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Synthesis and Binding Studies of Some Epibatidine Analogues

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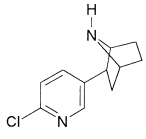
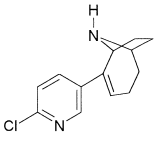
Abstract—A series of epibatidine analogues and their positional isomers bearing an 8-azabicyclo[3.2.1]octane moiety is described. Some of the compounds, especially those containing 8-azabicyclo[3.2.1]oct-2-ene moiety show high affinity for the nicotinic cholinergic receptor.

Epibatidine (**1**) and some of its analogues have been shown to possess high analgesic activity, which was found to be mediated through nicotinic cholinergic receptor.[1,2] We found especially interesting reports on high activity of unsaturated anatoxin–epibatidine hybrid **2** (ref 3) and diazabicyclo[3.2.1]octane derivative **3** (ref 4). The therapeutic potential of neuronal nicotinic acetylcholine receptor ligands is not limited to analgesics, it also includes possible prevention of neurodegeneration in Alzheimer's disease.[5–7] This area is, therefore, one of the most studied areas of medicinal chemistry.



Epibatidine (**1**)



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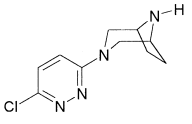

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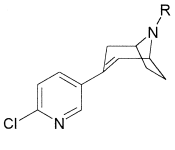
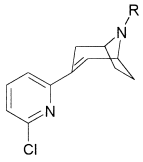
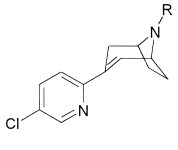
These facts inspired us to prepare similar compounds **4a**, **4b**, as well as their positional isomers **5** and **6.** As expected, compounds **4** were found to be the most active nicotinic receptor ligands; therefore, we decided to prepare also simplified analogues **7**.



4a; R = Me
4b; R = H

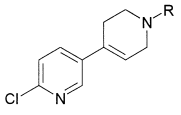


5a; R = Me
5b; R = H

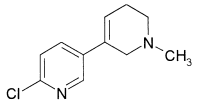


6a; R = Me
6b; R = H

It is a well known fact that some 3-piperideine analogues, especially those bearing suitable heterocyclic rings in the position 3, are good muscarinic M_1 acetylcholine receptor ligands.[8–12] To have some compounds with strong binding both to nicotinic and M_1 muscarinic receptors we extended the series and prepared also compound **8**.



7a; R = Me
7b; R = H



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Chemistry

The following starting compounds were prepared according to the previously described methods: 5-bromo-2-chloropyridine (**9a**) (ref 13), 2-bromo-6-chloropyridine (**9b**) (ref 14), and 2-bromo-5-chloropyridine (**9c**) (ref 15).

The general route for the preparation of compounds **4**–**6** is illustrated in Scheme 1. A solution of starting bromopyridine **9a**–**9c** in diethyl ether was treated at −78 °C with butyl lithium. After stirring for 1 h at this temperature, the formed intermediate was treated with a solution of tropinone in diethyl ether to provide the corresponding alcohol **10a**, **10b** and **10c** in yields of 70, 64 and 57%, respectively. Although the addition could, theoretically, provide two possible stereoisomers, only the isomers shown in the scheme were formed. The identification was based on the NMR spectral measurement.[16] Upon prolonged heating with trifluoroacetic acid at 130 °C in a sealed tube the alcohols **10a**–**10c** gave

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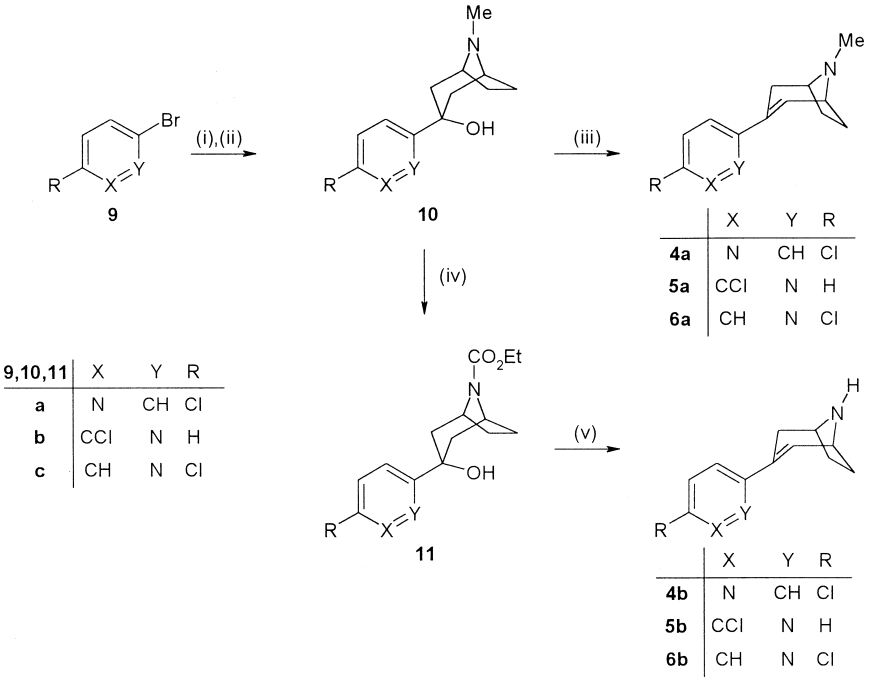
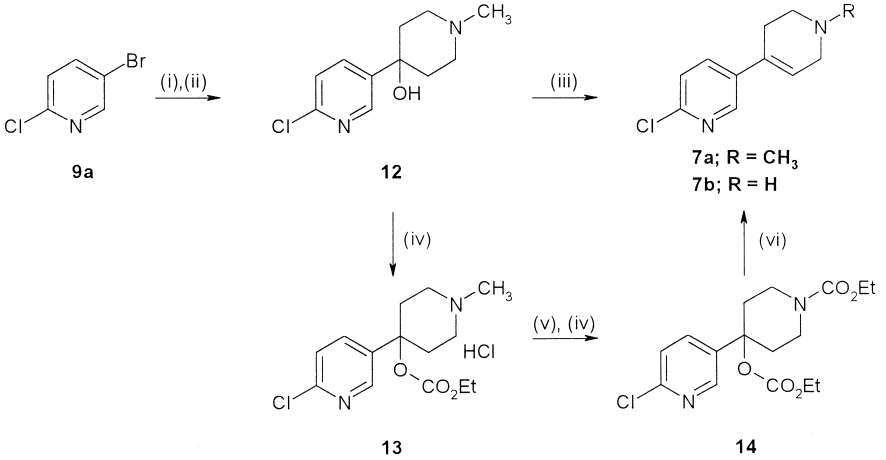


