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[a] Abbreviations: ACh, acetylcholine; αBgtx, αBungarotoxin; Boc, *t*-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, tetrahydrofurane.

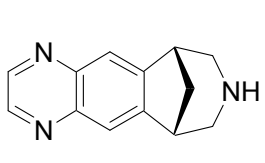
ABSTRACT. A series of unichiral analogues of 2*R*,2'*S*-2-(1'-methyl-2'-pyrrolidinyl)-7-hydroxy-1,4-benzodioxane, a potent and selective α4β2-nAChR partial agonist, were designed by opening the dioxane ring and replacing hydroxyl carbon with nitrogen. The resulting 3-pyridyl ethers and *meta*-hydroxyphenyl ethers have high α4β2 affinity and good subtype selectivity, which get lost if OH is

removed from phenyl or the position of pyridine nitrogen is changed. High α4β2 affinity and selectivity are also attained by *meta* hydroxylating the 3-pyridyl ether of (*S*)-N-methylprolinol, the corresponding phenyl ether and the phenyl ether of (*S*)-2-azetidinemethanol, known α4β2 agonists, although, on the basis of reported docking analyses, the interaction mode of the aryloxymethylene substructure of these compounds cannot be assimilated to that of benzodioxane. Indeed, the functional tests on the α4β2 and α3β4 subtypes well differentiate behaviors that the binding tests tend to make similar; in particular, they reveal that both the 3-hydroxyphenyl and the 5-hydroxy-3-pyridyl ether of N-methylprolinol are α4β2 full agonists, but only the latter highly α4β2/α3β4 selective, while potent and selective partial α4β2 agonism characterizes the hydroxybenzodioxane derivative and its two opened semi-rigid analogues.

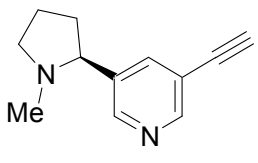
Introduction

The widespread distribution in the CNS and the key role in a number of CNS functions make α4β2 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 α4β2 agonist indicated for smoking cessation, has been approved for human use [7-10] and a number of selective α4β2 nAChR agonists have passed preclinical evaluations and the early stages of clinical trials before being abandoned. [6,11]

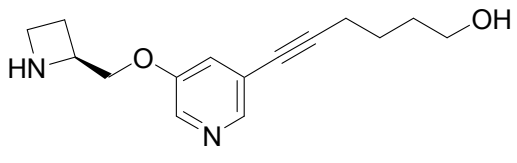
Chart 1. α4β2-nAChR partial agonists: Varenicline and 3,5-disubstituted pyridines



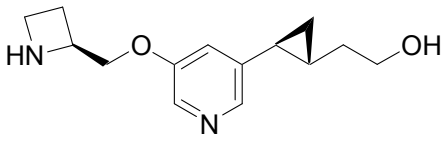
varenicline

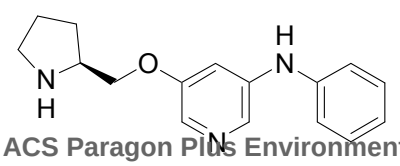


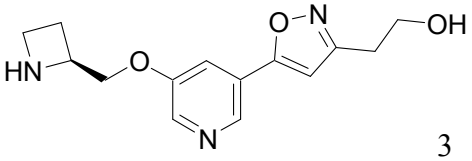
altinicline



sazetidine-A



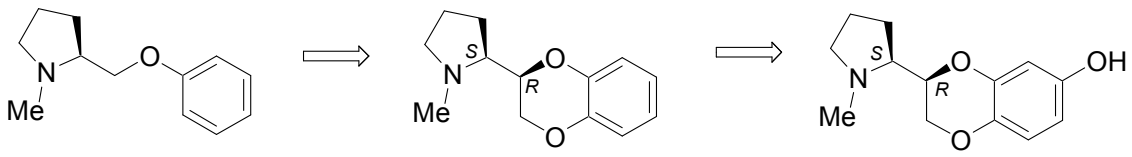




More recently, selective α4β2 partial agonists have received considerable attention as novel antidepressants.[12] Common features of their structures are 3-pyridyl bound to the alpha carbon of pyrrolidine or azetidine directly or through a flexible oxymethylene bridge, and the presence of a second substituent at the 5-position of pyridine nucleus, that confers selectivity for α4β2- over α3β4-nAChR subtype without prejudicing the high functional potency at the α4β2 receptor proper to the parent monosubstituted pyridines (Chart 1).[13-18] Reducing the side effects due to activation or blockade of peripheral α3β4-nAChR is a central issue when developing centrally acting nicotinoids, and docking studies indicate that the substitution pattern at the pyridyl residue is determinant of very high α4β2/α3β4 binding selectivity because it directly influences the close interactions of pyridyl with non-conserved 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 α4β2-nAChR partial agonist and such a feature is shared by the majority of the α4β2-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 α4β2-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 α4β2-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]

Chart 2. Design of conformationally restricted prolinol phenyl ethers



(*S*)-**1**
K_i (α4β2) = 42 nM

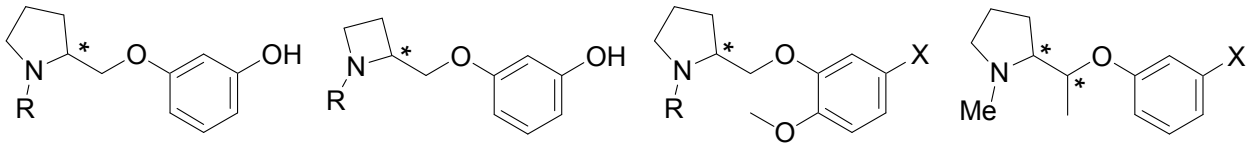
(*R,S*)-**2**
K_i (α4β2) = 260 nM

(*R,S*)-**3**
K_i (α4β2) = 12 nM
EC_{50} (α4β2) = 82 nM
EC_{50} (α3β4) = 18 μM

The considerable enhancement of α4β2 affinity caused by the 7-OH substituent at the benzodioxane nucleus and the high α4β2 versus α3β4 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 semi-rigid 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 *m*-hydroxylated analogues **4**, **9** and **10**, we also considered the azetidinyl 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 α4β2-nAChRs ligands and an SAR analysis based on the comparison between the affinity and activity profiles of the conformationally uncostrained 3-pyridyl and 3-hydroxyphenyl ethers **4**, **5** and **6** on one side and of the hydroxybenzodioxane **3** and its semi-rigid opened analogues **7**-**13** on the other.

