

Chemistry and Pharmacology of a Series of Unichiral Analogues of 2-(2-Pyrrolidinyl)-1,4-benzodioxane, Prolinol Phenyl Ether, and Prolinol 3-Pyridyl Ether Designed as $\alpha 4\beta 2$ -Nicotinic Acetylcholine Receptor Agonists

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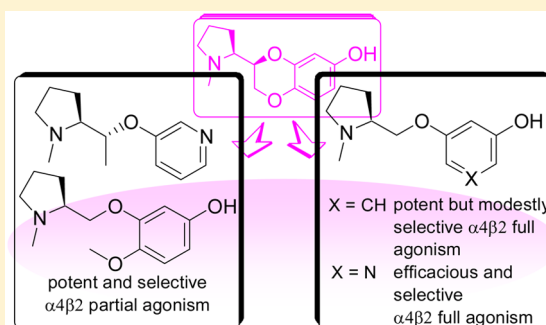
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Supporting Information

ABSTRACT: Some unichiral analogues of 2*R*,2'*S*-2-(1'-methyl-2'-pyrrolidinyl)-7-hydroxy-1,4-benzodioxane, a potent and selective $\alpha 4\beta 2$ -nAChR partial agonist, were designed by opening dioxane and replacing hydroxyl carbon with nitrogen. The resulting 3-pyridyl and *m*-hydroxyphenyl ethers have high $\alpha 4\beta 2$ affinity and good subtype selectivity, which get lost if OH is removed from phenyl or the position of pyridine nitrogen is changed. High $\alpha 4\beta 2$ affinity and selectivity are also attained by meta hydroxylating the 3-pyridyl and the phenyl ethers of (*S*)-*N*-methylprolinol and the phenyl ether of (*S*)-2-azetidinethanol, known $\alpha 4\beta 2$ agonists, although the interaction mode of the aryloxymethylene substructure cannot be assimilated to that of benzodioxane. Indeed, the $\alpha 4\beta 2$ and $\alpha 3\beta 4$ functional tests well differentiate behaviors that the binding tests homologize: both the 3-hydroxyphenyl and the 5-hydroxy-3-pyridyl ether of *N*-methylprolinol are $\alpha 4\beta 2$ full agonists, but only the latter is highly $\alpha 4\beta 2/\alpha 3\beta 4$ selective, while potent and selective partial $\alpha 4\beta 2$ agonism characterizes the hydroxybenzodioxane derivative and its two opened semirigid analogues.



INTRODUCTION

The widespread distribution in the CNS and the key role in a number of CNS functions make $\alpha 4\beta 2$ neuronal nicotinic acetylcholine receptors (nAChRs) promising therapeutic targets in the treatment of CNS disorders such as neurodegenerative conditions, cognitive dysfunctions, pain, and nicotine dependence.^{1–6} Varenicline (Chart 1), an $\alpha 4\beta 2$ agonist indicated for smoking cessation, has been approved for human use,^{7–10} and a number of selective $\alpha 4\beta 2$ -nAChR agonists have passed preclinical evaluations and the early stages of clinical trials before being abandoned.^{6,11}

More recently, selective $\alpha 4\beta 2$ partial agonists have received considerable attention as novel antidepressants.¹² Common features of their structures are 3-pyridyl bound to the α carbon of pyrrolidine or azetidine directly or through a flexible oxymethylene bridge, and the presence of a second substituent at the 5-position of pyridine nucleus, which confers selectivity for $\alpha 4\beta 2$ - over $\alpha 3\beta 4$ -nAChR subtype without prejudicing the

high functional potency at the $\alpha 4\beta 2$ receptor proper to the parent monosubstituted pyridines (Chart 1).^{13–18} Reducing the side effects due to activation or blockade of peripheral $\alpha 3\beta 4$ -nAChR is a central issue when developing centrally acting nicotinoids. Docking studies indicate that the substitution pattern at the pyridyl residue is determinant of very high $\alpha 4\beta 2/\alpha 3\beta 4$ binding selectivity because it directly influences the close interactions of pyridyl with nonconserved residues on the β -side of the ligand-binding pocket and, in some cases, indirectly also the pose of the protonated amino group interacting with the conserved residues of the α -side.^{19–21} Coupled with such a subtype selectivity, potent partial agonism with low intrinsic activity seems to have higher therapeutic potential than full agonism or antagonism for reasons under debate. Indeed varenicline is an $\alpha 4\beta 2$ -nAChR partial agonist, and such a

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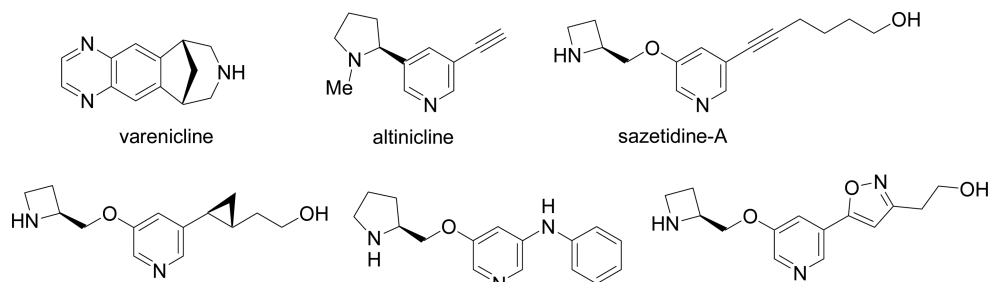
Chart 1. $\alpha 4\beta 2$ -nAChR Partial Agonists: Varenicline and 3,5-Disubstituted Pyridines

Chart 2. Design of Conformationally Restricted Prolinol Phenyl Ethers

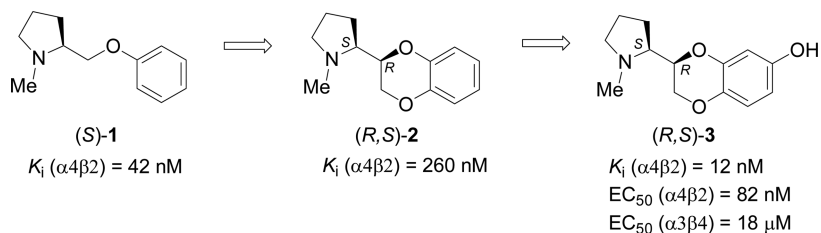
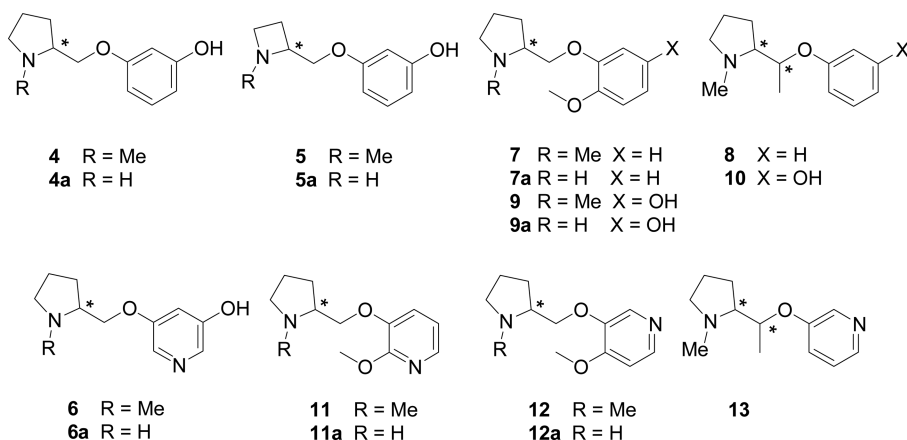
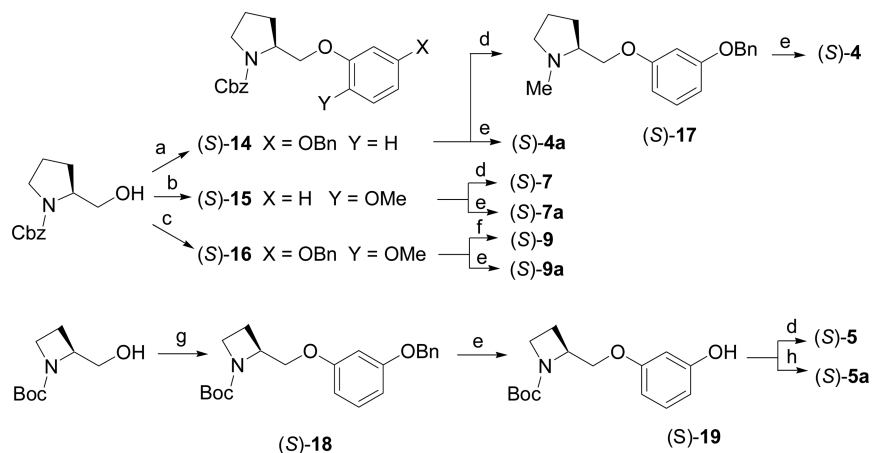


Chart 3. Target Phenyl and 3-Pyridyl Ethers of Prolinol and 2-Azetidinemethanol

Scheme 1. Synthesis of Compounds 4, 4a, 5, 5a, 7, 7a, 9, and 9a^a

^aReagents and conditions: (a) 3-benzyloxyphenol, PPh₃, DIAD, THF, reflux, 18 h; (b) 2-methoxyphenol, PPh₃, DIAD, THF, 140 °C, 30 min, microwave; (c) 2-methoxy-5-benzyloxyphenol, PPh₃, DIAD, THF, 120 °C, 15 min, microwave; (d) LiAlH₄, THF, reflux, 2 h; (e) H₂, Pd/C, MeOH, rt, 2 h; (f) H₂, Pd/C, CH₂O, MeOH, rt, 2 h; (g) 3-benzyloxyphenol, PPh₃, DIAD, THF, 140 °C, 30 min, microwave; (h) 1.25 N HCl, MeOH, rt, 16 h.

feature is shared by the majority of the $\alpha 4\beta 2$ -nAChR targeting ligands submitted to preclinical and clinical evaluation, for instance as antidepressants.¹²

We have recently identified the 2*R*,2'*S* diastereomer of 2-(1'-methyl-2'-pyrrolidinyl)-1,4-benzodioxane hydroxylated at the benzodioxane C(7) [(*R,S*)-3] as a new potent $\alpha 4\beta 2$ -nAChR partial agonist.²² The only one in a series of analogues with different 7-substituents to have nanomolar binding affinity, (*R,S*)-3 resulted from a SAR study on 2-(1'-methyl-2'-pyrrolidinyl)-1,4-benzodioxane (**2**), an $\alpha 4\beta 2$ -nAChR ligand with moderate submicromolar affinity when *S* configured at the pyrrolidine stereocenter, in turn designed by rigidifying the phenoxymethylene portion of the known nicotinic agonist *N*-methylprolinol phenyl ether **1**²³ in the 1,4-benzodioxane system (Chart 2).^{24,25}

The considerable enhancement of $\alpha 4\beta 2$ affinity caused by the 7-OH substituent at the benzodioxane nucleus and the high $\alpha 4\beta 2$ versus $\alpha 3\beta 4$ functional selectivity of (*R,S*)-3 prompted us to study the effect of such a beneficial substitution also at the meta position of the conformationally free parent compound of **3**, namely, the prolinol phenyl ether **1**, and of two semirigid analogues of **3**, the prolinol 2-methoxyphenyl ether **7** and the α -methylprolinol phenyl ether **8** (Chart 3). In addition to the phenyl ethers **1**, **7**, and **8** and their respective meta-hydroxylated analogues **4**, **9**, and **10**, we also considered the azetidinyll analogue **5** of **4** and the pyridines **6**, **11**, **12**, and **13**, which are isosteres of **4**, **7**, and **8** (Chart 3).

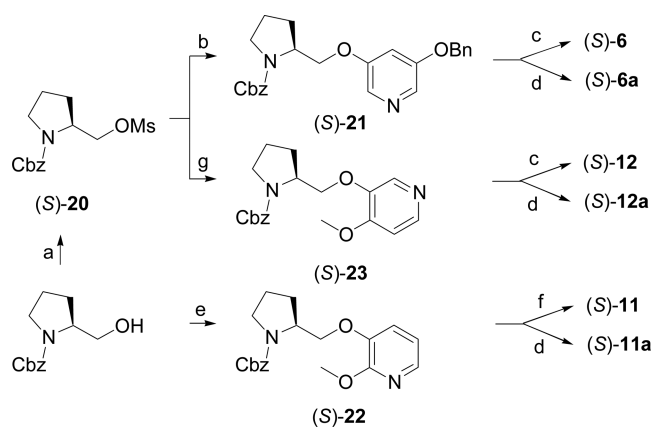
Here, we report the synthesis of the compounds **4**–**13** and of the *N*-desmethyl analogues **4a**–**7a**, **9a**, **11a**, and **12a**, in particular of the stereoisomers with *S* configuration at the alicyclic amine stereocenter, their pharmacological evaluation as $\alpha 4\beta 2$ -nAChRs ligands, and an SAR analysis based on the comparison between the affinity and activity profiles of the conformationally unconstrained 3-pyridyl and 3-hydroxyphenyl ethers **4**, **5**, and **6** on one side and of the hydroxybenzodioxane **3** and its semirigid opened analogues **7**–**13** on the other.

