Synthesis and Biological Activity of the Metabolites of N-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl]-2-nitroaniline Fumarate (SK-946)\(^1\)

Tomoo SUZUKI,* Hideaki INAGAKI,* Hitoshi HAMAJIMA,* Hiroshi UESAKA,\(^{b}\) Kohsuke HORI,\(^{b}\) and Takao IKAMI\(^{a}\)

Drug Discovery Research Laboratory\(^{a}\) and Drug Development Laboratory,\(^{b}\) Sanwa Kagaku Kenkyusho Co., Ltd., 363 Shiosaki, Hokusai-cho, Inabe-gun, Mie 511-04, Japan. Received December 7, 1998; accepted March 3, 1999

Three metabolites of N-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-2-nitroaniline fumarate (SK-946), a novel central muscarinic cholinergic receptor agonist, were prepared to confirm their proposed structures, and tested for muscarinic receptor affinity \textit{in vitro}.

Key words metabolite; SK-946; muscarinic receptor affinity; 1-azabicyclo[3.3.0]octane

We have been studying cognition activators in order to develop a new drug to treat Alzheimer’s disease (AD),\(^2\) and recently reported a new compound, N-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-2-nitroaniline fumarate (SK-946) (1), which has highly selective affinity for the muscarinic M\(_1\) receptor. This compound increased inositol phosphate production in primary cultured rat fetal hippocampal neuronal cells, and improved scopolamine-induced dementia in a mouse model.\(^1\)

SK-946 is under preclinical investigations as a candidate for the treatment of AD. In a study of the pharmacokinetics of SK-946 (1), N-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-2-nitroaniline (free base of 1) and four metabolites (2—5) were isolated from urine following intravenous administration to dogs. Compounds 3 was the major metabolite. Also, 1 and the same four metabolites were found in dog and rat urine following oral administration. Their structures were proposed to be a hydroxylated derivative (2), its glucuronide (3), and two oxidized and hydrolyzed derivatives (4, 5).\(^3\) (Chart 1).

In this paper, we describe the synthesis of these metabolites to confirm their structures and to test the biological activity of compound 2.

Synthesis

N-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl]-4-hydroxy-2-nitroaniline (2) was synthesized from 4-chloro-3-nitrophenol (6) and 5-(2-aminoethyl)-1-azabicyclo[3.3.0]octane (7)\(^2,4\) in pyridine with NaHCO\(_3\) in 8.5% yield (Chart 2). Metabolite 2 was shown to be identical with this synthetic compound by means of TLC, \(^1\)H-NMR and MS spectroscopy.

Metabolite 4 was prepared as shown in Chart 3. Key compound, 5-(2-benzyloxycarbonylaminoethyl)-1-azabicyclo[3.3.0]octane-2-one (9), was obtained in 45.2% yield by \(\text{KMnO}_4\) oxidation of 5-(2-benzyloxycarbonylaminoethyl)-1-azabicyclo[3.3.0]octane (8), which was derived from amine 7 and benzyloxy carbonyl chloride. Condensation of 5-(2-aminoethyl)-1-azabicyclo[3.3.0]octane-2-one (10), obtained by deprotection of 9, and 4-chloronitrobenzene produced N-[2-(1-azabicyclo[3.3.0]octan-2-on-5-yl)ethyl]-2-nitroaniline (11) in 26.2% yield, which led to 3-[(2-(o-nitroanilino)pyrrolidin-2-yl)propanoic acid (4) by hydrolysis in 84.3% yield. Metabolite 4 was identical with this authentic compound in all respects as far as of TLC, \(^1\)H-NMR and MS spectral data were concerned.

Metabolite 5 was prepared as shown in Chart 4. 1-Chloro-4-(4-methoxybenzoyloxy)-2-nitrobenzene (12) was obtained by benzylation of 6 with \(p\)-methoxybenzyl chloride. Condensation of 12 and 10 gave N-[2-(1-azabicyclo[3.3.0]octan-2-on-5-yl)ethyl]-4-(4-methoxybenzoyloxy)-2-nitroaniline (14) in very low yield (3.3%). Therefore, a two-step synthesis was carried out. Condensation of 12 with 7 gave N-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-4-(4-methoxybenzoyloxy)-2-nitroaniline (13) in 58.2% yield, and then oxidation of 13 produced 14 in 13.7% yield. Deprotection of 14 produced the 4-hydroxyaniline derivative (15), which led to 3-[(2-(4-hydroxy-2-nitroanilino)ethyl]pyrrolidin-2-yl)propanoic acid (5).

* To whom correspondence should be addressed.

© 1999 Pharmaceutical Society of Japan
by hydrolysis in 59.8% yield. Metabolite 5 was identical with this synthetic sample following comparison of their TLC, \(^1\)H-NMR and MS spectral properties.

