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3-(5-Alkylamino-4-isoxazolyl)-1,2,5,6-tetrahydropyridines: a Novel Class of Central Nicotinic Receptor Ligands

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Abstract—A novel class of central nicotinic acetylcholine receptor ligands, 3-(5-alkylamino-4-isoxazolyl)-1,2,5,6-tetrahydropyridine **4a**–**f**, was synthesized. Several of the compounds showed high affinity for central nicotinic receptors (**4c**: $IC_{50} = 50$ nM), with more than a 100-fold selectivity for nicotinic over muscarinic receptors. The compounds showed up to a 10-fold selectivity for the central nicotinic subtype combination α4β2 (**4c**: $IC_{50} = 4.6$ nM), as compared to the major ganglionic subtype composed of α3 containing subunits (**4c**: $IC_{50} = 48$ nM). The compounds were further evaluated in a dopamine release assay in vitro, and in a drug discrimination assay in vivo. Compound **4a** is an effective nicotinic agonist with a potency 50–100 times lower than nicotine. Extending the alkylamino chain beyond one, compound (**4b**–**f**), changed the pharmacological profile of the compounds in an antagonistic direction.

Introduction

Neuronal nicotinic receptors are members of the ligand-gated ion channel family and are composed of a pentameric combination of subunits; the combination of subunits present in a given pentamer appears to dictate the pharmacology of the receptor.[1] New ligands that are cholinergic channel modulators and selectively activate or inhibit subtypes of the central cholinergic nicotinic receptors (nAChR) may be interesting tools for examination of the functional activity of the different nACh receptor subtypes, and may have therapeutic potential for the treatment of central nervous system disorders.[1–4] The natural product arecoline, methyl 1,2,5,6-tetrahydro-1-methylnicotinate has been shown to possess weak affinity for nicotinic receptors compared to muscarinic receptors,[5] and the compound has been used extensively as a lead compound for the preparation of ligands with high affinity and selectivity for muscarinic receptors.[6–9] Only a few examples have been reported, in which the tetrahydropyridine azacyclic unit of arecoline has been used in order to produce ligands with affinity for central nicotinic receptors, (e.g. arecholone and isarecholone).[10–12]

Acetylcholine is the endogenous transmitter for both the nicotinic and the muscarinic cholinergic receptor systems. For the stimulation of muscarinic receptors, acetylcholine can be replaced by arecoline and it would appear that the tetrahydropyridine azacycle in arecoline can be used as a cationic head for preparing ligands with high affinity and selectivity for nicotinic ACh receptors as well.

Using this working hypothesis we have discovered a novel series of 3-(5-alkylamino-4-isoxazolyl)-1,2,5,6-tetrahydropyridines with high affinity and selectivity for central nicotinic receptors. Evaluation of these compounds in cell lines expressing nicotinic receptor subtypes, showed that the compounds had up to a 10-fold higher affinity for the α4β2 receptor subtype than the α3β2 receptor.

Chemistry

The 3-(5-amino-4-isoxazolyl)pyridines **2** (Scheme) were obtained by reaction of 3-pyridylacetonitril **1** with dimethylform-amide dimethylacetal (DMFDMA) or dimethy-

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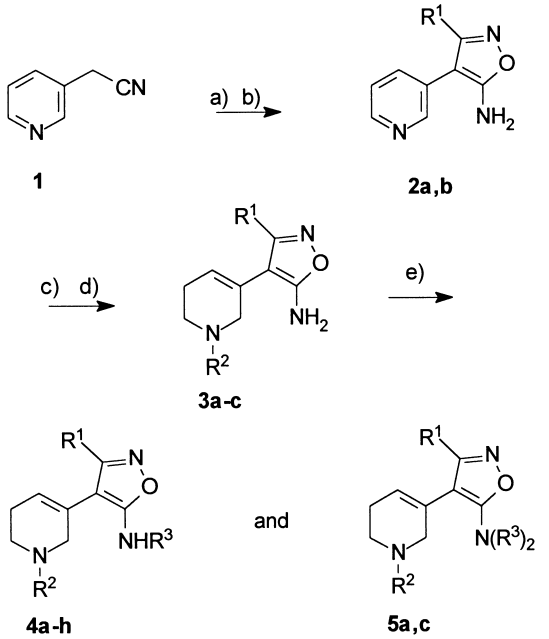