Chart 3. Target phenyl and 3-pyridyl ethers of prolinol and 2-azetidinemethanol.

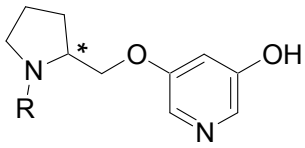


4 R = Me
4a R = H

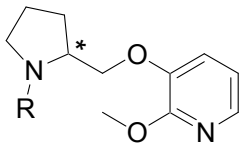
5 R = Me
5a R = H

7 R = Me X = H
7a R = H X = H
9 R = Me X = OH
9a R = H X = OH

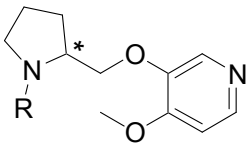
8 X = H
10 X = OH



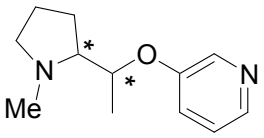
6 R = Me
6a R = H



11 R = Me
11a R = H



12 R = Me
12a R = H



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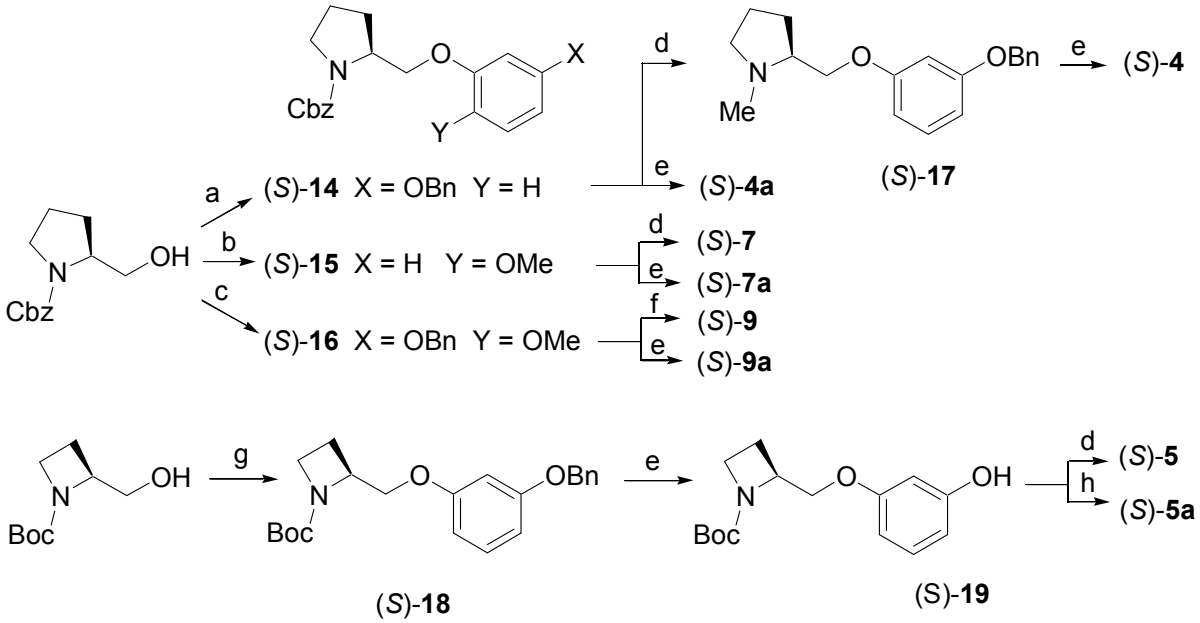
Chemistry

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 $LiAlH_4$ 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 azetidinyl analogues of **4** and **4a**, namely (*S*)-**5** and (*S*)-**5a**, were prepared from N-Boc protected (*S*)-azetidinylmethanol,[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 $LiAlH_4$

converted (*S*)-**19** into (*S*)-**5**, while Boc removal into (*S*)-**5a**.

Scheme 1. Synthesis of compounds **4**, **4a**, **5**, **5a**, **7**, **7a**, **9** and **9a**.