CHEMISTRY

Scheme 1 reports the synthesis of the *S* stereoisomers of the phenyl ethers **4**, **7**, and **9** and of the corresponding *N*-desmethyl analogues **4a**, **7a**, and **9a**, which started from the Mitsunobu reaction between *N*-Cbz protected (*S*)-prolinol and 3-benzyloxyphenol, 2-methoxyphenol, and 2-methoxy-5-benzyloxyphenol to give, respectively, (*S*)-**14**, (*S*)-**15**, and (*S*)-**16**. The successive reduction of the Cbz protecting group with LiAlH₄ converted (*S*)-**14** into the *N*-methylprolinol 3-benzyloxyphenyl ether (*S*)-**17** and (*S*)-**15** into the 2-methoxyphenyl ether (*S*)-**7**. Hydrogenolysis of (*S*)-**17**, (*S*)-**14**, (*S*)-**15**, and (*S*)-**16** provided (*S*)-**4**, (*S*)-**4a**, (*S*)-**7a**, and (*S*)-**9a**, respectively, while the 2-methoxy-5-hydroxyphenyl ether (*S*)-**9** was obtained from the intermediate (*S*)-**16** by treatment with hydrogen in the presence of formaldehyde. The azetidinyll analogues of **4** and **4a**, namely, (*S*)-**5** and (*S*)-**5a**, were prepared from *N*-Boc protected (*S*)-azetidinyllmethanol,^{26,27} which was condensed with 3-benzyloxyphenol to give the *m*-benzyloxyphenyl ether (*S*)-**18** and then debenzylated to the *m*-hydroxyphenyl ether (*S*)-**19**. Reduction with LiAlH₄ converted (*S*)-**19** into (*S*)-**5**, while Boc removal converted it into (*S*)-**5a**.

N-Cbz protected (*S*)-prolinol was the starting material to synthesize the three pyridyl ethers (*S*)-**6**, (*S*)-**11**, and (*S*)-**12** and the respective desmethyl analogues (*S*)-**6a**, (*S*)-**11a**, and (*S*)-**12a** (Scheme 2). Its mesyl ester (*S*)-**20** was reacted with the sodium salt of 3-benzyloxy-5-hydroxypyridine to give (*S*)-

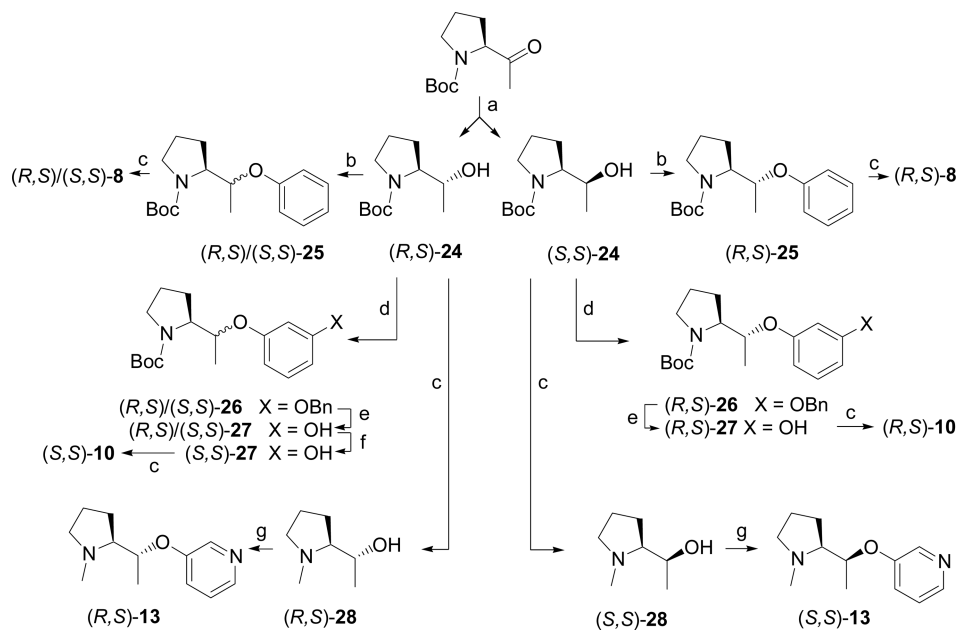
Scheme 2. Synthesis of Compounds **6**, **6a**, **11**, **11a**, **12**, and **12a**^a



^aReagents and conditions: (a) MsCl, TEA, DCM, rt, 2 h; (b) 3-benzyloxy-5-hydroxypyridine, NaH, DMF, reflux, 4 h; (c) H₂, Pd/C, CH₂O, MeOH, rt, 2 h; (d) H₂, Pd/C, MeOH, rt, 2 h; (e) 2-methoxy-3-hydroxypyridine, PPh₃, DIAD, THF, 140 °C, 30 min, microwave; (f) LiAlH₄, THF, reflux, 2 h; (g) 3-hydroxy-4-methoxypyridinium trifluoroacetate, K₂CO₃, DMF, reflux, 24 h.

21, whose O- and N-deprotection by hydrogenolysis provided (*S*)-**6a** or, in the presence of formaldehyde, (*S*)-**6**. The reaction of (*S*)-**20** with the potassium salt of 3-hydroxy-4-methoxypyridine gave the intermediate 4-methoxy-3-pyridyl ether (*S*)-**23**, which was converted into (*S*)-**12** and (*S*)-**12a** by hydrogenolysis in the presence and absence of formaldehyde, respectively. The 2-methoxy-3-pyridyl ethers (*S*)-**11** and (*S*)-**11a** were also synthesized from *N*-Cbz protected (*S*)-prolinol: Mitsunobu reaction with 2-methoxy-3-hydroxypyridine yielded (*S*)-**22**, which was reduced to (*S*)-**11** and (*S*)-**11a** by treatment with LiAlH₄ and hydrogen, respectively.

The phenyl and pyridyl ethers of α -methylprolinol **8**, **10**, and **13** were prepared from *N*-Boc protected (*S*)-2-acetylpyrrolidine (Scheme 3). The reduction with LiAlH₄ in THF at -10 °C afforded a near equimolar mixture of the diastereomeric alcohols (*R,S*)-**24** and (*S,S*)-**24**, epimers at the exocyclic secondary carbon. The two diastereomers were separated by chromatography, and the respective configurations were assigned on the basis of literature data.²⁸ The (*S,S*)-**24** diastereomer reacted with phenol in the presence of DIAD/triphenylphosphine to give, in moderate yield, the phenyl ether (*R,S*)-**25**. The reaction between (*S,S*)-**24** and 3-benzyloxyphenol led to (*R,S*)-**26**, which was debenzylated to (*R,S*)-**27**. The Boc protecting group of both (*R,S*)-**25** and (*R,S*)-**27** was reduced to methyl with LiAlH₄ to give (*R,S*)-**8** and (*R,S*)-**10**, respectively. The *R* absolute configuration was assigned to the exocyclic stereocenter of **8** and **10** prepared from (*S,S*)-**24** and, retrospectively, to the same carbon of the respective precursors **25**, **26**, and **27** with the aid of ¹H NMR and conformational analysis. As reported in the Experimental Section, the signal of the exocyclic methine is a quartet of doublets, resulting from coupling to the three methyl protons with a larger *J* value (~6 Hz) and to the endocyclic methine proton with a smaller *J* value (~3 Hz). Such a pattern indicates a relative synclinal disposition of the two methinic protons, which is favored in the *R,S* diastereomer and virtually forbidden in the *S,S* diastereomer because of repulsive interaction between the two methyl groups. This means that the two Mitsunobu reactions between (*S,S*)-**24** and, respectively, phenol and 3-benzyloxyphenol

Scheme 3. Synthesis of Compounds 8, 10, and 13^a

^aReagents and conditions: (a) LiAlH₄, THF, -10 °C, 1 h, chromatographic separation; (b) phenol, PPh₃, DIAD, THF, 150 °C, 30 min, microwave; (c) LiAlH₄, THF, reflux, 2 h; (d) 3-benzyloxyphenol, PPh₃, DIAD, THF, 150 °C, 30 min, microwave; (e) H₂, Pd/C, MeOH, rt, 2 h; (f) chromatographic separation; (g) 3-hydroxypyridine, PPh₃, DIAD, THF, reflux, 16 h.

Table 1. Nicotine and Compounds 1–13: Affinity for Native $\alpha 4\beta 2$ and $\alpha 7$ nAChR Subtypes in Rat Brain Membranes, Respectively Labeled by [³H]Epibatidine and [¹²⁵I] α -Bungarotoxin, and Heterologously Expressed Human $\alpha 3\beta 4$ nAChRs, Labeled by [³H]Epibatidine^a

compd	K _i (μM)			compd	K _i (μM)		
	$\alpha 4\beta 2$ nAChR [³ H]Epi	$\alpha 7$ nAChR [¹²⁵ I] α Bgtx	$\alpha 3\beta 4$ nAChR [³ H]Epi		$\alpha 4\beta 2$ nAChR [³ H]Epi	$\alpha 7$ nAChR [¹²⁵ I] α Bgtx	$\alpha 3\beta 4$ nAChR [³ H]Epi
nicotine	0.004 (18)	0.234 (29)	0.261 (30)	(R,S)-8	1.55 (29)	15.20 (21)	1.30 (32)
(S)-1	0.042			(R,S)-8 + (S,S)-8	4.59 (29)	8.90 (88)	1.40 (30)
(R,S)-2	0.26 (32)	21 (44)	1.2 (28)	(S)-9	0.0189 (33)	1.15 (61)	0.271 (37)
(R,S)-3	0.012 (13)	0.427 (34)	0.310	(S)-9a	1.08 (37)	nd	7.7 (25)
(S)-4	0.0011 (29)	0.119 (25)	0.074 (100)	(R,S)-10	0.0111 (18)	0.057 (32)	0.257 (38)
(S)-4a	0.407 (26)	0.212 (42)	3.8 (119)	(S,S)-10	0.192 (20)	0.743 (60)	0.752 (39)
(S)-5	0.0085 (49)	0.029 (37)	0.102 (37)	(S)-11	7.28 (29)	nd	0.793 (25)
(S)-5a	0.0071 (48)	1.20 (82)	0.040(42)	(S)-11a	8.96 (37)	nd	5.3 (28)
(S)-6	0.0037 (29)	0.321 (27)	0.235 (57)	(S)-12	0.255 (22)	9.40 (23)	2.10 (39)
(S)-6a	0.0025 (19)	3.50 (60)	0.100 (46)	(S)-12a	0.613 (16)	21.50 (26)	2.90 (40)
(S)-7	9.4 (34)	nd	0.749 (27)	(R,S)-13	0.027	0.388 (21)	5.0 (32)
(S)-7a	53.5 (38)	nd	3.6 (30)	(S,S)-13	0.877	6.100 (28)	5.6 (39)

^aThe K_d and K_i values were derived from three [³H]epibatidine and [¹²⁵I] α -bungarotoxin saturation and three competition binding experiments using rat cortex ($\alpha 4\beta 2$) and hippocampus ($\alpha 7$) membranes and the membrane of human $\alpha 3\beta 4$ transfected cells as described in refs 22 and 31. 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, 2, and 3 are those previously reported in refs 23, 25, and 22.

occurred with the same expected S_N2 mechanism, which implies configuration inversion of the hydroxyl carbon. On the other hand, the (R,S)-24 diastereomer reacted with phenol in the presence of DIAD/triphenylphosphine to give, in very low yield, an equimolar (R,S)-25/(S,S)-25 mixture, thus excluding a reaction mechanism based on the S_N2 displacement by the phenol. The mixed (R,S)-25 and (S,S)-25 diastereomers were reduced to an equimolar mixture of (R,S)-8 and (S,S)-8, which was submitted to the binding tests as such, while postponing its resolution to the finding of high affinities for pure (R,S)-8 and/or for its mixture with (S,S)-8.

