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Synthesis and α4β2 nicotinic affinity of 2-pyrrolidinylmethoxyimines and prolinal oxime ethers

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Abstract—Homochiral *E* and *Z* isomers of *N*-methylprolinal *O*-isopropyloxime and (1-methyl-2-pyrrolidinyl)methoxyimines were synthesized as candidate bioisosteres of nicotine and its isoxazolic analogue ABT 418. Two of them, namely (*S*)-2-isopropylideneaminooxymethyl- and (*Z*)-(*S*)-2-ethylideneaminooxymethyl-1-methylpyrrolidine, proved to bind at α4β2 nicotinic acetylcholine receptor with submicromolar affinity and remarkable selectivity over α7 and muscarinic receptors thus supporting the hypothesized bioisosteric relationship between their methyloxyimino group and the aromatic heterocycles of the reference ligands.

The neuronal nicotinic acetylcholine receptors (nAChRs) play an important role in several brain functions and they are also involved in severe brain pathologies such as Parkinson's and Alzheimer's diseases, schizophrenia, anxiety, some forms of epilepsy and tobacco smoking addiction. nAChRs are a heterogeneous family of pentameric subtypes and this subtype variety is mainly due to the diversity of combinations of α and β subunits encoded by at least 12 different genes (α2–α10, β2–β4).[1–3] In vertebrate brain, the most abundant subtypes are the α4β2 subtype, which accounts for most of the high affinity ^{3}H-agonist binding sites, and the α7 subtype, which binds αBungarotoxin (αBgtx) with high affinity.[1–3] The generation of potent and selective α4β2 ligands has thus become an important objective of pharmaceutical research, which has been intensively pursued over the last decade leading to the formulation of reliable α4β2 pharmacophores and QSAR models.[4–6]

Common elements of these pharmacophoric patterns, based on the structures of nicotine and other nicotinic agonists, are (i) a protonated nitrogen (N^+) and (ii) a hydrogen bond acceptor (HBA) and/or a π-electron rich moiety (π) with a relative separation of cationic and HBA/π groups of ca. 4–6 Å (Fig. 1). More subtle differences between the proposed nicotinic pharmacophores derive from the individuation of additional elements, which can be a ring centroid (C) or an atom in the ligand defining the direction of the ligand–receptor hydrogen bond,[7] a planar area on the receptor recognizing the π-electron rich moiety of the ligand,[8] one or two points (*a* and *b*) on the receptor, with which HBA and N^+ interact, and the angle between two distance vectors chosen out of those joining the various pharmacophore elements.[5,9,10] From most of these models, the directionality of the HBA moiety relative to the $N^+ \rightarrow$ HBA/π vector emerges as a critical feature and the flat area proposed by Barlow and Johnson[8] or the ring centroid of the Sheridan model[7] would be different ways to make the pharmacophore tridimensional implicitly acknowledging the importance of such a directionality.

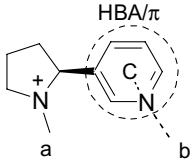

Figure 1. The proposed pharmacophoric elements illustrated with (*S*)-nicotine.