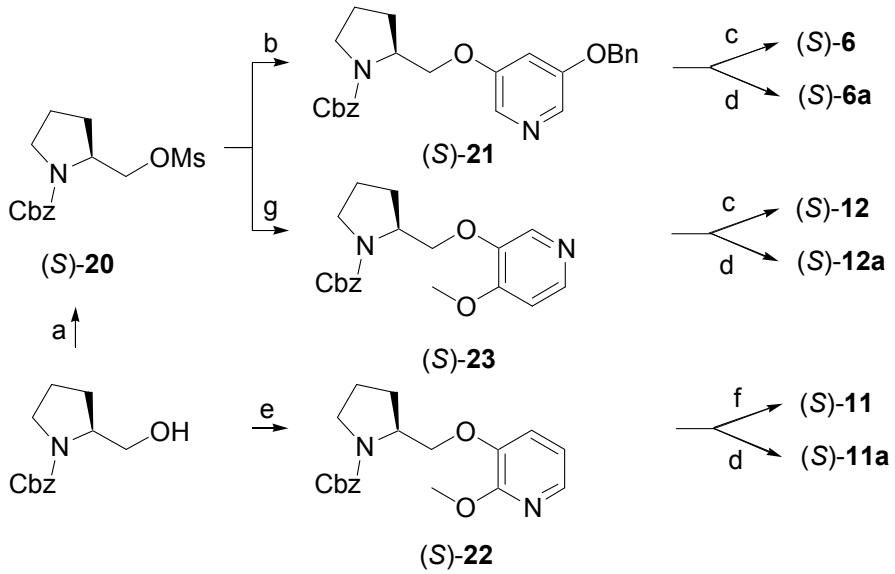


Reagents and conditions: (a) 3-Benzyloxyphenol, PPh_3, DIAD, THF, reflux, 18 h; (b) 2-Methoxyphenol, PPh_3, DIAD, THF, 140 °C, 30 min, MW; (c) 2-Methoxy-5-benzyloxyphenol, PPh_3, DIAD, THF, 120 °C, 15 min, MW; (d) $LiAlH_4$, THF, reflux, 2 h; (e) H_2, Pd/C, MeOH, rt, 2 h; (f) H_2, Pd/C, CH_2O, MeOH, rt, 2 h; (g) 3-Benzyloxyphenol, PPh_3, DIAD, THF, 140 °C, 30 min, MW; (h) 1.25 N HCl, MeOH, rt, 16 h.

N-Cbz protected (*S*)-prolinol was the starting material also 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*)-**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 or 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 $LiAlH_4$ or hydrogen respectively.

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



Reagents and conditions: (a) MsCl, TEA, DCM, rt, 2 h; (b) 3-Benzyloxy-5-hydroxypyridine, NaH, DMF, reflux, 4 h; (c) H_2, Pd/C, CH_2O, MeOH, rt, 2 h; (d) H_2, Pd/C, MeOH, rt, 2 h; (e) 2-Metoxy-3-hydroxypyridine, PPh_3, DIAD, THF, 140 °C, 30 min, MW; (f) $LiAlH_4$, THF, reflux, 2 h; (g) 3-Hydroxy-4-methoxypyridinium trifluoroacetate, K_2CO_3, DMF, reflux, 24 h.

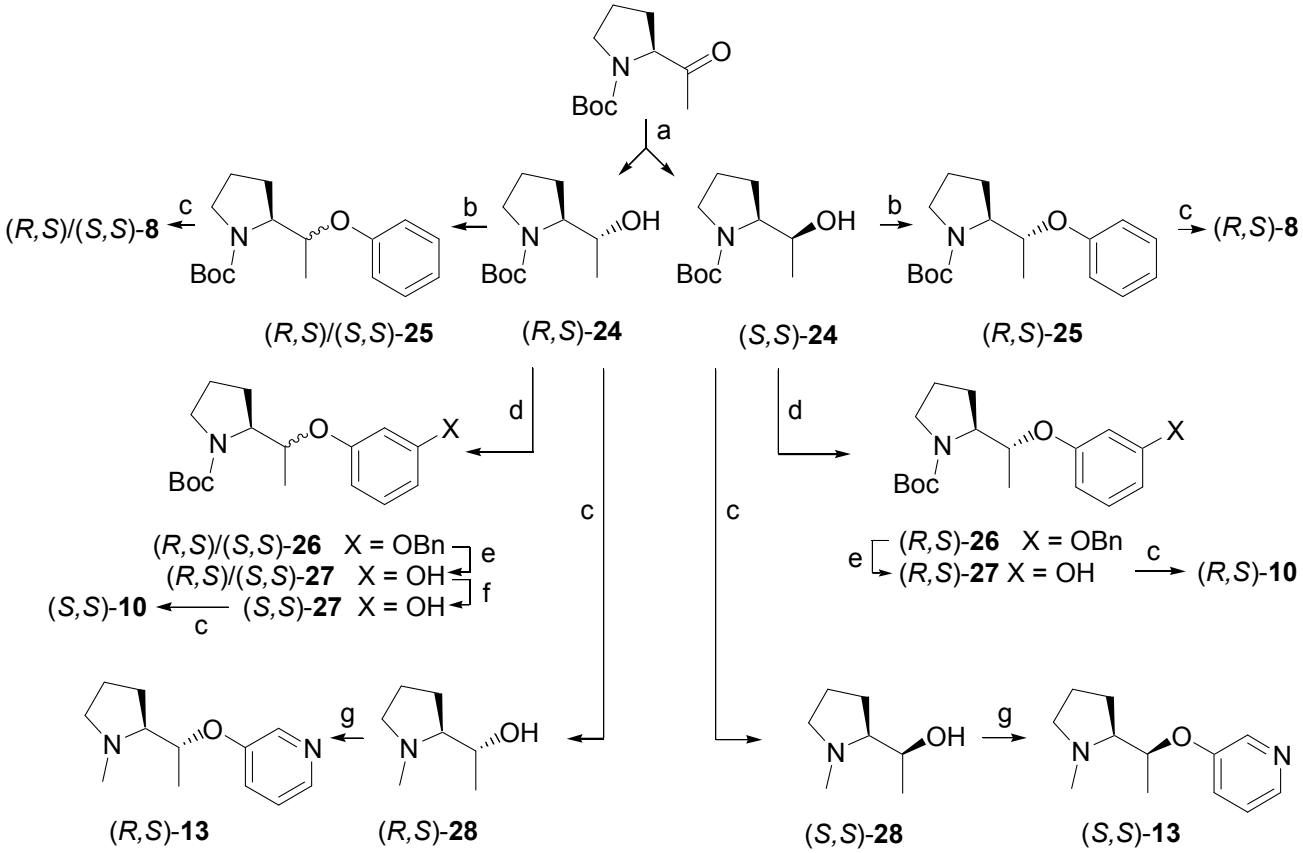
The phenyl and pyridyl ethers of α-methylprolinol **8**, **10** and **13** were prepared from N-Boc protected (*S*)-2-acetylpyrrolidine (Scheme 3). The reduction with $LiAlH_4$ in THF at -10 °C afforded a near equimolar mixture of the diastereomeric alcohols (*R,S*)-**24** and (*S,S*)-**24**, epimers 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 $LiAlH_4$ 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 ^{1}H 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 *sin* disposition of the two methinic protons, which is favoured in the *R,S* diastereomer and virtually forbidden in the *S,S* diastereomer. This means that the two Mitsunobu reactions between (*S,S*)-**24** and, respectively, phenol and 3-benzyloxyphenol 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 debenzylation, 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 ^{1}H 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 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 *anti* disposition of the two methinic protons, which is favoured in the *S,S* diastereomer and disfavoured in the *R,S* diastereomer.

