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,*[,a] Hideaki INAGAKI,[a] Hitoshi HAMAJIMA,[a] 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, Hokusei-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 *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 treatmnet 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. Compound **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]octan-2-one (**9**), was obtained in 45.2% yield by $KMnO_4$ oxidation of 5-(2-benzyloxycarbonylaminoethyl)-1-azabicyclo[3.3.0]octane (**8**), which was derived from amine **7** and benzyloxycarbonyl chloride. Condensation of 5-(2-aminoethyl)-1-azabicyclo[3.3.0]octan-2-one (**10**), obtained by deprotection of **9**, and *o*-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)-ethyl]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-methoxybenzyloxy)-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-methoxybenzyloxy)-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-methoxybenzyloxy)-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**)

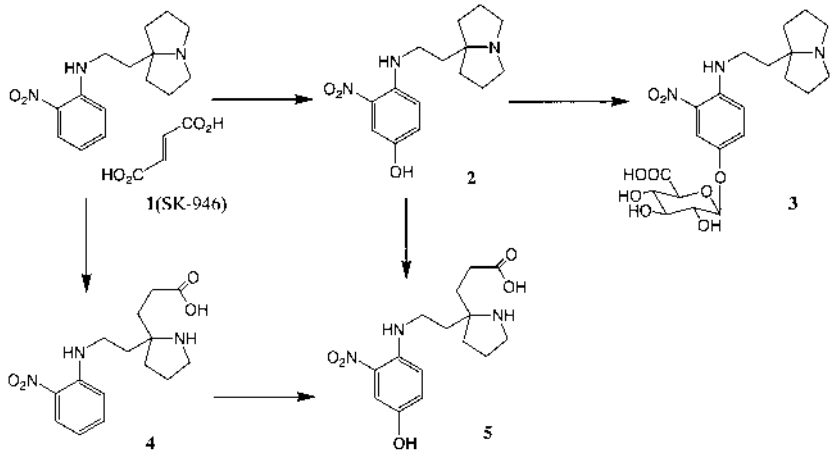

Chart 1

* To whom correspondence should be addressed.

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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}H]pirenzepine, an M_1-selective ligand, from rat cerebral cortex membrane and [^{3}H]quinuclidinyl 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 muscarine receptors. The M_1 affinity of **2** was among the strongest of all the aniline derivatives,[1b] 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.

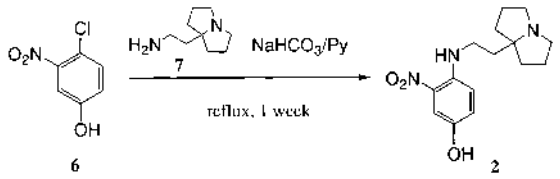
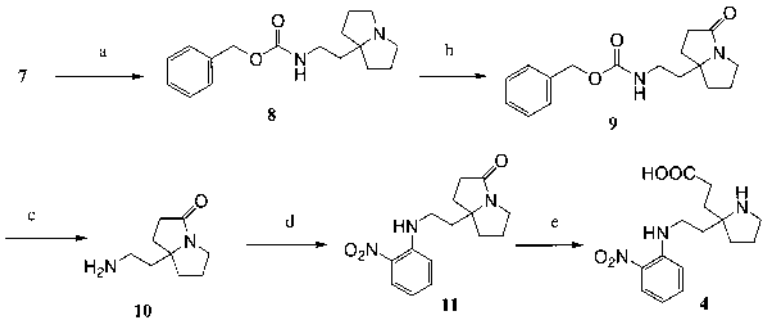