**Results and Discussion**

The affinity of metabolite 2 for the M\(_1\) and M\(_2\) receptors was evaluated in terms of its ability to displace \(^{[3]}\)Hpirenzipine, a M\(_1\)-selective ligand, from rat cerebral cortex membrane and \(^{[3]}\)Hquinuclidinyl benzilate (QNB) from rat cerebellum membrane, respectively.

Metabolite 2, possessing a characteristic amine, 1-azabicyclo[3.3.0]octane ring, had strong affinity for the muscarinic receptors. The M\(_1\) affinity of 2 was among the strongest of all the aniline derivatives,\(^{10}\) but weaker than SK-946. Metabolites 4 and 5 have different structures from SK-946 and compound 2 in terms of the cleavage of the 1-azabicyclo[3.3.0]octane ring. Therefore, we considered that compounds 4 and 5 have little affinity for muscarinic receptors.

**Experimental**

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. \(^1\)H-NMR spectra were recorded on a JEOL JNM-GSX270 spectrometer (270 MHz for \(^1\)H and 68 MHz for \(^{13}\)C). Chemical shifts are expressed in parts per million downfield from tetramethylsilane (TMS), the internal standard, and the following abbreviations are used:

\[\begin{align*}
\text{a) \text{Pyridine}} & \text{acetone-H}2O, \\
\text{b) KMnO}_4, \text{NaHCO}_3, \text{acetone-H}2O, \\
\text{c) H}_2\text{O, 10% H}2\text{O}-\text{EtOH,} \\
\text{d) \text{Chloroform-benzene, NaHCO}_3, \text{acetone-H}2O,} \\
\text{e) TFA/\text{CH}_2\text{Cl}_2,} \\
\text{f) 2 \times \text{NaOH/MeOH}}
\end{align*}\]

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Muscarinic receptor affinities K(_i) ((\mu)M)</th>
<th>Ratio of (^{[3]})HQN/ (^{[3]})Hpirenzipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.19</td>
<td>2.9</td>
</tr>
<tr>
<td>SK-946</td>
<td>0.12</td>
<td>1.4</td>
</tr>
<tr>
<td>(-)-YM796</td>
<td>1.8</td>
<td>7.7</td>
</tr>
</tbody>
</table>

\(^{a)}\) K\(_i\) value (\(\mu\)M) calculated from the respective IC\(_{50}\) using the Cheng–Prusoff equation, K\(_i\)=IC\(_{50}\)/1+[L]/Kd, where [L] and Kd are ligand concentration and dissociation constant, respectively. Kd values: \(^{[3]}\)Hpirenzipine, cortex, 7.1 ns; \(^{[3]}\)HQN, cerebellum, 0.041 ns.
N-[2-(1-azabicyclo[3.3.0]oct-5-yl)ethyl]-4-hydroxy-2-nitroaniline (2)