Scheme 1. Synthesis of 3-(5-alkylamino-4-isoxazolyl)-1,2,5,6-tetrahydropyridines: (a) **2a**: DMFDMA **2b**: DMADMA; (b) NH_2OH, HCl, AcOH; (c) Alkyl iodide, acetone; (d) $NaBH_4$, MeOH; (e) NaOH, alkyl iodide, DMF.

lacetamide dimethylacetal (DMADMA) followed by a ring closure reaction with hydroxylamine under acidic conditions to give compound **2** as the only isomer. Compound **2** was quaternized with the appropriate alkyl iodide, and the quaternary salt formed was reduced with sodium boro-hydride in methanol to give the crucial intermediates **3**. These compounds were stable to neutral and acidic conditions, whereas under alkaline conditions or, upon heating the compounds slowly decomposed, via ring opening of the isoxazole heterocycle.

Due to this decomposition process the next reaction step had to be rapidly performed, involving successive addition of powdered potassium hydroxide followed by rapid addition of the appropriate alkyl halogenide in DMF. The reaction mixture was immediately quenched with 1 N hydrochloric acid to give a mixture of the monoalkylated compound **4** as the major product and the dialkylated compound **5** as the minor product, together with some starting material. This mixture of compounds was separated by column chromatography to give the free bases of the compounds **4** and **5**. In most cases, only compound **4** was isolated from the reaction mixture (see Methods). The final products were crystallized as the oxalate salts. The crystalline compounds could be stored for prolonged periods of time without decomposition, and no decomposition of the compounds was detected in aqueous solution at neutral pH, or as weakly acidic solutions with pH down to 2.0, no decomposition of the compounds was detected after 8 days at ambient temperature.

All the compounds shown in Table 1 gave satisfactory elemental analyses and spectral data (MS and ^1H NMR)

Table 1. In vitro binding data for 3-(5-alkylamino-4-isoxazolyl)-1,2,5,6-tetrahydropyridines

				Receptor binding to rat brain homogenates[a]		Receptor binding to cloned cell lines[a]	
Compd	R^1	R^2	R^3	[^{3}H]-MCC cortex IC_{50} (nM)	[^{3}H]-Oxo-M cortex IC_{50} (nM)	[^{3}H]-MCC α4β2(Sf 21) IC_{50} (nM)	[^{3}H]-MCC α3β2(Sf 9) IC_{50} (nM)
3a	H	CH_3	H	1800	4300	n.d.	n.d.
3b	CH_3	CH_3	H	> 10000	> 10000	n.d.	n.d.
3c	H	Ethyl	H	> 10000	> 10000	n.d.	n.d.
4a	H	CH_3	$CH_{3\backslash 10}$	230	4400	63	92
4b	H	CH_3	Ethyl	62	1400	17	140
4c	H	CH_3	Propyl	38	4600	4.6	48
4d	H	CH_3	Butyl	55	2800	14	95
4e	H	CH_3	Pentyl	45	3000	15	130
4f	H	CH_3	Benzyl	85	4000	200	300
4g	CH_3	CH_3	Butyl	> 10000	> 10000	n.d.	n.d.
4h	H	Ethyl	Propyl	> 10000	> 10000	n.d.	n.d.
5a	H	CH_3	CH_3	> 10000	> 10000	n.d.	n.d.
5c	H	CH_3	Propyl	> 10000	> 10000	n.d.	n.d.
nicotine				3.6	28000	4.2	3.8
lobeline				6.5	990	3.8	25

[a]Each point in each dose–response curve is the mean of duplicate or triplicate determinations. IC_{50}'s were calculated from 5–10 point dose–response curves. n.d. = not determined.

were consistent with the structures proposed.

Biological Results

The affinities of the compounds for the central nicotinic receptor sites in rat cortex were determined using competitive radioligand binding studies with [^{3}H]methylcarbachol (MCC).[13,14] MCC has been shown to be a nicotinic agonist[15] and the affinity for the MCC binding sites has correlated well with psychotropic effects in rats.[16] The affinities of the compounds for muscarinic receptor sites in rat cortex were determined using competitive radioligand binding assays employing [^{3}H]oxotremorine-M (Oxo-M).[13,17] Oxo-M is a potent muscarinic agonist lacking muscarinic subtype selectivity.