Chart 2

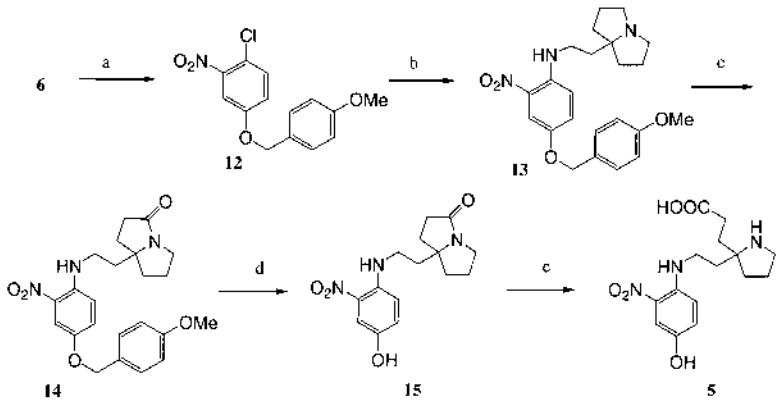
Experimental

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:



a)$PhCH_2OCOCl$,triethylamine/CH_2Cl_2; b)$KMnO_4$,$NaHCO_3$/acetone-H_2O; c)H_2,10%Pd-C/EtOH; d)*o*-Chloronitrobenzene,$NaHCO_3$/pyridine; e)2 N-NaOH/MeOH

Chart 3



a) *p*-Methoxybenzyl chloride, *n*-Bu_4NI,K_2CO_3/acetone; b) **7**/pyridine; c) $KMnO_4$, $NaHCO_3$ /acetone-H_2O; d) TFA/CH_2Cl_2; e) 2 N NaOH/MeOH

Chart 4

Table 1. Affinities of SK-946 and **2** for M_1 and M_2 Receptors

Compd.	Muscarinic receptor affinities Ki (μM)[a]		Ratio of [^{3}H]QNB/[^{3}H]pirenzepine
	[^{3}H]Pirenzepine (M_1 receptor)	[^{3}H]QNB (M_2 receptor)	
2	0.19	2.9	15.3
SK-946	0.12	1.4	11.7
(−)-YM796	1.8	7.7	4.3

a) Ki value (μM) calculated from the respective IC_{50} using the Cheng–Prusoff equation, $Ki = IC_{50}/1+[L]/Kd$, where [L] and Kd are ligand concentration and dissociation constant, respectively. Kd values: [^{3}H]pirenzepine, cortex, 7.1 nM; [^{3}H]QNB, cerebellum, 0.041 nM.

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-SX102. FAB-MS were recorded on a JEOL JMS-SX 102A mass spectrometer/JMA-DA7000 data system. Each sample was mixed with a glycerol or *m*-nitrobenzyl alcohol matrix [low-resolution MS (LR-MS)] and PEG 600 matrix [high-resolution MS (HR-MS)] on a target. Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 spectrometer.

***N*-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-4-hydroxy-2-nitroaniline (2)** A suspension of 4-chloro-3-nitrophenol (**6**) (4.05 g, 23.3 mmol), 5-(2-aminoethyl)-1-azabicyclo[3.3.0]octane (**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–triethylamine and DIAION HP-20 eluting MeOH to give 575 mg (8.5%) **2** as an amorphous mass. IR (KBr) cm^{-1}: 2956, 2871, 1508, 1291, 1139. ^{1}H-NMR (CD_3OD) δ: 1.71—1.98 (10H, m, $NHCH_2C\underline{H}_2$ and 3,4,6,7-CH_2 of azabicyclooctane), 2.68 (2H, dt, J=10, 6 Hz, 2,8-CH_2 of azabicyclooctane), 3.05 (2H, dt, J=10, 6 Hz, 2,8-CH_2 of azabicyclooctane), 3.37 (2H, t, J=8 Hz, $NHC\underline{H}_2CH_2$), 6.90 (1H, d, J=9 Hz, 6-H of aniline), 7.12 (1H, dd, J=9, 3 Hz, 5-H of aniline), 7.50 (1H, d, J=3 Hz, 3-H of aniline). LR-MS *m/z*: 291 (M^+), 154, 110 (base peak). HR-MS Calcd for $C_{15}H_{21}N_3O_3$: 291.1583. Found: 291.1575.

