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Synthesis and nicotinic acetylcholine receptor binding affinities of 2- and 3-isoxazolyl-8-azabicyclo[3.2.1]octanes

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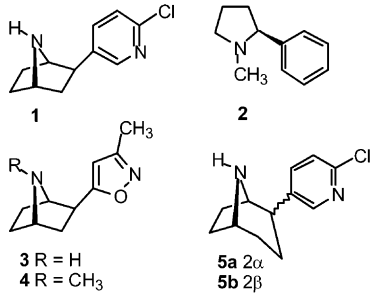
Abstract—A series of epiboxidine homologues, 2- and 3-isoxazole substituted 8-azabicyclo[3.2.1]octane derivatives was synthesized and evaluated as potential ligands for neuronal nicotinic acetylcholine receptors in [^{3}H]cytisine labeled rat brain. The 2β-isoxazolyl-8-azabicyclo[3.2.1]octane **9b** ($K_i = 3$ nM) was the most potent compound of the series with a binding affinity twice that of nicotine. The 3β-isoxazolyl-8-azabicyclo[3.2.1]octane **15b** ($K_i = 148$ nM) exhibited moderate affinity while the corresponding 2α- and 3α-isomers exhibited micromolar binding affinity.

The amphibian alkaloid (−)-epibatidine (**1**)[1] is a highly potent nicotinic acetylcholine receptor (nAChR) agonist at neuronal receptor subtypes (α4β2 and α7) as well as at nAChRs in peripheral autonomic ganglia and skeletal muscle.[2–5] The binding affinity of **1** at nAChRs is several orders of magnitude greater than nicotine (**2**) and the remarkable analgesic activity of epibatidine is 200 times more potent than morphine.[1] The analgesic properties of **1** are thought to be mediated through α4β2-subtype nAChRs,[6] and in mice, this effect is thought to be mediated primarily by spinal nAChRs.[7,8] However, the therapeutic potential of epibatidine is limited due to its acute toxicity at doses only slightly higher than its effective analgesic dose.[9–12]

Numerous investigations have focused on the synthesis and biological screening of structurally similar analogues of epibatidine and nicotine to discover compounds that possess low toxicity and greater selectivity.[4,5] Early studies revealed that substitution of the chloropyridyl ring of **1** with an isoxazole ring afforded epiboxidine (**3**), which was reported to exhibit potent binding affinity in [^{3}H]nicotine labeled rat brain but was 10-fold less potent than (−)-epibatidine (**1**).[13] The analgesic activity of **3** was also diminished relative to epibatidine in rat hot-plate assays. However, **3** was much less lethal in mice, giving epiboxidine a superior activity/toxicity ratio to that of epibatidine. In addition, the *N*-methyl analogue of epiboxidine **4** has been reported to exhibit similar potency and analgesic efficacy to **3**.[5]



In the course of our studies aimed at the development of less toxic analgesic analogues of epibatidine with diminished side-effects, we recently reported that the (1*R*,5*S*)-2-[3-(5-chloropyridyl)]-8-azabicyclo[3.2.1]octanes **5a**,**b**[14] exhibited a higher potency ratio of spinally mediated analgesia/side effects than epibatidine.[15] The favorable pharmacological profile of **5a** and **5b** has prompted recent studies to explore the structure–activity relationships of the 8-azabicyclo[3.2.1]octane ring system as a scaffold for the design of less toxic epibatidine analogues. To this end a series of 2- and 3-isoxazolyl-8-

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azabicyclo[3.2.1]octanes have been prepared. Herein we wish to report the synthesis and in vitro binding affinity of novel homologues of epiboxidine.