The highest concentration used for screening the compounds in the displacement of [^{3}H]MCC and [^{3}H]OXO-M from cortical rat brain homogenates was 10 μM. As seen in Table 1, only the monoalkyl amino-substituted compounds **4a–f** had high affinity for the central nicotinic receptors. Extending the substitution pattern in these heterocycles with a methyl substituent in the 3-position of the isoxazole heterocycle, (e.g. **4g**), dialkylation of the aminoisoxazole, (e.g. **5a–5b**), or even by replacing the *N*-methyl substituent in the tetrahydropyridine with an *N*-ethyl substituent as in **3c**, dramatically reduced the affinities of the compounds for the central nACh and muscarinic receptors.

For the monoalkyl substituted compounds **4a–f**, the affinity increased with increasing alkyl side chain length, with the maximum affinity obtained with propyl **4c** (IC_{50} = 38 nM). However, even bulkier substituents such as pentyl **4e** and benzyl **4f** gave compounds with high affinity for nACh receptors (IC_{50} = 45 nM and 85 nM, respectively). The SAR for the affinity to the muscarinic ACh receptors followed the same pattern as observed for the affinity to nACh receptors. Only the monoalkyl substituted compounds **4a–f** gave affinities for the muscarinic ACh receptors with IC_{50}'s in the 1400–4000 nM range.

Only compounds having binding affinities less than 1000 nM to cortical homogenates were further evaluated in receptor binding to subtypes of nicotinic receptors. The compounds were tested for affinity to two different subtype combinations of nACh receptors: (a) the α4β2 subunit combination that supposedly is the major subtype in the brain,[18] and (b) the α3β2 subunit combination. The α3 unit is supposedly the major ganglionic nACh receptor type[19] (Table 1). The affinity for the α4β2 subtype increased with increasing alkyl side chain length, reaching a maximum with the propyl amino substituent, compound **4c**, (IC_{50} = 4.6 nM). With an alkyl side chain beyond 4 the affinity decreased slightly, and with the bulkier benzyl substituent **4f** the affinity decreased to 200 nM. The affinity of the compounds for the α3β2 subtype was in general 10 times lower than for the α4β2 subtype, but the SAR for the affinity to both subtypes follows the same pattern as described for the α4β2 subtype. As shown in Table 1, all the monoalkylamino substituted isoxazoles **4a–f** were selective for the brain (α4β2) subtype, with the highest binding ratio obtained for the propylamino compound **4c**, α3β2/α4β2 = 10. To determine whether the new ligands behaved as agonists or antagonists, compounds with an affinity less than 100 nM for α4β2 nicotinic receptor subtype were evaluated in two functional assays (i.e. dopamine release from striatal slices in vitro and a nicotinic drug discrimination assay in vivo).

Activation of nicotinic receptors located on presynaptic, dopaminergic terminals in the striatum induces the release of dopamine. The induction of dopamine release from striatal slices has previously been shown for nicotine and other nicotinic agonists.[13,20,21] The compounds were evaluated for their ability to induce dopamine release from striatal slices at 1 μM and 10 μM concentrations. As shown in Table 2, only the compounds **4c** and **4d** induced a small release compared to nicotine at the highest dose tested (10 μM). A dose–response curve was obtained for the ability of compound **4a** to induce dopamine release and compared to nicotine (Fig. 1). As shown, compound **4a** is a partial agonist compared to nicotine, with a potency 40-fold less than nicotine.

Nicotinic agonists produce nicotine-like responses in nicotinic drug discrimination assays.[22] Using standard drug discrimination procedures,[23] it has been shown

Table 2. In vitro and in vivo functional data for 3-(5-alkylamino-4-isoxazolyl)-1,2,5,6-tetrahydropyridines

	Drug induced [^{3}H] dopamine release from striatal slices[a]		Nicotine drug discrimination	
Compd	1 μM	10 μM	% of max score[b]	ED_{50} mg/kg sc[c]
4a	0	13	72	3.5
4b	0	0	40	> 30
4c	1.2	44	39	30
4d	1.5	23	55	9.0
4e	0.21	10	nd	> 30
nicotine	100	180	96	0.04
lobeline	1.2	5	40	> 6

[a]Results are given in % of release induced by 1 μM nicotine. Data were obtained from a single determination.
[b]Results are given in % of maximal score relative to nicotine.
[c]Results are calculated from means of 8–10 rats per dose.