5-(2-Benzyloxycarbonylaminoethyl)-1-azabicyclo[3.3.0]octane (8) To a solution of 5-(2-aminoethyl)-1-azabicyclo[3.3.0]octane (**7**) (20.0 g, 0.13 mol) and triethylamine (13.1 g, 0.13 mol) in CH_2Cl_2 (200 ml) was added dropwise benzyloxycarbonyl chloride (24.3 g, 0.14 mol) in an ice bath. After stirring the reaction mixture at 25 °C for 16 h, 1 N HCl (100 ml) was added and the whole mixture washed with CH_2Cl_2 (100 ml). The aqueous phase was adjusted to pH 12 with 1 N NaOH, extracted with CH_2Cl_2 (100 ml ×3), and concentrated *in vacuo*. The residue was chromatographed on aluminum oxide eluting with CH_2Cl_2–MeOH to give 33.7 g (90.2%) **8** as a colorless oil. IR (neat) cm^{-1}: 2950, 2868, 1713, 1558, 1260. ^{1}H-NMR ($CDCl_3$) δ: 1.50—1.95 (10H, m, $NHCH_2C\underline{H}_2$, 3,4,6,7-CH_2 of azabicyclooctane), 2.57 (2H, dt, J=10, 6 Hz, 2,8-CH_2 of azabicyclooctane), 2.96 (2H, dt, J=10, 6 Hz, 2,8-CH_2 of azabicyclooctane), 3.28 (2H, dt, J=11, 6 Hz, $NHC\underline{H}_2CH_2$), 5.02 (1H, br s, NH), 5.09 (2H, s, $PhCH_2$), 7.20—7.39 (5H, m, aromatic). LR-MS *m/z*: 288 (M^+), 110 (base peak). HR-MS Calcd for $C_{17}H_{24}N_2O_2$: 288.1838. Found: 288.1831.

5-(2-Benzyloxycarbonylaminoethyl)-1-azabicyclo[3.3.0]octan-2-one (9) To a suspension of **8** (11.7 g, 40.6 mmol) and $NaHCO_3$ (34.1 g, 410 mmol) in acetone (120 ml) and H_2O (120 ml) was added slowly $KMnO_4$ (19.3 g, 120 mmol). After the reaction mixture had been stirred at reflux temperature for 1 h, MeOH (50 ml) was added and the mixture was stirred for 30 min. The resulting mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was extracted with CH_2Cl_2 (200 ml ×2). The extracts were washed with saturated NH_4Cl (100 ml), dried, and evaporated *in vacuo*. The residue was chromatographed on silica-gel eluting with AcOEt–MeOH to give 5.60 g (45.2%) **9** as a colorless oil. IR (neat) cm^{-1}: 3307, 2949, 1681, 1538, 1455, 1258. ^{1}H-NMR ($CDCl_3$) δ: 1.45—2.20 (6H, m, 4,6,7-CH_2 of azabicyclooctanone), 1.76 (2H, t, J=8 Hz, $NHCH_2C\underline{H}_2$), 2.41 (1H, ddd, J=17, 9, 2 Hz, 3-CH_2 of azabicyclooctanone), 2.73 (1H, dt, J=17, 10 Hz, 3-CH_2 of azabicyclooctanone), 2.97 (1H, dt, J=12, 5 Hz, 8-CH_2 of azabicyclooctanone), 3.14—3.31 (2H, m, $NHC\underline{H}_2CH_2$), 3.70 (1H, dt, J=12, 6 Hz, 8-CH_2 of azabicyclooctanone), 5.09 (2H, s, $PhCH_2$), 7.31—7.39 (5H, m, aromatic). LR-MS *m/z* 302 (M^+), 274, 124 (base peak). HR-MS Calcd for $C_{17}H_{22}N_2O_3$: 302.1630. Found: 302.1647.

5-(2-Aminoethyl)-1-azabicyclo[3.3.0]octan-2-one (10) A suspension of **9** (9.50 g, 31.5 mmol) and 10% Pd–C (950 mg) in EtOH (100 ml) was stirred under a stream of hydrogen at 25 °C for 16 h. The resulting mixture was filtered, and the filtrate was evaporated *in vacuo* to give 5.16 g (97.6%) **10** as a colorless oil. IR (neat) cm^{-1}: 3359, 2941, 2886, 1681, 1668, 1416. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (2H, br s, NH_2), 1.45—2.21 (6H, m, 4,6,7-CH_2 of azabicyclooctanone), 1.67—1.75 (2H, m, $NHCH_2C\underline{H}_2$), 2.41 (1H, ddd, J=17, 9, 2 Hz, 3-CH_2 of azabicyclooctanone), 2.76 (1H, dt, J=17, 10 Hz, 3-CH_2 of azabicyclooctanone), 2.79 (2H, dd, J=9, 7 Hz, $NHC\underline{H}_2CH_2$), 3.01 (1H, dt, J=12, 6 Hz, 8-CH_2 of azabicyclooctanone), 3.69 (1H, dt, J=12, 7 Hz, 8-CH_2 of azabicyclooctanone). LR-MS *m/z*: 168 (M^+), 124 (base peak). HR-MS Calcd for $C_9H_{16}N_2O$: 168.1263. Found: 168.1254.

