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Novel 5-substituted 3-hydroxyphenyl and 3-nitrophenyl ethers of *S*-prolinol as α4β2-nicotinic acetylcholine receptor ligands

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ABSTRACT

A series of 3-nitrophenyl and 3-hydroxyphenyl ethers of (*S*)-*N*-methylprolinol bearing bulky and lipophilic substituents at phenyl C5 were tested for affinity at α4β2 and α3β4 nAChRs. The two phenyl ethers 5-substituted with 6-hydroxy-1-hexynyl showed high α4β2 affinity and significantly increased α4β2/α3β4 selectivity compared to the respective unsubstituted parent compounds. Within the two series of novel phenyl ethers, we observed parallel shifts in affinity and, furthermore, the increase in α4β2/α3β4 selectivity resulting from the hydroxyalkynyl substitution parallels that reported for the same modification at the 3-pyridyl ether of (*S*)-*N*-methylprolinol (A-84543), a well-known potent α4β2 agonist. On the basis of these results, our nitrophenyl and hydroxyphenyl prolinol ethers can be considered bioisosteres of the pyridyl ether A-84543 and lead compounds candidable to analogous optimization processes.

Agonists at neuronal nicotinic acetylcholine receptors (nAChRs), in particular those at the α4β2 subtype, are intensively studied nowadays because of their potential application in the therapy of a number of nervous-system disorders, tobacco addiction and alcohol dependence [1–3]. Varenicline, a partial α4β2 agonist, is widely used as an aid to smoking cessation [4–7], while another partial and highly selective α4β2 agonist, sazetidine-A [8,9], has been studied as an antidepressant within a wide number of 3-pyridyl ethers of N-methylprolinol or 2-azetidinemethanol bearing different substituents at the pyridine 5-position [1]. Recently, VMY-2-95 has been proved to have the high and selective α4β2 affinity typical of these 5-substituted 3-pyridyl ethers and an interesting character of low-efficacy agonist and potent desensitizer of α4β2 nAChR. Such a profile and good drug like properties make this molecule a good candidate for the treatment of nicotine addiction and other nAChRs related CNS disorders [2,10] (see Fig. 1).

Sazetidine-A and VMY-2-95 descend from the N-methylprolinol 3-pyridyl ether A-84543 (**1**) and its azetidinyl analogue A-85380 (**2**). These were discovered at the end of the nineties [11] as potent α4β2 agonists with selectivity over α7 and muscle-type nAChRs. From 2005, the pyridyl ethers A-84543 and A-85380 were reconsidered in order to increase their selectivity also over the ganglionic α3β4 nAChR subtype, in particular by introducing a series of sterically bulky substituents at the pyridyl C5 [12–15]. Our previous researches have been also focused on modifications of 3-pyridyl ether A-84543 aimed at modulating its activity profile. [16–19] As shown in Fig. 2, the first notable result of these investigations has been the finding that conformational blocking of the aryloxymethyl portion of **1** inside a pyridodioxane [20] or benzodioxane [17–19] system or its conformational restriction by *ortho*-methoxy substitution [16] results into selective partial α4β2 agonism, but on condition that pyridine nitrogen is correctly positioned in the pyridodioxane system (**3**) [20] or the benzene ring is hydroxylated at a certain position (**5** and **6**). [16,17] Isosteric replacement of pyridine nitrogen with CH or removal of the OH substituent transforms the selective α4β2 partial agonists **3** and **5** into the unselective α4β2 antagonist **4**. [20] On the other hand, the same modification, namely the *meta*-hydroxylation, made on the conformationally unrestricted **1** and its phenyl isoster is associated to potent α4β2 full agonism (**7** and **8**), but selective only in the case of the *meta*-hydroxylated derivative **7** [16]. These results suggest different receptor interactions of the aromatic portion between the forcedly extended bicyclic derivatives **3** and **5** and the aryl ethers **7** and **8**, for which a folded conformation of the oxymethylene linker is allowed [16].

