Pharmacological Profile of Lurasidone, a Novel Antipsychotic Agent with Potent 5-HT7 and 5-HT1A Receptor Activity

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List of Abbreviations:
5-HT, serotonin; APO, apomorphine; CAR, conditioned avoidance response; CNS, central nervous system; DOPAC, 3,4-dihydroxyphenylacetic acid; EPS, extrapyramidal side effects; FRT, forepaw retraction time; Lurasidone, (3a\text{R},4\text{S},7\text{R},7\text{aS})-2-{(1\text{R},2\text{R})-2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-ylmethyl]cyclohexylmethyl}hexahydro-4,7-methano-2\text{H}-isoindo1-1,3-dione hydrochloride, SM-13496; MAP, methamphetamine; MED, minimum effective dose; p-CAMP, p-chloroamphetamine; TRY, tryptamine

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Abstract

Lurasidone ((3aR,4S,7R,7aS)-2-[(1R,2R)-2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-ylmethyl]cyclohexylmethyl]hexahydro-4,7-methano-2H-isoindole-1,3-dione hydrochloride, SM-13496) is an azapirone derivative, and a novel antipsychotic candidate. The objective of the current studies was to investigate the in vitro and in vivo pharmacological properties of lurasidone. Receptor binding affinities of lurasidone and several antipsychotic drugs were tested under comparable assay conditions using cloned human receptors or membrane fractions prepared from animal tissue. Lurasidone was found to have potent binding affinity for dopamine D₂, 5-HT₂A, 5-HT₇, 5-HT₁A and noradrenaline α₂C receptors. Affinity for noradrenaline α₁, α₂A and 5-HT₂C receptors was weak, while affinity for histamine H₁ and muscarinic acetylcholine receptors was negligible. In vitro functional assays demonstrated that lurasidone acts as an antagonist at D₂ and 5-HT₇ receptors and a partial agonist at the 5-HT₁A receptor subtype. Lurasidone showed potent effects predictive of antipsychotic activity such as inhibition of methamphetamine (MAP)-induced hyperactivity and apomorphine (APO)-induced stereotyped behavior in rats, similar to other antipsychotics. Furthermore, lurasidone had only weak extrapyramidal effects in rodent models. In animal models of anxiety disorders and depression, treatment with lurasidone was associated with significant improvement. Lurasidone showed a preferential effect on the frontal cortex (vs. striatum) in increasing dopamine turnover. Anti-α₁-noradrenergic, anticholinergic and central nervous system (CNS) depressant actions of lurasidone were also very weak. These results demonstrate that lurasidone possesses antipsychotic activity, and antidepressant- or anxiolytic-like effects with potentially reduced liability for extrapyramidal and CNS depressant side effects.
Introduction

Schizophrenia is a heterogeneous disease with diverse symptomatology including positive symptoms (e.g., hallucinations, delusions and excitement), negative symptoms (e.g., flattened affect, apathy and social withdrawal), dysphoric mood symptoms (e.g., anxiety and depression) and cognitive dysfunction (e.g., deficit in working memory, executive function, attentional processing and memory) (Andreasen et al., 1990; Meltzer, 1999).

First generation antipsychotics such as haloperidol, which mainly have dopamine D$_2$ antagonist action, are effective against positive symptoms, but have relatively less benefit in treating negative and associated mood symptoms (Lieberman, 1993). In addition, the D$_2$ antagonists frequently induce extrapyramidal side effects (EPS, e.g., parkinsonism, dystonia, akathisia and tardive dyskinesia) that are thought to reflect blockade of D$_2$ receptors in the basal ganglia (Casey, 1996). Second generation antipsychotics, which commonly have combined 5-HT$_{2A}$ and D$_2$ blocking activity, may offer greater improvement in negative symptoms (Meltzer et al., 2003; Leucht et al., 2009), and have a more favorable tolerability profile, with markedly reduced risk of EPS; however several (e.g., clozapine, olanzapine) are associated with significant weight gain and metabolic dysfunction (Haddad, 2005).

