VOL. 53 NO. 5, MAY 2000 THE JOURNAL OF ANTIBIOTICS pp. 467 — 473

(2*R-trans*)-2-Butyl-5-heptylpyrrolidine as a Potent Sigma Receptor Ligand Produced by *Streptomyces longispororuber*

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(Received for publication February 18, 2000)

A potent sigma (σ) receptor ligand was isolated from the culture broth of *Streptomyces longispororuber* #525. The active compound was identified to be (2*R-trans*)-2-butyl-5-heptylpyrrolidine by spectroscopic and chemical studies. The compound exhibited high affinity and selectivity for σ receptors. The IC_{50} values toward σ_1, σ_2 and dopamine D_2 receptors were 2.0, 22.7 and more than 40,000 nM, respectively. Its (2*S-trans*)- and (±)-*cis*-isomers, both synthesized, were also found to be high affinity σ ligands.

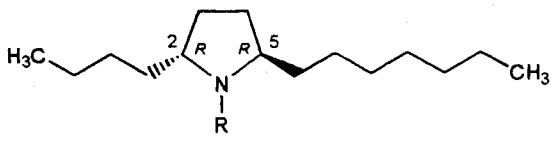
The sigma (σ) receptors are membrane-bound protein receptors which are distributed in the central nervous system and some in peripheral tissues[1]. Although the functions of σ receptors are not yet completely known, their high affinity for several antipsychotic drugs and unique tissue distribution suggest that they may be a potential target for the treatment of psychotic disorders such as schizophrenia and depression[2,3]. The σ receptors can be classified into at least two subtypes, namely σ_1 and σ_2[4,5]. The cloning of σ_1 receptor has revealed that the receptor had no significant homology with any known proteins except for yeast sterol C8-C7 isomerase[6], suggesting that it may play a role in sterol biosynthesis[7].

In the search for new ligands specific for σ receptors from microorganisms, we have isolated an active compound from the culture broth of *Streptomyces longispororuber* #525. The compound was identified as (2*R-trans*)-2-butyl-5-heptylpyrrolidine (**1**) by spectroscopic and chemical studies. Here we report the taxonomy of the producing organism, fermentation, isolation, structure elucidation, and σ receptor binding affinity of **1** along with its synthetic isomers. Since many of known σ ligands exhibit relatively high affinity for dopamine D_2 receptors[8], we also examined their selectivity against D_2 receptors.

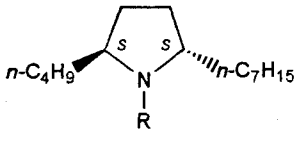
Taxonomic Characterization of the Producing Strain #525

The strain #525 formed well-branched substrate mycelia without fragmentation on agar media. Aerial mycelia were

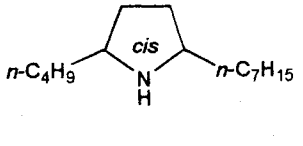
Fig. 1. Structures of (2*R-trans*)-2-butyl-5-heptylpyrrolidine (**1**), its isomers and derivatives.



1 R = H
2 R = SO_2Ph



3 R = H
4 R = SO_2Ph



(±)-*cis*-**5**

Table 1. Cultural characteristics of strain #525.

Medium	Growth	Aerial mycelium	Reverse side color	Soluble pigment
Yeast extract-malt extract agar (ISP No. 2)	Good	Scant, pink	Reddish pink	None
Oatmeal agar (ISP No. 3)	Good	Abundant, pinkish white	Yellowish red	None
Inorganic salts-starch agar (ISP No. 4)	Good	Abundant, pale pink	Pink	None
Glycerol-asparagine agar (ISP No. 5)	Good	Scant, pink	Reddish orange	None

abundant on oatmeal and inorganic salts-starch agar, but scant on yeast extract-malt extract and glycerol-asparagine agar (Table 1). The color of aerial mycelia was white to pink, while that of reverse side of colony was pink to orange or red. The mature spore chain consisted of 20~50 or more spores and bore 2~5 spiral turns. The spore was oval and 0.4~0.5×0.9~1.2 μm in size with a smooth surface. The whole-cell hydrolysate contained LL-diaminopimelic acid. Melanoid pigment was formed in peptone-yeast extract-iron agar, but not in tyrosine agar (Table 2). L-Arabinose, D-fructose, D-glucose, inositol, D-mannitol and D-xylose were utilized as a carbon source, but raffinose, L-rhamnose or sucrose were not. These characteristics are very similar to those of *Streptomyces longispororuber* (Krasil'nikov) Waksman[9]. Therefore, the strain #525 was identified as *S. longispororuber*.