Keywords: Neuronal nicotinic acetylcholine receptor; nAChR; Nicotine; Ligand; Affinity; Oxime ether; Bioisostere.
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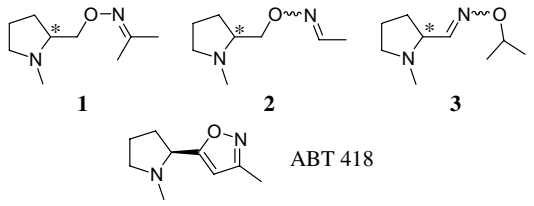
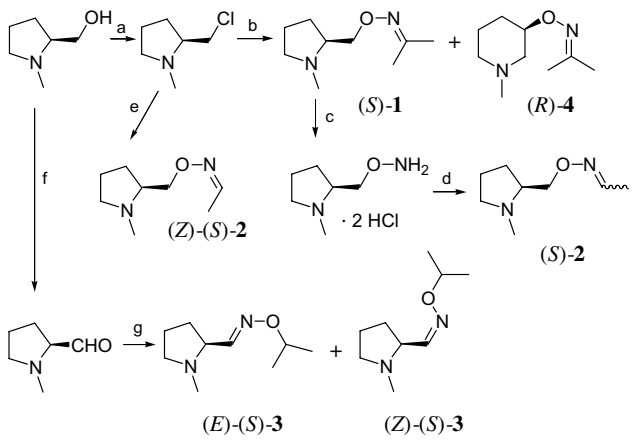
On the ground of these postulates, the oximethereal function (C=N–O–R) should suitably act as HBA/π group promoting the interaction with the receptor. In fact, it presents π electrons and hydrogen bond acceptor atoms, in particular the weakly basic sp^2 hybridized nitrogen. Due to its planarity, such a function could favourably interact with that flat area suggested by Barlow. Furthermore, the presence of the double bond and the consequent geometric isomerism give the opportunity of restraining and differentiating the possible directions of the ligand–receptor hydrogen bond.

The bioisosteric replacement of the ester function of acetylcholine by an oxyimino group dates back to the 1960s, when *O*-(β-dimethylamino)ethyl acetaldoxime iodomethylate was found to show both muscarinic and nicotinic activity by Rossi and co-workers.[11] More recently, arylalkenyl ethers of seven- to nine-membered azabicyclic ketone oximes and alkyliden hydroxylamines etherified with seven- to nine-membered azabicyclic residues have been described as muscarinic agonists.[12–14] Although nicotine has been known to chemists for decades, surprisingly enough such a bioisosteric replacement of its HBA/π moiety, namely the pyridine ring, has never been undertaken. This prompted us to design the oxime ethers **1**–**3** with the intention of evaluating the affinity for the α4β2 nAChR of all their optical and geometric isomers. Acetone and acetaldehyde were chosen to construct the oxyimino ethers **1** and **2** with the intent to mimic the 3-methyl-5-isoxazolyl residue of ABT 418, a potent and rather selective α4β2 nAChR agonist,[15] while the formal inversion of the methyloxyimino group (C–O–N=C) of **1** led to the methyleneaminoxy group (C=N–O–C) of prolinal oxime isopropyl ether **3**. This latter modification was made on the basis of the hypothesized bioisosteric relationship between the O–N=C and C=N–O atomic sequences.



The synthesis of the *S* isomers of oxime ethers **1**–**3** was accomplished as outlined in Scheme 1. (*S*)-*N*-Methylprolinol was converted into (*S*)-1-methyl-2-chloromethylpyrrolidine by treatment with thionyl chloride in toluene. Successive nucleophilic displacement of chloride by acetone oximate anion was performed by reacting with acetone oxime and potassium *tert*-butoxide in DMSO. As anticipated, on the basis of the known formation of a bicyclic aziridinium ion intermediate,[16] the reaction gave two substitution products, the 2-pyrrolidinemethyloxime (*S*)-**1** and its 3-piperidinyloxime isomer (*R*)-**4**, in an approximately 2:1 ratio. Repeated MPLC purifications on silica gel allowed (*R*)-**4**, which exhibited a slightly higher mobility than (*S*)-**1**, to be completely separated from this latter.[17] Finally, (*R*)-**4** was easily transformed into the corresponding crystal-



Scheme 1. Synthesis of oxime ethers. Reagents and conditions: (a) $SOCl_2$, toluene, 70 °C, 3 h; (b) acetone oxime, $(CH_3)_3COK$, DMSO, 18 h; (c) 10% aq HCl, reflux, 16 h; (d) CH_3CHO, EtOH and TEA (pH 4); (e) (*Z*)-acetaldoxime, $(CH_3)_3COK$, DMSO, 18 h; (f) $(COCl)_2$, DMSO, DCM, −50 °C, 30 min, then TEA; (g) $(CH_3)_2CHONH_2{\cdot}HCl$, DCM, 18 h.

line hydrochloride, while (*S*)-**1** was salified with picric acid, because its salts with hydrochloric acid or other commonly used acids were found too hygroscopic to be isolated.[18]