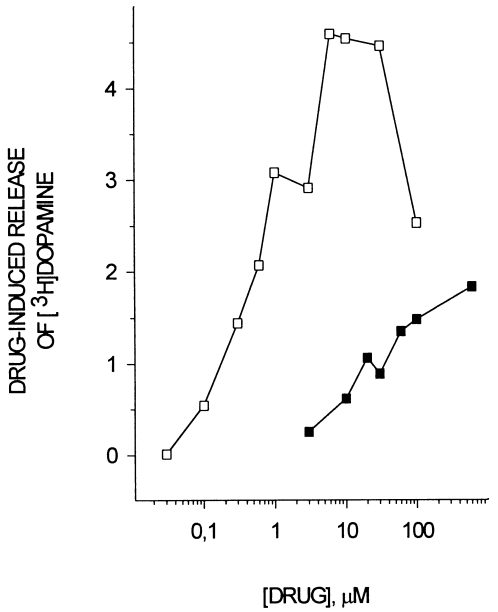

Figure 1. Dopamine release from striatal slices induced by nicotine (□—□, $ED_{50} = 0.48\,\mu M$) and compound **4a** (■—■, $ED_{50} = 19\,\mu M$).

that the nicotinic discriminative stimulus is selective for nicotinic receptors in that muscarinic compounds do not produce nicotine responses. Data from the drug discrimination assay (Table 2) show that compound **4a** generalized to nicotine with an $ED_{50} = 3.7\,mg/kg$. For compounds **4b**–**e**, only partial generalization to nicotine was observed at the highest doses tested. As shown, nicotine is 90 times more potent than compound **4a** in the drug discrimination assay.

Discussion and Conclusion

The data from the binding assay show that only small variations in the substitution pattern of the parent compound **3a** are allowed, and the introduction of even small space filling substituents (e.g. a methyl sustituent in the isoxazole ring for compound **4g**), or replacement of an *N*-methyl group with an *N*-ethyl, immediately decreases the affinity for the nicotinic receptor. This observation is in accordance with data obtained for other series of nicotinic receptor ligands, where high affinity for the receptor site was limited to small compact substituents.[12,24,25] For the compounds described in this paper, even large and bulky substituents are allowed in the 5-position of the isoxazole ring, which suggests that this position could possibly be associated with a region of bulk tolerance on the receptor.

A comparison of the biological data for binding, dopamine release, and drug discrimination assays for compound **4a** and nicotine shows that nicotine is 60 times more potent in binding to rat brain homogenates, 40 times more potent in dopamine release and 90 times more potent in the nicotine drug discrimination assay. Although compound **4a** is less potent and less efficacious in the dopamine release assay (Fig. 1), this may indicate that compound **4a** activates the nicotinergic system through some of the same receptor subtypes as nicotine itself, but this point needs more clarification.

In addition to determining that our compound acted as agonists in the dopamine release assay, the ability of the compounds to block the dopamine release induced by 1.0 μM of nicotine was also examined for some of the compounds (data not shown). Compound **4a** at 1 μM and 10 μM concentrations did not antagonize the dopamine release induced by 1 μM nicotine, whereas **4c** and **4e** at the same concentrations blocked the dopamine release induced by 1 μM of nicotine. As previously described[26] we also showed that lobeline at 1 μM antagonized the release of dopamine from striatal slices induced by 1 μM nicotine. Neither compounds **4b**–**f** nor lobeline generalized to nicotine in the drug discrimination assay, and in the binding assays compounds **4b**–**f** have a binding profile similar to lobeline with an $\alpha3\beta2/\alpha4\beta2$ ratio of about 10. These data may indicate that compounds **4b**–**f** have a pharmacological profile similar to lobeline, but further work has to be done to address this point.

In conclusion, a novel class of compounds with a cationic head composed of the tetrahydropyridine azacycle has been synthesised. The compounds are selective for central nicotinic receptors compared to muscarinic receptors. Although 50–100 times less potent, compound **4a** has the same profile as nicotine in the binding, dopamine release and drug discrimination assays. Extending the alkylamino chain beyond one carbon changed the pharmacological profile of the compounds to become more similar to that of lobeline.

Further work will be necessary on these compounds to specify their mechanism of action and to clarify the functional activity.