Table 2. Physiological characteristics of strain #525.

Temperature range for growth (°C)	14~41
Production of melanoid pigment	
Peptone-yeast extract-iron agar (ISP No. 6)	+
Tyrosine agar (ISP No. 7)	−
Carbohydrate utilization	
L-Arabinose	+
D-Fructose	+
D-Glucose	+
Inositol	+
D-Mannitol	+
Raffinose	−
L-Rhamnose	−
Sucrose	−
D-Xylose	+

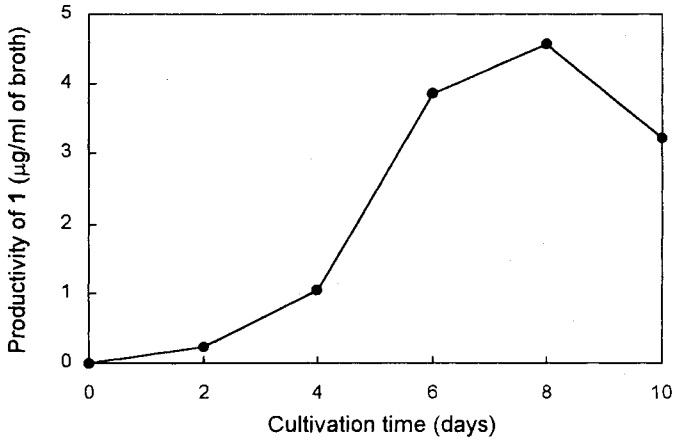
Fermentation

The strain #525 was inoculated into a 500-ml Sakaguchi flask containing 75 ml of liquid medium composed of glucose 3%, soy bean flour 1.5%, yeast extract 0.3% and $CaCO_3$ 0.2%, pH 7.2. After incubation with reciprocal shaking at 130 rpm at 27°C for 5 days, 2 ml aliquots of the seed culture were transferred into 500-ml Sakaguchi flasks containing 100 ml of the same medium and incubated with reciprocal shaking at 130 rpm at 27°C. The production of **1** began at day 2 and reached the maximum level at day 8 (Fig. 2).

Isolation

The culture broth (27 liters) harvested at day 8 of fermentation was centrifuged at 10,000 *g* for 15 minutes at 4°C to collect supernatant. The supernatant was extracted three times with 9 liters of ethyl acetate. The extract (27

Fig. 2. Time course of production of **1** by strain #525.



liters) was dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The resultant residue was dissolved in *n*-hexane - ethyl acetate (1 : 1) and applied to a column of silica gel (500 ml). The column was washed with *n*-hexane - ethyl acetate (19 : 1) containing 1% triethylamine, then eluted with *n*-hexane - ethyl acetate (9 : 1 to 8 : 2) containing 1% triethylamine. Active fractions were concentrated *in vacuo* to dryness, dissolved in methanol, and applied to a column of Sephadex LH-20 (80 ml). The column was eluted with methanol, and active fractions were concentrated *in vacuo* to dryness to give **1** (2.7 mg) as a pale yellow oil.