In order to obtain (*S*)-**2**, (*S*)-**1** was hydrolyzed to (*S*)-(1-methyl-2-pyrrolidinyl)methoxylamine and reacted with acetaldehyde yielding a mixture of (*E*)-(*S*)-**2** and (*Z*)-(*S*)-**2**, whose ^1H NMR analysis indicated a nearly equimolar composition. Unfortunately, such a mixture could not be resolved by chromatography, neither could it be significantly enriched in one of the two isomer by preferential crystallization of the respective picrates. Therefore, we decided to directly assess the binding affinity of the purified mixture of the two free amines.[19] This notwithstanding, the challenge to isolate almost one of the two geometric isomers of (*S*)-**2** was not dropped. Analogously to the preparation of (*S*)-**1**, (*S*)-1-methyl-2-chloromethylpyrrolidine was reacted with (*Z*)-acetaldoxime and potassium *tert*-butoxide in DMSO. Under such reaction conditions, the oxime configuration was preserved and, after chromatographic removal of the piperidine substitution product, isolated (*Z*)-(*S*)-*O*-(1-methyl-2-pyrrolidinyl)methyl acetaldoxime [(*Z*)-(*S*)-**2**] contained only traces of (*E*)-(*S*)-**2**,[17] which disappeared upon conversion into picrate.[20]

Finally, the inverted oxime ethers (*E*)-(*S*)-**3** and (*Z*)-(*S*)-**3** were prepared from (*S*)-*N*-methylprolinal, in turn obtained by Swern oxidation of (*S*)-*N*-methylprolinol. Reaction of the aldehyde with isopropoxyamine hydrochloride afforded a mixture of the two oxyimine ethers with an approximately 2:1 *E*/*Z* ratio. The two isomers were separated by chromatography[21] and subjected to the biological tests as free amines like the *E*/*Z* mixture of (*S*)-**2**.[22]

(*S*)-**4** and the *R* isomers of **1**, **2** (*E*/*Z* mixture), (*Z*)-**2**, (*E*)-**3** and (*Z*)-**3** were prepared by the same strategy illustrated in Scheme 1 but starting from (*R*)-*N*-methylprolinol.[23]

The geometric configuration of the methyleneaminoxy molecular portion (C=N–O) of **2** and **3** was assigned by ^{1}H NMR spectroscopy. The *syn* relationship between the oxime oxygen and the hydrogen linked to the iminic carbon atom of the aldehyde in the *E* isomers resulted, due to the paramagnetic effect of the spatially proximal oxygen, in the resonance of such a proton at a lower field with respect to the same proton of the corresponding *Z* isomers. This assumption was consistent with the literature spectral data for (*E*)- and (*Z*)-acetaldoxime[24,25] and the corresponding methyl ethers[26,27] and, in the case of **3**, led to the reasonable assignment of the presumably more stable *E* configuration to the prevalent oxime isomer produced by the reaction of prolinal with isopropoxyamine.

We evaluated the affinity towards the α4β2 subtype present in rat cortex membranes by binding studies using, as a ligand, [^{3}H]-epibatidine and the results are shown in Table 1. For the most interesting compounds, the affinities towards the α7-containing receptors, that bind [^{125}I]-αBgtx, and muscarinic receptors, that bind [^{3}H]-*N*-methylscopolamine, were also determined. Nicotine and ABT 418 were included in the series for comparison.

We found that, as shown in Table 1, the *S* isomers of oximes **1** and **2** are the most potent compounds in the series with a moderate submicromolar affinity for α4β2 nAChR. For (*S*)-**2**, such an affinity seems imputable to the *Z* isomer, which is twice more potent than the respective 1:1 *E*/*Z* mixture. This is also suggested by the α4β2 affinity of (*Z*)-(*R*)-**2**, which is the double of that displayed by the respective *E*/*Z* mixture. Otherwise, a micromolar affinity for the α4β2 receptor is shown by both the *E* and *Z* isomers of the inverted oxime (*S*)-**3**.