On the basis of these observations, we were induced to postulate similar interactions with the receptor counterpart for **1** and

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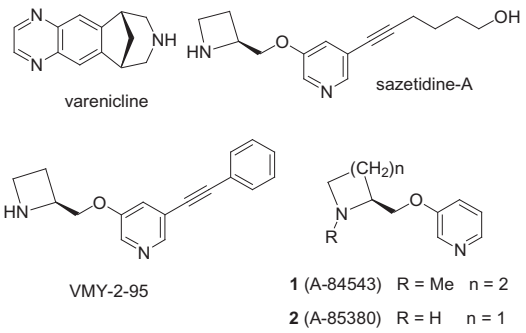


Fig. 1. α4β2 nAChR agonists: chemical structures of varenicline and of some reference 3-pyridyl ethers.

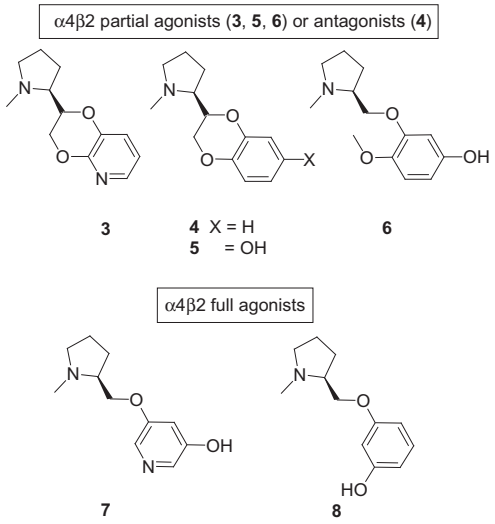


Fig. 2. Analogues of **1** (A-84543) with different activity and selectivity profiles at α4β2 nAChR.

8. The known bioisosteric relationship between pyridine and phenol further supported such a hypothesis and prompted us to consider also the isosteric replacement of 3-pyridyl with 3-nitrophenyl and to synthesize **9** [21–23]. Indeed, this compound had been already characterized for its α4β2 affinity by displacement of [^{3}H]-cytisine twenty years ago, [24] but we needed new binding data, fit to be compared with those of our other α4β2 nicotinic ligands, all tested against epibatidine. Furthermore, for the same reason, we prepared also the 3-methoxyphenyl analogue **10** [25] as a comparison term with **8**. The aim of the research was to explore the effect of the introduction of some *meta*-substituents, which have been described to preserve the high α4β2 affinity of **1** and **2** and, in some cases, also to confer α4β2 vs α3β4 selectivity, into the superimposable *meta* position of the supposedly bioisosteric aryl ethers **8** and **9**. This was done in order to substantiate our hypothesis of bioisosteric relationship and to find new α4β2 ligands with good α4β2 vs α3β4 selectivity. We selected, as *meta*-substituents, 6-hydroxy-1-hexynyl, conferring high α4β2 vs α3β4 selectivity to **2** (sazetidine-A), and bromine and phenyl, which are reported to maintain the high α4β2 affinity of **1** and **2** when linked to the pyridyl C5 [26,27]. Moreover, we thought that a terminal hydroxyl could reinforce the interaction of the *meta*-substituent, as in sazetidine-A and in **5**. Therefore, we enclosed into the series also *p*-hydroxyphenyl and planned the synthesis of compounds **9**–**19** (Fig. 3).

The title compounds have the same *S* configuration as the literature reference pyridyl ethers (see sazetidine-A, VMY-2-95, **1** and **2**) and our previous prolinol ethers with higher α4β2 affinity [16–19,28].