An equimolar diastereomeric mixture of phenyl ethers, (R,S)-26 and (S,S)-26, resulted, in low yield, also from the Mitsunobu reaction between (R,S)-24 and 3-benzyloxyphenol. After hydrogenolytic debenylation, the two phenols (R,S)-27 and (S,S)-27 were separated by chromatography and the purified (S,S)-27 was converted into (S,S)-10. Again, the configuration, *S* in this case, was assigned to the exocyclic stereocenter of 10 and, retrospectively, to the same carbon of the stereoisomer of its precursor 27 isolated by chromatography with the aid of ¹H NMR and conformational analysis. Instead of the quartet of doublets observed in (R,S)-10 spectrum, the signal of the exocyclic methine was now a quintet, resulting from coupling to

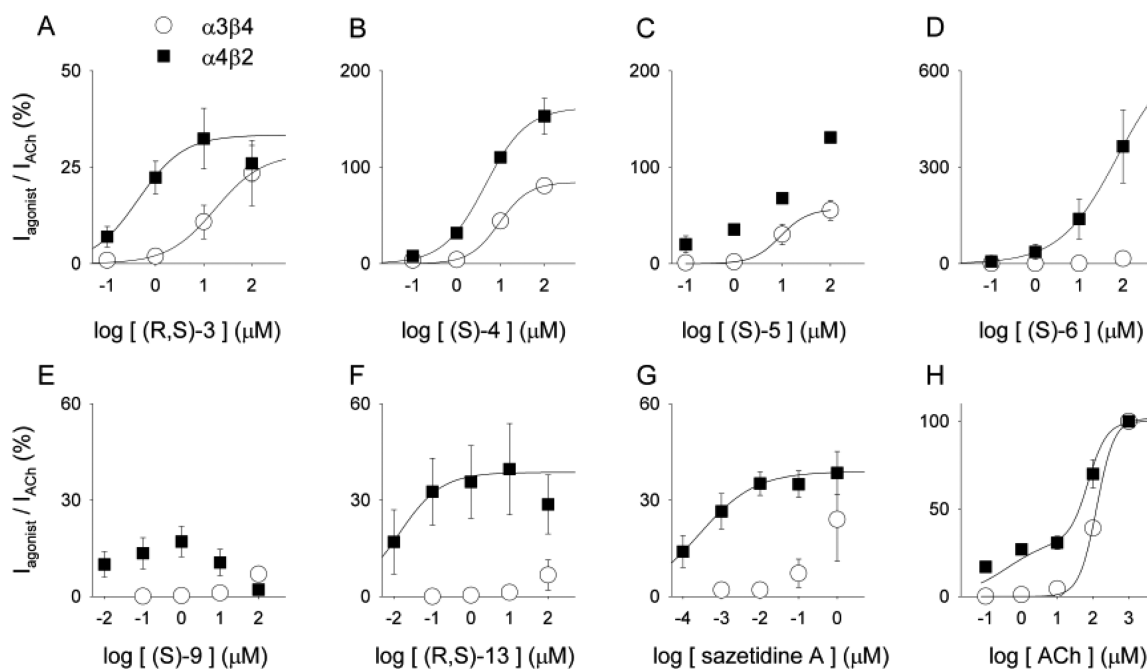


Figure 1. Agonist effects of (R,S)-3, (S)-4, (S)-5, (S)-6, (S)-9, (R,S)-13, sazetidine, and ACh on transfected human $\alpha 4\beta 2$ and $\alpha 3\beta 4$ subtypes. Human $\alpha 4\beta 2$ (■), $\alpha 3\beta 4$ (○) subtypes were transiently transfected into the GH4C1 rat anterior pituitary cell line, and the activation responses are normalized to the maximal response to 1 mM ACh.

the three methyl protons and to the endocyclic methine proton with the same relatively large J value (~ 6 Hz). Such a multiplicity pattern is consistent with a relative anticlinical disposition of the two methinic protons, which is favored in the S,S diastereomer and disfavored in the R,S diastereomer again because of the too close position of the two methyl groups.

Lastly, to prepare (R,S)-13 and (S,S)-13, which are described as an unresolved diastereomeric mixture,²⁹ the Boc protecting group of the secondary alcohols (R,S)-24 and (S,S)-24 was reduced to methyl. The ^1H NMR signal of the exocyclic methine of the resulting N -methylamino alcohols (R,S)-28 and (S,S)-28, whose configurations are known because they are derived from (R,S)-24²⁸ and (S,S)-24, was a quartet of doublets and a quintet, respectively, thus confirming the previous configurations assignments to **8** and **10** diastereomers. The Mitsunobu reaction was carried out on (R,S)-28 and (S,S)-28 by treatment with 3-hydroxypyridine in the presence of DIAD/triphenylphosphine. Different from the hydroxyl carbon inversion and racemization observed in the etherification of (S,S)-24 and (R,S)-24, respectively, (R,S)-13 and (S,S)-13 were formed with configuration retention from (R,S)-28 and (S,S)-28, respectively. Indeed, the signal of the exocyclic methinic proton in the ^1H NMR spectrum of both (R,S)-28 and its derived pyridyl ether **13** is a quartet of doublets, as for (R,S)-**8** and (R,S)-**10**, while the same signal is a quintet in the ^1H NMR spectrum of both (S,S)-28 and its derived pyridyl ether **13**, as for (S,S)-**10**. This can be explained by the formation of a bicyclic aziridinium ion intermediate: a double S_N2 , also called neighboring group participation, in this case of the pyrrolidine basic nitrogen, would be responsible for the conservation of the configuration of the hydroxyl carbon. The isolation of 1-methyl-3-(3-pyridyloxy)piperidine as a byproduct proves such a mechanism, which we had previously observed also in the chloride displacement from N -methyl-2-chloromethylpyrrolidine by acetone oximate anion.³⁰

■ BIOLOGY

Binding Studies. The synthesized compounds were tested in vitro on rat cerebral cortex or hippocampus membranes in order to evaluate their affinity at the $\alpha 4\beta 2$ and $\alpha 7$ central nicotinic receptors labeled by [^3H]epibatidine ([^3H]Epi) and [^{125}I] α -bungarotoxin ([^{125}I] α Bgtx) and to evaluate their affinity on heterologously expressed human $\alpha 3\beta 4$ receptors labeled with [^3H]Epi. Nicotine was included in the series for comparison. The results are listed in Table 1 together with those previously reported for (S)-**1**,²³ (R,S)-**2**,²⁵ and (R,S)-**3**.²²

On the basis of their $\alpha 4\beta 2$ affinity, two groups of compounds can be distinguished. The first includes the compounds **3**, **4**, **5**, **6**, **9**, **10**, and **13**, which have high $\alpha 4\beta 2$ affinity (ranging from 1.1 to 27 nM) when S configured at the alicyclic amine stereocenter and whose respective N -desmethyl analogues have considerably lower affinity except for **5a** and **6a**, which also have nanomolar $\alpha 4\beta 2$ affinity. Their affinity for $\alpha 7$ is lower than their affinity for $\alpha 4\beta 2$ (except for (S)-**4a**), with (S)-**4**, (S)-**5a**, (S)-**6**, (S)-**6a**, and (S)-**9** showing the greatest difference in affinity for $\alpha 4\beta 2$ over $\alpha 7$. Their $\alpha 3\beta 4$ affinity is also lower, with (S)-**4**, (S)-**6**, (S)-**6a**, and (R,S)-**13** showing the greatest difference in sensitivity for $\alpha 4\beta 2$ over $\alpha 3\beta 4$.

The second group includes the compounds **7**, **8**, and **11** which all have very low $\alpha 4\beta 2$ affinity, ranging from 1 to 10–50 μM K_i , regardless of N -methylation (**7** and **11**) or configuration (**8**), and with no or even reversed $\alpha 4\beta 2/\alpha 3\beta 4$ selectivity. Compounds (S)-**12** and (S)-**12a** fall between these two groups, having moderate submicromolar $\alpha 4\beta 2$ affinity values, which are higher than those for $\alpha 7$ and $\alpha 3\beta 4$ subtypes.

In Vitro Functional Activity at nAChR. The affinity measurements indicate that the stereoisomers with S configuration at the alicyclic amine stereocenter of compounds **4**, **5**, **6**, **9**, **10**, and **13** have high or very high affinity for $\alpha 4\beta 2$ -nAChRs and moderate or very low affinity for $\alpha 3\beta 4$ and $\alpha 7$ nAChRs. These measurements give no indication of their possible functional activity and, consequently, neither of their

actual functional selectivity between receptor subtypes. We have previously reported that (R,S)-3 shows 26-fold lower $\alpha 3\beta 4$ affinity ($0.310 \mu\text{M } K_i$) than that determined for the $\alpha 4\beta 2$ subtype ($0.012 \mu\text{M } K_i$) against 214-fold lower $\alpha 3\beta 4$ activity ($\text{EC}_{50} = 17.6 \mu\text{M}$) than that measured for the $\alpha 4\beta 2$ subtype ($\text{EC}_{50} = 0.082 \mu\text{M}$). In order to determine the potency, efficacy, and selectivity toward the $\alpha 4\beta 2$ and $\alpha 3\beta 4$ subtypes, we tested functionally compounds (S)-4, (S)-5, (S)-6, (S)-9, and (R,S)-13 and we compared the results with those of the compound (R,S)-3.

Figure 1 shows electrophysiological effects of (S)-4, (S)-5, (S)-6, (S)-9, (R,S)-13, and (R,S)-3 on heterologously expressed human $\alpha 4\beta 2$ and $\alpha 3\beta 4$ nAChRs. Table 2 shows all of the data normalized to those obtained upon superfusion of the same cell with 1 mM ACh. The electrophysiological study confirmed that (R,S)-3 (Figure 1A) behaves as a partial agonist that is 57 times more potent on the $\alpha 4\beta 2$ subtype ($\text{EC}_{50} = 0.3 \pm 0.2 \mu\text{M}$) than

on the $\alpha 3\beta 4$ subtype ($17 \pm 6 \mu\text{M}$) and showed that the compound (R,S)-13 (Figure 1F) is a more potent and selective partial agonist than (R,S)-3, with an EC_{50} of $0.013 \mu\text{M}$ for the $\alpha 4\beta 2$ subtype and no effect on the $\alpha 3\beta 4$ subtype.

We also determined that (S)-4 (Figure 1B) behaves as a full agonist with a greater efficacy (166%) than ACh on the $\alpha 4\beta 2$ but similar potency on the $\alpha 4\beta 2$ ($\text{EC}_{50} = 4.4 \pm 0.6 \mu\text{M}$) and $\alpha 3\beta 4$ subtype ($9 \pm 2 \mu\text{M}$). (S)-5 (Figure 1C) also is a full agonist for the $\alpha 4\beta 2$ and a partial agonist for the $\alpha 3\beta 4$ subtype with an EC_{50} of $9.2 \mu\text{M}$. Compound (S)-6 (Figure 1D) is a very efficacious ($661 \pm 46\%$ of 1 mM ACh) and selective $\alpha 4\beta 2$ agonist with relatively low potency ($73 \pm 16 \mu\text{M}$). Compound (S)-9 (Figure 1E) has an inverted U shaped curve of activation for the $\alpha 4\beta 2$ subtype (probably because it has a channel blocker activity at high concentration) and no effect on the $\alpha 3\beta 4$ subtype.

It is known that the heteromeric $\alpha 4\beta 2$ and $\alpha 3\beta 4$ can exist in two alternative stoichiometries: $2\alpha/3\beta$ or, conversely, $3\alpha/2\beta$. In each receptor stoichiometry the two agonist orthosteric binding sites are at the two α/β interfaces and the presence of the fifth accessory subunit may confer differences to the receptor's pharmacological properties.³² In the case of the $\alpha 4\beta 2$ subtype the two stoichiometries differ greatly in pharmacology: ACh has an EC_{50} value of $\sim 1 \mu\text{M}$ ³² and the agonist sazetidine is a full agonist for the $(\alpha 4)_2(\beta 2)_3$ stoichiometry,³³ whereas toward the $(\alpha 4)_3(\beta 2)_2$ stoichiometry ACh has an EC_{50} value of $\sim 100 \mu\text{M}$ and sazetidine has a very low efficacy (6%). Analysis of the functional response of the $\alpha 4\beta 2$ subtype expressed in our cells shows that ACh has biphasic concentration–effect curve with the high affinity ($0.4 \mu\text{M}$) representing 33% and the low affinity ($100 \mu\text{M}$) 67% of the total ACh current (Table 2 and Figure 1H), and sazetidine (Figure 1G) has a maximum response of 40% that of 1 mM ACh. These data indicate that in our cells both $\alpha 4\beta 2$ stoichiometries are present and represent $1/3$ [$(\alpha 4)_2(\beta 2)_3$] and $2/3$ [$(\alpha 4)_3(\beta 2)_2$] of the functional receptors.

In the case of the $\alpha 3\beta 4$ subtype, it was previously reported that the two stoichiometries have very similar EC_{50} values and potency for almost all nicotinic agonists,¹⁴ and in agreement with this we only determined a single EC_{50} value of $127 \pm 1 \mu\text{M}$.