Table 1. Affinity of nicotine, ABT 418 and compounds **1**–**4** for native receptor subtypes, present in rat cortex membranes, labeled by [^{3}H]-epibatidine, [^{125}I]-αBungarotoxin and [^{3}H]-*N*-methylscopolamine

	[^{3}H]-Epi K_i (μM)	[^{125}I]-αBgtx K_i (μM)	[^{3}H]-NMS K_i (μM)
(−)-Nicotine	0.002 (59)	0.469 (33)	ND
ABT-418	0.020 (14)	1.20 (85)	>50
(*S*)-**1**	0.52 (14)	49 (18)	>50
(*R*)-**1**	16.0 (9)	ND	ND
(*S*)-**2** (1:1 *E*/*Z*)	0.68 (23)	>50	>50
(*R*)-**2** (1:1 *E*/*Z*)	10.5 (15)	ND	ND
(*Z*)-(*S*)-**2**	0.33 (24)	>50	>50
(*Z*)-(*R*)-**2**	5.79 (14)	ND	ND
(*E*)-(*S*)-**3**	1.52 (22)	>50	17 (23)
(*E*)-(*R*)-**3**	5.5 (13)	47 (19)	ND
(*Z*)-(*S*)-**3**	1.9 (23)	ND	ND
(*Z*)-(*R*)-**3**	14.5 (16)	ND	ND
(*S*)-**4**	>50	ND	ND
(*R*)-**4**	>50	ND	ND
K_d (nM)	0.026 (12)	661 (41)	0.076 (19)

The K_d and K_i values were derived from [^{3}H]-epibatidine, [^{125}I]-αBungarotoxin and [^{3}H]-*N*-methylscopolamine saturation and two competition binding experiments on rat cortex membranes. The curves were fitted using a nonlinear least squares analysis program and the *F* test. The numbers in brackets represent the %CV (ND = not determined).

Apart from **4**, whose *S* and *R* forms exhibit similarly low α4β2 affinities, the *S* isomers are invariably more potent than their *R* enantiomers. As expected, such a trend is more evident for **1** and **2** (eudismic index > 1) than for the less potent oximes **3** (eudismic index < 1).

Compared with ABT-418, (*S*)-**1** and (*S*)-**2** exhibit 15- to 30-fold lower α4β2 affinity, but higher selectivity over the α7 nAChR. Analogous subtype selectivity is shown by (*E*)-(*S*)-**3**.

Finally, we also determined whether the most potent compounds of our series bind at muscarinic AchRs. As shown in Table 1, (*S*)-**1** and (*S*)-**2** had very low affinity towards muscarinic receptors thus displaying a good α4β2 selectivity, whereas (*E*)-(*S*)-**3**, with only 11-fold higher affinity to α4β2 subtype, did not exhibit such a selectivity.

In summary, this study shows that replacement of the nicotine pyridine or the ABT 418 isoxazole by an isopropyliden- or ethylideneaminooxymethyl group leads to two new nicotinoids, (*S*)-**1** and (*Z*)-(*S*)-**2**, with (i) a moderate submicromolar affinity for α4β2 nAChR and compared to those compounds, (ii) the same stereoselectivity (the *S* isomers have higher affinity than their *R* enantiomer) and (iii) analogous or better α4β2 versus α7 subtype selectivity, thus supporting the initially hypothesized bioisosterism.

Furthermore, the double bond geometry, which should be more determinant for the direction of the ligand–receptor hydrogen bond relatively to the $N^+ \rightarrow$ HBA/π vector in the compounds **3** than in the compounds **2**, conversely affects the affinity of these latter in greater degree. This some unexpected result is probably imputable to the fact that the loss of affinity, due to the reduced internitrogen distance in the inverted oximes **3**, not only makes their chirality little influential but also their double bond configuration.