Scheme 1. Reagents: (i) BuLi, Et_2O, −78 °C; (ii) tropinone; (iii) CF_3CO_2H, 130 °C; (iv) $ClCO_2Et$, 90 °C; (v) concd HCl, CH_3CO_2H, reflux.

the required *N*-methyl derivatives **4a**, **5a** and **6a** in low yields (about 40%). When alcohols **10a**–**10c** were dissolved in toluene and treated with an excess of ethyl chloroformate at 90 °C, the corresponding *N*-ethoxycarbonyl derivatives **11a**–**11c** were formed. Flash chromatography of the crude reaction mixtures provided the compounds as white crystalline solids in yields in the range of 70–82%. Refluxing these compounds in a mixture of acetic and hydrochloric acids results in the hydrolysis of the carbamate group and the dehydration of the hydroxy group, thus yielding directly *N*-unsubstituted unsaturated compounds **4b**, **5b**, and **6b**.

The synthetic procedure leading to compounds **7** (Scheme 2) is similar to the procedure described above. The starting bromo derivative **9a** yielded the corresponding lithiated pyridine, which upon treatment with 1-methyl-4-piperidone gave **12** in good yields. Prolonged heating of this alcohol with trifluoroacetic acid yielded the corresponding dehydration product **7a**. Reaction of **12** with ethyl chloroformate occurred at the hydroxy group and not at the *N*-methyl group as in compounds **10**. Attempts to demethylate alcohol **12** by this reagent without a protection of the hydroxy group failed and hydrochloride **13** was the only isolable product. So the compound was first converted into its base, then, without purification, treated with ethyl chloroformate to give compound **14**. Upon prolonged heating in a mixture of acetic and hydrochloric acids, the latter product underwent the hydrolysis of the carbamate group and the elimination of the ethoxycarbonyloxy group, to yield directly **7b**.



Scheme 2. Reagents: (i) BuLi, Et_2O, −78 °C; (ii) 1-methyl-4-piperidone; (iii) CF_3CO_2H, 130 °C; (iv) $ClCO_2Et$, 90 °C; (v) $NaHCO_3$; (vi) concd HCl, CH_3CO_2H, reflux.

Preparation of compound **8** again started from bromo derivative **9a**. Its palladium catalyzed cross-coupling reaction with 3-trimethylstannylpyridine[17,18] yielded selectively the corresponding bipyridine **15**. When a catalytic amount of tetrakis(triphenylphosphine)-palladium was used in boiling xylene for 18 h, yields in the range of 75–85% were obtained repeatedly. The bipyridine **15** was treated with methyl iodide in acetone at room temperature to be selectively converted to the corresponding less hindered monopyridinium iodide **16** (isolated yields of about 85–90%). Its reduction with sodium borohydride in ethanol gave the target compound **8** in about 80% yield (Scheme 3).

Caution

Unsaturated compounds **4–6,** and, especially compound **7a,** are structurally similar to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a neurotoxin known for its ability to reproduce parkinsonian-like symptoms in animals as well as in humans.[19] Therefore special care is advisable during the synthesis and handling of these compounds.

Biological Results and Discussion

The compounds were evaluated in binding studies on serotonin 5-HT_{1A} and 5-HT_{1B} subtypes, on muscarinic cholinergic M_1 and M_2 subtypes, and neuronal nicotinic cholinergic receptors. The compounds **5b**, **6b**, **7a**, **12**, and **13** were tested as hydrochlorides, compounds **4a**, **4b**, **5a**, **6a**, **7b**, **8**, **10a**, **10b**, and **10c** as maleates. Carbamates **11a**–**11c** and bipyridine **15** were used as neutral molecules for the studies.