DISCUSSION

The introduction of OH into the 7-position of pyrrolidinylbenzodioxane (R,S)-2 results in a 22-fold increase in $\alpha 4\beta 2$ affinity: (R,S)-3 has $12 \text{ nM } K_i$ against the $0.26 \mu\text{M } K_i$ of (R,S)-2.^{22,25} Moreover, (R,S)-3 is a potent $\alpha 4\beta 2$ partial agonist, 200-fold selective over the ganglionic $\alpha 3\beta 4$ subtype, as previously proved by a test of dopamine release from rat striatum ($\text{EC}_{50} = 82 \text{ nM}$)²² and now confirmed on human $\alpha 4\beta 2$ nicotinic receptors ($\text{EC}_{50} = 0.3 \mu\text{M}$). Consistent with such results, our previous docking analysis showed similar interactions of the protonated pyrrolidine ring and of the benzodioxane substructure of (R,S)-2 and (R,S)-3 with $\alpha 4$ and $\beta 2$ amino acid residues of the $\alpha 4\beta 2$ -nAChR binding pocket and, only for (R,S)-3, additional H-bonds between the 7-hydroxyl and two serines, Ser(111) and Ser(115) of the $\beta 2$ subunit.^{22,25} These additional bonds would be responsible for the increased potency and the $\beta 2$ selectivity, while substitutions other than OH are found unproductive or deleterious apparently because only small substituents can be accepted at benzodioxane 7-position.²²

Compared to our pyrrolidinylbenzodioxanes, nicotinoids with a flexible oxymethylene bridge between the aromatic

Table 2. Effects of (R,S)-3, (S)-4, and (S)-5 on Transfected Human Nicotinic Receptor Subtypes^a

compd	parameter	$\alpha 4\beta 2$	$\alpha 3\beta 4$
(R,S)-3	$I/I_{\text{max ACh}}$ (%)	29 ± 4 (8)	28 ± 3 (4)
	EC_{50} (μM)	0.3 ± 0.2	17 ± 6
	nH	1.1 ± 0.7	0.9 ± 0.2
(S)-4	$I/I_{\text{max ACh}}$ (%)	162 ± 6 (4)	85 ± 5 (7)
	EC_{50} (μM)	4.4 ± 0.6	9 ± 2
	nH	0.91 ± 0.08	1.3 ± 0.4
(S)-5	$I/I_{\text{max ACh}}$ (%)	131 ± 1 (4)	57 ± 1 (5)
	EC_{50} (μM)	9.2 ± 0.5	9.2 ± 0.5
	nH	1.5 ± 0.2	
(S)-6	$I/I_{\text{max ACh}}$ (%)	661 ± 46 (4)	15 ± 2 (5)
	EC_{50} (μM)	73 ± 16	
	nH	0.66 ± 0.03	
(S)-9	$I/I_{\text{max ACh}}$ (%)	17 ± 5 (7)	7.0 ± 0.5 (8)
	EC_{50} (μM)		
	nH		
(R,S)-13	$I/I_{\text{max ACh}}$ (%)	39 ± 1 (7)	7 ± 4 (5)
	EC_{50} (μM)	0.013 ± 0.003	
	nH	0.7 ± 0.1	
sazetidine A	$I/I_{\text{max ACh}}$ (%)	39 ± 1	24 ± 13 (4)
	EC_{50} (μM)	0.00027 ± 0.00006	
	nH	0.6 ± 0.1	
acetylcholine	$I/I_{\text{max ACh}}$ (%)	33 ± 8 (4)	100 (4)
		67 ± 8	
	EC_{50} (μM)	0.4	127 ± 1
	nH	100	
		0.6 ± 0.5	1.5 ± 0.3
		1.6 ± 0.9	

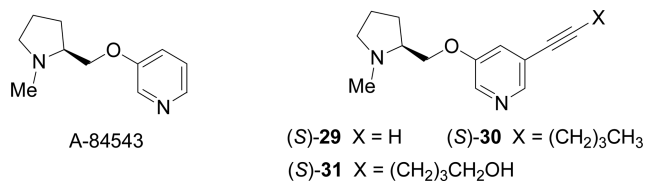
^aDose–response curves were constructed by sequentially applying different concentrations of the indicated compounds and normalizing the current amplitudes to the values obtained using 1 mM ACh on the same cell. For the quantitative estimates of agonist activity, the dose–response relationship was fitted when possible using the equation

$$I = I_{\text{max}} \left(\frac{[C]^{\text{nH}}}{\text{EC}_{50}^{\text{nH}} + [C]^{\text{nH}}} \right)$$

where I is the peak current amplitude induced by the agonist at concentration C , I_{max} is the maximum response of the cell, nH the Hill coefficient, and EC_{50} the concentration at which a half-maximum response is induced. Numbers in parentheses in Table 2 represent the number of tested cells.

ring and the pyrrolidine residue, such as the 3-pyridyl ether of *S*-prolinol A-84543^{20,34} and its 5-alkynyl substituted analogues (*S*)-29,²⁰ (*S*)-30,²⁰ and (*S*)-31¹⁹ (Chart 4), similarly bind to the

Chart 4. 3-Pyridyl Ether of (*S*)-*N*-Methylprolinol (A-84543) and Some of Its 5-Alkynyl Substituted Derivatives



carbonyl group of Trp147, a conserved residue of the $\alpha 4$ subunit, through the pyrrolidine N⁺ but differently place the aromatic portion in the $\alpha 4/\beta 2$ -nAChR binding pocket. In fact, though variously oriented, the pyridine ring of A-84543, (*S*)-29, (*S*)-30, and (*S*)-31 always maintains π - π and strong hydrophobic interaction with Phe117, a nonconserved residue of the $\beta 2$ subunit, with which the benzodioxane aromatic ring of (*R,S*)-2 and (*R,S*)-3 cannot interact because of the conformation of the oxymethine bridge, necessarily extended as a part of the dioxane ring (Figure 2).

On the other side, the pivotal role in the benzodioxane arrangement is played by the dioxane O(4) which replaces pyridyl nitrogen in interaction with nonconserved Lys77- $\beta 2$).^{22,25} Consistent with such a difference in the pose of the aromatic ring between pyridyl ethers and benzodioxanes, the pyridine ring is far from the mentioned $\beta 2$ serine residues and tolerates elongated and bulky substituents at the 5-position,^{15–18} whereas the benzodioxane benzene approximates the serine residues and does not tolerate bulky substituents at the 7-position but only the small hydroxyl function eliciting beneficial H-bonds with those hydroxylamino acids.²² Although all these observations suggested that the unoccupied meta position of the 3-pyridyl ethers and reasonably the meta position of the isosteric phenyl ethers are not assimilable to the 7-position of pyrrolidinebenzodioxane, it was mandatory to investigate the effect of the meta-hydroxy substitution on the $\alpha 4/\beta 2$ affinity, activity, and selectivity of the phenyl and 3-pyridyl ethers of prolinol, which are the parent opened analogues of 2.

Indeed, these effects are different and absolutely not negligible. Compared to the unsubstituted phenyl ether of (*S*)-*N*-methylprolinol (*S*)-1, the meta-hydroxy analogue (*S*)-4 shows a 40-fold higher $\alpha 4/\beta 2$ affinity (1.1 nM K_i) with a good selectivity over the $\alpha 7$ and $\alpha 3/\beta 4$ subtypes. Similar $\alpha 4/\beta 2$ affinity increase is produced also by the hydroxylation of the phenyl ether of 1-methyl-2-azetidinmethanol: (*S*)-5 has a 12-fold

higher affinity (8.5 nM K_i) than its nonhydroxylated analogue (101 nM K_i),²³ and (*S*)-5a is 7-fold higher (7.1 nM versus 52 nM K_i).²³ Moreover, functional tests indicate that (*S*)-4 is a potent full $\alpha 4/\beta 2$ agonist ($EC_{50} = 4.4 \mu\text{M}$; 162% maximal efficacy relative to 1 mM ACh) and a partial $\alpha 3/\beta 4$ agonist ($EC_{50} = 9 \mu\text{M}$; 85% maximal efficacy relative to 1 mM ACh) showing, however, a functional $\alpha 4/\beta 2$ vs $\alpha 3/\beta 4$ selectivity sensibly lower than that found in the binding experiments. Again, the behavior of the azetidiny analogue (*S*)-5 is near identical to that of (*S*)-4 as shown by the almost superimposable dose–response curves (Figure 1B and Figure 1C). On the other hand, the meta hydroxylated (*S*)-prolinol 3-pyridyl ether (*S*)-6 maintains, without further improvements, the high $\alpha 4/\beta 2$ affinity and selectivity displayed by A-84543 in binding assays, but interestingly it behaves as a full $\alpha 4/\beta 2$ agonist with a very high functional $\alpha 4/\beta 2$ vs $\alpha 3/\beta 4$ selectivity, much higher than that of A-84543 and of other unsubstituted 3-pyridyl ethers.³⁴ Such a behavior resembles that of 2,5-diazabicyclo[2.2.1]heptane 5-hydroxy-3-pyridyl substituted at the 2-position.³⁵

In summary, as shown in Table 1, the five hydroxylated compounds (*R,S*)-3, (*S*)-4, (*S*)-5, (*S*)-5a, and (*S*)-6 have very similar $\alpha 4/\beta 2$ nicotinic affinities and more or less pronounced $\alpha 4/\beta 2$ selectivity in binding experiments; because of hydroxylation, both the benzodioxane (*R,S*)-3 and the phenyl ethers (*S*)-4, (*S*)-5 and (*S*)-5a are upgraded to the rank of pyridyl ethers A-84543 and (*S*)-6, selective $\alpha 4/\beta 2$ ligands with nanomolar affinity, although similar interaction modes, as previously explained, cannot be postulated for the aryl ethers and the benzodioxanes. It is reasonable that the 3-hydroxyphenyl derivatives can interact better than the unsubstituted phenyl ether (*S*)-1 and similarly to the 3-pyridyl ether A-84543, while the 5-hydroxy-3-pyridyl ether (*S*)-6 behaves as a wide number of 5-substituted 3-pyridyl ethers of (*S*)-*N*-methylprolinol,³⁶ which generally maintain the nanomolar $\alpha 4/\beta 2$ affinity of A-84543. Anyhow, the apparent similarity of the hydroxylated compounds in binding assays is unmasked by the functional tests, which give three quite different profiles for (*R,S*)-3, the pair of (*S*)-4 and (*S*)-5, and (*S*)-6: (a) the 7-hydroxybenzodioxane (*R,S*)-3 is confirmed to be a partial $\alpha 4/\beta 2$ and $\alpha 3/\beta 4$ agonist with high selectivity over the ganglionic subtype; (b) the 3-hydroxyphenyl ethers (*S*)-4 and (*S*)-5 are full $\alpha 4/\beta 2$ and partial $\alpha 3/\beta 4$ agonist with modest $\alpha 4/\beta 2$ selectivity, thus assimilable to A-84543; (c) the 5-hydroxy-3-pyridyl ether (*S*)-6 is a full $\alpha 4/\beta 2$ agonist with practically no $\alpha 3/\beta 4$ activity.