Finally, it is noteworthy that the 3-piperidinyloximes **4**, in which the optimal internitrogen distance of four bonds[28] is realized like in **1** and **2**, are virtually devoid of nicotinic affinity. Conformational analysis[29] of the protonated form of the *S* enantiomers of **1**, (*E*)-**2**, (*Z*)-**2** and of (*R*)-**4** with both *R* and *S* configuration at the ammonium nitrogen revealed two significant differences between the lowest energy structures of the 2-pyrrolidine and the 3-piperidine derivatives: (a) the ammonium proton is engaged in an intramolecular hydrogen bond with the oxygen atom in **1** and **2** to form a five-membered cycle, while the same interaction is obviously precluded in **4**; (b) the N^+–N distance is around 4.2 Å in all the *S* forms of the 2-pyrrolidine derivatives regardless of the configuration of the protonated tetrahedral nitrogen, whereas this stereolabile center sensibly influences the same distance in (*R*)-**4** (4.3 Å in the more stable conformer having N^+ with *S* configuration and both the piperidinyl ring substituents *equatorially* oriented and only 3.8 Å in the less stable conformer with N^+ in *R* configuration). These observations are consistent with the already underlined importance of the directionality of

the HBA moiety in addition to a suitable distance of this latter from the charged nitrogen. Furthermore, they draw attention to the fact that, in some cases, such a distance can conform to the nicotinic pharmacophore on condition of a given ammonium nitrogen configuration, which is another important, but often neglected determinant of the affinity of ligands containing protonation-induced stereocenters.