Scheme 3. Synthesis of compounds **8**, **10** and **13**.



Reagents and conditions: (a) $LiAlH_4$, THF, -10 °C, 1 h, chromatographic separation; (b) Phenol, PPh_3, DIAD, THF, 150 °C, 30 min, MW; (c) $LiAlH_4$, THF, reflux, 2 h; (d) 3-Benzyloxyphenol, PPh_3, DIAD, THF, 150 °C, 30 min, MW; (e) H_2, Pd/C, MeOH, rt, 2 h; (f) Chromatographic separation; (g) 3-Hydroxypyridine, PPh_3, DIAD, THF, reflux, 16 h.

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 and the Mitsunobu reaction was carried out on the resulting N-methyl aminoalcohols (*R,S*)-**28** and (*S,S*)-**28** by treatment with 3-hydroxypyridine in the presence of DIAD/triphenylphosphine. Differently 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 ^{1}H 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 ^{1}H 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 neighbouring 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 by-product 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 α4β2 and α7 central nicotinic receptors labeled by [^{3}H]-epibatidine ([^{3}H]-Epi) and [^{125}I]-αBungarotoxin ([^{125}I]-αBgtx) and to evaluate their affinity on heterologously expressed human α3β4 receptors labeled with [^{3}H]-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]

Table 1. Nicotine and compounds **1**-**13**: affinity for native α4β2 and α7 nAChR subtypes, in rat brain membranes, respectively labeled by [^{3}H]-epibatidine and [^{125}I]-αBungarotoxin, and heterologously

expressed human α3β4 nAChRs, labeled by [^{3}H]-epibatidine.

	α4β2 nAChR [^{3}H]-Epi K_i (μM)	α7 nAChR [^{125}I]-αBgtx K_i (μM)	α3β4 nAChR [^{3}H]-Epi K_i (μM)		α4β2 nAChR [^{3}H]-Epi K_i (μM)	α7 nAChR [^{125}I]-αBgtx K_i (μM)	α3β4 nAChR [^{3}H]-Epi K_i (μM)
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)

The K_d and K_i values were derived from three [^{3}H]-epibatidine and [^{125}I]-αBungarotoxin saturation and three competition binding experiments using rat cortex (α4β2) and hippocampus (α7) membranes and the membrane of human α3β4 transfected cells as described in ref. 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 ref. 23, 25 and 22.

On the basis of their α4β2 affinity, two groups of compounds can be distinguished. The first includes the compounds **3**, **4**, **5**, **6**, **9**, **10**, and **13**, which have high α4β2 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 α4β2 affinity. Their affinity for α7 is lower than their affinity for α4β2, (except for (*S*)-**4a**), with (*S*)-**4**, (*S*)-**5a**, (*S*)-**6**, (*S*)-**6a**

and (*S*)-**9** showing the greatest difference in affinity for α4β2 over α7. Their α3β4 affinity is also lower, with (*S*)-**4**, (*S*)-**6**, (*S*)-**6a** and (*R,S*)-**13** showing the greatest difference in sensitivity for α4β2 over α3β4.

The second group includes the compounds **7**, **8**, and **11** which all have very low α4β2 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 α4β2/α3β4 selectivity. Compounds (*S*)-**12** and (*S*)-**12a** fall between these two groups, having moderate submicromolar α4β2 affinity values, which are higher than those for α7 and α3β4 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 α4β2 nAChRs and moderate or very low affinity for α3β4 and α7 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 α3β4 affinity (0.310 μM K_i) than that determined for the α4β2 subtype (0.012 μM K_i) against 214-fold lower α3β4 activity (EC_{50}17.6 μM) than that measured for the α4β2 subtype (EC_{50} 0.082 μM). In order to determine the potency, efficacy and selectivity towards the α4β2 and α3β4 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 α4β2 and α3β4 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** (Fig. 1A) behaves as a partial agonist that is 57 times more potent on the α4β2 subtype (EC_{50} 0.3 ± 0.2 μM) than on the α3β4 subtype (17 ± 6 μM) and showed that the compound

(*R,S*)-**13** (Fig. 1F) is a more potent and selective partial agonist than (*R,S*)-**3**, with an EC_{50} of 0.013 μM for the α4β2 subtype and no effect on the α3β4 subtype.

We also determined that (*S*)-**4** (Fig. 1B) behaves as a full agonist with a greater efficacy (166%) than ACh on the α4β2, but similar potency on the α4β2 (EC_{50} 4.4 ± 0.6 μM) and α3β4 subtype (9 ± 2 μM). (*S*)-**5** (Fig. 1C) also is a full agonist for the α4β2 and a partial agonist for the α3β4 subtype with an EC_{50} of 9.2 μM. Compound (*S*)-**6** (Fig. 1D) is a very efficacious (661 ± 46% of 1 mM Ach) and selective α4β2 agonist with relatively low potency (73 ± 16 μM). Compound (*S*)-**9** (Fig. 1E) has an inverted U shaped curve of activation for the α4β2 subtype (probably because it has a channel blocker activity at high concentration) and no effect on the α3β4 subtype.

It is known that the heteromeric α4β2 and α3β4 can exist in two alternative stoichiometries: 2α/3β or, conversely, 3α/2β. 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 α4β2 subtype the two stoichiometries differ greatly in pharmacology: ACh has an EC_{50} value of ≈ 1 μM [32] and the agonist sazetidine is a full agonist for the $(\alpha4)_2(\beta2)_3$ stoichiometry [33] whereas towards the $(\alpha4)_3(\beta2)_2$ stoichiometry ACh has an EC_{50} value of ≈ 100 μM and sazetidine has a very low efficacy (6%). Analysis of the functional response of the α4β2 subtype expressed in our cells shows that ACh has biphasic concentration-effect curve with the high affinity (0.4 μM) representing 33% and the low affinity (100 μM) 67% of the total ACh current (Table 3 and Fig. 1H) and sazetidine (Fig. 1G) has a maximum response of 40% that of 1 mM ACh. These data indicate that in our cells both α4β2 stoichiometries are present and represent 1/3 $[(\alpha4)_2(\beta2)_3]$ and 2/3 $[(\alpha4)_3(\beta2)_2]$ of the functional receptors.

