Synthesis of (1-Azabicyclo[3.3.0]octanyl)methyl-Substituted Aromatic Heterocycles and Their Muscarinic Activity1)

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In our development of drugs effective against Alzheimer’s disease, we have researched a series of aromatic compounds having a characteristic cyclic amine, 1-azabicyclo[3.3.0]octane ring.

In this report, we describe synthesis of a series of aromatic heterocycles with the 1-azabicyclo[3.3.0]octane ring and their pharmacological evaluation. 3-Amino-5-(1-azabicyclo[3.3.0]octan-5-yl)methyl-1,2,4-oxadiazole (2b) showed the highest M1 selectivity.

Key words Alzheimer’s disease; 1-azabicyclo[3.3.0]octane; SK-946; heterocycle; muscarinic cholinergic receptor binding affinity

Certain biochemical deficiencies and pathological changes have been well documented in brains of Alzheimer’s disease (AD) patients. Most consistent among them is the selective loss of certain neuronal populations. In particular, the cholinergic neurons that project from the basal forebrain to the cerebral cortex and hippocampus are at risk in AD.1) This selective cholinergic neurodegeneration is the basis of a cholinergic hypothesis, which triggered research efforts aimed at restoring the defective cholinergic transmission.

We have been studying the cognition activators in order to develop a new medicine for AD, and recently found a new compound, N-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-2-nitroaniline fumarate (SK-946), which is highly efficacious and functionally selective for muscarinic M1 affinity.1)

In a recent pharmacokinetic study of SK-946 on rats and dogs, it was found that 4-position of the aniline ring is hydroxylated easily.3) In addition, SK-946 displayed comparable affinities for cortical muscarinic M1 receptors using either N-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-2-nitroaniline fumarate (SK-946), which is highly efficacious and functionally selective for muscarinic M1 affinity.1)

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Studies by Showell and coworkers indicated that the potency of affinities for muscarinic M₁ receptor (by displacement of [3H]N-methylscopolamine) of 3-amino and 3-methyl-1,2,4-oxadiazole derivatives having tetrahydropyridine were almost equal. Wadsworth and coworkers reported that the affinities of tetrazole derivatives with quinuclidine for muscarinic M₁ receptor were strong, but not as active as 1,2,4-oxadiazole derivatives (by displacement of [3H]oxotremorine-M). In our study, the affinity for muscarinic M₁ receptor of tetrazole derivatives 4a and 4b was as active as 1,2,4-oxadiazole derivatives 2a and 2b (Table 1).

The affinity for muscarinic M₁ receptor of 1,2,5-thiadiazole derivatives 8a and 8b with 1-azabicyclo[3.3.0]octane moiety was examined (Table 1). Studies by Sauerberg and coworkers showed that the affinities for muscarinic M₁ receptor of 3-n-pentyloxy-1,2,5-thiadiazole derivative having tetrahydropyridine were about ten times as active as the 3-ethoxy derivative (by displacement of [3H]pirenzepine). In 1-azabicyclo[3.3.0]octane derivatives, compound 8b (3-ethoxy derivative) had stronger affinity for M₁ receptor than 8a (3-n-pentyloxy).

1,2,4-Oxadiazole derivative 2b exhibited the highest M₁ selectivity in our compounds. Thus, 2b was chosen as the most desirable sample for the in vivo test, and ameliorated scopolamine induced impairment in passive avoidance tasks.

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**Table 1. Affinities for M₁ and M₂ Receptors of Heterocyclic Compounds with 1-Azabicyclo[3.3.0]octane Ring**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Receptor affinities $K_i$ (μM)$^a$</th>
<th>Ratio of [3H]QNB/ [3H]pirenzepine</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>C₁₀H₁₇N₃O</td>
<td>5.0</td>
<td>&gt;50</td>
</tr>
<tr>
<td>2b</td>
<td>C₁₀H₁₆N₄O</td>
<td>4.1</td>
<td>&gt;50</td>
</tr>
<tr>
<td>4a</td>
<td>C₁₀H₁₇N₅</td>
<td>4.3</td>
<td>&gt;50</td>
</tr>
<tr>
<td>4b</td>
<td>C₁₀H₁₇N₅</td>
<td>4.0</td>
<td>26.6</td>
</tr>
<tr>
<td>8a</td>
<td>C₁₅H₂₅N₃OS</td>
<td>0.23</td>
<td>0.84</td>
</tr>
<tr>
<td>8b</td>
<td>C₁₂H₁₉N₃OS</td>
<td>0.076</td>
<td>0.57</td>
</tr>
<tr>
<td>SK-946</td>
<td>—</td>
<td>0.12</td>
<td>1.4</td>
</tr>
<tr>
<td>(-)YM-796</td>
<td>—</td>
<td>1.8</td>
<td>7.7</td>
</tr>
</tbody>
</table>