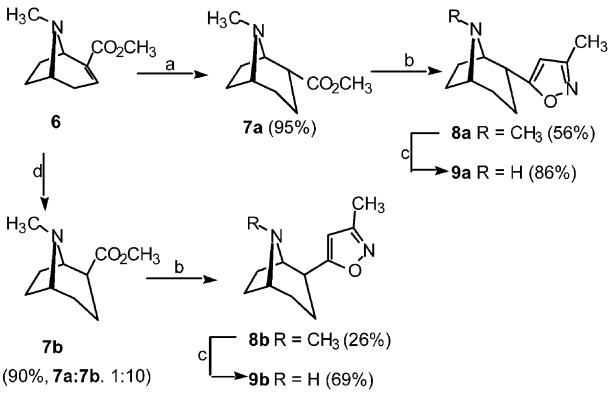
As illustrated in Scheme 1, a practical and efficient approach was employed for the preparation of the 2-isoxazolyl 8-azabicyclo[3.2.1]octane derivatives. The stereoselective hydrogenation (1 atm, 10% Pd–C) of (1*R*)-(−)-anhydroecgonine methyl ester (**6**)[16] furnished the 2α-dihydroecgonine ester (**7a**) in 95% yield. The 2α-ester **7a** was treated with dilithiated acetone oxime at 0 °C to form a β-ketone oxime intermediate, which was subjected to cyclodehydration conditions to generate the isoxazole ring and furnish **8a** in 56% overall yield.[17] The demethylation of **8a** was achieved with diethyl azodicarboxylate (DEAD) in benzene at reflux for 6 h, followed by treatment with HCl in aqueous ethanol to furnish the secondary amine **9a** in 86% isolated yield. This method routinely has proven to be successful in our laboratories for the demethylation of bicyclic amines when classical reagents (e.g., ACE-Cl) failed to give good yields.[18]

The *N*-methyl and *N*-H 2β-isoaxole derivatives **8b** and **9b** were prepared by stereoselective reduction of **6** with magnesium in methanol to predominantly yield (2α:2β, 1:10) the 2β-dihydroecgonine ester (**7b**) in 90% yield. Subsequent formation of the isoxazole ring furnished **8b** in 26% yield. Demethylation of **8b** provided **9b** in 86% yield.

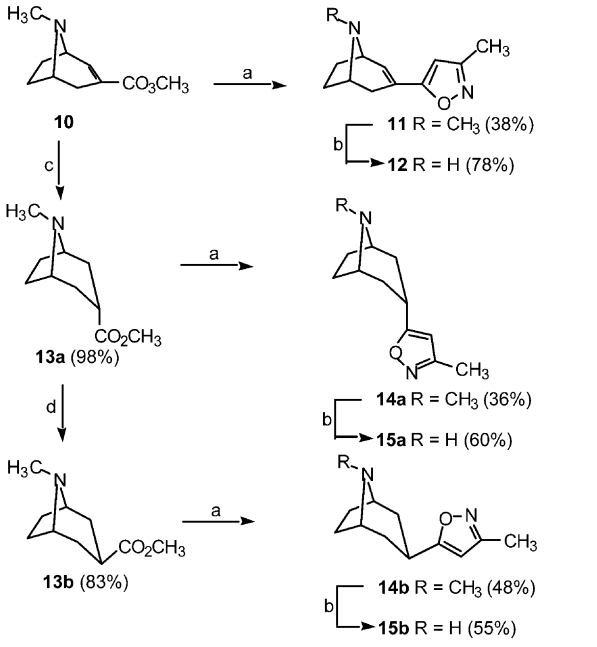
Readily available methyl trop-2-ene-3-carboxylate (**10**)[19] was converted into the 3-isoxazolylderivatives **11** and **12** in a similar fashion to that described above (Scheme 2). In addition, **10** was stereoselectively converted into the 3α-ester **13a**, which was readily epimerized to furnish the 3β-ester **13b** using procedures previously reported by our laboratories.[19] It was found that the isoxazole ring formation could be achieved by a more simple procedure than that employed for the preparation of the 2-homoepiboxidines **8** and **9**. The reaction of corresponding carboxylic esters **10**, **13a**, and **13b** with lithiated acetone oxime followed by treatment with dilute HCl afforded the desired 3-isoxazolyl derivatives **11**, **14a**, and **14b** in one-pot with moderate yields (36–48%).[20] This procedure eliminated isolation of β-ketone oxime intermediate since concomitant cyclodehydration could be carried out in situ. It was determined that an excess amount of the acetone oxime (2 equiv) and *n*-BuLi (4 equiv) was necessary to achieve good yields of the isoxazole derivatives. For example, 1.2 equiv of acetone oxime and 2.4 equiv of *n*-BuLi gave only 12% yield of **11**. However, use of more than 2 equiv of dilithiated acetone oxime proved less effective and gave rise to the formation of side-products. Finally, the demethylation of **14a** and **14b** with DEAD/10% HCl afforded the *N*-H derivatives **15a** and **15b** in 55% and 60% yields, respectively (Scheme 2).