Interestingly, also some pyrrolidinefuopyridines, designed by rigidifying the 3-pyridyloxymethylene portion of A-8453 in a bicyclic system as our benzodioxanes, differ from the prolinol pyridyl ether in potency profile and SAR trends.³⁷ In our series

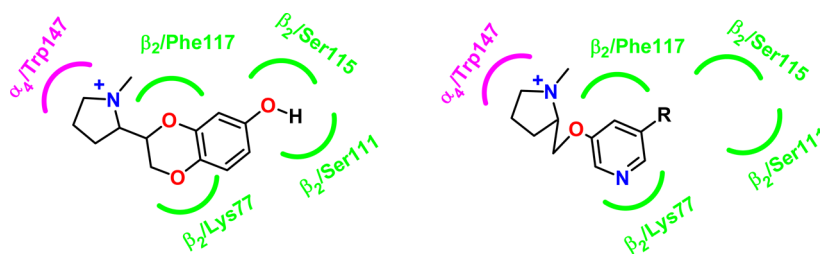


Figure 2. Bidimensional schematic representation of difference in the binding modes of (*R,S*)-3 and of (*S*)-prolinol 3-pyridyl ethers docked in a model of $\alpha 4/\beta 2$ -nAChR receptor.^{19,20,22}

of compounds, the statement that the 7-hydroxybenzodioxane derivative has its own interaction mode, quite different from those of the 3-hydroxyphenyl ether and 5-hydroxy-3-pyridyl ether and highly advantaged by hydroxylation in proper position, is substantiated by the binding results obtained for compounds 7–13. These are phenyl and 3-pyridyl ethers of prolinol, formally obtained by opening the dioxane cycle of benzodioxane or pyridodioxane and thus maintaining some rigidity of the parent bicycle due to the residual α -methyl (compounds 8, 10, and 13) or *o*-methoxyl (compounds 7, 9, 11, and 12). Compared to the unsubstituted benzodioxane (*R,S*)-2 ($K_i = 0.26 \mu\text{M}$), the 2-methoxyphenyl ether (*S*)-7, the phenyl ether of (*S*)- α -methylprolinol (*R,S*)-8, and the 2-methoxy-3-pyridyl ether (*S*)-11 show much lower 1–10 μM $\alpha 4\beta 2$ affinities and no $\alpha 4\beta 2$ selectivity. This indicates that they lose the interaction abilities of benzodioxane system without acquiring those of flexible phenoxymethylene and 3-pyridyloxymethylene substructures. However, the phenyl meta-hydroxylation again increases the $\alpha 4\beta 2$ affinity: 500 times in the case of the 2-methoxy-5-hydroxyphenyl ether (*S*)-9 (cf. 18.9 nM K_i with 9.4 μM K_i of (*S*)-7) and 140 times in the case of the 3-hydroxyphenyl ether of (*S*)- α -methylprolinol (*R,S*)-10 (cf. 11.1 nM K_i with 1.55 μM K_i of (*R,S*)-8). Also the $\alpha 4\beta 2$ selectivity is similar to that of benzodioxanes (*R,S*)-2 and (*R,S*)-3. It is evident that the *m*-hydroxyl plays a pivotal role and it can be presumed that such a substituent drives the aryloxy fragment of (*S*)-9 and (*R,S*)-10 to interact similarly to the 7-hydroxybenzodioxane substructure of (*R,S*)-3.

In the series of the three pyridyl ethers 11–13, a trend is observed analogous to that of the phenyl ethers 7, 9, and 10, if the position of the pyridine nitrogen is considered instead of the meta-hydroxylation: (a) binding profile of (*S*)-11 identical to that of its carba isostere (*S*)-7, namely, very modest $\sim 10 \mu\text{M}$ $\alpha 4\beta 2$ affinity, even lower than $\alpha 3\beta 4$ affinity; (b) moderate and high $\alpha 4\beta 2$ affinity/selectivity of (*S*)-12 and (*R,S*)-13, respectively, which are both 3-pyridyl ethers and whose pyridine nitrogen is superimposable to the hydroxylated carbon, respectively, of (*S*)-9 and (*R,S*)-10. The functional tests were performed on (*S*)-9 and (*R,S*)-13, selected among the opened benzodioxane analogues 7–13 because of their highest and most selective $\alpha 4\beta 2$ affinity. As shown in Figure 1E and Figure 1F, the *o*-methoxylated hydroxyphenyl ether (*S*)-9 and the pyridyl ether of α -methylated prolinol (*R,S*)-13 behave as partial $\alpha 4\beta 2$ agonists like (*R,S*)-3, thus supporting the hypothesis of the similar interaction mode with $\alpha 4\beta 2$ receptor suggested by the binding results. What is more, the deconstruction of 7-hydroxybenzodioxane to (*S*)-9 and, especially, to (*R,S*)-13 increases the potency of the partial $\alpha 4\beta 2$ agonism and suppresses $\alpha 3\beta 4$ activity.

For the compounds with only one stereocenter, namely, 4–7, 9, 11, and 12, the *N*-desmethyl analogues were also considered. In the case of the only azetidinyll derivative, the *N*-desmethyl compound (*S*)-5a shows the same nanomolar $\alpha 4\beta 2$ affinity as its *N*-methyl analogue (*S*)-5. On the other hand, all the *N*-methylpyrrolidinyll derivatives, especially those OH-substituted at the benzene nucleus, display higher $\alpha 4\beta 2$ affinity than the respective *N*-desmethyl analogues, as previously observed for the benzodioxane (*R,S*)-1 and (*R,S*)-3. The 5-hydroxy-3-pyridyl ether is an exception: it shows nanomolar $\alpha 4\beta 2$ affinity whether methylated [(*S*)-6] or nonmethylated [(*S*)-6a] and, interestingly, a very high $\alpha 4\beta 2$ vs $\alpha 7$ selectivity in the latter case.

CONCLUSIONS

The structures of pyrrolidinyll benzodioxane 2 and of the prolinol phenyl and 3-pyridyl ethers, 1 and A-84543, are superimposable, and all display $\alpha 4\beta 2$ -nAChR affinity. The OH substituent at the 3-position of phenyl in 1 and at the 7-position of benzodioxane in 2 enhances the $\alpha 4\beta 2$ affinity to 1 and 10 nM levels, respectively (compounds 4 and 3), and it is analogously beneficial when positioned meta to the oxymethylene or oxymethine linker in a series of phenyl ethers that we have designed as benzodioxane opened analogues (compounds 7–10). In a ligand-based approach, we have considered also the 5-OH substituted 3-pyridyl ether of *N*-methylprolinol (compound 6), which maintains the nanomolar $\alpha 4\beta 2$ affinity of its nonhydroxylated analogue A-84543, and some 3-pyridyl ethers, the compounds 11–13, which differently mimic opened 2-pyrrolidinyllbenzodioxane and show moderate or high $\alpha 4\beta 2$ affinity when the pyridine nitrogen can be superimposed to the benzodioxane hydroxylated C(7) as in compounds 12 and 13.

Though internally coherent in appearance, the high $\alpha 4\beta 2$ affinities of all our compounds hydroxylated or with properly positioned pyridine nitrogen cannot be explained by a common interaction mode. On the basis of previously reported docking studies and of the present binding data, the pyrrolidinyllbenzodioxanes 2 and 3 and their semirigid opened analogues 9, 10, 12, and 13 would similarly interact with the $\alpha 4\beta 2$ -nAChR binding pocket and in a different manner from the flexible phenyloxymethyl and pyridyloxymethyl ethers 4–6, in particular by differently positioning the aromatic cycle. Such similarity and diversity are confirmed by the profiles of $\alpha 4\beta 2$ agonism and selectivity over the ganglionic nicotinic subtype shown by the six compounds we have selected for functional tests, namely, (a) the pyrrolidinyll hydroxybenzodioxane (*R,S*)-3 and its opened analogues (*S*)-9 and (*R,S*)-13, which are all potent and selective $\alpha 4\beta 2$ partial agonists with potency and $\alpha 4\beta 2/\alpha 3\beta 4$ selectivity increasing in the order (*R,S*)-3 < (*S*)-9 < (*R,S*)-13, (b) the hydroxyphenyl ethers (*S*)-4 and (*S*)-5, potent and moderately selective $\alpha 4\beta 2$ full agonists, and the hydroxypyridyl ether (*S*)-6, a highly efficacious and selective $\alpha 4\beta 2$ full agonist.

EXPERIMENTAL SECTION

Chemistry. All chemicals and solvents were used as received from commercial sources or prepared as described in the literature. Flash chromatography purifications were performed using KP-Sil 32–63 μm 60 Å cartridges. TLC analyses were carried out on alumina sheets precoated with silica gel 60 F254 and visualized with UV light; R_f values are given for guidance. ^1H and ^{13}C NMR spectra were recorded at 300 and 75 MHz using an FT-NMR spectrometer. Chemical shifts are reported in ppm relative to residual solvent (CHCl_3 , MeOH, or DMSO) as internal standard. Thermal analyses were performed on 2–5 mg samples in closed pans at 5 $^\circ\text{C}/\text{min}$ using DSC 2010, TA Instruments. Melting points correspond to the peak maximum. Optical rotations were determined in a 1 dm cell of 1 mL capacity. Results from elemental analyses (CHN) are within $\pm 0.40\%$ of theoretical values. The results of elemental analyses indicated that the purity of all tested compounds was higher than 95%. In each described preparation, the moles of reagents are given for 1 mol of substrate.

General Procedure for Synthesis of Compounds 13–16, 18, 22, 25, and 26. The *S* isomers of 14, 15, 16, and 22 were obtained by treatment of (*S*)-*N*-Cbz-2-hydroxymethylpyrrolidine with PPh_3 (1.2 mol), DIAD (1.2 mol), and equimolar 3-benzyloxyphenol (14), 2-methoxyphenol (15), 2-methoxy-5-benzyloxyphenol (16), or 2-methoxy-3-hydroxypyridine (22). The *S* isomer of 18 was obtained by treatment of (*S*)-*N*-Boc-2-hydroxymethylazetidide with PPh_3 (1.2 mol), DIAD (1.2 mol), and equimolar 3-benzyloxyphenol. The *R,S*

isomer and the (*R,S*)/(*S,S*) diastereomeric mixture of **25** were prepared by combining PPh₃ (2.2 mol), DIAD (2.2 mol), and equimolar phenol with (*S,S*)-**24** and (*R,S*)-**24**, respectively; the same reaction with 3-benzyloxyphenol in place of phenol gave the *R,S* isomer and the (*R,S*)/(*S,S*) diastereomeric mixture of **26**, respectively. The compounds (*R,S*)-**13** and (*S,S*)-**13** were obtained from (*R,S*)-**28** and (*S,S*)-**28**, respectively, by treatment with PPh₃ (1.2 mol), DIAD (1.2 mol), and equimolar 3-hydroxypyridine. The reactions were carried out in THF at 66 °C for 16–18 h (**14** and **13**) or under microwave irradiation at 140 °C for 30 min (**15** and **18**), at 120 °C for 15 min (**16**), and at 150 °C for 30 min (**25** and **26**). The Mitsunobu condensations carried out on (*R,S*)-**24** gave (*R,S*)/(*S,S*) diastereomeric mixtures of **25** and **26** due to the racemization of the *R* stereocenter involved in the reaction. The diastereomeric mixture of (*R,S*)-**25** and (*S,S*)-**25** was not resolved, while that of (*R,S*)-**26** and (*S,S*)-**26** was resolved by chromatography on silica gel after debenzoylation.

(*R,S*)-*N*-Methyl-2-[1-(3-pyridyloxy)ethyl]pyrrolidine [(*R,S*)-13**].** Obtained as a yellow oil in 15% yield after chromatography on silica gel (3% TEA in acetone), *R_f* = 0.42; [α]_D²⁵ –87.6 (*c* 1, MeOH); ¹H NMR (CDCl₃) δ 8.27 (d, 1H, *J* = 2.0 Hz), 8.14 (dd, 1H, *J* = 4.1, 2.0 Hz), 7.20–7.12 (m, 2H), 4.42 (dq, 1H, *J* = 6.3, 3.0 Hz), 3.06 (m, 1H), 2.41 (dt, 1H, *J* = 7.7, 3.0 Hz), 2.39 (s, 3H), 2.27–2.18 (m, 1H), 1.90–1.66 (m, 4H), 1.26 (d, 3H, *J* = 6.3 Hz); ¹³C NMR (CDCl₃) δ 16.3, 23.4, 26.5, 42.2, 58.1, 69.4, 74.9, 122.9, 123.9, 139.9, 142.1, 154.6.

(*S,S*)-*N*-Methyl-2-[1-(3-pyridyloxy)ethyl]pyrrolidine [(*S,S*)-13**].** Obtained as a yellow oil in 18% yield after chromatography on silica gel (3% TEA in acetone), *R_f* = 0.39; [α]_D²⁵ +18.3 (*c* 1, MeOH); ¹H NMR (CDCl₃) δ 8.30 (d, 1H, *J* = 1.9 Hz), 8.17 (dd, 1H, *J* = 3.9, 1.9 Hz), 7.23–7.15 (m, 2H), 4.41 (quint, 1H, *J* = 6.1 Hz), 3.08 (m, 1H), 2.55 (m, 1H), 2.44 (s, 3H), 2.33–2.24 (m, 1H), 1.94–1.65 (m, 4H), 1.26 (d, 3H, *J* = 6.1 Hz); ¹³C NMR (CDCl₃) δ 15.9, 23.5, 27.5, 42.9, 58.4, 68.6, 77.7, 122.5, 124.0, 139.6, 142.1, 154.4.

(*S*)-*N*-Cbz-2-(3-benzyloxyphenoxymethyl)pyrrolidine [(*S*)-14**].** Obtained as a light yellow oil in 81% yield after chromatography on silica gel (toluene/EtOAc 95:5), *R_f* = 0.32; [α]_D²⁵ –49.6 (*c* 1, CHCl₃); ¹H NMR (CDCl₃) δ 7.42–7.20 (m, 11H), 6.64–6.48 (m, 3H), 5.15 (s, 2H), 5.04 (s, 2H), 4.28–4.19 (m, 1H), 4.05–3.75 (m, 2H), 3.50–3.40 (m, 2H), 2.05–1.85 (m, 4H).