***N*-[2-(1-Azabicyclo[3.3.0]octan-2-on-5-yl)ethyl]-2-nitroaniline (11)** A suspension of **10** (266 mg, 3.17 mmol), 1-chloro-2-nitrobenzene (250 mg, 3.17 mmol), and $NaHCO_3$ (133 mg, 3.17 mmol) in pyridine (100 ml) was stirred at reflux temperature for 16 h. The cooled mixture was concentrated *in vacuo*, and water was added to the residue. The mixture was extracted with CH_2Cl_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 (26.2%) **11** as orange needles. mp 98—99 °C. IR (KBr) cm^{-1}: 3386, 2961, 1698, 1616, 1506. ^{1}H-NMR ($CDCl_3$) δ: 1.57—2.30 (8H, m, $NHCH_2C\underline{H}_2$, 4,6,7-CH_2 of azabicyclooctanone), 2.48 (1H, ddd, J=17, 10, 2 Hz, 3-CH_2 of azabicyclooctanone), 2.79 (1H, ddd, J=17, 10, 9 Hz, 3-CH_2 of azabicyclooctanone), 3.05 (1H, dt, J=12, 6 Hz, 8-CH_2 of azabicyclooctanone), 3.35—3.43 (2H, m, $NHC\underline{H}_2CH_2$), 3.79 (1H, dt, J=12, 7 Hz, 8-CH_2 of azabicyclooctanone), 6.70 (1H, ddd, J=9, 7, 2 Hz, 4-H of aniline), 6.82 (1H, d, J=8 Hz, 6-H of aniline), 7.46 (1H, ddd, J=8, 7, 2 Hz, 5-H of aniline), 8.00 (1H, br s, NH), 8.18 (1H, dd, J=9, 2 Hz, 3-H of aniline). LR-MS *m/z*: 289 (M^+), 259, 124 (base peak). HR-MS Calcd for $C_{15}H_{19}N_3O_3$: 289.1426. Found: 289.1442.

3-{2-[2-(2-Nitrophenylamino)ethyl]pyrrolidinyl}propanoic Acid (4) A solution of **11** (105 mg, 0.36 mmol) in MeOH (10 ml) and 2 N NaOH (10 ml) was refluxed for 16 h. The reaction mixture was concentrated *in vacuo*, washed with CH_2Cl_2 (20 ml), and acidified with 1 N HCl. The resulting mixture was chromatographed on DIAION HP-20 eluting with MeOH to give 94 mg (84.3%) **4** as an orange amorphous mass. IR (KBr) cm^{-1}: 3381, 2956, 1619, 1573, 1511, 1419. ^{1}H-NMR (D_2O) δ: 1.81—2.10 (8H, m, $NHCH_2C\underline{H}_2$, $C\underline{H}_2CH_2COOH$ and 3,4-CH_2 of pyrrolidine), 2.22 (2H, dd, J=10, 7 Hz, $CH_2C\underline{H}_2COOH$), 2.90—3.05 (2H, m, 5-CH_2 of pyrrolidine), 3.36 (2H, dd, J=16, 9 Hz, $NHC\underline{H}_2CH_2$), 6.67 (1H, ddd, J=9, 7, 2 Hz, 4-H of aniline), 6.97 (1H, d, J=8 Hz, 6-H of aniline), 7.51 (1H, ddd, J=8, 7, 2 Hz, 5-H of aniline), 8.06 (1H, dd, J=9, 2 Hz, 3-H of aniline). LR-MS *m/z*: 289 [$(M-H_2O)^+$], 259, 124 (base peak). (FAB) *m/z*: 308 [$(M+H)^+$]. HR-MS (FAB) Calcd for $C_{15}H_{22}N_3O_4$ $(M+H)^+$: 308.1610. Found: 308.1593.