The binding affinities of the isoxazolyl-8-azabicyclo[3.2.1]-octanes and isoxazolyl-8-azabicyclo-[3.2.1]oct-2-enes summarized in Table 1, were determined by the inhibition of [^{3}H]cytisine binding in homogenates of rat striatum.[21] There are a variety of nACHRs subtypes that exist in the central nervous system; however, the α4β2-subtype is the predominant nAChR in rat striatum tissue. Therefore, the binding affinities reported in Table 1 most likely correspond to the α4β2-subtype affinity of epibatidine and related compounds.

In general, as expected all of the isoxazolyl-8-azabicyclo[3.2.1]octane derivatives exhibited diminished binding affinity relative to epibatidine (**1**). Within the series of the isoxazolyl-8-azabicyclo[3.2.1]octane derivatives both the positional attachment and stereochemical orientation of the isoxazolyl group had a dramatic effect upon the binding affinity of these compounds. The β-isomers were generally at least two-orders of magnitude more potent than the corresponding α-isomers, while substitution at the 2-position was significantly favored



Scheme 1. Reagents and conditions: (a) H_2 (1 atm), 10% Pd/C, MeOH; (b) (1) *n*-BuLi (2 equiv), acetone oxime, THF, 0 °C; (2) MsCl, Et_3N, CH_2Cl_2, 0 °C; (c) (1) DEAD, C_6H_6, reflux, 6 h; (2) 10% HCl, EtOH, reflux; (d) Mg, MeOH, rt.



Scheme 2. Reagent and conditions: (a) (1) *n*-BuLi (4 equiv), acetone oxime (2 equiv), THF, 0 °C; (2) 10% HCl, 0 °C; (b) (1) DEAD, C_6H_6, reflux, 6 h; (2) 10% HCl, EtOH, reflux; (c) H_2 (1 atm), 10% Pd/C, MeOH; (d) NaOMe, MeOH, reflux.

Table 1. Inhibition of [^{3}H]cytisine binding at nAChR in rat brain

Compd[a]	K_i (nM)[b]	
1	0.79	±0.02
2	8.0	±4.5
5a	4800	±300
5b	1.04	±0.040
8a	7500	±500
8b	1470	±50
9a	26,000	±3000
9b	3.0	±0.6
11	720	±70
12	320	±30
14a	40% inhibition[c]	
14b	194	±15
15a	18,000	±3000
15b	148	±14

[a] All compounds were tested as the oxalate salt.
[b] All values are the mean ±SEM of three experiments performed in triplicate.
[c] Percent inhibition at highest dose tested (100 μM).

over the 3-position. The 2β-isoxazolyl-8-azabicyclo[3.2.1]-octane **9b** ($K_i = 3$ nM) was the most potent compound of the isoxazole derivatives and exhibited 50-fold greater affinity than the 3β-isomer **15b**. In addition, consistent with the structure–activity relationship studies of epibatidine and epiboxidine, the *N*-H analogues (e.g., **9b**) were found to be slightly more potent than the corresponding *N*-methyl analogues (e.g., **8b**). Only the 2α-isomer **8a** ($K_i = 7{,}450$ nM) was more potent than the corresponding demethylated congener **9a** ($K_i = 26{,}200$ nM), although neither compound exhibited a very potent affinity.