(*S*)-*N*-Cbz-2-(2-methoxyphenoxymethyl)pyrrolidine [(*S*)-15**].** Obtained as a light yellow oil in 52% yield after chromatography on silica gel (toluene/EtOAc 8:2), *R_f* = 0.56; [α]_D²⁵ –54.1 (*c* 1, CHCl₃); ¹H NMR (CDCl₃) δ 7.43–7.22 (m, 5H), 7.10–6.64 (m, 4H), 5.16 (s, 2H), 4.38–4.24 (m, 2H), 4.10–3.98 (m, 1H), 3.82 (s, 3H), 3.58–3.38 (m, 2H), 2.21–1.83 (m, 4H).

(*S*)-*N*-Cbz-2-(2-methoxy-5-benzyloxyphenoxymethyl)pyrrolidine [(*S*)-16**].** Obtained as a light yellow oil in 51% yield after chromatography on silica gel (toluene/EtOAc 8:2), *R_f* = 0.47; [α]_D²⁵ –33.1 (*c* 1, CHCl₃); ¹H NMR (CDCl₃) δ 7.45–7.21 (m, 10H), 6.81 (m, 1H), 6.60 (m, 1H), 6.45 (m, 1H), 5.12 (s, 2H), 5.02 (m, 2H), 4.28–4.08 (m, 3H), 3.81 (s, 3H), 3.58–3.40 (m, 2H), 2.10–1.85 (m, 4H).

(*S*)-*N*-Boc-2-(3-benzyloxyphenoxymethyl)azetidide [(*S*)-18**].** Obtained as a light yellow oil in 74% yield after chromatography on silica gel (cyclohexane/EtOAc 8:2), *R_f* = 0.61; [α]_D²⁵ –73.3 (*c* 0.9, CHCl₃); ¹H NMR (CDCl₃) δ 7.25–7.45 (m, 5H), 7.18 (m, 1H), 6.50–6.62 (m, 3H), 5.04 (s, 2H), 4.52–4.45 (m, 1H), 4.23 (m, 1H), 4.05 (dd, 1H, *J* = 10.2 and 2.7 Hz), 3.99–3.86 (m, 2H), 2.39–2.17 (m, 2H), 1.26 (s, 9H).

(*S*)-*N*-Cbz-2-(2-methoxy-3-pyridyloxymethyl)pyrrolidine [(*S*)-22**].** Obtained as a light yellow oil in 48% yield after chromatography on silica gel (toluene/EtOAc 9:1), *R_f* = 0.22; [α]_D²⁵ –41.6 (*c* 1, CHCl₃); ¹H NMR (CDCl₃) δ 7.70 (m, 1H), 7.36–7.26 (m, 5H), 6.80–6.91 (m, 1.5H), 6.57 (m, 0.5H), 5.15 (s, 2H), 4.28–4.04 (m, 2H), 3.98 (s, 3H), 3.84–3.72 (m, 1H), 3.50–3.31 (m, 2H), 2.20–1.80 (m, 4H).

(*R,S*)- and (*S,S*)-*N*-Boc-2-(1-Phenoxyethyl)pyrrolidine [(*R,S*)-25** + (*S,S*)-**25**].** Obtained as a yellow oil in 10% yield after chromatography on silica gel (toluene/EtOAc 9:1) in 1:1 diastereomeric ratio, as proved by the presence of two equally intense singlets,

integrating for 9 H, due to the *tert*-butyl and two equally intense doublets, integrating for 3 H, due to the methyl, *R_f* = 0.47; ¹H NMR (CDCl₃) δ 7.29–7.07 (m, 6H), 6.89 (m, 4H), 5.03–4.70 (m, 2H), 4.21–4.12 (m, 1H), 3.86–3.79 (m, 1H), 3.47–3.16 (m, 4H), 2.32–1.79 (m, 8H), 1.56 (s, 9H), 1.42 (s, 9H), 1.25 (d, 3H, *J* = 6.3 Hz), 1.21 (d, 3H, *J* = 6.3 Hz).

(*R,S*)-*N*-Boc-2-(1-Phenoxyethyl)pyrrolidine [(*R,S*)-25**].** Obtained as a yellow oil in 46% yield after chromatography on silica gel (toluene/EtOAc 9:1), *R_f* = 0.47; [α]_D²⁵ –120.7 (*c* 1, CH₃OH); ¹H NMR (CDCl₃) δ 7.29–7.07 (m, 3H), 6.89 (m, 2H), 5.03 (m, 0.6 H), 4.70 (m, 0.4 H), 3.86–3.79 (m, 1H), 3.47–3.16 (m, 2H), 2.23, 2.15–(m, 1H), 2.02–1.79 (m, 3H), 1.42 (s, 9H), 1.25 (d, *J* = 6.3 Hz, 3H).

(*R,S*)- and (*S,S*)-*N*-Boc-2-[1-(3-benzyloxyphenoxy)ethyl]pyrrolidine [(*R,S*)-26** + (*S,S*)-**26**].** Obtained as a yellow oil in 7% yield after two chromatographies on silica gel, first with 9:1 cyclohexane/EtOAc and then with 9:1 toluene/acetone, in 1:1 diastereomeric ratio as proved by the two equally intense singlets, integrating for 9 H, due to *tert*-butyl and the two equally intense doublets, integrating for 3 H, due to the methyl: ¹H NMR (CDCl₃) δ 7.44–7.29 (m, 10H), 7.13 (m, 2H), 6.52 (m, 6H), 5.04 (s, 2H), 5.02 (s, 2H), 4.25–4.12 (m, 2H), 3.79–3.86 (m, 2H), 3.47–3.16 (m, 4H), 2.06–1.70 (m, 8H), 1.56 (s, 9H), 1.44 (s, 9H), 1.24 (d, *J* = 6.3 Hz, 3H), 1.20 (d, *J* = 6.3 Hz, 3H).

(*R,S*)-*N*-Boc-2-[1-(3-benzyloxyphenoxy)ethyl]pyrrolidine [(*R,S*)-26**].** Obtained as an oil in 44% yield after chromatography on silica gel (cyclohexane/EtOAc 9:1), *R_f* = 0.43; [α]_D²⁵ –75.1 (*c* 0.5, CH₃OH); ¹H NMR (CDCl₃) δ 7.44–7.29 (m, 5H), 7.13 (m, 1H), 6.52 (m, 3H), 5.02 (s, 2H), 4.09–3.86 (m, 2H), 3.42–3.08 (m, 2H), 2.07–1.65 (m, 4H), 1.44 (s, 9H), 1.24 (d, *J* = 6.3 Hz, 3H).

(*S*)-*N*-Cbz-2-mesyloxymethylpyrrolidine [(*S*)-20**].** Obtained as a solid in 90% yield by treatment with mesyl chloride (1.1 mol) and TEA (1.1 mol) in DCM at room temperature for 2 h, 1 N HCl/DCM extraction and chromatography on silica gel (cyclohexane/EtOAc 7:3), *R_f* = 0.30; mp 43.7 °C; [α]_D²⁵ –51.0 (*c* 1, CHCl₃); ¹H NMR (CDCl₃) δ 7.38–7.30 (m, 5H), 5.13 (s, 2H), 4.32–4.05 (m, 3 H), 3.48–3.43 (m, 2 H), 2.95–2.83 (m, 3 H), 2.17–1.85 (m, 4 H).

(*S*)-*N*-Cbz-2-(5-benzyloxy-3-pyridyloxymethyl)pyrrolidine [(*S*)-21**].** Obtained as a light yellow oil in 42% yield by treatment of (*S*)-**20** with 3-benzyloxy-5-hydroxypyridine (1 mol) and NaH (1 mol) in boiling DMF for 4 h and chromatography on silica gel (DCM/acetone 95:5), *R_f* = 0.42 [α]_D²⁵ –50.1 (*c* 0.5, CHCl₃); ¹H NMR (CDCl₃) δ 8.05–7.91 (m, 2H), 7.26–7.14 (m, 10H), 6.93 (m, 0.7H), 6.78 (m, 0.3H), 5.03–5.22 (m, 4H), 4.22 (m, 2H), 4.19 (m, 0.7H), 3.82 (m, 0.3H), 3.56 (m, 2H), 2.17–1.88 (m, 4H).

(*S*)-*N*-Cbz-2-(4-methoxy-3-pyridyloxymethyl)pyrrolidine [(*S*)-23**].** Obtained as a light yellow oil in 51% yield by treatment of (*S*)-**20** with the trifluoroacetate salt of 3-hydroxy-4-methoxypyridine (0.8 mol) and K₂CO₃ (1.6 mol) in boiling DME overnight and chromatography on silica gel (DCM/acetone 9:1), *R_f* = 0.34; [α]_D²⁵ –31.4 (*c* 1, CHCl₃); ¹H NMR (CDCl₃) δ 8.21–8.08 (m, 2H), 7.33–7.26 (m, 5H), 6.77 (d, *J* = 5.2 Hz, 1H), 5.14 (m, 2H), 4.26–4.04 (m, 3H), 3.87 (s, 3H), 3.48 (m, 2H), 2.17–1.85 (m, 4H).

(*R,S*)-*N*-Boc-2-(1-Hydroxyethyl)pyrrolidine [(*R,S*)-24**].** ²⁸ Obtained as an oil in 42% yield by treatment of (*S*)-*N*-Boc-2-acetylpyrrolidine with LiAlH₄ (2 mol) in THF at –10 °C for 1 h and successive chromatography on silica gel (toluene/acetone 7:3), *R_f* = 0.57; [α]_D²⁵ –74.2 (*c* 1, CH₃OH); ¹H NMR (CDCl₃) δ 3.96–3.84 (m, 2H), 3.54 (m, 1H), 3.24 (m, 1H), 3.13 (bs, 1H, exchange with D₂O), 2.17–1.95 (m, 1H), 1.87–1.79 (m, 1H), 1.77–1.68 (m, 2H), 1.47 (s, 9H), 1.08 (d, 3H, *J* = 6.3 Hz); ¹³C NMR (CDCl₃) δ 17.9, 24.3, 27.9, 28.7, 48.3, 63.6, 69.9, 80.3, 156.6.

(*S,S*)-*N*-Boc-2-(1-Hydroxyethyl)pyrrolidine [(*S,S*)-24**].** Obtained as a white solid in 51% yield as described for (*R,S*)-**24**, *R_f* = 0.53, mp = 62.3 °C; [α]_D²⁵ –54.8 (*c* 1, CH₃OH); ¹H NMR (CDCl₃) δ 4.31 (bs, 1H, exchange with D₂O), 3.75–3.61 (m, 2H), 3.52–3.44 (m, 1H), 3.24 (m, 1H), 1.99–1.87 (m, 1H), 1.84–1.70 (m, 2H), 1.68–1.54 (m, 1H), 1.47 (s, 9H), 1.13 (d, 3H, *J* = 6.0 Hz); ¹³C NMR (CDCl₃) δ 21.1, 24.2, 28.7, 29.1, 47.6, 64.5, 72.4, 80.7, 158.1.

General Procedure for Synthesis of Compounds 4, 4a, 6a, 7a, 9a, 11a, 12a, 19, and 27. The *S* isomers of **4**, **4a**, **6a**, **7a**, **9a**, **11a**,

12a, and **19** and the *R,S* isomer of **27** were obtained by hydrogenolysis (H_2 , 1 atm, Pd/C) of (*S*)-**17**, (*S*)-**14**, (*S*)-**21**, (*S*)-**16**, (*S*)-**15**, (*S*)-**22**, (*S*)-**23**, (*S*)-**18**, and (*R,S*)-**26**, respectively, in MeOH at room temperature for 2 h. Under the same conditions, the *S,S* isomer of **27** was obtained by hydrogenation of the (*R,S*)/(*S,S*) diastereomeric mixture of **26** and successive chromatographic separation.

(S)-N-Methyl-2-(3-hydroxyphenoxymethyl)pyrrolidine [(S)-4]. Obtained as a yellow oil in 84% yield; $[\alpha]_D^{25} +15.0$ (*c* 0.5, $CHCl_3$); 1H NMR ($CDCl_3$) δ 7.05 (t, *J* = 8.4 Hz, 1H), 6.41 (m, 3H), 5.30 (s, 1H, exchange with D_2O), 3.99–3.82 (m, 2H), 3.21–3.17 (m, 1H), 2.82–2.77 (m, 1H), 2.59 (s, 3H), 2.41–2.32 (m, 1H), 2.18–1.56 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 23.0, 28.6, 42.5, 58.1, 64.9, 70.6, 103.2, 105.6, 109.2, 130.1, 158.3, 160.0.

