Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 15, pp. 1963-1968, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00347-8

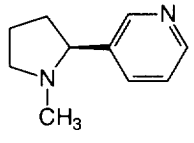
IDENTIFICATION OF NOVEL (ISOXAZOLE)METHYLENE-1-AZABICYCLIC COMPOUNDS WITH HIGH AFFINITY FOR THE CENTRAL NICOTINIC CHOLINERGIC RECEPTOR.

P.H. Olesen,* M.D.B. Swedberg, K. Eskesen, M.E. Judge, J. Egebjerg,
J.E. Tønder, T. Rasmussen, M.J. Sheardown, and K. Rimvall.
Health Care Discovery, Novo Nordisk, Novo Nordisk Park, DK-2760 Maaloev Denmark.

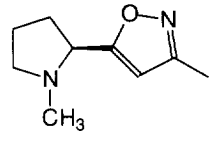
Abstract: A novel class of compounds with high affinity (IC_{50} = 3.4 - 500 nM) for the central nicotinic cholinergic receptor was synthesized. The compounds were characterized *in vitro* and *in vivo* and compared to ABT 418 and nicotine. The new ligands are effective nicotinic compounds with biological profiles distinguishable from reference compounds. © 1997 Elsevier Science Ltd.

Recent findings suggesting a palliative role for nicotine in the treatment of diseases such as Alzheimer's disease, Parkinson's disease and Tourette's syndrome have led to a search for novel nicotinic agents with an increased CNS selectivity, without the side effects of nicotine such as, e.g. abuse potential.[1-3] Furthermore, the therapeutic potential for a nicotine-like drug for obesity as suggested by observations in animals and humans has also contributed to the increased interest in nicotinic compounds over the last few years.[4]

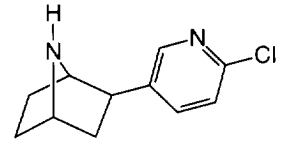
A number of new ligands for the central nicotinic acetylcholine receptor have recently been described, e.g. epibatidine[5] and ABT 418.[6] The existence of receptor subtypes and the availability of the corresponding cDNA's may make it possible to design new drugs that are subtype-specific. Many of the beneficial effects described for nicotine may be related to specific subtypes of the nicotinic receptor system.



Nicotine



ABT 418



Epibatidine

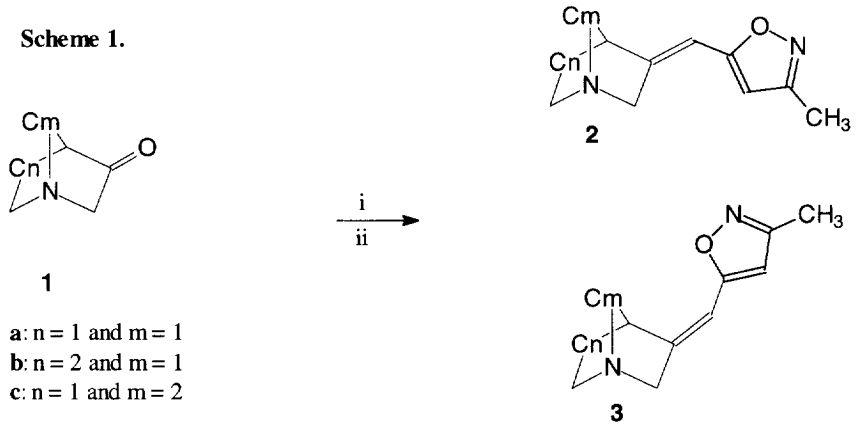
In the present report the synthesis and some preliminary *in vivo* and *in vitro* data on a new series of (isoxazole)methylene-1-azabicyclic compounds are presented. These novel structures may be useful lead compounds for the design and synthesis of subtype selective nicotinic ACh receptor ligands.