Materials and Methods

Chemistry

Melting points were determined with a Büchi capillary melting point apparatus and are uncorrected. ^1H NMR was recorded at 200 MHz, on a Bruker BZH-400/52 MHz FT-NMR instrument. Chemical shifts are given in ppm (δ) relative to tetramethylsilane. Mass spectra were

recorded with a Finnigan MAT TSQ 70 mass spectrometer with electron impact (EI) ionization. Reactions were followed by thin-layer chromatography performed on silica gel 60 F_{254} (Merck) TLC aluminium sheets. Elemental analyses were performed by Novo Nordisk, Microanalytical Laboratory, Denmark.

3-(5-Amino-4-isoxazolyl)-pyridine (2a). 1-(3-Pyridyl)-acetonitrile (5.9 g, 50 mmol) was dissolved in DMFDMA (10 mL) and stirred at 100 °C for 0.5 h. After cooling to ambient temperature, diethyl ether was added and the precipitated 2-dimethylaminomethylene-2-(3-pyridyl)-acetonitrile was collected by filtration. Yield 8.2 g.

To a solution of 2-dimethylaminomethylene-2-(3-pyridyl)-acetonitrile (5.19 g, 30 mmol) in AcOH (40 mL) was added NH_2OH, HCl (2.8 g, 40 mmol). The reaction mixture was stirred at 100 °C for 1 h, cooled to ambient temperature and concentrated in vacuo. H_2O (50 mL) was added and the reaction mixture neutralized with solid K_2CO_3. The precipitated compound was filtered washed with H_2O and dried giving the title compound in 4.2 g (52%) yield. Mp 158–59 °C; ^{1}H NMR (DMSO-d_6) δ 7.31 (s, 2H, br), 7.35 (m, 1H), 7.90 (m, 1H), 8.40 (m, 1H), 8.70 (s, 1H), 8.80 (m 1H); EI-ms m/z 161 (M^+); Anal. calcd for $C_8H_7N_3O$: C, 59.62; H, 4.38; N, 26.07. Found: C, 59.83; H, 4.40; N, 26.16.

3-(5-Amino-3-methyl-4-isoxazolyl)-pyridine (2b). A solution of 3-pyridylacetonitrile (2.0 g, 17 mmol) in DMADMA (3 mL) was stirred at 100 °C for 0.5 h. The reaction mixture was evaporated in vacuo. The residue was dissolved in AcOH (30 mL) and NH_2OH, HCl (2.8 g, 40 mmol) was added. The reaction mixture was stirred at 100 °C for 1 h, cooled to ambient temperature and evaporated in vacuo. H_2O (30 mL) was added to the residue and the reaction mixture was neutralized with solid K_2CO_3. The precipitated compound was filtered, washed with H_2O and dried. Yield 1.8 g (61%). Mp 179–80 °C; ^{1}H NMR (DMSO-d_6) δ 2.10 (s, 3H), 7.31 (s, 2H, br), 7.35 (m, 1H), 7.90 (m, 1H), 8.40 (m, 1H), 8.80 (m, 1H); EI-ms m/z 175 (M^+); Anal. calcd for $C_9H_9N_3O$: C, 61.70; H, 5.18; N, 23.99. Found: C, 61.95; H, 5.31; N, 24.29.

3-(5-Amino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine oxalate (3a). To a solution of 3-(5-amino-4-isoxazolyl)-pyridine (1.0 g, 6.25 mmol) in acetone (25 mL) was added iodomethane (2 mL). The reaction mixture was stirred overnight at ambient temperature and then evaporated in vacuo. The crude compound was dissolved in MeOH (30 mL) and reduced by adding $NaBH_4$ (0.5 g, 13.5 mmol) in small portions. The reaction mixture was concentrated in vacuo and H_2O (40 mL) was added. The H_2O phase was extracted with ether (4×30 mL). The combined ether extracts were dried over $MgSO_4$ and evaporated. The crude compound was crystallized as the oxalate salt from acetone to give the title compound in 0.6 g (36%) yield. Mp 138–39 °C; ^{1}H NMR (DMSO-d_6) δ 2.45 (m, 2H), 2.75 (s, 3H), 3.18 (m, 2H), 3.80 (m, 2H), 5.85 (m, 1H), 7.00 (s, 2H, br), 7.75 (s, 2H, br), 8.20 (s, 1H); EI-ms m/z 179 (M^+); Anal. calcd for $C_9H_{13}N_3O$, $C_2H_2O_4$: C, 49.07; H, 5.62; N, 15.61. Found: C, 49.42; H, 5.64; N, 15.39.