In the case of the α3β4 subtype, in agreement with the fact that the two stoichiometries have very similar EC_{50} values and potency for almost all nicotinic agonists,[14] we could not discriminate between

the expressed stoichiometries.

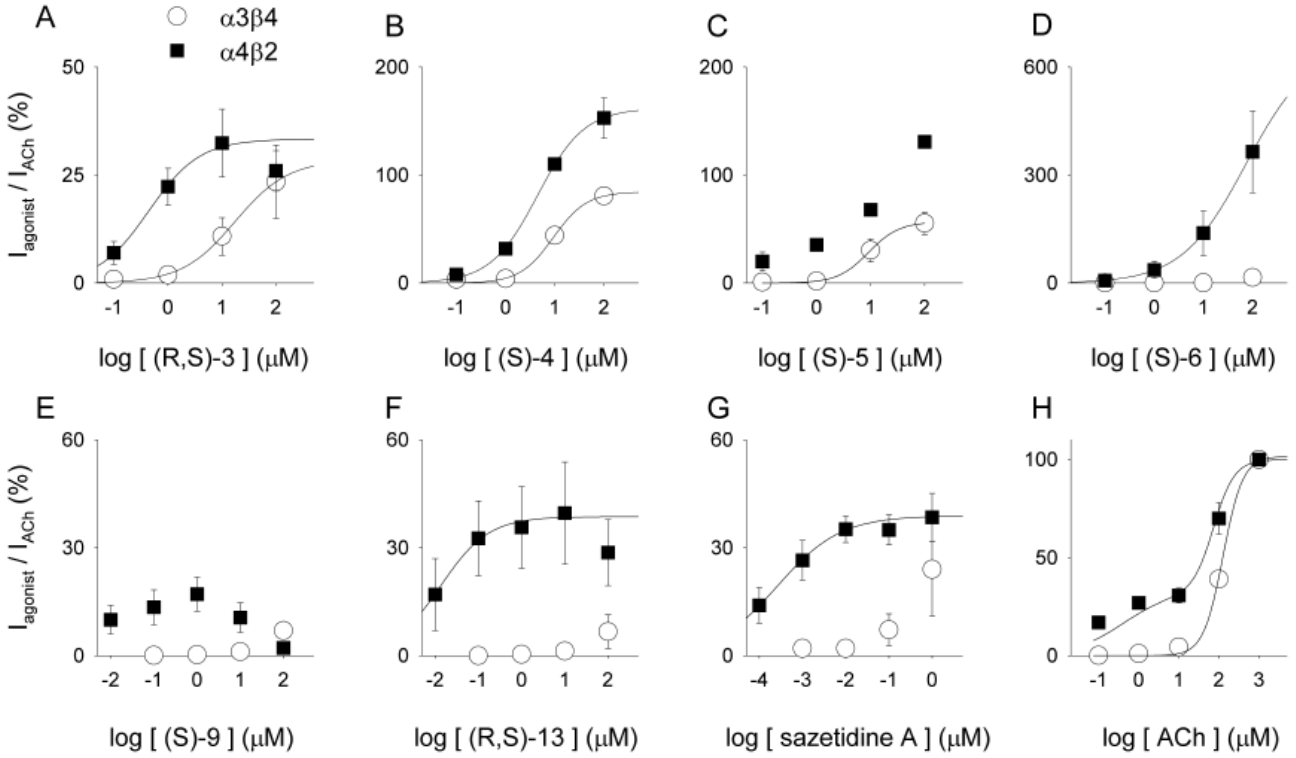


Figure 1. Agonist effects of (*R,S*)-**3**, (*S*)-**4**, (*S*)-**5**, (*S*)-**6**, (*S*)-**9**, (*R,S*)-**13**, sazetidine and ACh on transfected human α4β2 and α3β4 subtypes. Human α4β2 (■), α3β4 (○) subtypes were transiently transfected into the GH4C1 rat anterior pituitary cell line and the activation responses are normalised to the maximal response to 1 mM ACh.

Table 2. Effects of (*R,S*)-**3**, (*S*)-**4** and (*S*)-**5** on transfected human nicotinic receptor subtypes

		α4β2	α3β4
(*R,S*)-**3**	$I / I_{MAX\ ACh}$ (%)	29±4 (8)	28±3 (4)
	EC_{50} (μM)	0.3±0.2	17±6
	n_H	1.1±0.7	0.9±0.2
(*S*)-**4**	$I / I_{MAX\ ACh}$ (%)	162±6 (4)	85±5 (7)
	EC_{50} (μM)	4.4±0.6	9±2

	n_H	0.91±0.08	1.3±0.4
(*S*)-**5**	I / $I_{MAX\ ACh}$ (%)	131±1 (4)	57±1 (5)
	EC_{50} (μM)	-	9.2±0.5
	n_H	-	1.5±0.2
(*S*)-**6**	I / $I_{MAX\ ACh}$ (%)	661±46 (4)	15±2 (5)
	EC_{50} (μM)	73±16	-
	n_H	0.66±0.03	-
(*S*)-**9**	I / $I_{MAX\ ACh}$ (%)	17±5 (7)	7.0±0.5 (8)
	EC_{50} (μM)	-	-
	n_H	-	-
(*R,S*)-**13**	I / $I_{MAX\ ACh}$ (%)	39±1 (7)	7±4 (5)
	EC_{50} (μM)	0.013±0.003	-
	n_H	0.7±0.1	-
Sazetidine A	I / $I_{MAX\ ACh}$ (%)	39±1	24±13 (4)
	EC_{50} (μM)	0.00027±0.00006	-
	n_H	0.6±0.1	-
Acetylcholine	I / $I_{MAX\ ACh}$ (%)	33±8 (4) 67±8	100 (4)
	EC_{50} (μM)	0.4 100	127±1
	n_H	0.6±0.5 1.6±0.9	1.5±0.3

Dose–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: $I = Imax\ [[C]^{nH}/(EC_{50}{}^{nH} + [C]^{nH})]$, 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 brackets represent the number of tested cells.