It is noteworthy that the C3-sp^2-hybridized derivative **12** ($K_i = 320$ nM) exhibited modest binding affinity, similar in magnitude to the 3β-isomer **15b** ($K_i = 148$ nM) rather than the 3α-isomer **15a** ($K_i = 18{,}000$ nM). This is consistent with structure–activity relationship studies of other 3-heteroaryl substituted 8-azabicyclo[3.2.1]oct-2-enes.[22,23]

The binding affinity of the epiboxidine homologue **9b** was determined to be 2-fold more potent than nicotine (**2**) in this assay. The low nanomolar binding affinity of **9b** was encouraging in that it was not significantly different than the binding affinity determined for the 2β-chloropyridyl analogue **5b** ($K_i = 1$ nM) and suggests that **9b** may exhibit similar potencies in analgesic paradigms. In addition, it is envisaged that the isoxazolyl derivative **9b** will have an improved activity/toxicity ratio relative to **1** and possibly **5b**, much the same as epiboxidine (**3**) has an improved therapeutic ratio relative to epibatidine (**1**). These studies are currently under investigation and will be reported in due course.

The 3β-isoxazolyl-8-azabicyclo[3.2.1]octanes **14b** and **15b**, and 3-isoxazolyl-8-azabicyclo[3.2.1]oct-2-ene **11** and **12**, exhibited moderate binding affinity at α4β2 nAChRs. Even though these compounds have much lower affinities for nAChRs compared to epibatidine, the affinities still fall within a useful pharmacological range. Epibatidine has exceptionally high affinity, both in producing nicotinic agonist effects and in producing side effects, and this contributes greatly to the problems with its clinical usefulness. As recently reported for the 2α-chloropyridyl-8-azabicyclo[3.2.1]octane **5a**, although it exhibited weak binding affinity it still exhibited spinally-mediate analgesia at doses only 3-fold less than epibatidine, yet side-effects were not observed at twice the ED_{50}.[15] Therefore compounds like **11**, **12**, **14b**, and **15b** with modest or weak binding affinities compared to epibatidine may still prove to have therapeutic value.

In summary, we have identified a new class of nAChR ligands and a potent homologue **9b** of epiboxidine. From these studies it is evident that the 8-azabicyclo-[3.2.1]octane ring system can be substituted for the 7-azabicyclo[2.2.1]heptane ring system of epibatidine to furnish compounds with reduced binding affinities relative to epibatidine but still exhibit pharmacologically relevant potencies. As a result these compounds may prove to exhibit a more favorable therapeutic profile than epibatidine.

Acknowledgements

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20. **General procedure for formation of isoxazole ring**. A solution of acetone oxime (590 mg, 8.0 mmol) in dry THF (20 mL) under argon at 0 °C was treated dropwise with *n*-BuLi (11.4 mL, 1.4 M in hexane, 16.0 mmol), and the reaction mixture was allowed to warm to room temperature and stirred for 1.5 h. Then a solution of ester (4 mmol) in dry THF (20 mL) was added dropwise via syringe pump over 1 h while the reaction mixture was stirred at 0 °C. The reaction mixture was allowed to warm to room temperature slowly and stirred overnight. The reaction mixture was added slowly into a vigorously stirred solution of 10% aq HCl (20 mL, pre-cooled to 0 °C). The mixture was washed with ether (2×20 mL), the aqueous layer was basified with saturated Na_2CO_3 solution to pH ≈10 and extracted with CH_2Cl_2 (3×30 mL). The organic layers were combined, washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, EtOAc:Et_3N, 20:1)
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