1-Chloro-4-(4-methoxybenzyloxy)-2-nitrobenzene (12) A suspension of **6** (10.0 g, 57.6 mmol), 4-methoxybenzyl chloride (10.8 g, 69.1 mmol), K_2CO_3 (15.9 g, 0.12 mol) and *n*-Bu_4NI (2.12 g, 5.76 mmol) in acetone (200 ml) was refluxed for 16 h. The reaction mixture was filtered, and the filtrate was evaporated *in vacuo*. To the residue was added saturated $NaHCO_3$ (200 ml), and the whole was extracted with AcOEt (200 ml ×3). The AcOEt extracts were dried and evaporated *in vacuo*. The residue was crystallized from CH_2Cl_2–ether to give 15.5 g (91.4%) of **12** as pale yellow needles. mp 86—87 °C. IR (KBr) cm^{-1}: 3104, 1612, 1523, 1254. ^{1}H-NMR ($CDCl_3$) δ: 3.83 (3H, s, OCH_3), 5.02 (2H, s, OCH_2), 6.94 (2H, d, J=9 Hz, aromatic), 7.10 (1H, dd, J=9, 3 Hz, 5-H of chlorobenzene), 7.34 (2H, d, J=9 Hz, aromatic), 7.42 (1H, d, J=9 Hz, 6-H of chlorobenzene), 7.47 (1H, d, J=3 Hz, 3-H of chlorobenzene). LR-MS *m/z*: 289 [$(M-NO)^+$], 121 (base peak).

***N*-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl]-4-(4-methoxybenzyloxy)-2-nitroaniline (13)** A suspension of **12** (12.0 g, 40.9 mmol) and 5-(2-aminoethyl)-1-azabicyclo[3.3.0]octane (**7**) (12.6 g, 81.2 mmol) in pyridine (240 ml) was stirred at reflux temperature for 16 h. The cooled mixture was concentrated *in vacuo*, and water was added to the residue. The mixture was extracted with CH_2Cl_2 (200 ml ×3), and the extracts were washed with brine, dried, and concentrated *in vacuo*. The residue was chromatographed on silica-gel with AcOEt to give an oily product, which was crystallized from AcOEt to give 9.60 g (58.2%) **13** as orange needles. mp 137—141 °C. IR (KBr) cm^{-1}: 3452, 2961, 2865, 1558, 1520, 1249. ^{1}H-NMR ($CDCl_3$) δ: 1.55—1.86 (10H, m, $NHCH_2C\underline{H}_2$ and 3,4,6,7-CH_2 of azabicyclooctane), 2.63 (2H, dt, J=10, 6 Hz, 2,8-CH_2 of azabicyclooctane), 3.06 (2H, dt, J=10, 6 Hz, 2,8-CH_2 of azabicyclooctane), 3.35 (2H, t, J=8 Hz, $NHC\underline{H}_2CH_2$), 3.81 (3H, s, CH_3O), 4.94 (2H, s, CH_2O), 6.80 (1H, d, J=9 Hz, 6-H of aniline), 6.92 (2H, d, J=9 Hz, aromatic), 7.18 (1H, dd, J=9, 3 Hz, 5-H of aniline), 7.36 (2H, d, J=9 Hz, aromatic), 7.72 (1H, d, J=3 Hz, 3-H of aniline), 9.05 (1H, br s, NH). LR-MS *m/z*: 411 (M^+), 154, 121 (base peak). HR-MS Calcd for $C_{23}H_{29}N_3O_4$: 411.2158. Found: 411.2141.