3-(5-Amino-3-methyl-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine oxalate (3b). To a solution of 3-(5-amino-3-methyl-4-isoxazolyl)-pyridine (**2b**) (0.9 g, 5 mmol) in a mixture of MeOH (15 mL) and acetone (15 mL) was added iodomethane (2 mL, 32 mmol). The reaction mixture was stirred overnight at ambient temperature and then evaporated in vacuo. The solid material obtained was dissolved in MeOH (40 mL) and reduced by adding $NaBH_4$ (0.5 g, 13.5 mmol) in small portions. The reaction mixture was concentrated in vacuo and H_2O (40 mL) was added. The H_2O phase was extracted with EtOAc (5×20 mL). The combined organic phases were dried and evaporated in vacuo. The crude compound was crystallized with oxalic acid from acetone in 800 mg yield (56%). Mp 189–190 °C; ^{1}H NMR (DMSO-d_6) δ 2.00 (s, 3H), 2.45 (m, 2H), 2.72 (s, 3H), 3.25 (m, 2H), 3.75 (m, 2H), 5.75 (m, 1H), 6.70 (s, 2H, br), 11.00 (s, 2H, br); EI-ms m/z 193 (M^+); Anal. calcd for $C_{10}H_{15}N_{3O}$, 1.5 $C_2H_2O_4$: C, 47.56; H, 5.53; N, 12.80. Found: C, 47.50; H, 5.71; N, 12.80.

3-(5-Amino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-ethylpyridine oxalate (3c). The title compound was obtained by the same procedure as described for compound **3a** starting from 3-(5-amino-4-isoxazolyl)-pyridine (**2a**) and 1-iodoethane. Yield 68%. Mp 112–13 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (t, 3H), 2.45 (m, 2H), 3.18 (q, 2H), 3.25 (m, 2H), 3.85 (m, 2H), 5.82 (m, 1H), 7.05 (s, 2H, br), 8.28 (s, 1H), 10.50 (s, 2H, br); EI-ms m/z 193 (M^+); Anal. calcd for $C_{10}H_{15}N_3O$: C, 50.88; H, 6.05; N, 14.83. Found: C, 50.71; H, 6.35; N, 14.52.

General procedures for the synthesis of 3-(5-dialkylamino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine oxalate **5a,c** and 3-(5-alkylamino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine oxalate **4a–h**.

3-(5-Dipropylamino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine 5c oxalate, and 3-(5-propylamino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine oxalate (4c). To a solution of 3-(5-amino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine (**3a**) (6.0 g, 33.2 mmol) in DMF (25 mL) was added powdered KOH (5.7 g, 100 mmol) in one portion, immediately followed by the addition of 1-iodopropane (5.64 g, 33.2 mmol). The reaction mixture was stirred for 1 min at ambient tem-

perature. The reaction mixture was acidified with 1 N hydrochloric acid solution, and the water phase extracted with ether (2×75 mL). The water phase was made alkaline with ammonium hydroxide (25% in H_2O) and extracted with ether (5×100 mL). The combined alkaline extracts were dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (SiO_2; eluent CH_2Cl_2/MeOH, 9:1.). The first fractions contained 3-(5-dipropylamino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine. The free base was crystallized as the oxalate salt giving **5c** 500 mg (5%) yield. Mp 139–40 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.82 (t, 6H), 1.45 (m, 4H), 2.42 (m, 2H), 2.75 (s, 3H), 3.14 (m, 2H), 3.20 (t, 4H) 3.68 (m, 2H), 5.78 (m, 1H), 6.00 (s, 2H, br), 8.20 (s, 1H); EI-ms m/z 263 (M^+); Anal. calcd for $C_{15}H_{25}N_3O,C_2H_2O_4$: C, 57.77; H, 7.70; N, 11.89. Found: C, 57.65; H, 7.96; N, 11.60.