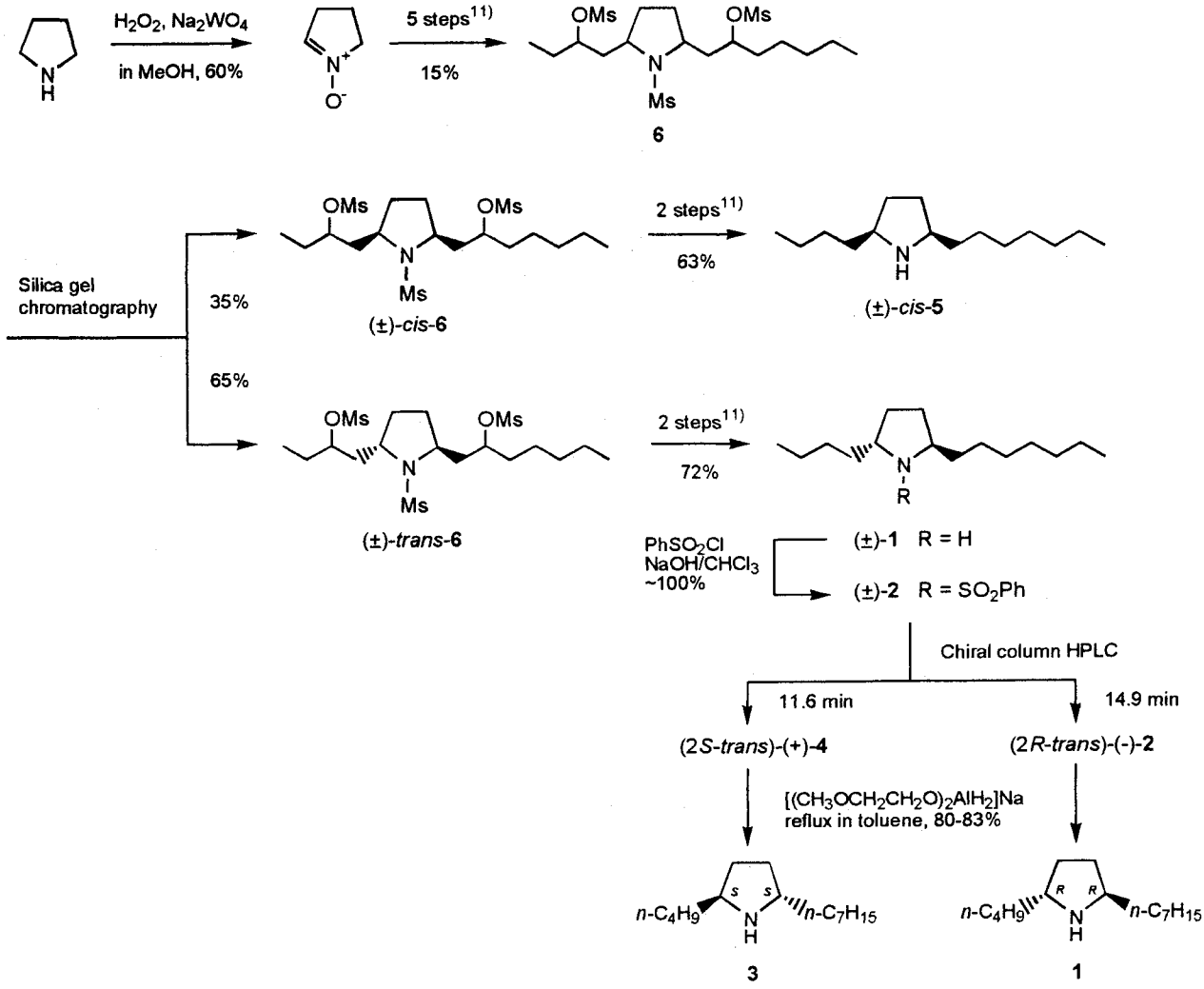
Structure Elucidation

The planar structure of **1** was identified as 2-butyl-5-heptylpyrrolidine based on ^{1}H and ^{13}C NMR, UV, IR and MS analyses. The relative configuration was determined to be *trans* by comparison of chemical shifts of **1** with those of known *trans*- and *cis*-2,5-dialkylpyrrolidines[10]. Owing to the poor yield of **1**, the confirmation of its structure and determination of absolute configuration were done by direct comparison with synthetic samples (Fig. 3). (±)-*trans*-2-Butyl-5-heptylpyrrolidine as well as its (±)-*cis*-isomer were synthesized according to the method of TUFARIELLO and PUGLIS[11]. The *trans* isomer was converted to its phenylsulfonamide derivative. The derivative gave two peaks in a chiral column HPLC (retention time 11.6 and 14.9 minutes). The optical rotation of the first peak was $[\alpha]_D^{25}$ +67.5° (*c* 0.20, CH_2Cl_2) and that of the second peak was $[\alpha]_D^{25}$ −59.9° (*c* 0.24, CH_2Cl_2). By comparison with reported optical rotations[12,13], the first peak was identified as (2*S*-*trans*)-enantiomer **4** and the second as (2*R*-*trans*)-enantiomer **2**. The phenylsulfonamide derivative similarly obtained from the natural sample of **1** showed the same retention time as the synthetic sample of **2** (Fig. 4). No peak corresponding to **4** was detected, indicating that strain #525 produced optically pure **1**. Thus microbial product **1** was determined to be (2*R*-*trans*)-2-butyl-5-heptylpyrrolidine. Interestingly, **1** is closely related to an alkaloid isolated from the venom of the ant *Solenopsis fugax*[14]. The absolute configuration of the venom alkaloid, *trans*-2-butyl-5-heptylpyrrolidine, has not been established. This is the first report describing the isolation of **1** from microorganism and determination of the absolute configuration of **1** as a natural product. Recently, CURRIE *et al.*[15]. have reported the symbiotic association between ants and *Streptomyces*. Compound **1** may add a new insight into chemical interaction of these two organisms.