Furthermore, most second generation antipsychotics possess relatively potent α1 antagonistic activity, associated with postural hypotension and sedation; muscarinic antagonistic activity, associated with impairment of the parasympathetic autonomic system and with impairment in cognitive function; and H$_1$ antagonistic activity, associated with additional sedative effects and weight gain (Kroeze et al., 2003). Existing drugs appear to have only limited efficacy in treating cognitive deficits, which are a core feature of schizophrenia (Harvey et al., 2004). Thus, there is a continued need for new agents with improved efficacy and safety profile for the treatment of schizophrenia, bipolar disorder and other types of psychosis.

Lurasidone ((3aR,4S,7R,7aS)-2-(((1R,2R)-2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-ylmethyl]cyclohexylmethyl)hexahydro-4,7-methano-2H-isooindole-1,3-dione hydrochloride, SM-13496, Fig. 1) is an azapirone derivative, based on structure-activity relationship studies,
and a novel antipsychotic candidate. Previous studies revealed that lurasidone reversed MK-801-induced impairment of learning and memory in the passive avoidance test, the Morris water maze test and radial-arm maze test in rats, suggesting that lurasidone might possess procognitive activity in addition to antipsychotic effects (Ishiyama et al., 2007, Enomoto et al., 2008). In the present study, we aimed to evaluate the pharmacological characteristics of lurasidone comprehensively other than its procognitive activity, which was considered to provide the rational of the clinical use in patients with psychiatric conditions. Thus, we firstly investigated the neurochemical profiles (receptor binding affinities and functional activities) of lurasidone in comparisons with typical and atypical antipsychotics under clinical use. Secondly, based on the assumption from in vitro profiles that lurasidone should have antipsychotic, anxiolytic, and antidepressant-like effects without causing central nervous system (CNS) adverse effects, we have chosen appropriate animal models and compared behavioral pharmacological characteristics of lurasidone with those of other antipsychotics.
Methods

Animals

Male Sprague-Dawley (SD) rats (Japan SLC Inc., Shizuoka, Japan or Charles River Laboratories Japan Inc., Yokohama, Japan), male Wistar rats (Charles River Laboratories Japan Inc.), male Lister hooded rats (Japan Lab Animals Co., Ltd., Osaka, Japan), male ddY mice and male Hartley guinea pigs (Japan SLC Inc.) were used. All animals were housed in a controlled environment (23 ± 2°C, 55 ± 10% humidity) with a 12-h light/dark cycle (light on at 8:00 AM). They had free access to food and water unless specified. All procedures related to housing conditions and cares complied with the institutional guidelines of Dainippon Sumitomo Pharma Drug Research Division.

Drugs

The following compounds were prepared by Dainippon Sumitomo Pharma: lurasidone, haloperidol, risperidone, olanzapine, clozapine, methamphetamine hydrochloride (MAP). Other agents were purchased as listed below; spiperone, 8-OH-DPAT (8-Hydroxy-2-(di-N-propylamino)tetralin) hydrobromide, ketanserin tartrate salt, serotonin (5-HT) hydrochloride, prazosin hydrochloride, WB4101 (2-[2-(2,6-Dimethoxyphenoxy)ethylaminomethyl]-1,4-benzodioxane), triprolidine hydrochloride, oxotremorine sesquifumarate, p-chloroamphetamine hydrochloride (p-CAMP), imipramine (Sigma-Aldrich Inc., St Louis, MO, USA), tryptamine hydrochloride (TRY), L-noradrenaline (Nacalai Tesque, Kyoto, Japan) and apomorphine hydrochloride (APO; Sandoz, Bazel, Switzerland or Sigma-Aldrich). [3H]Radioligands were purchased from GE Healthcare UK (Buckinghamshire, UK) or Perkin Elmer (Waltham, MA, USA). Human recombinant 5-HT$_7$, α$_{2A}$ and α$_{2C}$ receptors were purchased from Perkin Elmer. Lurasidone, risperidone, olanzapine, clozapine, haloperidol and imipramine were suspended in 0.5% methylcellulose solution and given orally, and other compounds were dissolved in saline. In in vitro experiments, compounds were dissolved in dimethylsulfoxide (DMSO, Nacalai Tesque).
**In vitro binding experiments**

Binding assays were carried out using standard protocols (Hirose et al., 1990; Kato et al., 1990). Assay conditions, membrane fractions, radioligands and displacers are summarized in Table 1. An IC₅₀ value of the drug, which inhibits specific binding of the ligand by 50%, was calculated from Hill plot analysis. The $K_i$ values were calculated by the following equation $K_i = IC_{50}/(1+S/K_d)$, where $S$ is the radioligand concentration used in the assay and $K_d$ is the dissociation constant.