A suspension of 4-chloro-3-nitrophenol (6.05 g, 23.3 mmol), 5-(2-aminoethyl)-1-azabicyclo[3.3.0]octanoic acid (7) (3.60 g, 23.3 mmol), and NaHCO$_3$ (1.96 g, 23.3 mmol) in pyridine (80 ml) was stirred at reflux temperature for 1 week. The cooled reaction mixture was then filtered and evaporated in vacuo. The residue was chromatographed on silica gel eluting with AcOEt–MeOH to give 11.7 g (92.6%) of 5-(2-chloroethyl)-1-azabicyclo[3.3.0]octan-2-one (3). The residue was chromatographed on silica gel eluting with CH$_2$Cl$_2$, washed with brine, dried, and concentrated in vacuo. The residue was chromatographed on silica gel eluting with AcOEt to give an oily product. The resulting oil was crystallized from AcOEt to give 120 mg (4.1% yield) of 13. The residue was chromatographed on silica gel eluting with CH$_2$Cl$_2$–MeOH to give 33.7 g (90.2%) of 5-(2-benzyloxycarbonylaminoethyl)-1-azabicyclo[3.3.0]octan-2-one (5). The residue was chromatographed on silica gel eluting with CH$_2$Cl$_2$–MeOH to give 588 mg (13.7%) of 14. To a suspension of 5-(2-aminomethyl)-1-azabicyclo[3.3.0]octan-2-one (10) (4.15 g, 10.3 mmol) and NaHCO$_3$ (3.41 g, 50 mmol) in acetone (200 ml) was added slowly triethylamine (13.1 g, 0.13 mol) in CH$_2$Cl$_2$ (200 ml) was added dropwise benzyloxycarbonyl chloride (24.3 g, 0.14 mol) in an ice bath. After stirring for 1 h, the reaction mixture was filtered, and the filtrate was evaporated in vacuo. The residue was chromatographed on silica gel eluting with CH$_2$Cl$_2$–ether to give 15.5 g (91.4%) of 15. The residue was chromatographed on silica gel eluting with CH$_2$Cl$_2$–MeOH to give 575 mg (8.5%) of 16. The residue was chromatographed on silica gel eluting with CH$_2$Cl$_2$–MeOH to give 5.16 g (97.6%) of 17. To a suspension of 1-Chloro-4-(4-methoxybenzyloxy)-2-nitrobenzene (12) (6.0 g, 27.6 mmol) and triethylamine (13.1 g, 0.13 mol) in CH$_2$Cl$_2$ (200 ml) was added dropwise benzyloxycarbonyl chloride (24.3 g, 0.14 mol) in an ice bath. After stirring for 1 h, the reaction mixture was filtered, and the filtrate was evaporated in vacuo. The residue was chromatographed on silica gel eluting with CH$_2$Cl$_2$ to give 5.97 g (90.2%) of 18. The residue was chromatographed on silica gel eluting with CH$_2$Cl$_2$–MeOH to give 3.37 g (84.3%) as an orange amorphous mass. IR (KBr) cm$^{-1}$: 3381, 2956, 2871, 1548, 1508, 1291, and 1139. 1H-NMR (CDCl$_3$) δ: 3.19—3.24 (8H, m, 5-CH$_2$ of pyrrolidine), 2.90—3.05 (2H, m, 5-CH$_2$ of pyrrolidine), 2.48 (1H, d, J = 12 Hz, 8-CH$_2$ of azabicyclooctane), 2.30—2.36 (2H, m, 5-CH$_2$ of pyrrolidine), 2.36 (2H, dd, J = 16, 9 Hz, NHCH$_2$), 2.67 (1H, d, J = 9 Hz, 3-H of aniline), 8.00 (1H, br, s, NH), 6.18 (1H, d, J = 9 Hz, 2-H of aniline), 7.08 (1H, d, J = 8 Hz, 5-H of aniline), 3.71 (1H, d, J = 8 Hz, 5-H of aniline), 8.06 (1H, d, J = 9 Hz, 3-H of aniline), 3.25 (1H, d, J = 7 Hz, 6-H of aniline), 3.71 (1H, d, J = 8 Hz, 5-H of aniline), 8.11 (1H, d, J = 9 Hz, 2-H of aniline), 7.08 (1H, d, J = 7 Hz, 6-H of aniline). LR-MS m/z: 289 (M$^{+}$), 259, 124 (base peak). HR-MS Calculated for C$_{17}$H$_{24}$N$_2$O$_2$: 289.1838. Found: 289.1831.

1-Chloro-4-(4-methoxybenzyl)zuberine (2) A suspension of 6 (10.0 g, 57.6 mmol), 4-methoxybenzyl chloride (10.8 g, 69.1 mmol), KH (1.93 g, 0.12 mol) and triethylamine (13.1 g, 0.13 mol) in CH$_2$Cl$_2$ (200 ml) was refluxed for 16 h. The reaction mixture was concentrated in vacuo, washed with CH$_2$Cl$_2$ (200 ml), and evaporated with 1 N HCl. The residue was washed with CH$_3$COOH (200 ml), and the whole was extracted with AcOEt (200 ml × 3). The AcOEt extracts were dried and evaporated in vacuo. The residue was chromatographed on silica gel eluting with CH$_2$Cl$_2$–ether to give 15.5 g (91.4%) of 15. 1H-NMR (CDCl$_3$) δ: 3.19—3.24 (8H, m, 5-CH$_2$ of pyrrolidine), 2.90—3.05 (2H, m, 5-CH$_2$ of pyrrolidine), 2.48 (1H, d, J = 12 Hz, 8-CH$_2$ of azabicyclooctane), 2.30—2.36 (2H, m, 5-CH$_2$ of pyrrolidine), 2.36 (2H, dd, J = 16, 9 Hz, NHCH$_2$), 2.67 (1H, d, J = 9 Hz, 3-H of aniline), 8.00 (1H, br, s, NH), 6.18 (1H, d, J = 9 Hz, 2-H of aniline), 7.08 (1H, d, J = 8 Hz, 5-H of aniline), 3.71 (1H, d, J = 8 Hz, 5-H of aniline), 8.11 (1H, d, J = 9 Hz, 2-H of aniline), 7.08 (1H, d, J = 7 Hz, 6-H of aniline). LR-MS m/z: 289 (M$^{+}$), 259, 124 (base peak). (FAB) m/z: 308 (M$^{+}$). HR-MS (FAB) Calculated for C$_{17}$H$_{24}$N$_2$O$_2$: 308.1810. Found: 308.1593.
6.92 (2H, d, J=9 Hz, aromatic), 7.20 (1H, dd, J=9, 3 Hz, 5-H of aniline),
7.34 (2H, d, J=9 Hz, aromatic), 7.72 (1H, d, J=3 Hz, 3-H of aniline), 7.92