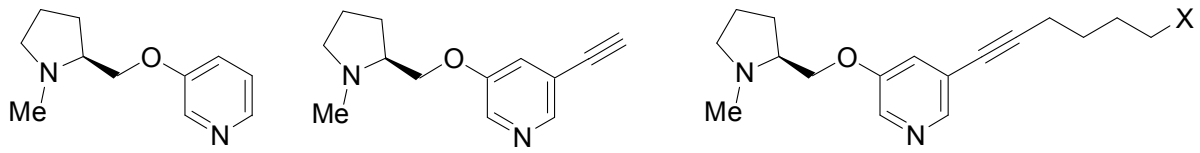
Discussion

The introduction of OH into the 7-position of pyrrolidinyl-benzodioxane (*R,S*)-**2** results in a 22-fold increase in α4β2 affinity: (*R,S*)-**3** has 12 nM K_i against the 0.26 μM K_i of (*R,S*)-**2**.[22,25] Moreover, (*R,S*)-**3** is a potent α4β2 partial agonist, 200-fold selective over the ganglionic α3β4 subtype, as previously proved by a test of dopamine release from rat striatum (EC_{50} 82 nM) [22] and now confirmed on human α4β2 nicotinic receptors (EC_{50} 0.3 μM). Consistently 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 α4 and β2 aminoacid residues of the α4β2-nAChR binding pocket, but, only for (*R,S*)-**3**, additional H-bonds between the 7-hydroxyl and two serines, Ser(111) and Ser(115) of the β2 subunit.[22,25] These additional bonds would be responsible for the increased potency and the β2 selectivity, while other substitutions than OH are found unproductive or deleterious apparently because only small substituents can be accepted at benzodioxane 7-position.[22]

Compared to our pyrrolidinyl-benzodioxanes, nicotinoids with a flexible oxymethylene bridge between the aromatic ring and the pyrrolidine residue, such as the 3-pyridyl ether of *S*-prolinol A-84543 [20,34] and its 5-alkinyl substituted analogues altinicline,[19,20] (*S*)-**29** [19,20] and (*S*)-**30** [19] (Chart 4) similarly bind to the carbonyl group of Trp147, a conserved residue of the α4 subunit, through the pyrrolidine N^+, but differently place the aromatic portion in the α4β2-nAChR binding pocket. In fact, though variously oriented, the pyridine ring of A-84543, altinicline, (*S*)-**29** and (*S*)-**30** always maintains π-π and strong hydrophobic interaction with Phe117, a non-conserved residue of the β2 subunit, with which the benzodioxane aromatic ring of (*R,S*)-**2** and (*R,S*)-**3** cannot interact due to the conformation of the oxymethine bridge, necessarily extended because part of the dioxane ring. On the other side, the pivotal role in the benzodioxane arrangement is played by the dioxane O(4) which replaces pyridyl nitrogen in interacting with non-conserved Lys77(β2).[22,25] Consistently with such a difference in the pose of the aromatic ring between pyridyl ethers and benzodioxanes, the pyridine ring is far from the mentioned β2

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 hydroxyl aminoacids.[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 is not assimilable to the 7-position of pyrrolidine-benzodioxane, it was mandatory to investigate the effect of the meta-hydroxy substitution on the α4β2 affinity, activity and selectivity also of the phenyl and 3-pyridyl ethers of prolinol, which are the parent opened analogues of **2**.

Chart 4. The 3-pyridyl ether of (*S*)-*N*-methylprolinol (A-84543) and some its 5-alkinyl substituted derivatives.



A-84543 altinicline (*S*)-**29** X = H (*S*)-**30** X = OH

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 α4β2 affinity (1.1 nM K_i) with a good selectivity over the α7 and α3β4 subtypes. Similar α4β2 affinity increase is produced also by the hydroxylation of the phenyl ether of 1-methyl-2-azetidinemethanol: (*S*)-**5** has a 12-fold higher affinity (8.5 nM K_i) than its non-hydroxylated analogue (101 nM K_i) [23] and (*S*)-**5a** 7-fold higher (7.1 nM versus 52 nM K_i [23]). Moreover, functional tests indicate that (*S*)-**4** is a potent full α4β2 agonist (EC_{50} 4.4 μM; 162% maximal efficacy relative to 1 mM ACh) and a partial α3β4 agonist

(EC_{50} 9 μM; 85% maximal efficacy relative to 1 mM ACh) showing, however, a functional α4β2 vs α3β4 selectivity sensibly lower than that found in the binding experiments. Again, the behavior of the azetidinyl analogue (*S*)-**5** is near identical to that of (*S*)-**5** as shown by the almost superimposable dose-response curves (Fig. 1B and 1C). On the other hand, the meta hydroxylated (*S*)-prolinol 3-pyridyl ether (*S*)-**6** maintains, without further improvements, the high α4β2 affinity and selectivity displayed by A-84543 in binding assays, but interestingly it behaves as a full α4β2 agonist with a very high functional α4β2 vs α3β4 selectivity, much higher than that of A-84543 and of other unsubstituted 3-pyridyl ethers [34] and resembling that of 2,5-diazabicylo[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 α4β2 nicotinic affinities and more or less pronounced α4β2 selectivity in binding experiments; thanks to 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 α4β2 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 better interact 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 α4β2 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 α4β2 and α3β4 agonist with high selectivity over the ganglionic subtype; (b) the 3-hydroxyphenyl ethers (*S*)-**4** and (*S*)-**5** are full α4β2 and partial α3β4 agonist with modest α4β2 selectivity, thus assimilable to A-84543; (c) the 5-hydroxy-3-pyridyl ether (*S*)-**6** is a full α4β2 agonist with practically no α3β4 activity.