**In vitro Functional Studies**

**³⁵S-GTPγS binding experiments for dopamine D₂ and 5-HT₁A receptors**

³⁵S-GTPγS binding experiments for the human dopamine D₂L or 5-HT₁A receptors stably expressed in the membranes of recombinant Chinese hamster ovary (CHO) cells were performed following the methods of Yabuuchi et al. (2004) with slight modifications. Shortly, after dopamine (or serotonin) and/or lurasidone was incubated for 20 min at room temperature with the cell membrane preparation containing ³⁵S-GTPγS (0.05 nM for D₂L or 0.2 nM for 5-HT₁A), the membranes were filtered through glass filters and the radioactivity bound to each filters was measured with a liquid scintillation counter. For non-specific binding, cold GTPγS (20 μM for D₂L or 10 μM for 5-HT₁A) was added together with ³⁵S-GTPγS. $K_b$ value for inhibition of dopamine-stimulated ³⁵S-GTPγS binding by lurasidone was calculated according to Cheng-Prusoff equation: $K_b = IC_{50}$ of lurasidone $/(1 + (agonist/EC_{50}))$, where agonist is the concentration of dopamine, and EC₅₀ is the EC₅₀ of dopamine alone. The maximum activity of lurasidone in stimulating [³⁵S]-GTPγS binding were calculated using the “Dx calculation (logistic curve fitting)” method in SAS Application for Preclinical Study Version 5.0.

**cAMP accumulation assay for 5-HT₇ receptor**

Cell lines with stable expression of recombinant human 5-HT₇ receptors (CHO/h5-HT₇) were incubated in a 96-well-plate at a density of 1 x 10⁴ cells/well for 24 hours and then
washed with PBS, the assay buffer (Hanks buffer, 20 mM HEPES (pH7.4); 1 mM ascorbic acid and 1 mM 3-isobutyl-1-methylxanthine) were added. After preincubation at 37 °C for 15 min, the cells were incubated with test drugs and the assay buffer at 37 °C for 30 min. Accumulation of the intracellular cAMP was measured with the cAMP HiRange kit (Cisbio bioassays). $K_B$ value was calculated according to the Cheng-Prusoff equation (see above).

**Levels of dopamine and metabolites**

SD rats were sacrificed by decapitation 2 hr after oral administration of test drugs and brain tissues were dissected on ice. The levels of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in brain tissues were determined with the high performance liquid chromatography with electrochemical detection (HPLC-ECD) system as described previously (Tatsuno et al., 1989).

**Behavioral Tests That Predict Antipsychotic Efficacy**

Most in vivo experiments were performed according to the methods of Hirose et al. (1990).

**Effects on MAP-induced hyperactivity in rats**

SD rats were individually isolated in clear plastic cages and injected with MAP (1 mg/kg, i.p.) 1 hr after the administration of drugs or vehicle. In the test of persistence of the effect, lurasidone was administered 1, 2, 4 and 8 hr before the MAP injection. Locomotor activity was measured for 80 min from 10 min after MAP injection. Four or five groups of six to thirteen rats were used to calculate the $ED_{50}$ value which inhibits the MAP-induced hyperactivity by 50% of animals tested.

**Effects on APO-induced stereotyped behavior in rats.**

SD rats were individually placed in stainless-steel mesh cages and given APO (1.25 mg/kg, i.v.) 1 hr after the administration of study drugs. Inhibition of APO-induced stereotypy
was judged to be positive unless stereotyped licking or biting was observed for 30 min after APO injection. Three or four groups of six to twelve rats were used to calculate the ED$_{50}$ value which inhibits the APO-induced stereotyped behavior in 50% of animals tested.

**Effects on APO-induced climbing behavior in mice**

Mice were placed in cylindrical cages made of stainless-steel rods. APO (1 mg/kg, s.c.) was administered at 1 hr after the administration of drugs or vehicle. The climbing behavior was observed for 20 minutes from 10 minutes after APO administration, and was scored by means of the 4 point-ranked scale as follows; 0: absent; 1: slight; 2: moderate; 3: pronounced. Four or five groups of five mice were used to calculate the ED$_{50}$ value which reduces score of the APO-induced climbing behavior by 50%.