(S)-2-(3-Hydroxyphenoxymethyl)pyrrolidine [(S)-4a]. Obtained as a beige solid in 88% yield; mp = 121.8 °C; $[\alpha]_D^{25} -5.86$ (*c* 0.5, $CHCl_3$); 1H NMR ($CDCl_3$) δ 7.10 (t, 1H, *J* = 8.3 Hz), 6.72 (m, 1H), 6.46 (dd, 1H, *J* = 8.3, 2.2 Hz), 6.33 (d, 1H, *J* = 8.3, 2.2 Hz), 5.42 (bs, 2H, exchange with D_2O), 4.13–3.89 (m, 1H), 3.79–3.61 (m, 2H), 3.02 (m, 2H), 2.12–1.96 (m, 1H), 1.93–1.79 (m, 2H), 1.61–1.55 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 25.6, 27.9, 45.9, 56.8, 70.5, 103.0, 103.5, 110.0, 130.3, 159.4, 159.9.

(S)-2-(5-Hydroxy-3-pyridoxymethyl)pyrrolidine [(S)-6a]. Obtained as a light yellow solid in 85% yield after chromatography on silica gel (DCM/MeOH/30% NH_3 90:10:2), R_f = 0.14; mp = 118.26 °C; $[\alpha]_D^{25} -5.10$ (*c* 1, $CHCl_3$); 1H NMR ($CDCl_3$) δ 7.82 (d, 1H, *J* = 2.2 Hz), 7.63 (d, 1H, *J* = 2.2 Hz), 6.83 (t, 1H, *J* = 2.2 Hz), 5.85 (bs, 2H), 4.01 (dd, 1H, *J* = 9.1, 3.3 Hz), 3.81 (t, 1H, *J* = 9.1 Hz), 3.64–3.57 (m, 1H), 3.12–2.99 (m, 2H), 2.05–1.81 (m, 3H), 1.56 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 25.5, 27.9, 46.2, 57.2, 70.8, 110.3, 125.5, 132.9, 155.9, 156.6.

(S)-2-(2-Methoxyphenoxymethyl)pyrrolidine [(S)-7a]. Obtained as a light brown oil in 81% yield; $[\alpha]_D^{25} +0.87$ (*c* 1, $CHCl_3$); 1H NMR ($CDCl_3$) δ 6.96–6.78 (m, 4H), 4.03–3.87 (m, 2H), 3.84 (s, 3H), 3.56 (m, 1H), 3.18–2.92 (m, 3H), 1.96–1.68 (m, 3H), 1.62–1.51 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 25.3, 28.1, 46.5, 56.1, 57.5, 72.7, 112.2, 114.3, 121.1, 121.6, 148.7, 149.9.

(S)-2-(2-Methoxy-5-hydroxyphenoxymethyl)pyrrolidine [(S)-9a]. Obtained as a light yellow oil in 68% yield after chromatography on silica gel (DCM/MeOH/30% NH_3 90:10:2), R_f = 0.27; $[\alpha]_D^{25} +4.9$ (*c* 1, $CHCl_3$); 1H NMR ($CDCl_3$) δ 6.67 (d, 1H, *J* = 8.6 Hz), 6.45 (d, 1H, *J* = 2.7 Hz), 6.35 (dd, 1H, *J* = 8.6, 2.7 Hz), 3.90 (m, 2H), 3.75 (s, 3H), 3.57 (m, 3H, 2 exchange with D_2O), 3.18–3.05 (m, 1H), 3.01–2.90 (m, 1H), 2.05–1.75 (m, 3H), 1.62–1.51 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 25.1, 27.8, 46.2, 57.1, 57.9, 71.5, 104.0, 108.1, 114.1, 143.2, 149.1, 151.7.

(S)-2-(2-Methoxy-3-pyridyloxymethyl)pyrrolidine [(S)-11a]. Obtained as a light yellow oil in 75% yield; $[\alpha]_D^{25} +5.84$ (*c* 1, $CHCl_3$); 1H NMR ($CDCl_3$) δ 7.72 (dd, 1H, *J* = 1.4, 4.9 Hz), 7.06 (dd, 1H, *J* = 1.4, 7.7 Hz), 6.80 (dd, 1H, *J* = 4.9, 7.7 Hz), 3.99 (s, 3H), 3.98–3.84 (m, 2H), 3.62–3.50 (m, 1H), 3.04–2.93 (m, 2H), 2.15 (bs, 1H, exchange with D_2O), 1.98–1.74 (m, 3H), 1.60–1.51 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 25.3, 28.2, 46.7, 53.7, 57.2, 72.6, 116.9, 119.3, 137.6, 143.8, 152.1.

(S)-2-(4-Methoxy-3-pyridyloxymethyl)pyrrolidine [(S)-12a]. Obtained as a light yellow oil in 91% yield after chromatography on silica gel (DCM/MeOH/30% NH_3 95:5:2), R_f = 0.17; $[\alpha]_D^{25} +3.5$ (*c* 1, $CHCl_3$); 1H NMR ($CDCl_3$) δ 8.16 (d, 1H, *J* = 5.2 Hz), 8.15 (s, 1H), 6.78 (d, 1H, *J* = 5.2 Hz), 4.03 (dd, 1H, *J* = 9.4, 4.9 Hz), 3.95 (dd, 1H, *J* = 9.4, 6.9 Hz), 3.88 (s, 3H), 3.63–3.46 (m, 1H), 3.06–2.96 (m, 2H), 2.82 (bs, 1H, exchange with D_2O), 1.99–1.77 (m, 3H), 1.63–1.54 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 25.3, 28.1, 46.6, 55.8, 57.5, 73.4, 106.9, 136.5, 144.9, 145.4, 155.9.

(S)-N-Boc-2-(3-Hydroxyphenoxymethyl)azetidine [(S)-19]. Obtained as a light brown oil in 96% yield; $[\alpha]_D^{25} -78.8$ (*c* 1, $CHCl_3$); 1H NMR ($CDCl_3$) δ 7.25–7.06 (m, 1H), 6.47–6.39 (m, 3H), 4.52–4.45 (m, 1H), 4.23 (dd, 1H, *J* = 10.2, 5.2 Hz), 4.05 (dd, 1H, *J* = 10.2, 2.7 Hz), 3.99–3.81 (m, 2H), 2.39–2.17 (m, 2H), 1.26 (s, 9H).

(R,S)-N-Boc-2-[1-(3-Hydroxyphenoxy)ethyl]pyrrolidine [(R,S)-27]. Obtained as an oil in 92% yield after chromatography on

silica gel (toluene/EtOAc 95:5), R_f = 0.12; $[\alpha]_D^{25} -101.0$ (*c* 0.5, CH_3OH); 1H NMR ($CDCl_3$) δ 7.10 (m, 1H), 6.41 (m, 3H), 4.98 (m, 0.7H), 4.64 (m, 0.3H), 3.86–3.77 (m, 1H), 3.32–3.18 (m, 2H), 2.22–2.16 (m, 1H), 2.14–1.78 (m, 3H), 1.55–1.38 (m, 9H), 1.21 (m, 3H).

(S,S)-N-Boc-2-[1-(3-Hydroxyphenoxy)ethyl]pyrrolidine [(S,S)-27]. Obtained as an oil in 42% yield after chromatography on silica gel (toluene/EtOAc 95:5), R_f = 0.18; $[\alpha]_D^{25} +12.2$ (*c* 0.6, $CHCl_3$); 1H NMR ($CDCl_3$) δ 7.08 (t, 1H, *J* = 8.25 Hz), 6.61–6.45 (m, 3H), 4.98–4.78 (m, 1H), 4.12–4.22 (m, 1H), 3.53–3.38 (m, 1H), 3.36–3.08 (m, 1H), 2.03–1.79 (m, 4H), 1.50 (s, 9H), 1.19 (d, 3H, *J* = 6.3 Hz).

General Procedure for Synthesis of Compounds 5, 7, 8, 10, 11, 17, and 28. The *S* isomers of **5**, **7**, **11**, **17** were obtained by treatment of (*S*)-**19**, (*S*)-**15**, (*S*)-**22**, and (*S*)-**14**, respectively, with $LiAlH_4$ (3 mol) in boiling THF for 2 h. Under the same conditions, the *R,S* isomers of **25**, **27**, and **24** gave (*R,S*)-**8**, (*R,S*)-**10**, and (*R,S*)-**28**, respectively, and the *S,S* isomers of **27** and **24** gave (*S,S*)-**10** and (*S,S*)-**28**, respectively. By the same reaction, the *S,S* isomer of **8** was obtained in mixture with (*R,S*)-**8** from the (*R,S*)/(*S,S*) diastereomeric mixture of **25**.

(S)-N-Methyl-2-(3-hydroxyphenoxymethyl)azetidine [(S)-5]. Obtained as a white solid in 63% yield after DCM/water extraction, adjustment of pH to 8 by formic acid, extraction with EtOAc, combination and concentration of the DCM and EtOAc extracts; mp 116.5 °C; $[\alpha]_D^{25} +34.3$ (*c* 1, $CHCl_3$); 1H NMR ($CDCl_3$) δ 6.96 (t, *J* = 8.5 Hz, 1H), 6.38–6.41 (m, 2H), 6.23 (dd, *J* = 8.5, 2.2 Hz, 1H), 3.98–3.89 (m, 2H), 3.59 (m, 2H), 3.04–2.92 (m, 1H), 2.58 (s, 3H), 2.06–2.19 (m, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 20.0, 45.6, 53.4, 66.5, 71.0, 103.9, 104.1, 109.5, 130.0, 158.2, 159.6.

(S)-N-Methyl-2-(2-methoxyphenoxymethyl)pyrrolidine [(S)-7]. Obtained as a light yellow oil in 80% yield after destruction of the excess of hydride by water, filtration through Celite, concentration of the filtrate, DCM/1 M NaOH extraction of the resulting residue, and concentration of the organic phase; $[\alpha]_D^{25} -42.7$ (*c* 1, $CHCl_3$); 1H NMR ($CDCl_3$) δ 6.90 (m, 4H), 4.04 (dd, 1H, *J* = 5.5, 9.4 Hz), 3.90 (dd, 1H, *J* = 6.3, 9.4 Hz), 3.85 (s, 3H), 3.10 (m, 1H), 2.79–2.68 (m, 1H), 2.51 (s, 3H), 2.35–2.25 (m, 1H), 2.10–1.95 (m, 1H), 1.85–1.69 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 23.2, 29.3, 41.9, 56.2, 57.9, 64.5, 72.6, 112.4, 113.8, 121.3, 121.6, 148.5, 149.9.

(R,S)-N-Methyl-2-(1-phenoxyethyl)pyrrolidine [(R,S)-8]. Obtained as a white amorphous solid in 60% yield after destruction of the excess of hydride by water, filtration through Celite, concentration of the filtrate, DCM/1 M HCl extraction of the resulting residue, alkalization of the aqueous phase by 1 M NH_3 , extraction with EtOAc, and concentration of the organic phase; $[\alpha]_D^{25} -72.0$ (*c* 0.5, CH_3OH); 1H NMR ($CDCl_3$) δ 7.31–7.22 (m, 2H), 6.93–6.82 (m, 3H), 4.40 (dq, 1H, *J* = 3.6, 6.1 Hz), 3.12 (m, 1H), 2.48 (m, 1H), 2.43 (s, 3H), 2.30–2.18 (m, 1H), 1.94–1.70 (m, 4H), 1.28 (d, 3H, *J* = 6.1 Hz); ^{13}C NMR ($CDCl_3$) δ 16.3, 23.35, 26.9, 42.4, 58.3, 69.3, 74.5, 116.4, 120.8, 129.6, 158.2.

(R,S)- and (S,S)-N-Methyl-2-(1-phenoxyethyl)pyrrolidine [(R,S)-8 + (S,S)-8]. Obtained, according to the procedure described for (*R,S*)-**8**, as a yellow oil in 80% yield and in 1:1 diastereomeric ratio; 1H NMR ($CDCl_3$) δ 7.33–7.18 (m, 2H), 6.98–6.80 (m, 3H), 4.50–4.37 (m, 1H), 3.12 (m, 1H), 2.57 (m, 0.5H), 2.47 (s, 2H), 2.43 (s, 1.5H), 2.31–2.18 (m, 1H), 1.92–1.72 (m, 4H), 1.28 (d, 1.5H, *J* = 6.3 Hz), 1.26 (d, 1.5H, *J* = 6.3 Hz); ^{13}C NMR ($CDCl_3$) δ 15.9, 16.3, 23.35, 23.36, 26.9, 27.3, 42.4, 42.7, 58.3, 58.4, 68.7, 69.3, 74.5, 76.4, 116.16, 116.4, 120.6, 120.8, 129.6, 129.7, 158.1, 158.2.