Interestingly, also some pyrrolidine-furopyridines, designed by rigidifying the 3-pyridyloxymethylene portion of A-8453, show analogue behavior differences from the prolinol pyridyl ether which would mimick.[37] In our series 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 *ortho*-methoxyl (compounds **7**, **9**, **11** and **12**). Compared to the unsubstituted benzodioxane (*R,S*)-**2** (K_i 0.26 μM), 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 micromolar α4β2 affinities and no α4β2 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 *m*-hydroxylation again increases the α4β2 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 α4β2 selectivity is similar to that of benzodioxanes (*R,S*)-**2** and (*R,S*)-**3** again. It is evident that the *meta*-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**, an analogous trend is observed 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 ~ 10 micromolar α4β2 affinity, even lower than α3β4 affinity; (b) moderate and high α4β2 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 α4β2 affinity. As shown in figures 1E and 1F, the *o*-methoxylated hydroxyphenyl ether (*S*)-**9** and the pyridyl ether of α-methylated prolinol (*R,S*)-**13** behave as partial α4β2 agonists like (*R,S*)-**3** thus supporting the hypothesis of the similar interaction mode with α4β2 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 α4β2 agonism and suppresses α3β4 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 azetidinyl derivative, the N-desmetyl compound (*S*)-**5a** shows the same nanomolar α4β2 affinity as its N-methyl analogue (*S*)-**5**. On the other hand, all the N-methyl-pyrrolidinyl derivatives, especially those OH-substituted at the benzene nucleus, display higher α4β2 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 α4β2 affinity whether methylated [(*S*)-**6**] or non-methylated [(*S*)-**6a**] and, interestingly, a very high α4β2 vs α7 selectivity in the latter case.

Conclusions

The structures of pyrrolidinyl benzodioxane **2** and of the prolinol phenyl and 3-pyridyl ethers, **1** and A-84543, are superimposable and all display α4β2-nAChR affinity. The OH substituent at the 3-position of phenyl in **1** and at the 7-position of benzodioxane in **2** enhances the α4β2 affinity to one and ten-nanomolar level respectively (compounds **4** and **3**) and it is analogously beneficial when positioned in meta to the oxymethylene or oxymethine linker in a series of phenyl ethers 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-methyl prolinol (compound **6**), which maintains the

nanomolar α4β2 affinity of its non-hydroxylated analogue A-84543, and some 3-pyridyl ethers, the compounds **11**-**13**, differently mimicking opened 2-pyrrolidinyl benzodioxane, which show moderate or high α4β2 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 α4β2 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 pyrrolidinyl benzodioxanes **2** and **3** and their semi-rigid opened analogues **9**, **10**, **12** and **13** would similarly interact with the α4β2-nAChR binding pocket and in a different manner from the flexible phenyloxmethyl and pyridyloxymethyl ethers **4**-**6**, in particular by differently positioning the aromatic cycle. Such similarity and diversity are confirmed by the profiles of α4β2 agonism and selectivity over the ganglionic nicotinic subtype showed by the six compounds we have selected for functional tests, namely (a) the pyrrolidinyl hydroxybenzodioxane (*R,S*)-**3** and its opened analogues (*S*)-**9** and (*R,S*)-**13**, which are all potent and selective α4β2 partial agonists with potency and α4β2/α3β4 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 α4β2 full agonists, and the hydroxypyridyl ether (*S*)-**6**, a higly efficacious and selective α4β2 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; Rf values are given for guidance. ^{1}H 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°C/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. Elemental analyses (CHN) are within ± 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 one mole of substrate.

General Procedure for Synthesis of Compounds 13, **14-16, 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-hydroxypiridine (**22**). The *S* isomer of **18** was obtained by treatment of (*S*)-*N*-Boc-2-hydroxymethylazetidine 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 PPh_3 (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 PPh_3 (1.2 mol), DIAD (1.2 mol) and equimolar 3-hydroxypyridine. The reactions were carried out in THF at 66 °C for 16-18 hours (**14** and **13**) or under microwave irradiation at 140 °C for 30 minutes (**15** and **18**), at 120 °C for 15 minutes (**16**) and at 150 °C for 30 minutes (**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 debenzylation.

(*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; $[\alpha]_D^{25}$ = -87.6 (*c* 1, MeOH); ^1H