**Effects on conditioned avoidance response (CAR) in rats**

SD rats were trained (6.5 min $\times$ 13 trials) once daily using a two-compartment shuttle-box (Medical Agent, Kyoto, Japan) to learn CAR. In each trial, rats were given a 5 seconds-warning sound and light stimulus (conditioned stimulus, CS) followed by a 5 seconds-electroshock (unconditioned stimulus, US) via a metal grid of the cage floor. CAR refers to the movement of rats to the other compartment during the CS-US interval (5 sec) in order to avoid the electroshock. Escape response refers as the movement of rats to the other compartment during the US. The number of CAR was measured in 10 trials excluding the first 3 trials from each day’s training (13 trials), and only animals that showed a CAR in at least 9 of the 10 trials were subjected for the drug testing. In the experiment, CAR was measured on 2 consecutive days, and the values on the first day used as the control. On the second day, animals were administered drugs at 1 hr before the assessment of CAR. Inhibition of the conditioned avoidance or escape response was expressed as a percentage of the control response. Three to five groups of seven to sixteen rats were used to calculate the ED$_{50}$ value, which reduces the number of CAR by 50%. Animals were tested repeatedly with a washout period of one week.
**Effect on TRY-induced forepaw clonic seizure**

SD rats were placed individually in clear plastic cages. TRY (40 mg/kg, i.v.) was given at 1 hr after the drugs administration. If no clonic seizure developed during 5 minutes immediately after administration of TRY, inhibition of TRY-induced seizure was judged as positive. Three groups of six rats were used to calculate the ED$_{50}$ value, which inhibits TRY-induced seizure in 50% of animals.

**Effect on p-CAMP-induced hyperthermia in rats**

SD rats were given with drug or vehicle, and p-CAMP (4mg/kg, s.c.) simultaneously, and the increase in rectal temperature between before and 1 hr after p-CAMP administration was measured (Sensortek; Model BAT-12). Four or Five groups of ten rats were used to calculate the ED$_{50}$ value, which inhibits the p-CAMP-induced hyperthermia by 50%.

**Behavioral Tests For Anxiolytic- and Antidepressant-like Efficacy**

**Vogel’s conflict test**

This experiment was performed according to the method of Shimizu et al. (1987). SD rats were deprived of water for 24 hr prior to the first training session. The animals showing 300-400 licks or more in the 3 min-training session were selected and subjected to a further 24 hr-water deprivation. The second session (pre-drug session) consisting of a 3 min period automatically started after completion of the first 20 licks and the first mild electric shock (0.35 mA, 0.5 sec) was delivered. After every 20 licks, subsequent licking delivered the mild electric shock. Only animals showing less than 260 licks during pre-drug session were selected and subjected to the test session. Lurasidone was administered at 1 hr before the test session, and the numbers of shocks delivered were measured during the 3 min-test session. In addition, the effect of lurasidone on spontaneous drinking behavior (number of licks) was also examined in a separate experiment.
Social interaction test

The experiments were performed according to the methods of Sakamoto et al. (1998). For 2 days before test session, Lister-hooded rats were individually housed and fully accustomed to handling. On the day of test session, two rats, previously unexposed to each other, were simultaneously treated with lurasidone. One hour after the administration, each pair was placed in a gray PVC observation chamber (50×50×35 cm) with 16.6 cm × 16.6 cm areas marked on the floor, and the social interactions of rats were recorded on a video tape recorder for 10 min. During the experiment, the illumination intensity of the floor of the chamber was maintained at about 1,200 lux using a white light and temperature inside the chamber was maintained constant using a fan. Measurement of social behavior recorded on tapes was performed manually on a later day. As the indicators of the spontaneous locomotor activity, the number of times the animals crossed over the lines marked on the floor (line crossing) and the number of rearing behavior were also counted. In this model, we have observed that the standard anxiolytic diazepam (5 mg/kg, p.o.) significantly increased the social interaction time (Sakamoto et al., 1998).