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17. MPLC on silica gel; eluent: DCM/MeOH/30% aq NH_3 95/5/1.
18. (*S*)-**1**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.05 (dd, J = 10.4, 6.2 Hz, 1H), 3.94 (dd, J = 10.4, 5.3 Hz, 1H), 3.06 (pseudo t, J = 7.9 Hz, 1H), 2.53 (m, 1H), 2.42 (s, 3H), 2.23 (dd, J = 17.6, 8.2 Hz, 1H), 1.86 (s, 3H), 1.84 (s, 3H), 1.51–1.99 (m, 4H). (*S*)-**1** Picrate: mp 94–95 °C; $[\alpha]_D^{25}$ +20.8 (*c* 2, EtOH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.83 (br s, 1H), 8.89 (s, 2H), 4.55 (dd, J = 13.2, 8.8 Hz, 1H), 4.26 (dd, J = 13.2, 3.3 Hz, 1H), 3.97 (m, 1H), 3.63 (m, 1H), 3.05 (s, 3H), 2.99 (m, 1H), 2.27 (m, 2H), 2.03 (m, 2H), 1.82 (s, 3H), 1.79 (s, 3H). (*R*)-**4** Hydrochloride: mp 130–131 °C; $[\alpha]_D^{25}$ +19.4 (*c* 2, EtOH); ^{1}H NMR (D_2O, 300 MHz): δ 4.28 (br s, 1H), 3.52 (pseudo d, J = 12.1 Hz, 1H), 3.30 (pseudo d, J = 12.1 Hz, 1H), 3.06 (pseudo d, J = 13.2 Hz, 1H), 2.85 (pseudo t, J = 11.7 Hz, 1H), 2.68 (s, 3H), 1.79 (s, 3H), 1.74 (s, 3H), 1.4–2.0 (m, 4H). ^{1}H NMR spectrum of (*R*)-**4** hydrochloride in $CDCl_3$ shows the presence of comparable quantities of both the ammonium and the oxyimmonium species.
19. MPLC on silica gel; eluent: DCM/MeOH/30% aq NH_3 95/5/1. (*S*)-**2** (*E*/*Z* mixture): ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.43 (q, J = 6.2 Hz, ~0.5H), 6.74 (q, J = 5.5 Hz, ~0.5H), 4.14 (dd, J = 11.0, 6.2 Hz, ~0.5H), 4.08 (dd, J = 10.6, 5.5 Hz, ~0.5H), 4.04 (dd, J = 11.0, 5.5 Hz, ~0.5H), 3.98 (dd, J = 10.6, 5.5 Hz, ~0.5H), 3.08 (m, 1H), 2.48–2.60 (m, 1H), 2.43 (s, ~1.5H), 2.41 (s, ~1.5H), 2.25 (m, 1H), 1.84 (d, J = 6.2 Hz, 3H), 2.00–1.56 (m, 4H).
20. (*Z*)-(*S*)-**2**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.74 (q, J = 5.5 Hz, 1H), 4.14 (dd, J = 11.0, 6.2 Hz, 1H), 4.04 (dd, J = 11.0, 5.5 Hz, 1H), 3.08 (m, 1H), 2.55 (m, 1H), 2.43 (s, 3H), 2.24 (m, 1H), 1.84 (d, J = 6.2 Hz, 3H), 1.56–2.00 (m, 4H). (*Z*)-(*S*)-**2** Picrate: mp 102–104 °C; $[\alpha]_D^{25}$ +12.9 (*c* 1, EtOH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.95 (br s, 1H), 8.89 (s, 2H), 6.79 (q, J = 5.5 Hz, 1H), 4.66 (dd, J = 8.8, 13.0 Hz, 1H), 4.34 (dd, J = 13.0, 2.9 Hz, 1H), 3.98 (m, 1H), 3.62 (m, 1H), 3.06 (s, 3H), 2.99 (m, 1H), 2.29 (m, 2H), 2.12 (m, 2H), 1.80 (d, J = 5.5 Hz, 3H).
21. MPLC on silica gel; eluent: DCM/MeOH/30% aq NH_3 97/3/0.5.
22. (*E*)-(*S*)-**3**: $[\alpha]_D^{25}$ −44.4 (*c* 2, EtOH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.18 (d, J = 7.7 Hz, 1H), 4.31 (septet, J = 6.2 Hz, 1H), 3.10 (m, 1H), 2.77 (dd, J = 15.8, 7.9 Hz, 1H), 2.31 (s, 3H), 2.22 (dd, J = 17.2, 8.8, 1H), 1.99 (m, 1H), 1.67–1.96 (m, 3H), 1.22 (d, J = 6.2, 6H). (*Z*)-(*S*)-**3**: $[\alpha]_D^{25}$ −60.6 (*c* 2, EtOH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.71 (d, J = 5.9 Hz, 1H), 4.30 (septet, J = 6.2 Hz, 1H), 3.39 (dd, J = 15.1, 7.0 Hz, 1H), 3.07 (m, 1H), 2.33 (s, 3H), 2.18 (dd, J = 18.6, 8.1 Hz, 1H), 2.10–2.20 (m, 1H), 1.72–1.92 (m, 2H), 1.61 (m, 1H), 1.22 (d, J = 6.2 Hz, 6H).
23. (*R*)-**1**: ^{1}H NMR identical to that of (*S*)-**1**. (*R*)-**1** Picrate: mp 92–93 °C; $[\alpha]_D^{25}$ −18.9 (*c* 2, EtOH); ^{1}H NMR identical to that of (*S*)-**1** picrate. (*S*)-**4** Hydrochloride: mp 128–129 °C; $[\alpha]_D^{25}$ −19.5 (*c* 2, EtOH); ^{1}H NMR identical to that of (*R*)-**4** hydrochloride. (*R*)-**2** (*E*/*Z* mixture): ^{1}H NMR identical to that of (*S*)-**2** (*E*/*Z* mixture) with similar *E*/*Z* ratio. (*Z*)-(*R*)-**2**: ^{1}H NMR identical to that of (*Z*)-(*S*)-**2**. (*Z*)-(*R*)-**2** Picrate: mp 104–105 °C; $[\alpha]_D^{25}$ −13.1 (*c*, 1 EtOH); ^{1}H NMR identical to that of (*Z*)-(*S*)-**2** picrate. (*E*)-(*R*)-**3**: $[\alpha]_D^{25}$ +40.9 (*c* 2, EtOH); ^{1}H NMR identical to that of (*E*)-(*S*)-**3**. (*Z*)-(*R*)-**3**: $[\alpha]_D^{25}$ +55.5 (*c* 2, EtOH); ^{1}H NMR identical to that of (*Z*)-(*S*)-**3**.
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29. The conformational analyses were performed using Monte Carlo approach that produced 1000 conformers for each molecule by rotating its flexible torsions. All the obtained geometries were optimized and clustered according to their similarity.