Biological Activity

In receptor binding assay, **1** showed high affinity at σ receptors. The IC_{50} values toward σ_1 and σ_2 receptors were 2.0 and 22.7 nM, respectively (Table 3). In contrast, **1** did not inhibit the binding of D_2 receptors even at 40 μM. Our preliminary experiments also revealed that **1** did not interact with many other receptors at 10 μM such as glutamate (α-amino-3-hydroxy-5-methylisoxazole-4-propionic, kainic and *N*-methyl-D-aspartic acids), muscarinic (M_1 and M_2), nicotinic acetylcholine, serotonin (5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4) and γ-aminobutyric acid

Fig. 3. Total synthesis of **1** and its isomers.



(GABA$_A$ and GABA$_B$) receptors (data not shown). Therefore the affinity of **1** for σ receptors is very specific. The (2*S-trans*)-enantiomer **3** and (±)-*cis*-isomer **5** were also found to have high affinity and specificity at σ receptors, suggesting that there is little steric selectivity in binding of σ receptors with these compounds.

Experimental

General

UV spectra were recorded on a Hitachi U-2000 spectrometer. IR spectra were recorded on a Perkin Elmer 1600 series FT-IR spectrometer. EI-MS spectra were obtained on a Hitachi M-80B spectrometer. HRFAB-MS spectra were measured on a JEOL JMS-SX-102-2 spectrometer. NMR spectra were obtained on a JEOL JNM α-500 or GX270 spectrometer. Optical rotations were measured on a JASCO DIP-370 polarimeter. TLC plates were observed under UV light of 254 nm or after spraying with Dragendroff reagent.

Microorganism

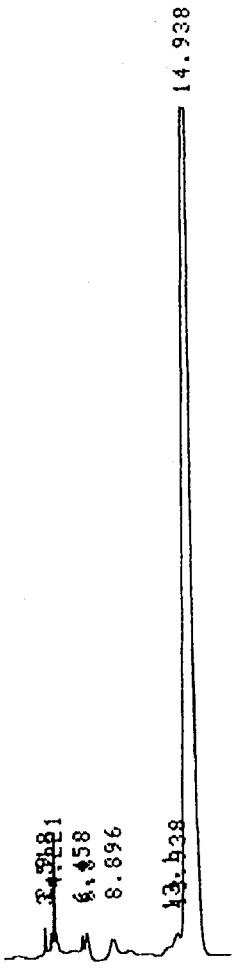
Strain #525 was isolated from a soil sample collected in Kansas, U.S.A. The strain has been deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan under the accession number FERM P-15557.

Taxonomical Characterization

Cultural characteristics of strain #525 were determined according to the method of SHIRLING and GOTTLIEB[16] after

Fig. 4. Chiral column HPLC chromatogram of the phenylsulfonamide derivative prepared from the natural product of **1**.

Column: Chiralcel OD (4.6×250 mm); mobile phase: *n*-hexane - 2-PrOH (100 : 1); flow rate: 1 ml/minute; Detection: UV 254 nm.



grown on various agar media at 27°C for 14 days. Color assignments were made by using the Color Tone Manual[17]. Analysis of diaminopimelic acid in the whole-cell hydrolysate was performed by the method of BECKER *et al.*[18]. The temperature range for growth was determined on inorganic salts - starch agar.