Olfactory bulbectomy model

Wistar rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic instrument (Narishige). The olfactory bulbs were removed using a blunt needle and a vacuum pump, according to the methods reported by Kelly et al. (1997). Sham-operated rats received similar surgical operation without removal of the olfactory bulb. Seven days after the operation, the rats were randomly assigned to the treatment groups according to the hyperemotionality score (Kelly et al., 1997), and then lurasidone (3 mg/kg, 2 weeks), imipramine (10 mg/kg, 1 week) or vehicle were repeatedly administered once a day. After the treatment, the rats were placed on the open field, and the locomotor activities were evaluated by counting the line crossed for 5 min.
Behavioral Tests That Predict EPS Liability

Catalepsy test

SD rats or mice were used. One hour after the administration of drugs, forepaws of animals were placed on a stainless-steel bar. If the animals keep this position for 30 seconds or longer, catalepsy was judged to be positive. Three to five groups of six rats or ten mice were used to calculate the ED$_{50}$ value which induces catalepsy in 50% of animals tested.

Pole test

The experiments were performed according to the method of Ohno et al. (1994). Mice were administered with drugs or vehicle. One hour after the administration, mice were placed head-upward at the top of a wooden pole. Then, the time to descend to the floor ($T_{total}$) was measured. The maximum observation period was 90 sec. The minimum effective dose (MED) to increase the $T_{total}$ values was determined using three to five groups of fourteen to thirty mice.

Paw test

The experiments were performed according to the method of Ellenbroek et al. (1987). Wistar rats were administered with drugs or vehicle. One hour after the administration, the four paws of each animal were placed in the holes on the surface of paw test platform. The latency for rats to withdraw their forepaws (Forepaw Retraction Time, FRT) was measured. The MED which significantly prolongs the FRT was determined using five groups of twelve rats.

Other CNS Actions

Ptosis

Mice were given drugs or vehicle. One hour later, ptosis was scored as follows: 4; complete closure, 3; marked closure, 2; moderate closure, 1; slight closure, 0; absent. Ptosis was judged to be positive when the mean score of both eyes was more than 2. Three or four
groups of ten mice were used to determine the ED$_{50}$ value which induced ptosis in 50% of animals.

**Oxotremorine-induced tremor**

Mice were placed individually, and oxotremorine (0.5 mg/kg, s.c.) was injected 1 hr after the administration of drugs or vehicle. Tremor which was observed for 10 min after oxotremorine injection was scored as follows: 2; pronounced, 1; moderate and 0; absent. Three or four groups of ten mice were used to determine the ED$_{50}$ value required to reduce the tremor score by 50%.

**Potentiation of hexobarbital-induced anesthesia**

Mice were given drugs or the vehicle. One hour later, hexobarbital (70 mg/kg, i.p.) was administered and the duration of the loss of righting reflexes was measured. If the duration was twice or more the mean duration of the vehicle group, potentiation of hexobarbital-induced anesthesia was judged to be positive. From the incidence rate of the enhancement at each dose, the ED$_{50}$ which enhances the anesthesia in 50% of animals was calculated using four or five groups of nine or ten mice. The maximum observation period was set at 3 hours.

**Muscle relaxation**

Mice were given drugs or vehicle. After 1 hr, the forepaws were placed on a wire stretched horizontally at a height of 30 cm. Three trials were performed sequentially and if the mouse did not place the hindpaws on the wire within 15 seconds in 2 trials or more, muscle relaxation was judged to be positive. Three or four groups of ten mice were used to determine the ED$_{50}$ value, which induces muscle relaxation in 50% of animals.

**Effect on motor coordination**

One day before the experiment, mice were placed on a rota-rod (a 3 cm-diameter rod
supported horizontally and rotated at 6 rpm) with their head against the direction of rotation.

Mice which could stay on the rod for 2 minutes or more were selected. On the day of the experiment, these mice were subjected to the same test, and only those which could stay on the rod for 1 minute or more were used. One hour after the administration of drugs or vehicle, each mouse was placed on the rota-rod again. If the mouse did not stay on the rod for 1 minute or more, inhibition of motor coordination was judged to be positive. Three or four groups of ten mice were used to determine the ED$_{50}$ value, which inhibits the incidence rate of motor coordination in 50% of animals.