(R,S)-N-Methyl-2-[1-(3-hydroxyphenoxy)ethyl]pyrrolidine [(R,S)-10]. Obtained as a light yellow oil in 86% yield according to the procedure described for (*R,S*)-**8**; $[\alpha]_D^{25} -47.3$ (*c* 1, CH_3OH); 1H NMR ($CDCl_3$) δ 7.05 (t, 1H, *J* = 8.2 Hz), 6.46–6.33 (m, 3H), 5.26 (bs, 1H, exchange with D_2O), 4.47 (qd, 1H, *J* = 6.3, 3.0 Hz), 3.16 (m, 1H), 2.57 (m, 1H), 2.46 (s, 3H), 2.38 (m, 1H), 1.97–1.76 (m, 4H), 1.25 (d, 3H, *J* = 6.3 Hz); ^{13}C NMR ($CDCl_3$) δ 16.6, 23.3, 26.1, 42.1, 58.1, 69.8, 73.0, 104.3, 107.4, 108.4, 130.1, 157.8, 159.0.

(S,S)-N-Methyl-2-[1-(3-hydroxyphenoxy)ethyl]pyrrolidine [(S,S)-10]. Obtained as a light oil in 70% yield according to the procedure described for (*R,S*)-**8**; $[\alpha]_D^{25} +9.4$ (*c* 1, CH_3OH); 1H NMR ($CDCl_3$) δ 6.91 (t, 1H, *J* = 8.3 Hz), 6.28–6.20 (m, 3H), 6.01 (bs, 1H,

exchange with D₂O), 4.39 (quintet, 1H, *J* = 6.3 Hz), 3.14 (m, 1H), 2.63–2.55 (m, 1H), 2.58 (s, 3H), 2.44–2.31 (m, 1H), 2.04–1.92 (m, 1H), 1.89–1.61 (m, 3H), 1.21 (d, *J* = 6.3 Hz, 3H); ¹³C NMR (CDCl₃) δ 17.2, 23.2, 28.5, 43.9, 58.5, 70.1, 76.8, 104.1, 106.9, 108.8, 129.9, 158.0, 158.8.

(S)-N-Methyl-2-(2-methoxy-3-pyridyloxymethyl)pyrrolidine [(S)-11]. Obtained as a light yellow oil in 85% yield after working up as described for (R,S)-8 and chromatography of the crude product on silica gel (DCM/MeOH/30% NH₃ 95:5:2), *R_f* = 0.38; [α]_D²⁵ –52.9 (c 1, CHCl₃); ¹H NMR (CDCl₃) δ 7.73 (dd, 1H, *J* = 1.7, 4.9 Hz), 7.06 (dd, *J* = 1.7, 7.7 Hz, 1H), 6.81 (dd, *J* = 4.9, 7.7 Hz, 1H), 3.99 (s, 3H), 4.02–3.97 (m, 1H), 3.91–3.86 (m, 1H), 3.18–3.09 (m, 1H), 2.80–2.70 (m, 1H), 2.50 (s, 3H), 2.35–2.26 (m, 1H), 2.17–2.01 (m, 1H), 1.85–1.67 (m, 3H); ¹³C NMR (CDCl₃) δ 23.3, 29.2, 42.0, 53.7, 57.9, 64.2, 72.2, 116.9, 119.0, 137.5, 143.9, 155.1.

(S)-N-Methyl-2-(3-benzyloxyphenoxymethyl)pyrrolidine [(S)-17]. Obtained as a yellow oil in 83% yield after working up as described for (S)-7 and chromatography of the crude product on silica gel (DCM/MeOH/30% NH₃ 90:10:2); [α]_D²⁵ –22.7 (c 1, CHCl₃); ¹H NMR (CDCl₃) δ 7.52–7.38 (m, 5H), 7.18 (m, 1H), 6.64–6.48 (m, 3H), 5.04 (s, 2H), 4.01–3.82 (m, 2H), 3.15 (m, 1H), 2.65–2.52 (m, 1H), 2.47 (s, 3H), 2.38–2.23 (m, 1H), 2.06–1.67 (m, 4H).

(R,S)-N-Methyl-2-(1-hydroxyethyl)pyrrolidine [(R,S)-28]. Obtained as a light yellow oil in 98% yield after chromatography on silica gel (DCM/MeOH/TEA 90:10:2), *R_f* = 0.25; ¹H NMR (CDCl₃) δ 3.89 (qd, 1H, *J* = 6.6, 2.2 Hz), 3.09 (m, 1H), 2.33 (s, 3H), 2.27 (m, 1H), 2.13 (td, 1H, *J* = 8.3, 2.2 Hz), 1.86–1.79 (m, 1H), 1.73–1.62 (m, 3H), 1.10 (d, 3H, *J* = 6.6 Hz).

(S,S)-N-Methyl-2-(1-hydroxyethyl)pyrrolidine [(S,S)-28]. Obtained as a light yellow oil in 97% yield after chromatography on silica gel (DCM/MeOH/TEA 90:10:2), *R_f* = 0.39; ¹H NMR (CDCl₃) δ 3.42 (quintet, 1H, *J* = 6.3 Hz), 3.07 (m, 1H), 2.48 (s, 3H), 2.44–2.34 (m, 2H), 1.95–1.83 (m, 1H), 1.82–1.68 (m, 2H), 1.55–1.42 (m, 1H), 1.15 (d, 3H, *J* = 6.3 Hz).

(S)-2-(3-Hydroxyphenoxymethyl)azetidine Hydrochloride [(S)-5a·HCl]. Obtained as a light yellow amorphous solid in 90% yield by treatment of (S)-19 with 1.25 M methanolic HCl at room temperature overnight; [α]_D²⁵ –2.9 (c 1, MeOH); ¹H NMR (CD₃OD) δ 7.10 (t, 1H, *J* = 8.3 Hz), 6.47 (m, 3H), 4.85 (m, 1H), 4.24 (d, 2H, *J* = 4.4 Hz), 4.11–3.98 (m, 2H), 2.57–2.67 (m, 2H); ¹³C NMR (CD₃OD) δ 20.7, 43.5, 59.7, 66.4, 102.0, 105.4, 108.8, 129.9, 158.8, 159.4.

(S)-N-Methyl-2-(5-hydroxy-3-pyridyloxymethyl)pyrrolidine [(S)-6]. Obtained as a light yellow oil in 92% yield by hydrogenation (H₂ 1 atm, Pd/C) of (S)-21 in methanol for 2 h and, after adding an excess of formalin, for additional 4 h and subsequent chromatography on silica gel (DCM/MeOH/30% NH₃ 90:10:2), *R_f* = 0.43; [α]_D²⁵ –30.1 (c 0.25, CHCl₃); ¹H NMR (CDCl₃) δ 7.81 (d, 1H, *J* = 2.2 Hz), 7.74 (d, 1H, *J* = 2.2 Hz), 6.70 (t, 1H, *J* = 2.2 Hz), 4.16 (bs, 1H), 4.00–3.88 (m, 2H), 3.16 (m, 1H), 2.78 (m, 1H), 2.52 (s, 3H), 2.33–2.42 (m, 1H), 2.18–1.98 (m, 1H), 1.86–1.65 (m, 3H); ¹³C NMR (CDCl₃) δ 23.3, 28.5, 42.4, 58.0, 64.8, 70.8, 110.1, 128.2, 131.3, 155.2, 156.2.

(S)-N-Methyl-2-(2-methoxy-5-hydroxyphenoxymethyl)pyrrolidine [(S)-9]. Obtained from (S)-16 in 80% yield as a light yellow solid under the reaction conditions described for (S)-6 and by chromatography on silica gel (DCM/MeOH/30% NH₃ 95:5:2), *R_f* = 0.47; mp 88.1 °C; [α]_D²⁵ –38.2 (c 1, CHCl₃); ¹H NMR (CDCl₃) δ 6.68 (d, 1H, *J* = 8.6 Hz), 6.44 (d, 1H, *J* = 2.7 Hz), 6.35 (dd, 1H, *J* = 8.6, 2.7 Hz), 4.05 (dd, 1H, *J* = 9.6, 6.1 Hz), 3.90 (dd, 1H, *J* = 9.6, 5.2 Hz), 3.76 (s, 3H), 3.82–3.41 (bs, 1H, exchange with D₂O), 3.23 (m, 1H), 2.92 (m, 1H), 2.60 (s, 3H), 2.43 (m, 1H), 2.24–2.05 (m, 1H), 1.95–1.61 (m, 3H); ¹³C NMR (CDCl₃) δ 22.9, 28.8, 42.0; 57.2, 57.9, 64.9, 71.6, 103.3, 107.8, 114.1, 143.3, 149.4, 151.3.

(S)-N-Methyl-2-(4-methoxy-3-pyridyloxymethyl)pyrrolidine [(S)-12]. Obtained from (S)-23 in 85% yield as a light yellow oil under the reaction conditions described for (S)-6 and by chromatography on silica gel (DCM/MeOH/30% NH₃ 95:5:2), *R_f* = 0.30; [α]_D²⁵ –17.7 (c 0.8 CHCl₃); ¹H NMR (CDCl₃) δ 8.15 (m, 2H), 6.78 (d, 1H, *J* = 5.2 Hz), 4.04 (dd, 1H, *J* = 9.4, 5.5), 3.94 (dd, 1H, *J* = 9.4, 5.8), 3.88 (s, 3H), 3.10 (m, 1H), 2.72 (m, 1H), 2.50 (s, 3H), 2.34–2.26 (m, 1H),

2.11–1.95 (m, 1H), 1.88–1.67 (m, 3H); ¹³C NMR (CDCl₃) δ 23.2, 28.9, 41.9, 55.8, 57.9, 64.5, 73.0, 106.9, 136.2, 144.8, 145.5, 155.9.

Biological Assays. Binding Studies. Details of the binding experiments to the nicotinic receptor subtypes have been published in ref 22 for the α4β2 and α7 subtypes and in ref 31 for the α3β4 subtype. The *K_i* values were obtained by simultaneously fitting three independent saturation and competition binding experiments for each compound on each subtype. The experimental data were analyzed by means of a nonlinear least-squares procedure using the LIGAND program.

Electrophysiological Experiments. The human α3β4 and α4β2 nAChRs were expressed by transient transfection in the rat anterior pituitary GH4C1 cell line.³⁸ Transient transfection was achieved by adding to each well 0.5 μg of each subunit cDNA, along with 2 μL of Magnetofection: NeuroMag (OZ Biosciences, France). All culture media were purchased from Invitrogen (Italy). Whole-cell current recordings were performed 2–3 days after transfection. Recordings and data analysis were performed by using borosilicate glass patch pipet (3–6 MΩ tip resistance) connected to an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Data were stored on a PC computer by using PCLAMP10 software (Molecular Devices). During the recording period, the cells were bathed in the following solution (mM): 140 NaCl, 2 CaCl₂, 2.8 KCl, 2 MgCl₂, 10 Hepes/NaOH, and 10 glucose; pH 7.3. The patch pipettes were filled with a solution containing the following (mM): 140 CsCl, 2 MgATP, 10 Hepes/CsOH, and 5 BAPTA; pH 7.3. Whole-cell capacitance and patch series resistance (5–15 MΩ) were estimated from slow transient compensations. A series resistance compensation of 85–90% was obtained in all cases. The cells were voltage-clamped at a holding potential of –70 mV and continuously perfused with a gravity-driven system using independent external tubes for the control and agonist-containing solutions. These tubes were positioned 50–100 μm from the patched cell and connected to a fast exchanger system (RSC-160, BioLogic, France). Dose–response relationships were constructed by sequentially applying different concentrations of agonists and normalizing the obtained current amplitudes to the value obtained by applying 1 mM μM ACh on the same cell. For quantitative estimations of agonist actions, dose–response relationship were fitted, when possible, by the equation

$$I = I_{\max} \left(\frac{[C]^{nH}}{EC_{50}^{nH} + [C]^{nH}} \right)$$

where *I* is the peak current amplitude induced by the agonist at concentration *C*, *I*_{max} is the maximum response of the cell, *nH* is the Hill coefficient, and *EC*₅₀ is the concentration for which a half-maximum response is induced.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00904.

¹H NMR and ¹³C NMR spectra and elemental analysis results for the final compounds 4–13, 4a–7a, 9a, 11a, and 12a; ¹H NMR spectra of the R,S and S,S diastereomers of the intermediates 24, 27, and 28; and the DSC trace of (S,S)-24 (PDF)
Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

ACh, acetylcholine; α Bgtx, α -bungarotoxin; Boc, *tert*-butoxycarbonyl; Cbz, carbobenzyloxy; CNS, central nervous system; DCM, dichloromethane; DIAD, diisopropyl azodicarboxylate; DME, dimethoxyethane; Epi, epibatidine; nAChR, nicotinic acetylcholine receptor; SAR, structure–activity relationship; TEA, triethylamine; THF, tetrahydrofuran

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