$^a$ $K_i$ value (μM) calculated from the respective IC₅₀ using the Cheng-Prusoff equation, $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] and $K_d$ are respectively ligand concentration and dissociation constant. $K_d$ values: [3H]pirenzepine, cortex, 7.1 nM; [3H]QNB, cerebellum, 0.041 nM. $^b$ [3H]quinuclidinyl benzilate.
Table 2. Effect of Compounds on Scopolamine Induced Failure of Step-through Passive Avoidance Response in ddY Mice

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>R.T. (%)</th>
<th>n</th>
<th>Criteria (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td>90 ± 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scopolamine control</td>
<td>20</td>
<td></td>
<td>90 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>0.1 (p.o.)</td>
<td>20</td>
<td>90 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 (p.o.)</td>
<td>20</td>
<td></td>
<td>90 ± 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK-946</td>
<td>0.1 (p.o.)</td>
<td>20</td>
<td>90 ± 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)YM-706</td>
<td>1.0 (p.o.)</td>
<td>20</td>
<td>90 ± 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 (p.o.)</td>
<td>20</td>
<td></td>
<td>90 ± 10</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

a) R.T.: Latency in retention trial. b) Criteria (%) = number of mice showing avoidance for more than 300 sec/total number of mice × 100.

at 1.0 mg/kg (p.o.) (Table 2).

In conclusion, our aromatic heterocycles having the characteristic cyclic amine, 1-azabicyclo[3.3.0]octane ring, had strong activity to muscarinic M1 receptor. Although this affinity to the receptor of 2b was weaker than that of SK-946 in vitro, 2b was about 10 times as active as SK-946 in passive avoidance tasks in vivo. This was thought to result from the fact shown below. 1,2-Oxadiazole ring of 2b is more metabolically stable than aniline ring of SK-946, and, muscarinic M1 agonistic property of 2b (QNB(IC50)/OXO-M(IC50) ratio equal to 15) is higher than that of SK-946. Previous reports by our group have found that SK-946 has other activity in addition to muscarinic M1 agonistic property: it increases acetylcholine release in relatively low concentrations.

It is possible that compound 2b and other heterocyclic derivatives also have acetylcholine releasing activity.

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. 1H-NMR spectra were taken at 60 MHz with a JEOL JNM-60 spectrometer, or at 270 MHz with a JEOL JNM-GSX270 spectrometer. Chemical shifts are expressed in δ (ppm) values with tetramethylsilane as an internal standard and the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = double doublet and dt = double triplet. Mass spectra (MS) were recorded on a JEOL JMS-DX300, or on a JEOL JMS-SX102. Infrared (IR) spectra were taken with JASCO IR-810, or Perkin-Elmer 1600. Elemental analyses were performed on Yanagimoto MT-5.

5-(1-Azabicyclo[3.3.0]octan-5-yl)methyl-3-methyl-1,2,4-oxadiazole (2a)

Acetamide oxime (2.42 g, 32.7 mmol) suspended in tetrahydrofuran (THF) (75.0 ml) was heated to 60 °C with NaH (980 mg of a 60% dispersion in oil, 32.7 mmol) for 1 h in the presence of 4A molecular sieves (5.00 g). Compound 1 (5.00 g, 27.3 mmol) in THF (25.0 ml) was added and the reaction mixture was heated under reflux for 2 h. After cooling, the mixture was filtered and the solvent was removed in vacuo. The residue was distilled under reduced pressure (120 °C/7 mmHg) to give 3.69 g (65.3%) of 2a as a colorless oil. 1H-NMR (CDCl3) δ: 1.55—2.04 (8H, m, 3,4-CH2-pyrrolidine), 2.39 (3H, s, CH3), 2.60 (2H, d, J = 11, 6H, 2-CH2-pyrrolidine), 2.96 (2H, s, CH2), 3.05 (2H, d, J = 11, 6H, 2-CH2-pyrrolidine). IR (neat) cm−1: 2958, 2868, 1579, 1393. CIMS m/z: 208 (M+1)−, 110 (base peak).