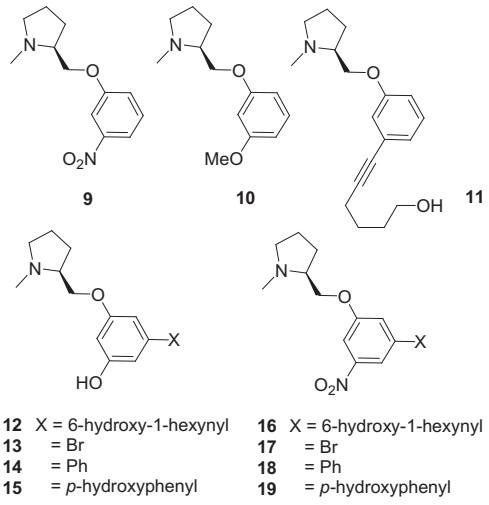


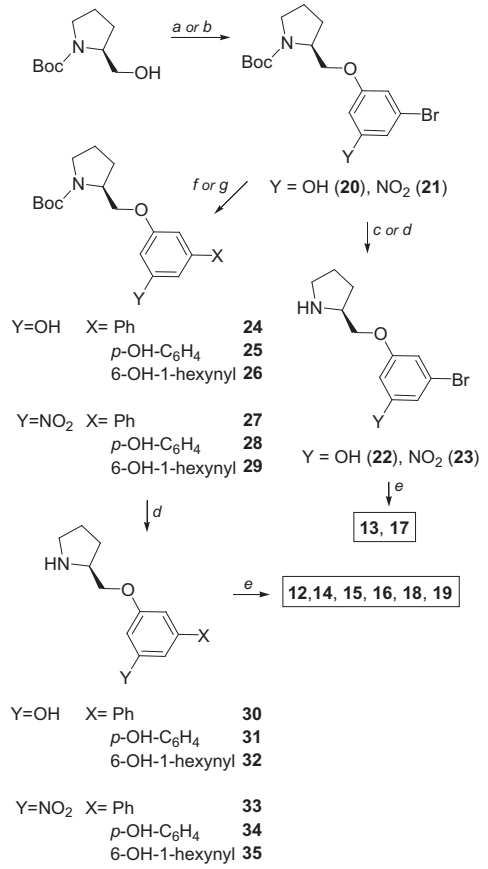
Fig. 3. Chemical structures of the designed phenyl ethers of (*S*)-*N*-methylprolinol.

The *meta*-bromo substituted hydroxyphenyl ether **13** [29] was synthesized from *N*-Boc protected *S*-prolinol by Mitsunobu reaction with 3-hydroxy-5-bromophenol, subsequent *N*-deprotection and final reductive alkylation with formaldehyde (Scheme 1). To obtain the hyroxyphenyl ethers **12**, [30] **14** [31] and **15**, [32] bearing an aryl or alkynyl residue at the *meta* position, the intermediate bromophenyl ether **20** was coupled with phenylboronic acid (**24**) or 4-hydroxyphenylboronic acid (**25**), according to previously reported methods, [33,34] or with 5-hexynol (**26**), according to the Sonogashira reaction, and then *N*-deprotected and *N*-methylated (Scheme 1). The corresponding *meta*-substituted nitrophenyl ethers **16**–**19** [35–38] were synthesized from *N*-Boc protected *S*-prolinol through the same synthetic sequences followed for **12**–**15**, but using 3-bromo-5-nitrophenol in the initial Mitsunobu reaction (Scheme 1).

The reference compounds **9** [39] and **11** [40] were prepared by Mitsunobu condensation of *N*-Boc protected *S*-prolinol with 3-nitrophenol (**36**) and 3-bromophenol (**37**). The nitrophenyl ether **36** was then deprotected and submitted to reductive alkylation with formaldehyde to yield **9**, while the bromophenyl ether **37** was coupled with 5-hexynol (**39**) before being *N*-deprotected and *N*-methylated to give **11** (Scheme 2). To prepare the 3-methoxyphenyl ether **10**, [41] *N*-Cbz protected *S*-prolinol was condensed with 3-methoxyphenol (**41**) and then reduced with $LiAlH_4$ (Scheme 2).

We evaluated the binding affinity of **9**–**19** towards the α4β2 nAChR present on rat cerebral cortex membranes and towards the human α3β4 nAChR transiently transfected on HEK 243 cells according to a previously described experimental protocol. [16] The α4β2 and α3β4 nAChRs were labelled by [^{3}H]-epibatidine and the binding affinities (K_i) of the compounds were determined with competition binding experiments. The results are listed on Table 1 together with the affinities of (*S*)-(−)-nicotine, determined as controls, and with those previously reported for the hydroxyphenyl ether **8** [16].