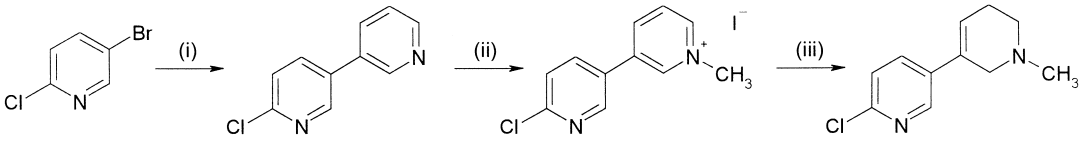
The serotonin radioligand displacement receptor binding assays were conducted in the hippocampus of the rat brain for 5-HT_{1A} receptors and in the rat striatum for 5-HT_{1B} receptors, according to the published procedures.[20,21] [^{3}H]-8-Hydroxy-2-dipropylamino-1,2,3,4-tetrahydronaphthalene ($c = 0.25$ nM) and [^{3}H]-5-hydroxytryptamine ($c = 2.00$ nM) were used for labeling of 5-HT_{1A} and 5-HT_{1B} receptors, respectively. Nonspecific binding was determined by incubation with serotonin ($c = 10$ μM).

Binding to muscarinic M_1 and M_2 receptors was carried out essentially as described previously.[22] Brain cortex for M_1 receptor assay or heart atria for M_2 receptor assay was used. [^{3}H]-Quinuclidinyl benzilate ($c = 0.1$ nM) was used for labeling both M_1 and M_2 receptors. Nonspecific binding was determined by incubation with atropine ($c = 1.0$ μM).

(S)-[^{3}H]-Nicotine binding to cholinergic receptors in rat brain membranes was determined by a modification of the literature procedure.[23,24] [^{3}H]-Nicotine ($c = 0.5$ nM) was used for labeling. Nonspecific binding was determined by incubation with nicotine ($c = 10$ μM).

Inhibition of specific radioligand binding by the tested compounds in 10^{-6} M concentrations was expressed as a percentage related to the specific binding of the radioligand in the absence of the tested compounds. The IC_{50} values (Table 1) were determined only for compounds with the values of the residual radioligand binding less than 50% (80% for M_1 and M_2 receptors). The IC_{50} values are means ± S.E.M. from 3 separate experiments performed in duplicate, the data were analyzed using nonlinear regression.

None of the tested compounds strongly bound to the 5-HT_{1A} and 5-HT_{1B} receptors, the most active compound being **7b**. Similarly, no substantial binding to the muscarinic M_1 and M_2 receptors was found. This finding is especially surprising for compound **8** since some very similar pyrazine derivatives are reported to bind to



9a **15** **16** **8**

Scheme 3. Reagents: (i) 3-trimethylstannylpyridine, $(Ph_4P)Pd$, xylene, reflux; (ii) MeI, acetone, r.t.; (iii) $NaBH_4$, ethanol, r.t.

Table 1. Binding data of the active compounds

Compound[b]	5-HT_{1A} IC_{50} [μM]	5-HT_{1B} IC_{50} [μM]	M_1 IC_{50} [μM]	M_2 IC_{50} [μM]	Nicotinic receptor IC_{50} [nM]
4a	NA[a]	NA	2.14 ± 0.08	4.18 ± 0.17	1.8 ± 0.3
4b	NA	NA	NA	NA	1.7 ± 0.2
5a	NA	NA	1.85 ± 0.24	NA	264 ± 15
5b	NA	NA	NA	NA	212 ± 18
7a	NA	NA	NA	NA	12.5 ± 1.4
7b	15.8[c]	2.69[c]	NA	NA	30 ± 3
8	NA	NA	NA	3.51 ± 0.23	2.2 ± 0.3
16	NA	NA	NA	NA	77 ± 14

[a]NA—The residual radioligand binding higher than 50% (80% for M_1 and M_2 receptors).
[b]Compounds **5b** and **7a** and were tested as hydrochlorides, compounds **4a**, **4b**, **5a**, **7b** and **8** as maleates.
[c]Only one determination.

the receptor in submicromolar concentrations.[10] As expected, some of the compounds are good neuronal nicotinic acetylcholine receptor ligands with IC_{50} in nanomolar concentrations. The most active compounds are **4a** and **4b**, which are structurally similar to epibatidine having the 8-azabicyclo[3.2.1]oct-2-ene moiety in the position 5. Compounds **5a** and **5b**, bearing the same bicyclic moiety in the vicinity of the pyridine nitrogen atom, are much weaker ligands with IC_{50} two orders of magnitude higher. The binding of compounds **6a** and **6b** is even weaker. On the other hand, compounds **7a** and **7b**, the simplified analogues of **4a** and **4b**, are only about 10-fold less active than the parent compounds. Compound **8**, which was prepared as a possible M_1 ligand, is a strong nicotinic receptor ligand instead, with IC_{50} comparable to those of compounds **4a** and **4b**.

Acknowledgements

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