The later fractions contained 3-(5-propylamino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine. The free base was crystallized as the oxalate salt giving **4c** in 2.5 g (24%) yield. Mp 132–33 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.82 (t, 3H), 1.55 (m, 2H), 2.42 (m, 2H), 2.75 (s, 3H), 3.20 (m, 2H), 3.20 (t, 2H) 3.68 (m, 2H), 5.82 (m, 1H), 7.08 (t, 1H, br), 8.25 (s, 1H); EI-ms m/z 221 (M^+); Anal. calcd for $C_{12}H_{19}N_3O,C_2H_2O_4$: C, 54.01; H, 6.80; N, 13.50. Found: C, 53.68; H, 6.98; N, 13.26.

3-(5-Dimethylamino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine oxalate (5a) and 3-(5-methylamino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine oxalate (4a). From 3-(5-amino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine (**3a**) and iodomethane. The first fractions contained 3-(5-dimethylamino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine. The free base was crystallized as the oxalate salt giving compound **5a** in 30% yield. Mp 106–107 °C; ^{1}H NMR (DMSO-d_6) δ 2.42 (m, 2H), 2.75 (s, 3H), 2.92 (s, 6H), 3.14 (m, 2H), 3.70 (m, 2H), 5.78 (m, 1H), 8.20 (s, 1H), 9.90 (s, 2H, br); EI-ms m/z 207 (M^+); Anal. calcd for $C_{11}H_{17}N_3O,C_2H_2O_4$: C, 52.52; H, 6.44; N, 14.13. Found: C, 52.80; H, 6.74; N, 14.09. The later fractions contained 3-(5-methylamino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine. The free base was crystallized as the oxalate salt giving **4a** in 10% yield. Mp 149–50 °C; ^{1}H NMR (DMSO-d_6) δ 2.42 (m, 2H), 2.75 (s, 3H), 2.92 (d, 3H), 3.14 (m, 2H), 3.70 (m, 2H), 5.78 (m, 1H), 7.10 (q, 1H), 7.50 (s, 2H, br), 8.20 (s, 1H), EI-ms m/z 193 (M^+); Anal. calcd for $C_{10}H_{15}N_3O$, $C_2H_2O_4$: C, 50.88; H, 6.05; N, 14.83. Found: C, 50.56; H, 6.20; N, 14.48.

3-(5-Ethylamino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine oxalate (4b). From 3-(5-amino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine **(3a)** and iodoethane in 25% yield. Mp 132–33 °C; ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3H), 2.40 (m, 2H), 2.70 (s, 3H), 3.17 (t, 2H), 3.27 (m, 2H), 3.78 (m, 2H), 5.82 (m, 1H), 6.70 (s, 2H, br), 7.12 (t, 1H), 8.25 (s, 1H); EI-ms m/z 207 (M^+); Anal. calcd for $C_{11}H_{17}N_3O,C_2H_2O_4$: C, 52.52; H, 6.44; N, 14.13. Found: C, 52.19; H, 6.62; N, 13.92.

3-(5-Butylamino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine oxalate (4d). From 3-(5-amino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine (**3a**) and 1-iodobutane in 30% yield. Mp 125–126 °C; ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3H), 1.30 (m, 2H), 1.60 (m, 2H), 2.45 (m, 2H), 2.70 (s, 3H), 3.25 (m, 2×2H), 3.82 (m, 2H), 5.82 (m, 1H), 7.12 (t, 1H), 8.25 (s, 1H), 9.20 (s, 2H, br); EI-ms m/z 235 (M^+); Anal. calcd for $C_{13}H_{21}N_3O$,-$C_2H_2O_4$: C, 55.37; H, 7.13; N, 12.91. Found: C, 55.44; H, 7.40; N, 12.80.

3-(5-Pentylamino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine oxalate (4e). From 3-(5-amino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine **(3a)** and 1-iodopentane in 27% yield. Mp 99–100 °C; ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3H), 1.30 (m, 4H), 1.60 (m, 2H), 2.45 (m, 2H), 2.70 (s, 3H), 3.25 (m, 2×2H), 3.82 (m, 2H), 5.82 (m, 1H), 7.12 (t, 1H), 8.25 (s, 1H), 9.20 (s, 2H, br); EI-ms m/z 249 (M^+); Anal. calcd for $C_{14}H_{23}N_3O$, $C_2H_2O_4$: C, 56.62; H, 7.42; N, 12.38. Found: C, 56.16; H, 7.74; N, 12.09.