N-[2-(1-Azabicyclo[3.3.0]octan-2-on-5-yl)ethyl]-4-hydroxy-2-nitroani-
line (15) To a solution of 14 (306 mg, 0.72 mmol) in CH₂Cl₂ (5.0 ml) in an
ice bath was added dropwise trifluoroacetic acid (3.0 ml). After stirring at
25 °C for 2 h, the reaction mixture was added to toluene and concentrated
in vacuo. The residue was chromatographed on silica gel eluting with CH ₂Cl₂–
MeOH to give 210 mg (95.6%) 15 as an amorphous mass. IR (KBr) cm⁻¹:
3376, 3120, 2964, 1657, 1524, 1232. ¹H-NMR (DMSO-
_d₆) d: 1.45—2.30 (9H, m, NHCH₂CH₂, 3,4,6,7-CH₂ of azabicyclooctanone), 2.69 (1H, dt,
J=5, 12, 6 Hz, 3-CH₂ of azabicyclooctanone), 2.86—2.97 (1H, m, 8-CH₂ of
azabicyclooctanone), 3.32—3.56 (3H, m, NHCH₂CH₂ and 8-CH₂ of azabi-
cyclooctanone), 6.99 (1H, d, J=9 Hz, 6-H of aniline), 7.15 (1H, dd, J=9, 3
Hz, 5-H of aniline), 7.41 (1H, d, J=3 Hz, 3-H of aniline), 7.85 (1H, br t,
J=6 Hz, NH), 9.41 (1H, br s, OH). LR-MS m/z: 305 (M⁺), 124 (base peak).
HR-MS Calcd for C₁₅H₁₉N₃O₄: 305.1376. Found: 305.1392.

3-{2-[2-(4-Hydroxy-2-nitrophenylamino)ethyl]pyrrolidinyl}propanoic
Acid (5) A solution of 15 (78.5 mg, 0.26 mmol), MeOH (10 ml) and 2 N
NaOH (10 ml) was refluxed for 16 h, and then concentrated in vacuo.
The residue was washed with CH₂Cl₂ (20 ml), and the washings were adjusted to
pH 1 with 1 N HCl. The aqueous layer was neutralized with 1 N NaOH, and
extracted with CH₂Cl₂. The extracts were dried and evaporated in vacuo.
The residual solid was chromatographed on DIAION HP-20 with MeOH to
give 47 mg (59.8%) 5 as a dark red amorphous mass. IR (KBr) cm⁻¹: 3377,
2964, 1638, 1578, 1521, 1224. ¹H-NMR (D₂O) d: 1.83—2.12 (8H, m,
NHCH₂CH₂, CH₂CH₂COOH, 3,4-CH₂ of pyrrolidine), 2.26 (2H, t, J=7 Hz,
CH₂CH₂COOH), 3.17—3.23 (2H, m, 5-CH₂ of pyrrolidine), 3.34 (2H, t,
J=7 Hz, NHCH₂CH₂), 6.84 (1H, d, J=9 Hz, 6-H of aniline), 7.10 (1H, dd,
J=9, 3 Hz, 5-H of aniline), 7.43 (1H, d, J=3 Hz, 3-H of aniline). LR-MS m/z: 305 [(M−H₂O)⁺], 124 (base peak). LR-MS (FAB) m/z: 324 [(M+H)⁺].

Biological Methods Preparation of Rat Brain Homogenate Rat
brain homogenate was prepared by a previously reported method.¹

¹[H]Pirenzepine Binding Inhibition The M₁ receptor binding assay
was carried out by a previously reported method.²

¹[H]QNB Binding Inhibition The M₂ receptor binding assay was car-
ried out by a previously reported method.²

Reference Compounds (−)-YM-796 was synthesized in our laboratory
as the fumarate salt.³

References
1) a) Suzuki T., Oka M., Maeda K., Furusawa K., Mitani T., Kataoka T.,
Chem. Pharm. Bull., 45, 1218—1220 (1997); b) Suzuki T., Oka M.,
Maeda K., Furusawa K., Uesaka H., Kataoka T., ibid., 47, 28—36
(1999).
3) Major basic metabolites in dog urine were prepared using a strong
cation exchange (SCX) solid-phase extraction (SPE) column (Bond
Elut, Analytichem International). Subsequently, the structures of the
metabolites were confirmed by NMR and MS analyses (unpublished
data).
4) Oka M., Baba K., Suzuki T., Matsumoto Y., Heterocycles, 45, 2317—
2320 (1997).
5) Tsukamoto S., Fujii M., Yasunaga T., Matsuda K., Wanibuchi F.,