NMR ($CDCl_3$) 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); ^{13}C NMR ($CDCl_3$) 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; $[\alpha]_D^{25}$ = +18.3 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) 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); ^{13}C NMR ($CDCl_3$) 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; $[\alpha]_D^{25}$ = -49.6 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 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; $[\alpha]_D^{25}$ = -54.1 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 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; $[\alpha]_D^{25}$ = -33.1 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): 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)azetidine [(S)-**18**]*. Obtained as a light yellow oil in 74% yield after chromatography on silica gel (cyclohexane/EtOAc 8:2): R_f = 0.61; $[\alpha]_D^{25}$ = -73.3 (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 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; $[\alpha]_D^{25}$ = -41.6 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 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 (tolune/EtOAc 9:1) in 1:1 distereomeric 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; ^{1}H NMR ($CDCl_3$) 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 (tolune/EtOAc 9:1): R_f = 0.47; $[\alpha]_D^{25}$ = -120.7 (*c* 1, CH_3OH); ^{1}H NMR ($CDCl_3$) 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: ^{1}H NMR ($CDCl_3$) 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; $[\alpha]_D^{25}$ = -75.1 (*c* 0.5, CH_3OH); ^{1}H NMR ($CDCl_3$) 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; $[\alpha]_D^{25}$ = -51.0 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 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 $[\alpha]_D^{25}$ = -50.1 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.05-7.91 (m, 2H), 7.26-7.41 (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 K_2CO_3 (1.6 mol) in boiling DME overnight and chromatography on silica gel (DCM/acetone 9:1): R_f = 0.34; $[\alpha]_D^{25}$ = -31.4 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 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 $LiAlH_4$ (2 mol) in THF at -10 °C for 1h and successive chromatography on silica gel (toluene/acetone 7:3): R_f = 0.57; $[\alpha]_D^{25}$ = -74.2 (*c* 1, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 3.96–3.84 (m, 2H), 3.54 (m, 1H), 3.24 (m, 1H), 3.13 (bs, 1H, exchange with D_2O), 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); ^{13}C NMR ($CDCl_3$) 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, m.p. = 62.3 °C; $[\alpha]_D^{25}$ = -54.8 (*c* 1, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 4.31 (bs, 1H, exchange with D_2O), 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); ^{13}C NMR ($CDCl_3$) 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 hours. 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, $CHCl_3$); ^{1}H 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: m.p. = 121.8 °C; $[\alpha]_D^{25}$ = -5.86 (*c* 0.5, $CHCl_3$); ^{1}H 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-pyridoxyymethyl)-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; m.p. = 118.26 °C; $[\alpha]_D^{25}$ = -5.10 (*c* 1, $CHCl_3$); ^{1}H 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 ($CDCl_3$): ppm 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, $CHCl_3$); ^{1}H 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, $CHCl_3$); ^{1}H 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-pyridyloxyymethyl-pyrrolidine [(S)-**11a**]*. Obtained as a light yellow oil in 75% yield: $[\alpha]_D^{25}$ = +5.84 (*c* 1, $CHCl_3$); ^{1}H 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, $CHCl_3$); ^{1}H 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, $CHCl_3$); ^{1}H 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 hours. 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 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/1M/NaOH extraction of the resulting residue and concentration of the organic phase: $[\alpha]_D^{25}$ = -42.7 (*c* 1, $CHCl_3$); ^{1}H 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/1M HCl extraction of the resulting residue, alkalinisation 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); ^{1}H 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: ^{1}H 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); ^{1}H 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 ($CDCl_3$) δ16.6, 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*)-*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); ^{1}H NMR ($CDCl_3$) δ 6.91 (t, 1H, J = 8.3, Hz), 6.28-6.20 (m, 3H), 6.01 (bs, 1H, exchange with D_2O), 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); ^{13}C-NMR ($CDCl_3$) 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-pyridyloxyymethyl)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% NH_3 95:5:2): R_f = 0.38; $[\alpha]_D^{25}$ = -52.9 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 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); ^{13}C-NMR ($CDCl_3$) 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% NH_3 90:10:2): $[\alpha]_D^{25}$ = -22.7 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 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, ^{1}H NMR ($CDCl_3$) 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 9:1:2%): R_f = 0.39, ^{1}H NMR ($CDCl_3$) 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** with1.25 M methanolic HCl at room temperature overnight: $[\alpha]_D^{25}$ = -2.9 (*c* 1, MeOH); ^{1}H NMR (CD_3OD) 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); ^{13}C-NMR (CD_3OD); ^{13}C-NMR ($CDCl_3$) 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 (H_2 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%NH_3 90:10:2): R_f = 0.43; $[\alpha]_D^{25}$ = -30.1 (*c* 0.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 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); ^{13}C-NMR ($CDCl_3$) 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%NH_3 95:5:2): R_f = 0.47; mp 88.1 °C; $[\alpha]_D^{25}$ = -38.2 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 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 D_2O), 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); ^{13}C-NMR ($CDCl_3$) 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%NH_3 95:5:2): R_f = 0.30; $[\alpha]_D^{25}$ = -17.7 (*c* 0.8 $CHCl_3$); ^{1}H NMR ($CDCl_3$) 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); ^{13}C-NMR ($CDCl_3$) 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 non-linear least square 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^{TM}: 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 pipette (3- to 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 $CaCl_2$, 2.8 KCl, 2 $MgCl_2$, 10 Hepes/NaOH and 10 glucose; pH 7.3. The patch pipettes were filled with a solution containing (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 construct 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:

$I = Imax ([C]^{nH} / (EC_{50}{}^{nH} + [C]^{nH}))$, where I is the peak current amplitude induced by the agonist at concentration [C], Imax 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.

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Supporting information available. ^{1}H NMR and ^{13}C NMR spectra and elemental analysis results for the final compounds **4-13**, **4a-7a**, **9a**, **11a** and **12a**. ^{1}H 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**. This material is available free of charge via the Internet at http://pubs.acs.org.

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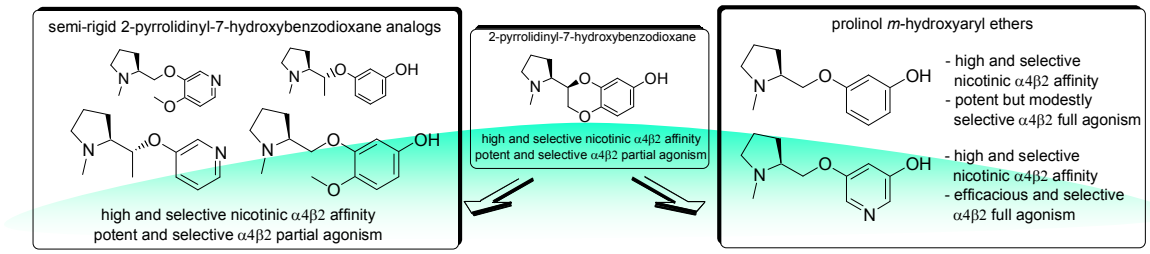
semi-rigid 2-pyrrolidinyl-7-hydroxybenzodioxane analogs
high and selective nicotinic α4β2 affinity
potent and selective α4β2 partial agonism
2-pyrrolidinyl-7-hydroxybenzodioxane
high and selective nicotinic α4β2 affinity
potent and selective α4β2 partial agonism
prolinol m-hydroxyaryl ethers
- high and selective nicotinic α4β2 affinity
- potent but modestly selective α4β2 full agonism
- high and selective nicotinic α4β2 affinity
- efficacious and selective α4β2 full agonism