3-(5-Benzylamino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine oxalate (4f). From 3-(5-amino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine (**3a**) and benzylbromide in 5% yield. Mp 110–112 °C; ^{1}H NMR (DMSO-d_6) δ 2.45 (m, 2H), 2.80 (s, 3H), 3.25 (m, 2H), 3.82 (m, 2H), 4.45 (d, 2H), 5.88 (m, 1H), 7.30 (m, 3H), 7.70 (t, 1H), 8.25 (s, 1H); EI-ms m/z 269 (M^+); Anal. calcd for $C_{16}H_{19}N_3O,C_2H_2O_4$, 1.5 H_2O: C, 56.72; H, 5.98; N, 10.72. Found: C, 56.10; H, 6.02; N, 10.90.

3-(5-Butylamino-3-methyl-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine oxalate (4g). From 3-(5-amino-3-methyl-4-isoxazolyl)-1,2,5,6-tetrahydro-1-methylpyridine (**3b**) and 1-iodobutane in 24% yield. Mp 116–117 °C; ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3H), 1.30 (m, 2H), 1.60 (m, 2H), 2.00 (s, 3H), 2.45 (m, 2H), 2.70 (s, 3H), 3.25 (m, 2×2H), 3.82 (m, 2H), 4.80 (s, 2H), 5.75 (m, 1H), 6.95 (t, 1H); EI-ms m/z 249 (M^+); Anal. calcd for $C_{14}H_{23}N_3O$, $C_2H_2O_4$: C, 56.63; H, 7.37; N, 12.39. Found: C, 56.31; H, 7.62; N, 12.10.

3-(5-Propylamino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-ethyl-pyridine oxalate (4h). From 3-(5-amino-4-isoxazolyl)-1,2,5,6-tetrahydro-1-ethylpyridine **3c** and 1-iodopropane in 28% yield. Mp 150–51 °C; ^{1}H NMR (DMSO-d_6) δ 0.87 (t, 3H), 1.25 (t, 3H), 1.55 (m, 2H), 2.45 (m, 2H), 3.15 (m, 3×2H), 3.80 (m, 2H), 5.82 (m, 1H), 7.15 (t, 1H), 8.28 (s, 1H), 8.50 (s, 2H, br); EI-ms $m/$

z 235 (M^+); Anal. calcd for $C_{13}H_{21}N_3O,C_2H_2O_4$: C, 55.37; H, 7.13; N, 12.91. Found: C, 55.19; H, 7.43; N, 13.03.

Biological Evaluation

Radioligand binding

The radioligand binding studies were conducted as previously described.[13] Briefly, binding of the nicotinic [^{3}H]MCC ligand to homogenates of rat cortex, or of cell lines expressing the α4β2 or the α3β2 nicotinic subunit combinations, in the presence of increasing amounts of competitive drugs, was carried out using a conventional filtration assay. IC_{50}s were determined using non-linear regression (InPlot, Graphpad).

The cDNA encoding for the nicotinic subunits was cloned into the baculo transfer vector pVL 1393. Recombinant AcMNPV baculovirus expressing the nicotinic subunits after a polyhedrin promoter was isolated. Sf9 or Sf21 insect cells were coinfected with vira expressing either the α or the β nicotinic subunits at a ratio of 1:1. Insect cells were infected at a MOI of three for each of the subunits and harvested 3–4 days postinfection.[13]

Dopamine release

Dopamine release from striatal slices was conducted as previously described.[13] Striatal sections from adult Wistar rats were loaded for 30 min with [^{3}H]dopamine and positioned in a Brandel superfusion apparatus. Each chamber was superfused in parallel. After a 30 min wash-out period, the fraction collection started, and after a baseline was obtained, the slices were stimulated with the test drug. The cpms in each fraction were normalized to the mean cpms in the first four fractions collected. The data are expressed as percentage of release induced by 1 μM nicotine.

Drug discrimination

Briefly, male Wistar rats (Møllegaard, Ry, Denmark) were trained to discriminate nicotine (0.1 mg/kg, s.cc., 15′) from no drug by using a standard FR10 food motivated task. Initial shaping, training and test procedures were similar to those described earlier.[13,23] Tests were typically run on Tuesdays and Fridays providing the animals had reached the criterion of at least 90% correct responding in the preceding training sessions and no more than nine incorrect responses were emitted before the first reinforcement. Results are given as means of 8–110 rats per dose.

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