E-mail: phol@novo.dk
Fax: +45 4466 3939

Chemistry:

The general route for making these compounds is illustrated in scheme 1. Peterson olefination of the appropriate 1-azabicyclic ketone **1** with 3-methyl-5-trimethylsilylmetylisoxazole provided a mixture (1:1) of the Z and E regioisomers. The products were then separated into their Z and E isomers by column chromatography to yield the geometrically pure compounds **2** and **3**.[7] The relative configuration of the compounds was assigned on the basis of NOE ^{1}H NMR experiments. Compounds **2 a,b** and **3 a,b** were obtained as racemic mixtures of compounds.

Scheme 1.



a: n = 1 and m = 1
b: n = 2 and m = 1
c: n = 1 and m = 2

i) 3-methyl-5-trimethylsilylmethylisoxazol/BuLi in THF, -78 ^{O}C, ii) Column chromatography.

Biology:

The ability of the compound to displace [^{3}H]methylcarbamoylcholine (MCC), a nicotinic agonist and [^{3}H]oxotremorine-M (Oxo-M), a muscarinic agonist, from native cholinergic binding sites in rat brain cortex was determined[8] (Table 1). All the compounds, except **3c**, bound with very high affinity to the nicotinic binding site and had high selectivity for central nicotinic receptors compared to central muscarinic receptors. For pairs of regioisomers a major difference in nicotinic binding affinity between the Z-**2c** and the E-**3c** isomers was observed, whereas for the regioisomers **2a,3a** and **2b,3b** equipotency in binding affinity was found.

Table 1: ***In vitro*** **binding data for (3-methyl-5-isoxazolyl)methylene-1-azabicycles compared to reference compounds.**

	Receptor binding to rat brain homogenates.[8]		Receptor binding to homogenates of cell lines expressing nicotinic receptors.[8,9]	
Compound (Isomer)	[^{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)
2a (Z)	23	1200	11	15
3a (E)	26	2800	6.5	14
2b (Z)	170	950	26	217
3b (E)	85	1700	41	117
2c (Z)	3.4	1000	1.4	1.9
3c (E)	500	1500	288	854
ABT 418	53	44000	37	62
Nicotine	4	28000	4.2	3.8

The compounds were tested for affinity to two different subtype combinations of nACh receptors: a) the major subtype in brain composed of the α4β2 subunit combination and b) the major ganglionic-type composed of α3 containing subunits[8,9] (Table 1). As shown, the affinities of the compounds for the receptor subtypes reflect their affinity to cortical receptors. For compound **2b** a 10-fold selectivity for the α4β2 receptor subtype compared to the α3β2 subtype is observed. Compounds with the highest binding affinity, i.e. **2a, 3a** and **2c,** were selected for further evaluation, and compared to ABT 418 and nicotine in functional *in vitro* and *in vivo* assays.

Activation of nicotinic receptors located on presynaptic, dopaminergic terminals in the striatum induces the release of dopamine.[10] As shown in Table 2, the compounds are agonists in inducing dopamine release from striatal slices, but are less efficacious compared to nicotine at comparable doses (Table 2).

The current response relative to nicotine was evaluated in Xenopus oöcytes injected with mRNA of the α4β2 and the α3β2 subtypes.[11] At equimolar concentrations the compounds tested are more efficacious than ABT 418

and nicotine at inducing stimulation of current response in Xenopus oocyte transfected with the human α4β2 receptor, but only **2c** is more efficacious than nicotine at inducing current response in the α3β2 subtype.

Table 2: ***In vitro* functional data for (3-methyl-5-isoxazolyl)methylene-1-azabicycles compared to reference compounds.**

	Drug induced [^{3}H]dopamine release from striatal slices.[a]		Drug induced stimulation of current response in Xenopus oocyte.	
Compound (Isomer)	1 μM	10 μM	α4β2[b] 1 μM	α3β2[c] 30 μM
2a (Z)	38	80	171	51
3a (E)	17	46	n.d.	38
2c (Z)	51	68	300	135
ABT 418	5	66	24	40
Nicotine	100	180	100	100

a) % of release induced by 1 μM nicotine,[10] b) % of response induced by 1μM nicotine,[11]
c) % of response induced by 30 μM nicotine.[11]

Nicotinic agonists produce nicotine-like responding in drug discrimination.[12] Using standard drug discrimination procedures[13] it has been shown that the nicotinic discriminative stimulus is selective for nicotinic receptors in that muscarinic compounds do not produce nicotine responding. In nicotine trained rats, drug discrimination data show that the compounds act centrally at very low doses s.c., and partially generalized to nicotine (Table 3).