**Statistical Analysis**

Data are expressed as mean ± SEM, MED, or ED$_{50}$ values with 95% confidence limits. The ED$_{50}$ values were calculated by the method of Litchfield and Wilcoxon (1949). To assess the significance in differences among the multiple groups, one-way ANOVA followed by Dunnett’s multiple comparison test (for a parametric analysis) or Kruskal-Wallis followed by Steel’s multiple comparison test (for a nonparametric analysis) were used.
Results

In vitro receptor binding profile

As shown in Table 2, in vitro receptor binding experiments revealed that lurasidone demonstrates affinity for dopamine D₂ and 5-HT₂A receptors higher than other tested to antipsychotics. In contrast to these other agents, lurasidone also displayed high affinity for 5-HT₇, 5-HT₁A and noradrenaline α₂C receptors (Ki values, 0.495, 6.75 and 10.8 nM, respectively).

Lurasidone had lower affinity for noradrenergic α₁ and α₂A receptors (Ki values, 47.9 and 40.7 nM, respectively), and only negligible affinities for histamine H₁ and muscarinic receptors (IC₅₀ values >1000nM). In contrast, olanzapine and clozapine showed high affinities for α₁, H₁ and muscarinic receptors, and risperidone showed potent affinities for α₁ and H₁ receptors. Haloperidol also displayed a marked affinity for the α₁ receptor.

Relative potency ratio of Ki values to dopamine D₂ receptors, which is one of the main targets of antipsychotic effects, are indicated in the parenthesis in Table 2. This ratio suggests that lurasidone primarily acts at 5-HT₇, 5-HT₂A and 5-HT₁A receptors in addition to dopamine D₂ receptor. Clozapine has shown relative potency ratio at 5-HT₁A, 5-HT₂A and 5-HT₇ receptors similar to lurasidone. Lurasidone has higher affinity (generally both absolute and relative to D₂) than risperidone, olanzapine, clozapine or haloperidol at 5-HT₇, 5-HT₁A and α₂C receptor types.

Lurasidone had only weak affinity for dopamine D₁ and 5-HT₂C receptors (Ki, 262 and 415 nM, respectively), and negligible affinity for 19 other receptor or 2 uptake sites including 5-HT₃, 5-HT₄, noradrenaline β₁, β₂, adenosine A₁, A₂, cholecystokinin CCKₐ, CCKₐ, γ-aminobutyric acid (GABA)ₐ, glutamate, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate, N-methyl-D-aspartate (NMDA), benzodiazepine, nicotine, opiate, sigma, L-type Ca²⁺ channel, N-type Ca²⁺ channel, 5-HT uptake site and dopamine uptake site (data not shown).

In vitro Functional Studies
In order to evaluate the intrinsic activities of lurasidone for dopamine D₂, 5-HT₇ and 5-HT₁A receptors, the effects of lurasidone on the intracellular signal in the recombinant receptors were investigated (Fig. 2). Lurasidone did not increase ³⁵S-GTPγS binding to the membrane preparations for dopamine D₂ receptors by itself (data not shown), but antagonized dopamine-stimulated ³⁵S-GTPγS binding in concentration-dependent manner with $K_B$ value of $2.8 \pm 1.1$ nM. Lurasidone also did not affect intracellular cAMP accumulation in CHO/h5-HT7 cells alone (data not shown), but antagonized 5-HT-stimulated cAMP accumulation in the cells with a $K_B$ value of $2.6 \pm 0.6$ nM. However, lurasidone partially stimulated ³⁵S-GTPγS binding to the membrane preparation for human 5-HT₁A receptors with a maximum effect of 33 % (versus 10 μM 5-HT).

**Dopamine turnover in frontal cortex and striatum in rats**

The effect of lurasidone on dopamine turnover, which is defined as a ratio of a dopamine metabolite to dopamine, in rat frontal cortex and striatum was compared with that of haloperidol or clozapine (Fig. 3). Lurasidone dose-dependently increased the ratio of DOPAC/dopamine in both regions, but it showed a preferential effect on the frontal cortex compared with the striatum, especially at higher doses. Clozapine also showed a similar tendency. On the other hand, haloperidol induced a preferential effect on the striatum, as compared with lurasidone (Fig. 3).