Natural Product of (2*R*-*trans*)-2-Butyl-5-heptyl-pyrrolidine (**1**)

The natural sample of **1** was obtained from the culture broth of strain #525 as described in the text: Pale yellow oil; HRFAB-MS *m/z* found 226.2526 $(M+H)^+$, calcd for $C_{15}H_{32}N$ 226.2536; EI-MS *m/z* 225 (M^+), 168, 126, 95, 81, 69, 55; UV (MeOH) no characteristic absorption; IR (KBr) cm^{-1} 3443, 2926, 2785, 2490, 2360, 1591, 1467, 1409; ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, t, J=7.0 Hz), 0.89 (3H, t, J=7.0 Hz), 1.25~1.35 (18H, m), 1.45 (2H, m), 1.72 (1H, br s), 1.93 (2H, m), 3.10 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 22.6, 22.8, 27.3, 29.3, 29.5, 29.7, 31.8, 32.5, 36.9, 37.2, 58.0; TLC (Silica gel) Rf 0.4 (CH_2Cl_2 - Me_2CO (3 : 1) with 1% triethylamine).

Synthesis of (±)-*trans*-2-Butyl-5-heptylpyrrolidine ((±)-**1**) and (±)-*cis*-2-Butyl-5-heptylpyrrolidine ((±)-*cis*-**5**)

Both (±)-*trans*- and (±)-*cis*-2-butyl-5-heptylpyrrolidines were synthesized in favor of the *trans* isomer according to the method of TUFARIELLO and PUGLIS[11] with a slight modification. The trimesylate **6** (4.3 g) was prepared in 6 steps from pyrrolidine, and chromatographed on silica gel using toluene - Me_2CO (20 : 1) as eluent to give (±)-*trans*-**6** (2.6 g) and (±)-*cis*-**6** (1.4 g). Treatment of (±)-*trans*- and

Table 3. Binding affinities of **1** and its isomers to σ and dopamine D_2 receptors.

Compound	IC_{50} (nM)			
	1,3-Di-(2-[5-^{3}H]tolyl)-guanidine (Non-selective σ)	[1,3-^{3}H]-(+)-Pentazocine (σ_1)	[*phenyl*-^{3}H]-Ifenprodil (σ_2)	[*phenyl*-4-^{3}H]-Spiperone (D_2)
1	6.6	2.0	22.7	> 40,000
3	10.1	NT	NT	> 40,000
(±)-*cis*-**5**	6.2	NT	NT	> 40,000
Haloperidol	8.0	2.2	43.0	5.6

NT = not tested.

(±)-*cis*-**6** with lithium triethylborohydride, then with sodium bis(2-methoxy-ethoxy)aluminum hydride afforded (±)-**1** and (±)-*cis*-**5**, respectively, in 63~72% yields.

(±)-*trans*-**6**: Pale yellow oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.88 (3H, t, J=7.0 Hz), 1.98 (3H, t, J=7.9 Hz), 1.25~1.45 (6H, m), 1.65~1.94 (8H, m), 2.07~2.15 (2H, m), 2.28~2.37 (2H, m), 2.93 (3H, s), 3.02 (6H, s), 3.78~3.86 (2H, m), 4.66~4.79 (2H, m). TLC (Silica gel) Rf 0.3 (toluene - Me_2CO (5 : 1)).

(±)-*cis*-**6**: Pale yellow oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.89 (3H, t, J=7.0 Hz), 1.98 (3H, t, J=8.0 Hz), 1.25~1.44 (6H, m), 1.66~1.97 (8H, m), 2.08~2.40 (4H, m), 2.79 (3H, s), 3.02 (6H, s), 3.68~3.79 (2H, m), 4.67~4.89 (2H, m). TLC (Silica gel) Rf 0.25 (toluene - Me_2CO (5 : 1)).

(±)-**1**: Colorless oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.89 (6H, q, two triplet overlapping), 1.23~1.48 (18H, m), 1.58~1.68 (2H, m), 1.84~1.98 (2H, m), 3.04~3.14 (2H, m); TLC (Silica gel) Rf 0.4 (CH_2Cl_2 - Me_2CO (3 : 1) with 1% triethylamine).