Effects on spontaneous locomotor activity can be a useful indicator of CNS activity, and can be utilised both to evaluate *in vivo* potency and efficacy. The drug discrimination technique is generally considered to be a more sensitive measure of CNS effects than inhibition of exploratory locomotor activity, and this is consistent with the results obtained in the present experiments. P.o/s.c ratios for exploratory locomotor activity show that compound **2a** has substantially better oral bioavailability than nicotine and ABT 418.

Table 3: ***In vivo*** **functional data for (3-methyl-5-isoxazolyl)methylene-1-azabicycles compared to reference compounds.**

	Drug discrimination[13]		Exploratory locomotor activity[14]	
Compound (Isomer)	% of max. score	ED_{50} (mg/kg) s.c.	ED_{50} (mg/kg) s.c.	ED_{50} (mg/kg) p.o.
2a (Z)	63	0.5	2.0	4.1
3a (E)	62	0.2	n.t.	n.t
2c (Z)	60	0.1	0.4	2.4
ABT 418	72	0.2	10.0	72
Nicotine	96	0.04	0.3	> 9.0

The present series of compounds have been shown to be effective nicotinic agonist compounds *in vivo* and *in vitro*, with biological profiles distinguishable from ABT 418 and nicotine. They may prove to be excellent lead structures for the design and the synthesis of new nicotinic cholinergic agonists with receptor subtype selectivity. An evaluation of the structure-activity relationships of an extended series of these (isoxazole)methylene-1-azabicycles is in progress and will be reported shortly.

References and Notes:

1. Benowitz, N. L. *Annu. Rev. Pharmacol. Toxicol.* **1996**, *36*, 597.
2. Lee, P. N. *Neuroepidemiology* **1994**, *13*, 131.
3. Jones, G. M.; Sahakian, B. J.; Levy, R.; Warburton, D. M., and Gray, J. A. *Psychopharmacol.* **1992**, *108*, 485.
4. a) Grunberg, N. E. *Psychopharmacol. Bull.* **1986**, *22*, 875. b) Grunberg, N. E.; Popp, K., and Winders, S. E. *Psychopharmacol. (Berl)* **1988**, *94*, 536.
5. a) Dukat, M.; Damaj, M. I.; Glassco, W.; Dumas, D.; May, E. L.; Martin, B. R.; Glennon, R. A. *Med Chem Res.* **1993**, *4*, 131. b) Qian, C. G.; Li, T. C.; Shen, T. Y.; Libertine-Garahan, L.; Eckman, J.; Ip, S. *European Journal of Pharmacology*, **1993**, *250*, R 13-R 14.
6. a) Garvey, D. S.; Wasicak, J. T.; Decker, M. W.; Brioni, M. W.; Sullivan, J. D.; Carrera, G. M.; Holladay, M. W.; Arneric, S. P.; Williams, M. J. *Med. Chem.* **1994**, *37*, 1055. b) Arneric, S. P.; Sullivan, J. P.; Briggs, C. A.; Donelly, D. R.; Anderson, D. J.; Raszkiewicz, J. L.; Hughes, M.; Cadman, E. C.; Adams, P.; Garvey, D. S.; Wasicak, J., and Williams, M. J. *J. Pharmacol. Exp. Therap.* **1994**, *270*, 310.
7. A typical experimental procedure is as follows: to a solution of 3-methyl-5-trimethylsilylmethyl-isoxazole (*J. Organomet. Chem.* **1980**, *195*, 275) (2.0 g, 12 mmol) in dry tetrahydrofuran (25 ml) cooled to -78 °C, was added butyllithium (1.64 M in hexane, 12 mmol). The reaction mixture was stirred at -78 °C for 1 h. A solution of 3-quinuclidinone (1.5 g, 12 mmol) in dry tetrahydrofuran (20 ml) was added dropwise at -78 °C. The reaction mixture was stirred at -78 °C for 2 hours and quenched with water. The reaction mixture was evaporated to half volume *in vacuo* and water (25 ml) was added. The water phase was extracted with ethyl acetate (3 x 25 ml). The organic extracts were dried over magnesium sulfate and evaporated. The crude compounds were purified by column chromatography on silica (eluent: ethyl acetate/methanol/ammonium