As shown in Table 1, both our lead compounds, namely the *meta*-hydroxyphenyl ether **8** and the *meta*-nitro phenyl ether **9** have high α4β2 affinity (1.1 and 31 nM K_i respectively), higher than that determined for the unsubstituted phenyl ether of *N*-methylated *S*-prolinol in 1995 (42 nM K_i) using [^{3}H]-cytisine. [25,42] On the other hand, compared to **1** (1.9 nM K_i), [12,41] **8** shows the same affinity, while **9** a lower one. Overall, it can be stated that

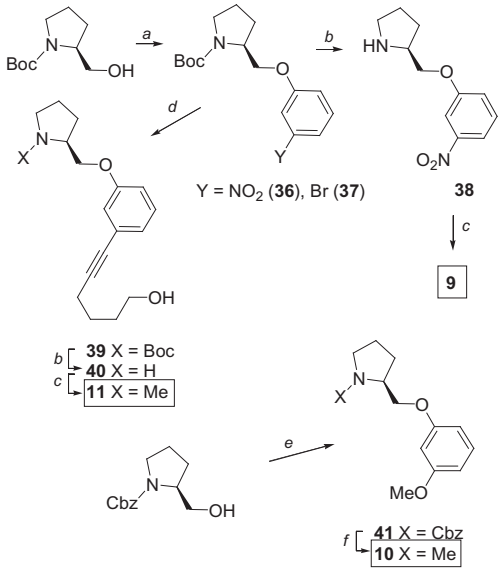


Scheme 1. Reagents, conditions and yields. (*a*) 3-Hydroxy-5-bromophenol, PPh_3, DIAD, THF, 130 °C, 30 min,, microwave, 34% (**20**); (*b*) 3-Bromo-5-nitrophenol, PPh_3, DIAD, THF, reflux, 12 h, 85% (**21**); (*c*) TFA, DCM, room temperature, 2 h, 80% (**22**); (*d*) Methanolic 1.25 N HCl, room temperature, 12 h, 42% (**23**), 100% (**30**), 96% (**31**), 80% (**32**), 78% (**33**), 81% (**34**), and 95% (**35**); (*e*) 37% CH_2O, CH_3COOH, pic-BH_3, CH_3OH, room temperature, 4 h, 87% (**12**), 75% (**13**), 80% (**14**), 51% (**15**), 80% (**16**), 98% (**17**), 86% (**18**) and 79% (**19**); (*f*) $Pd(PPh_3)_4$, toluene, 2 M aqueous Na_2CO_3, $XB(OH)_2$, ethanol, reflux, 12 h, 75% (**24**), 97% (**25**), 93%. (**27**) and 63% (**28**); (*g*) $Pd(PPh_3)_4$, CuBr, 5-hexyn-1-ol, TEA, reflux, 12 h, 80% (**26**) and 61% (**29**).

the introduction of NO_2 and OH at the *meta* position favours the α4β2 nAChR interaction of the phenyl and promotes, in the case of the *meta* hydroxylation, the phenyl ether to the rank of the corresponding pyridyl ether. The importance of the *m*-OH is further demonstrated by the drop of α4β2 affinity resulting from its methylation (see compound **10**) and from its removal (see compound **11** compared to **12**). Furthermore, both **8** and **9** show a moderate α4β2 vs α3β4 subtype selectivity.

Based on these premises, the same *meta* substituents (Br, Ph, *p*-hydroxyphenyl and 6-hydroxy-1-hexynyl) were introduced into **8** and **9** and, as shown in Table 1, parallel trends of affinity were found between the series of the *m*-hydroxyphenyl ethers **12**–**15** and that of the corresponding *m*-nitrophenyl analogues **16**–**19**. *Meta*-bromination (**13** and **17**) and, to a greater extent, *meta*-phenylation (**14** and **18**) lower the α4β2 affinity and cancel the α4β2/α3β4 selectivity. On the contrary, both the *meta*-6-hydroxy-1-hexynyl substituted derivatives **12** and **16** show high ten-nanomolar α4β2 affinities and significantly higher α4β2/α3β4 selectivity than the respective parent compounds **8** and **9**, and the *meta*-4-hydroxyphenyl substituted analogues **15** and **19** have the highest α4β2 affinities (3 and 12 nM K_i respectively), though with a modest α4β2/α3β4 selectivity.