***N*-[2-(1-Azabicyclo[3.3.0]octan-2-on-5-yl)ethyl]-4-(4-methoxybenzyloxy)-2-nitroaniline (14)** To a suspension of **13** (4.15 g, 10.3 mmol) and $NaHCO_3$ (8.65 g, 0.10 mol) in a mixture of acetone (250 ml) and H_2O (150 ml) was added slowly $KMnO_4$ (4.89 g, 30.9 mmol). After stirring the reaction mixture at reflux temperature for 1 h, MeOH (5 ml) was added. The mixture was stirred for 30 min, and then filtered. The filtrate was evaporated *in vacuo*, and the residue was extracted with CH_2Cl_2 (20 ml ×3), dried, and evaporated *in vacuo*. The residual solid was chromatographed on silica gel eluting with AcOEt–MeOH to give 588 mg (13.7%) **14** as a red amorphous mass. IR (KBr) cm^{-1}: 3328, 2958, 2901, 1698, 1519, 1253. ^{1}H-NMR ($CDCl_3$) δ: 1.54—2.27 (8H, m, $NHCH_2C\underline{H}_2$, 4,6,7-CH_2 of azabicyclooctanone), 2.48 (1H, ddd, J=17, 10, 2 Hz, 3-CH_2 of azabicyclooctanone), 2.78 (1H, ddd, J=17, 10, 9 Hz, 3-CH_2 of azabicyclooctanone), 3.04 (1H, dt, J=12, 6 Hz, 8-CH_2 of azabicyclooctanone), 3.31—3.42 (2H, m, $NHC\underline{H}_2CH_2$), 3.76 (1H, dt, J=12, 7 Hz, 8-CH_2 of azabicyclooctanone), 3.81 (3H, s, OCH_3), 4.95 (2H, s, OCH_2), 6.79 (1H, d, J=9 Hz, 6-H of aniline),

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 (1H, br t, J=6 Hz, NH). LR-MS *m/z*: 425 (M^+), 121 (base peak). HR-MS Calcd for $C_{23}H_{27}N_3O_5$: 425.1951. Found: 425.1972.

***N*-[2-(1-Azabicyclo[3.3.0]octan-2-on-5-yl)ethyl]-4-hydroxy-2-nitroaniline (15)** To a solution of **14** (306 mg, 0.72 mmol) in CH_2Cl_2 (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_2Cl_2–MeOH to give 210 mg (95.6%) **15** as an amorphous mass. IR (KBr) cm^{-1}: 3376, 3120, 2964, 1657, 1524, 1232. ^{1}H-NMR (DMSO-d_6) δ: 1.45—2.30 (9H, m, $NHCH_2C\underline{H}_2$, 3,4,6,7-CH_2 of azabicyclooctanone), 2.69 (1H, dt, J=12, 6 Hz, 3-CH_2 of azabicyclooctanone), 2.86—2.97 (1H, m, 8-CH_2 of azabicyclooctanone), 3.32—3.56 (3H, m, $NHC\underline{H}_2CH_2$ and 8-CH_2 of azabicyclooctanone), 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 $C_{15}H_{19}N_3O_4$: 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 CH_2Cl_2 (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 CH_2Cl_2. 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. ^{1}H-NMR (D_2O) δ: 1.83—2.12 (8H, m, $NHCH_2C\underline{H}_2$, $C\underline{H}_2CH_2COOH$, 3,4-CH_2 of pyrrolidine), 2.26 (2H, t, J=7 Hz, $CH_2C\underline{H}_2COOH$), 3.17—3.23 (2H, m, 5-CH_2 of pyrrolidine), 3.34 (2H, t, J=7 Hz, $NHC\underline{H}_2CH_2$), 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 [$(M-H_2O)^+$], 124 (base peak). LR-MS (FAB) *m/z*: 324 [$(M+H)^+$]. HR-MS (FAB) Calcd for $C_{15}H_{22}N_3O_5$ $(M+H)^+$: 324.1559. Found: 324.1583.

Biological Methods Preparation of Rat Brain Homogenate Rat brain homogenate was preparated by a previously reported method.[1b)]

[^{3}H]Pirenzepine Binding Inhibition The M_1 receptor binding assay was carried out by a previously reported method.[1b)]

[^{3}H]QNB Binding Inhibition The M_2 receptor binding assay was carried out by a previously reported method.[1b)]

Reference Compounds (−)-YM-796 was synthesized in our laboratory as the fumarate salt.[5)]

References

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