Anal. Calc. for C15H25N3OS: C, 60.98; H, 8.53; N, 14.22. Found: C, 60.74; H, 8.34; N, 13.95.

3-Amino-5-(1-azabicyclo[3.3.0]octan-5-yl)methyl-1,2,4-oxadiazole (2b)

Sodium metal (1.26 g, 54.8 mmol) was added to absolute EtOH (50 ml) stirred in the presence of 4A molecular sieves (12.0 g). After 15 min at room temperature, hydrogenosuudine hemisulfate hemihydrate (4.36 g, 32.8 mmol) was added and the stirring was continued for another hour. Compound 1 (1.00 g, 5.46 mmol) was added to the mixture, which was then heated under reflux for 2 h. After cooling, the reaction mixture was filtered and the solvent was removed in vacuo. Water (40 ml) was added to the residue, which had been extracted with CHCl3 (50 ml×4). The extracts were dried and evaporated in vacuo. The residue was recrystallized from iso-octane/CH2Cl2 ether to give 480 mg (42.2%) of 2b as colorless prisms. 1H-NMR (CDCl3) δ: 1.55—2.03 (8H, m, 3,4-CH2-pyrrolidine), 2.59 (2H, d, J = 11, 5H, 2-CH2-pyrrolidine), 2.85 (2H, s, CH3) 3.06 (2H, d, J = 11, 5H, 2-CH2-pyrrolidine), 4.37 (2H, s, brs, NH). IR (KBr) cm−1: 3348, 3207, 2966, 1611, 1591. MS m/z: 209 (M+1)−, 110 (base peak). Anal. Calc. for C10H17N3O: C, 63.74; H, 8.27; N, 20.27. Found: C, 63.58; H, 8.16; N, 20.37.

3-Amino-5-(1-azabicyclo[3.3.0]octan-5-yl)methyl-1,2,4-oxadiazole (2h)

Sodium metal (1.26 g, 54.8 mmol) was added to absolute EtOH (50 ml) stirred in the presence of 4A molecular sieves (12.0 g). After 15 min at room temperature, hydrogenosuudine hemisulfate hemihydrate (4.36 g, 32.8 mmol) was added and the stirring was continued for another hour. Compound 1 (1.00 g, 5.46 mmol) was added to the mixture, which was then heated under reflux for 2 h. After cooling, the reaction mixture was filtered and the solvent was removed in vacuo. Water (40 ml) was added to the residue, which had been extracted with CHCl3 (50 ml×4). The extracts were dried and evaporated in vacuo. The residue was recrystallized from iso-octane/CH2Cl2 ether to give 480 mg (42.2%) of 2b as colorless prisms. 1H-NMR (CDCl3) δ: 1.55—2.03 (8H, m, 3,4-CH2-pyrrolidine), 2.59 (2H, d, J = 11, 5H, 2-CH2-pyrrolidine), 2.85 (2H, s, CH3) 3.06 (2H, d, J = 11, 5H, 2-CH2-pyrrolidine), 4.37 (2H, s, brs, NH). IR (KBr) cm−1: 3348, 3207, 2966, 1611, 1591. MS m/z: 209 (M+1)−, 110 (base peak). Anal. Calc. for C10H17N3O: C, 63.74; H, 8.27; N, 20.27. Found: C, 63.58; H, 8.16; N, 20.37.
Biological Method

Preparation of Rat Brain Homogenate  The rat brain homogenate was prepared as to previously reported.1

[^3H]Pirenzepine Binding Inhibition  Assay for M₁ receptor was carried out as reported.1

[^3H]Quinuclidinyl Benzilate (QNB) Binding Inhibition  Assay for M₂ receptors was carried out as reported method.1

Reference Compounds  (+)-YM-796 was synthesized at our laboratory as fumarate salt.12

Passive Avoidance Performance in Scopolamine-Treated Mice  A passive avoidance learning test using mice was carried out according to the previously reported method.1

Reference

3) In metabolic study of SK-946, four metabolites were isolated from dog urine. The hydroxylated derivative and its glucuronide were major metabolites (unpublished data).
11) The affinities of 1,2,4-oxadiazole derivatives 2a, 2b and SK-946 for cortical muscarinic M₁ receptors were evaluated with[^3H]quinuclidinyl benzilate (QNB) and[^3H]oxotremorine-M (OXO-M) as radioligands (unpublished data).