With regard to the parallelism with **1**, this can be drawn only for **42**, namely the 5-(6-hydroxy-1-hexynyl)-substituted analogue



Scheme 2. Reagents, conditions and yields. (*a*) 3-Y-phenol, PPh_3, DIAD, THF, reflux, 12 h, 82% (**36**) and 48% (**37**); (*b*) Methanolic 1.25 N HCl, room temperature, 12 h, 100% (**38**), 71% (**39**) and 71% (**40**); (*c*) 37% CH_2O, CH_3COOH, pic-BH_3, CH_3OH, room temperature, 4 h, 98% (**9**); (*d*) $Pd(PPh_3)_4$, CuBr, 5-hexyn-1-ol, TEA, reflux, 12 h, 79%; (*e*) 3-Methoxy-phenol, PPh_3, DIAD, THF, reflux, 12 h, 63%; (*f*) $LiAlH_4$, THF, reflux, 90 min, 67%.

Table 1
Nicotine and compounds **9**–**19**: affinity for native α4β2 nAChR, present in rat brain membranes, labelled by [^{3}H]-epibatidine, and for heterologously expressed human α3β4 nAChR, labelled by [^{3}H]-epibatidine and affinity ratios.

	α4β2-nAChR [^{3}H]Epi K_i (μM)	α3β4-nAChR [^{3}H]Epi K_i(μM)	α3β4 *Ki*/ α4β2 K_i
8	0.0011 (29)	0.074 (100)	67
9	0.0312 (32)	0.946 (33)	30
10	0.600 (32)	4.5 (41)	7
11	4.4(44)	8.3 (44)	2
12	0.0237 (21)	3.1 (34)	131
13	0.186 (25)	0.147 (30)	0.8
14	0.528 (32)	0.200 (28)	0.4
15	0.0038 (47)	0.030 (53)	8
16	0.0142 (31)	1.2 (32)	85
17	0.055 (26)	0.415 (49)	8
18	0.330 (45)	0.947 (38)	3
19	0.012 (43)	0.122 (41)	10
(-)-65	Nicotine	0.004 (18)	0.261 (30)
1	0.0019	1.4	737
42	0.0009	63	74,000
K_d (nM)	0.040 (18)	0.194 (24)	

The K_d and K_i values were derived from[^{3}H]epibatidine saturation and competition binding experiments using rat cortex membranes (α4β2) and the membrane of human α3β4 transfected cells as described in Ref. [16]. The curves were fitted using a nonlinear least squares analysis program and the *F* test. The numbers in brackets represent the% coefficient of variation (CV). The affinities of **8**, **1** and **42** are those previously reported in Refs. [16] and [12].

of **1** (Fig. 4), [12] because the 5-(4-hydroxyphenyl)-substituted analogue of **1** is not reported and the binding data for the 5-bromo- and 5-phenyl analogues are limited to the α4β2 affinity and not directly comparable to the 1.9 nM K_i of **1**. [42] Nevertheless, though circumscribed to only one substituent, the parallelism with **1** is revealing: **42**, compared to **1**, maintains high α4β2 affinity (0.9 nM K_i), and shows increased α4β2/α3β4 selectivity with a behaviour analogous to that of **12** and **16**, compared to **8** and **9** respectively. The *meta*-6-hydroxy-1-hexynyl substitution similarly affects the nicotinic affinity profile of **1** and those of **8** and **9**, thus suggesting that the two prolinol phenyl ethers **8** and **9** share a

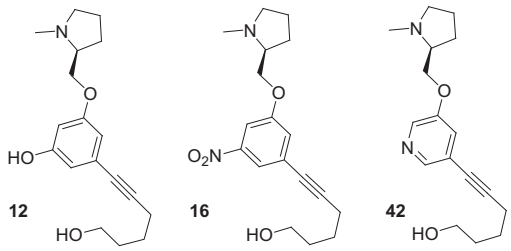


Fig. 4. Hydroxyhexynyl-aryl ethers of *S*-prolinol.

similar binding mode at α4β2 nAChR with the pyridyl ether **1**, of which they are veritable bioisosteres.