hydroxide, 25% in water: 2/1/2%). The first fractions contained the (Z)-3-(3-methyl-5-isoxazolyl)methylene-1-azabicyclo[2.2.2]octane (**2c**) which was crystallized as the oxalate salt in 1.0 g (28%) yield. Mp 134-137°C. The last fractions contained pure (E)-3-(3-methyl-5-isoxazolyl)methylene-1-azabicyclo[2.2.2]octane (**3c**) which was crystallized as the oxalate salt in 1.1 g (32 %) yield. Mp 135-36 °C. All new compounds were characterized by ^{1}H NMR and MS, and spectra were consistent with the proposed structures. Elemental analysis was performed by Novo Nordisk microanalytical laboratory, Denmark.

8. Binding of the nicotinic [^{3}H]MCC ligand to homogenates of rat cortex, or of cell lines expressing the $\alpha4\beta2$ or the $\alpha3\beta2$ 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).
9. 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. Sf 9 or Sf 21 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 3 for each of the subunits and harvested 3-4 days postinfection.
10. a) Wonnacott, S.; Drasdo, A.; Sanderson, E., and Rowell, P. in the biology of nicotine dependence, ed. by G. Bock and J. Marsh, Ciba foundation, Chichester, **1990**, *87*. b) Grady, S. R.; Marks, M. J., and Collins, A. C. J. *Neurochem.* **1994**, *62*, 1390. c) Striatal sections from adult Wistar rats were loaded for 30 minutes with [^{3}H]dopamine and positioned in a Brandel superfusion apparatus. Each chamber was superfused in parallel. After a 30 minutes wash-out period, the fraction collection started and after a baseline was obtained, the slices were stimulated with the test drug. The cpm's in each fraction were normalized to the mean cpm's in the first four fractions collected. The data are expressed as percentage of release induced by 1 μM nicotine.
11. mRNA encoding $\alpha4\beta2$ and $\alpha3\beta2$ were injected into defolliculated Xenopus oocytes and used 3-10 days later to test for responses to nicotinic agonists. The experiments were performed in a normal Ringer solution with Ca^{2+}. Superfusion of agonist and sampling of current responses measured with the oocytes voltage clamped at -60 mV were all controlled by a customized program. The data are expressed as percentage of current response induced by 1 μM and 30 μM nicotine for the $\alpha4\beta2$ and the $\alpha3\beta2$ subtype, respectively.
12. Reavill, C. and Stolerman, I. P. *J. Psychopharmacol.* **1987**, *1*, 264.
13. Male Wistar rats were trained to discriminate nicotine (0.1 mg/kg, s.c., 15') from no drug by using a standard FR10 food motivated task. Initial shaping, training and test procedures were similar to those described earlier. Swedberg, M. D. B.; Jacobsen, P., and Honore, T. *J. Pharmacol. Exp. Ther.* **1995**, *274*, 1113.
14. Mice were injected subcutaneously with the test compounds 30 min prior to testing. Testing occurred in sound isolated photo cell activity boxes. Animals were tested individually for 10 min.

(*Received in Belgium* 14 *April* 1997; *accepted* 23 *June* 1997)