50 μL of Optiphase Supermix was added to each well and the radioactivity was counted in Microbeta 3 counter. The amount of [^{3}H]catecholamine released was expressed as a percentage of the total radioactivity taken up in the slices before stimulation (i.e., the amount of tritium released + tritium remaining in the slices after stimulation). Each experiment was performed in six replicates and repeated at least three times.

Data Analysis. The K_i values of all the tested compounds were determined by means of the LIGAND program using the data obtained from three independent saturation and competition binding experiments and compared by means of the F-test. The striatal DA release data and hippocampal NA release data were expressed as the fractional percentage of total radioactivity for each well. Basal release was subtracted from stimulated release for each sample, and the results were normalized to the maximal release evoked by nicotine 10 μM (striatum) or 100 μM (hippocampus). Each concentration of control or peptide was applied in quadruplicate replicates. Concentration−response parameters were determined by nonlinear regression analysis using Prism 4.0 (GraphPad, San Diego, CA). Pooled normalized data were analyzed in Prism to determine EC_{50}, IC_{50}, and E_{max} as a percentage of nicotine response.

Computational Methods. The ligand's conformational space was explored by systematically rotating the rotatable bond connecting the two rings. The docking and scoring procedures were performed by GriDock, a parallel tool based on the AutoDock4.0 engine,[31] using the rat $\alpha4\beta2$ nicotinic model (PDB ID: 1OLE).[31] In detail, the grid box was set to include all residues within a 15 Å radius sphere around the highly conserved Trp147 residue, thus comprising the entire binding cavity. The resolution of the grid was 60 × 60 × 60 points with a grid spacing of 0.450 Å. For the docking simulations, the flexible bonds of the ligand were automatically recognized by GriDock and left free to rotate so as to account for ligand flexibility within the binding cavity. Each substrate was docked with the Lamarckian algorithm as implemented in AutoDock. The genetic-based algorithm ran 30 simulations per substrate with 2000000 energy evaluations and a maximum number of generations of 27000. The crossover rate was increased to 0.8, and the number of individuals in each population to 150. All other parameters were left at the AutoDock default settings. The obtained complexes were finally minimized to favor the mutual adaptability between ligand and receptor, and the optimized complexes were then used to recalculate AutoDock docking scores and the VEGA energy scores.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H NMR spectra and elemental analysis results for the final compounds **2**−**9** and **7a**. Preparations of 2-MEM-4-bromophenol and of 2-benzyloxyphenol *p*-substituted with $COCH_3$ and Ph. The alignment of six rat nicotinic subunits ($\alpha4$, $\alpha3$, $\alpha6$, $\alpha7$, $\beta2$, $\beta4$). The complexes between $\alpha4\beta2$ nAChR binding site and (*R*,*S*)-**4** and (*S*,*S*)-**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

Boc, *t*-butoxycarbonyl; Cbz, carbobenzyloxy; CNS, central nervous system; DA, dopamine; DBU, diazabicycloundecene; DCM, dichloromethane; DEAD, diethyl azodicarboxylate; DIAD, diisopropyl azodicarboxylate; DME, dimethoxyethane; HBA, hydrogen bond acceptor; HBA/π, hydrogen bond acceptor-π-electron rich group; HBD, hydrogen bond donor; HEK, human embryonic kidney; KB, Krebs buffer; MEM, methoxy ethoxy methyl; NA, noradrenaline; nAChR, nicotinic acetylcholine receptor; SAR, structure−activity relationship; TEA, triethylamine

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