As previously discussed, [43] the long and flexible alkynyl substituent at C5 of prolinol pyridyl ethers would be able to reach β-side areas relatively remote from the binding site of the charged pyrrolidine nitrogen and there interact, respectively, with those non-conserved β2- and β4-residues which are mainly responsible for the difference between α4β2 and α3β4 nAChRs. Such different remote interactions of the alkynyl appendage would result in a correct positioning of pyrrolidine N^+ relative to an α-conserved Trp residue in the α4β2 [43,44], but not in the α3β4 binding site and this would justify the high α4β2/α3β4 selectivity. On the basis of our binding data, we think that these structure–activity and structure-selectivity relationships, formulated for the alkynyl substituted pyridyl ethers of prolinol, [12,43] can be applied also to **12** and **16**, alkynyl substituted hydroxyphenyl and nitrophenyl ethers of prolinol, which are in fact new potent and selective α4β2 nAChR ligands.

Acknowledgements

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[29] (*S*)-**13**: obtained as a waxy solid: $[\alpha]_D^{25} = -18.36$ (c 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.56 (t, *J* = 1.9 Hz 1H); 6.47 (t, *J* = 1.9 Hz, 1H); 6.28 (t, *J* = 1.9 Hz, 1H); 4.80 (bs, 1H, exchange with D_2O); 4.00–3.81 (m, 2H); 3.21–3.14 (m, 1H); 2.86–2.79 (m, 1H); 2.60 (s, 3H); 2.42–2.31 (m, 1H); 2.12–2.00 (m, 1H); 1.93–1.89 (m, 2H); 1.82–1.61 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.9, 158.7, 122.8, 113.1, 108.4, 102.6, 70.7, 64.6, 58.0, 42.8, 28.2, 23.1.
[30] (*S*)-**12**: obtained as a yellow waxy solid after chromatography on silica gel (eluent 90:10:1 DCM/methanol/conc NH_3); $R_f = 0.42$; $[\alpha]_D^{25} = -19.02$ (c 0.5, methanol); ^{1}H NMR (300 MHz, CD_3OD) δ 6.39 (m, 2H); 6.31 (m, 1H); 3.90 (m, 2H); 3.60 (t, *J* = 6.4 Hz, 2H); 3.10–3.02 (m, 1H); 2.73–2.64 (m, 1H); 2.48 (s, 3H); 2.38–2.29 (m, 3H); 2.09–1.99 (m, 1H); 1.84–1.75 (m, 2H); 1.73–1.61 (m, 5H); ^{13}C NMR (75 MHz, CD_3OD) δ 159.8, 158.1, 125.3, 110.9, 108.4, 101.7, 88.5, 80.5, 69.7, 64.4, 61.1, 57.3, 40.6, 31.4, 27.7, 24.9, 22.1, 18.3.
[31] (*S*)-**14**: obtained as a waxy yellow solid after chromatography on silica gel (eluent 90:10:1 DCM/methanol/conc NH_3); $R_f = 0.38$; $[\alpha]_D^{25} = -27.80$ (c 0.5, methanol); ^{1}H NMR (300 MHz, CD_3OD) δ 7.54 (d, *J* = 7.5 Hz, 2H); 7.39 (t, *J* = 7.5 Hz, 2H); 7.29 (t, *J* = 7.5 Hz, 1H); 6.63 (m, 2H); 6.38 (m, 1H); 4.01 (m, 2H); 3.12–3.07 (m, 1H); 2.76–2.73 (m, 1H); 2.42 (s, 3H); 2.40–2.33 (m, 1H); 2.13–2.07 (m, 1H); 1.88–1.70 (m, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 160.4, 158.6, 143.3, 141.1, 128.3, 127.0, 126.5, 106.6, 104.4, 100.4, 69.8, 64.6, 57.3, 40.6, 27.7, 22.1.