(±)-*cis*-**5**: Colorless oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.89 (6H, q, two triplet overlapping), 1.25~1.38 (14H, m), 1.47~1.68 (6H, m), 1.82~1.90 (2H, m), 2.92~3.00 (2H, m); TLC (Silica gel) Rf 0.4 (CH_2Cl_2 - Me_2CO (3 : 1) with 1% triethylamine).

Separation of Phenylsulfonamide Derivatives by HPLC

Compound (±)-**1** was reacted with phenylsulfonyl chloride as described[12] to yield phenylsulfonamide derivative (±)-**2** in nearly quantitative yield. The separation of (+)- and (−)-enantiomers from the racemic phenylsulfonamide was performed on HPLC using a Chiralcel OD column (4.6×250 mm, Daicel Chemical Industry) with *n*-hexane - 2-PrOH (100 : 1) as eluent at a flow rate of 1 ml/minute with a detection of UV at 254 nm. (±)-**2** (6 mg) was injected, and two major peaks with Rt 11.6 and 14.9 minutes were collected and concentrated *in vacuo* to give (+)-**4** (2.0 mg) and (−)-**2** (2.4 mg), respectively. By comparison with literatures[12,13], (+)-**4** and (−)-**2** were assigned as 2*S-trans* and 2*R-trans*, respectively. The phenylsulfonamide derivative prepared from the natural sample of **1** was analyzed by the same procedure.

(2*R-trans*)-2-Butyl-5-heptyl-1-phenylsulfonylpyrrolidine ((−)-**2**): Colorless oil; $[\alpha]_D^{25}$ −59.9° (*c* 0.24, CH_2Cl_2), lit. $[\alpha]_D^{20}$ −59.8° (*c* 0.9, CH_2Cl_2)[13]; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.86 (3H, t, J=7.0 Hz), 0.88 (3H, t, J=7.0 Hz), 1.07~1.33 (16H, m), 1.63~1.68 (2H, m), 1.84~1.96 (4H, m), 3.78~3.87 (2H, m), 7.48 (3H, m), 7.85 (2H, m); TLC (Silica gel) Rf 0.4 (*n*-hexane - 2-PrOH (10 : 1)).

(2*S-trans*)-2-Butyl-5-heptyl-1-phenylsulfonylpyrrolidine ((+)-**4**): Colorless oil; $[\alpha]_D^{25}$ +67.5° (*c* 0.20, CH_2Cl_2), lit. $[\alpha]_D^{26}$ +62.0° (*c* 1.79, CH_2Cl_2)[12]; ^{1}H NMR and TLC identical to those of (−)-**2**.

Dephenylsulfonylation of (−)-**2** and (+)-**4** to **1** and its (2*S-trans*)-Enantiomer **3**

To a solution of (−)-**2** or (+)-**4** (2 mg) in toluene (20 ml) was added 65% sodium bis(2-methoxy-ethoxy)aluminum hydride in toluene (0.1 ml) and refluxed for 1 hour. The product was purified by preparative TLC to give **1** and its (2*S*,5*S*)-enantiomer **3**, respectively, in 80~83% yields. Spectral data of synthetic **1** was identical to those of the natural sample of **1**. Spectral data of **3** were also in good agreement with those reported in literatures[10,12].

Receptor Binding Assay

Radioligand competition binding assay for non-selective σ receptor binding was performed using 4 nM 1,3-di-(2-[5-^{3}H]tolyl)guanidine (NEN) and guinea pig brain membranes[19]. σ_1 receptor binding assay was performed using 1 nM [1,3-^{3}H]-(+)-pentazocine (NEN) and guinea pig brain membranes[20]. σ_2 receptor binding assay was performed using 3 nM [*phenyl*-^{3}H]ifenprodil (NEN) and rat brain membranes[21]. D_2 receptor binding assay was performed using 0.5 nM [*phenyl*-4-^{3}H]spiperone (Amersham) and rat striatal membranes[22]. Nonspecific binding was measured in the presence of 100 μM haloperidol, 10 μM 3-(3-hydroxyphenyl)-*N*-propylpiperidine, 10 μM ifenprodil or 10 μM spiperone for non-selective σ, σ_1, σ_2 and D_2 binding assays, respectively. An antipsychotic drug haloperidol (Sigma Chemical) was used as a reference compound.

Acknowledgments

We thank Dr. HIROKI TAKAHATA of Toyama Medical and Pharmaceutical University for his helpful discussions.

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