**Behavioral studies**

**Evidence of antipsychotic mechanisms**

Lurasidone and other antipsychotics dose-dependently inhibited dopamine D₂ receptor-mediated behaviors such as MAP-induced hyperactivity in rats, APO-induced stereotyped behavior in rats and APO-induced climbing behavior in mice (Table 3). In these experiments, lurasidone (ED₅₀ values; 2.3 to 5.0 mg/kg) showed a comparable potency to olanzapine (ED₅₀ values; 1.1 to 5.1 mg/kg), and higher than clozapine (ED₅₀; 9.5 to 290 mg/kg), but slightly lower potency than haloperidol (ED₅₀ values; 0.44 to 1.7 mg/kg).
Risperidone showed more potent efficacy in APO-induced climbing in mice, compared with lurasidone, equivalent potency in MAP-induced hyperactivity test and less potent efficacy in APO-induced stereotyped behavior test. The inhibitory actions of lurasidone on MAP-induced hyperactivity persisted for over 8 hr, and the ED\textsubscript{50} values of the action at 1, 2, 4 and 8 hr after the treatment were 2.3, 0.87, 1.6 and 5.0 mg/kg, respectively (data not shown).

Lurasidone (1 to 10 mg/kg) dose-dependently inhibited CAR in rats and the ED\textsubscript{50} values were 6.3 mg/kg (Table 3). In contrast, the inhibitory effects of lurasidone on escape behavior at these doses were weak (data not shown), indicating that the drug selectively inhibited CAR. The potency order of the CAR test of the drugs tested in the present study is consistent with that of the D\textsubscript{2} receptor-mediated behavior test; lurasidone showed slightly lower potency than haloperidol and risperidone, but higher than clozapine.

Lurasidone dose-dependently inhibited TRY-induced forepaw clonic seizure and \(p\)-CAMP-induced hyperthermia with the ED\textsubscript{50} values of 5.6 and 3.0 mg/kg, respectively (Table 3). Risperidone, olanzapine or clozapine also showed dose-dependent inhibitory effects in these tests. The potency of antiserotonergic activity of lurasidone was higher than that of haloperidol, but slightly lower than that of risperidone or olanzapine.

**Anxiolytic- and Antidepressant-like actions**

**Vogel’s conflict test**

Lurasidone (0.3 to 30 mg/kg) dose-dependently and significantly increased the number of shocks received by rats in the Vogel’s conflict test with MED of 10 mg/kg (\(p<0.01\), Fig. 4A). Lurasidone (3 to 100 mg/kg) did not affect the spontaneous water-drinking in a separate experiment without shocks (data not shown).

**Social interaction test**

Lurasidone (0.1 to 6 mg/kg) prolonged the time spent in social interaction, and this prolongation was significant at 1 and 3 mg/kg (\(p<0.01\) and \(p<0.05\), respectively) as compared with the control group (Fig. 4B). Lurasidone did not affect the spontaneous
locomotor activity at doses used in this test (data not shown).

**Olfactory bulbectomy model**

Olfactory bulbectomy model in rats is known as a useful animal model of depression. In the present study, olfactory bulbectomy treatment increased average number of line cross per 5 min compared with sham operated rats. Chronic treatment with an antidepressant imipramine (10 mg/kg, 1 week) significantly suppressed olfactory bulbectomy-induced hyperactivity ($p<0.01$, Fig. 5A). As with imipramine, chronic treatment of lurasidone (3 mg/kg, 2 week) also significantly suppressed the hyperactivity behavior ($p<0.001$, Fig. 5B). Neither treatment affected the line cross in sham operated-rats (Fig. 5).

**EPS Liability**

**Catalepsy test**

Lurasidone at doses up to 1000 mg/kg did not induce catalepsy either in rats or mice (Table 4). In contrast, haloperidol, risperidone and olanzapine dose-dependently induced catalepsy and potencies of catalepsy induction of these drugs were much higher than that of lurasidone. Clozapine induced practically no catalepsy at doses up to 300 mg/kg in rats, but higher doses were unable to be tested due to marked muscle relaxation. Similarly, higher doses of olanzapine (>10 mg/kg) and clozapine (>30 mg/kg) were unable to be tested in mice for the same reason.

**Induction of bradykinesia (pole test)**

We have previously shown that antipsychotics specifically induce bradykinesia as indexed by prolongation of the pole-descending time of mice in the pole test, and that these indices are well correlated with propensity of EPS (Ohno et al., 1994). As shown in Table 4, lurasidone showed no significant effects on the pole-descending time at doses up to 1000 mg/kg. Risperidone (0.3–10 mg/kg), olanzapine (3–10 mg/kg) and haloperidol (0.3–10 mg/kg) increased the time in a dose-dependent manner with MEDs of 3, 10 and 1 mg/kg,
respectively. Clozapine did not prolong the descending time at doses up to 30 mg/kg, but
could not be evaluated at higher doses due to severe muscle relaxation and impaired motor
coordination.