[32] (*S*)-**15**: obtained as a yellow oil after chromatography on silica gel (eluent 90:10:2 DCM/methanol/conc NH_3); $R_f = 0.30$; $[\alpha]_D^{25} = -20.93$ (c 1, methanol); ^{1}H NMR (300 MHz, CD_3OD) δ 7.39 (m, 2H); 6.81 (m, 2H); 6.58 (m, 2H); 6.30 (t, *J* = 1.8 Hz, 1H); 3.99 (m, 2H); 3.10 (m, 1H); 2.75 (m, 1H); 2.50 (s, 3H); 2.36 (m, 1H); 2.09 (m, 1H); 1.81 (m, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 160.3, 158.4, 156.9, 143.2, 132.4, 130.0, 127.5, 115.0, 105.9, 103.8, 99.5, 69.8, 64.5, 57.3, 40.6, 27.8, 22.1.
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[35] (*S*)-**16**: obtained as a light yellow oil after chromatography on silica gel (eluent 90:10:2 DCM/methanol/conc NH_3); $R_f = 0.57$; $[\alpha]_D^{25} = -30.23$ (c 1, methanol); ^{1}H NMR (300 MHz, CD_3OD) δ 7.76 (s, 1H); 7.70 (s, 1H); 7.31 (s, 1H); 4.07 (d, *J* = 5.3 Hz, 2H); 3.61 (t, *J* = 6.4 Hz, 2H); 3.01 (m, 1H); 2.75 (m, 1H); 2.49 (m, 5H); 2.38 (m, 1H); 2.05 (m, 1H); 1.86–1.67 (m, 7H); ^{13}C NMR (75 MHz, CD_3OD) δ 159.3, 149.1, 126.3, 123.0, 118.0, 108.4, 92.3, 78.3, 70.6, 64.2, 61.0, 57.3, 40.6, 31.4, 27.6, 24.6, 22.2, 18.3.
[36] (*S*)-**17**: obtained as a waxy yellow solid after chromatography on silica gel (eluent 90:10:2 DCM/methanol/conc NH_3); $R_f = 0.71$; $[\alpha]_D^{25} = -6.67$ (c 1, methanol); ^{1}H NMR (300 MHz, CD_3OD) δ 7.96 (t, *J* = 1.8 Hz, 1H); 7.76 (t, *J* = 1.8 Hz, 1H); 7.55 (t, *J* = 1.8 Hz, 1H); 4.08 (d, *J* = 5.3 Hz, 2H); 3.12–3.06 (m, 1H); 2.80–2.71 (m, 1H); 2.49 (s, 3H); 2.42–2.33 (q, *J* = 9.4 Hz, 1H); 2.13–2.03 (m, 1H); 1.88–1.70 (m, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 160.1, 149.6, 123.7, 122.6, 118.4, 108.3, 70.9, 64.1, 57.2, 40.6, 27.6, 22.2.
[37] (*S*)-**18**: obtained as a yellow oil after chromatography on silica gel (eluent 95:5 DCM/methanol); $R_f = 0.24$; $[\alpha]_D^{25} = -38.42$ (c 0.5, methanol); ^{1}H NMR (300 MHz, $CHCl_3$) δ 8.08 (s, 1H); 7.71 (s, 1H); 7.62 (m, 2H); 7.50 (m, 4H); 4.12 (m, 2H); 3.18 (m, 1H); 2.68 (m, 1H); 2.49 (s, 3H); 2.36 (m, 1H); 2.15–2.02 (m, 1H); 1.92–1.75 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.8, 149.5, 143.6, 138.7, 129.0, 128.6, 127.1, 120.2, 114.5, 107.3, 71.5, 64.0, 57.7, 41.7, 28.5, 23.1.
[38] (*S*)-**19**: obtained as a yellow solid after chromatography on silica gel (eluent 95:5:2 DCM/methanol/conc NH_3); $R_f = 0.33$; m.p. = 163.77 °C, $[\alpha]_D^{25} = -23.45$ (c 1, methanol); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.75 (bs, 1H, exchange with D_2O); 7.92 (s, 1H); 7.58 (m, 4H); 6.85 (m, 2H); 4.18 (m, 1H); 4.02 (m, 1H); 2.96 (m, 1H); 2.61 (m, 1H); 2.39 (s, 3H); 2.31–2.15 (m, 1H); 2.01–1.85 (m, 1H);

1.78–1.62 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.2, 157.7, 149.3, 143.1, 129.5, 128.0, 119.6, 116.5, 113.9, 105.7, 69.5, 64.8, 57.7, 42.0, 27.6, 22.8.

[39] (*S*)-**9**: obtained as a light yellow oil after chromatography on silica gel (eluent 90:10:2 DCM/methanol/conc NH_3); R_f = 0.64; $[\alpha]_D^{25}$ = −33.90 (c 1, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (dd, *J* = 8.2, 2.4 Hz, 1H); 7.77 (m, 1H); 7.52 (t, *J* = 8.2 Hz, 1H); 7.37 (m, 1H); 4.08 (d, *J* = 4.7 Hz, 2H); 3.08 (m, 1H); 2.75 (m, 1H); 2.42 (s, 3H); 2.37 (q, *J* = 9.4 Hz, 1H); 2.04–2.13 (m, 1H); 1.88–1.70 (m, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 159.5, 149.2, 130.0, 120.9, 115.3, 108.6, 70.5, 64.2, 57.3, 40.6, 27.7, 22.2.

[40] (*S*)-**11**: obtained as a yellow oil after chromatography on silica gel (eluent 90:10:2 DCM/methanol/conc NH_3); R_f = 0.24; $[\alpha]_D^{25}$ = −5.07 (c 1, $CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.21 (m, 1H); 6.90 (m, 3H); 4.40 (bs, 1H, exchange with D_2O); 3.94 (dd, *J* = 5.3, 9.6 Hz, 1H); 3.80 (dd, *J* = 5.9, 9.6 Hz, 1H); 3.30 (m, 2H); 2.94 (m, 1H); 2.51 (m, 1H); 2.40 (m, 2H), 2.38 (m, 3H); 2.17 (m, 1H); 1.96–1.86 (m, 1H); 1.71–1.50 (m, 7H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.9, 130.1, 124.8, 124.0, 117.1, 115.2, 91.0, 81.0, 71.3, 64.0, 60.6, 57.5, 41.8, 32.1, 28.9, 25.3, 23.0, 18.9.

[41] (*S*)-**10**: obtained as a light yellow oil after chromatography on silica gel (eluent Ethyl Acetate/TEA 3%); R_f = 0.40; $[\alpha]_D^{25}$ = −52.67 (c 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17 (t, *J* = 7.7 Hz, 1H); 6.50 (m, 3H); 4.00 (dd, *J* = 9.3, 5.1 Hz, 1H); 3.87 (dd, *J* = 9.3, 6.0, 1H); 3.78 (s, 3H); 3.12 (m, 1H); 2.66 (m, 1H); 2.49 (s, 3H); 2.32 (m, 1H); 2.07–1.98 (m, 1H); 1.89–1.71 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.0, 160.4, 130.1, 106.9, 106.7, 101.2, 70.7, 64.7, 57.9, 55.5, 41.7, 28.9, 23.1.

[42] The $\alpha4\beta2$ binding affinity (K_i) determined by using [^{3}H]-cytisine is 42 nM for the unsubstituted phenyl ether of *N*-methylated *S*-prolinol (Ref. [25]), 0.15 nM for **1** (Ref. [11]), 0.27 nM for the 5-bromo substituted derivative of **1** (Ref. [26]), 0.11 nM for the 5-phenyl substituted derivative of **1** (Ref. [27]), 9 nM for **9** (Ref. [24]), and 175 nM for **10** (Ref. [25]). Instead, by using [^{3}H]-epibatidine, significantly lower affinities were found: 1.9 nM for **1** (Ref. [12]), 31.2 nM for **9** (Table 1), and 600 nM for **10** (Table 1). Considering such unidirectional discrepancies, we think that the only literature affinity data with which we can compare our results are the 1.9 nM K_i of **1** and the 0.9 nM K_i of **42** and that the 1.1 and 31 nM affinities of **8** and **9**, determined by using [^{3}H]-epibatidine, can be rightly considered sensibly higher than the 'overvalued' 42 nM affinity of the unsubstituted phenyl ether, determined against [^{3}H]-cytisine .

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