**Induction of muscle rigidity (paw test)**

Forepaw retraction time (FRT) in the rat paw test is thought to be a useful index for
prediction of EPS risk of antipsychotics (Ellenbroek BA et al., 1987). Lurasidone had no
effect on FRT at doses up to 300 mg/kg in the paw test. At dose of 1000 mg/kg lurasidone
slightly increased FRT, but the effect was not statistically significant. In contrast, risperidone
(1–30 mg/kg), olanzapine (3–100 mg/kg), clozapine (10–300 mg/kg) and haloperidol (1–30
mg/kg) prolonged FRT in a dose-dependent manner, and MEDs of these antipsychotics are
indicated in Table 4.

**Safety ratio**

Table 4 also summarizes the potency ratio of EPS signs evaluated here to that of D₂
antagonism (i.e., inhibition of MAP hyperactivity in rats or that of APO climbing behavior in
mice). The ratio of lurasidone was calculated to more than 430 in rats and 240 in mice, but
the ratio of the other antipsychotics was between 1.1 and 21 (except for unfixed values).

**Other CNS actions**

**Potentiation of hexobarbital-induced anesthesia**

Lurasidone at relatively high doses (700 to 1000 mg/kg) slightly prolonged the
duration of loss of righting reflexes elicited by hexobarbital (anesthesia) in a dose-dependent
manner. However, even at a dose of 1000 mg/kg, only 4 out of 10 mice showed an
anesthesia duration at least twice or more the mean duration of the control group (ED₅₀,
>1000 mg/kg; Table 5). Other antipsychotics also potentiated the anesthesia time at even
relatively low doses and their ED₅₀ values were 1.5 mg/kg for risperidone, 8.3 mg/kg for
olanzapine, 8.2 mg/kg for clozapine and 11 mg/kg for haloperidol (Table 5).
Induction of muscle relaxation

Lurasidone failed to induce muscle relaxation even at a dose of 1000 mg/kg (one positive out of 10 mice; ED$_{50}$ value, > 1000 mg/kg; Table 5). However, other antipsychotics dose-dependently induced muscle relaxation in mice; the ED$_{50}$ values were 11 mg/kg for risperidone, 11 mg/kg for olanzapine, 32 mg/kg for clozapine and 33 mg/kg for haloperidol (Table 5).

Impairment of motor coordination

Lurasidone impaired motor coordination at relatively high doses (100 to 700 mg/kg) with an ED$_{50}$ value of 250 mg/kg (Table 5). Risperidone, olanzapine, clozapine and haloperidol impaired motor coordination with ED$_{50}$ values of 1.8 mg/kg, 5.2 mg/kg, 8.7 mg/kg and 2.7 mg/kg, respectively (Table 5).

Anti-noradrenergic action

In order to evaluate the noradrenaline $\alpha_1$-blocking action of lurasidone, we examined ptosis induction in mice. Lurasidone at relatively higher doses (100 to 1000 mg/kg) induced ptosis in a dose-dependent manner. Ptosis was observed in 5 out of 10 mice tested at 1000 mg/kg of lurasidone, and then the ED$_{50}$ value is estimated to approximately 1000 mg/kg (Table 5). In contrast, risperidone, olanzapine and haloperidol significantly induced ptosis with ED$_{50}$ values of 0.68, 9.2 and 6.3 mg/kg, respectively (Table 5).

Anticholinergic action

Lurasidone did not inhibit oxotremorine-induced tremor even at a dose of 1000 mg/kg (Table 5). On the contrary, olanzapine significantly inhibited oxotremorine-induced tremor with the ED$_{50}$ value of 4.9 mg/kg. Haloperidol also inhibited the tremor induced by oxotremorine, but its potency was relatively low (ED$_{50}$ value, 23 mg/kg).
Discussion

The present study evaluated the receptor binding affinities, functional activities, and behavioral pharmacological characteristics of lurasidone, in various animal models. The current results demonstrate that lurasidone possesses potent antipsychotic, anxiolytic- and antidepressant-like activity, with a low propensity for EPS, motor impairment and CNS depressant side effects.

In vitro receptor binding experiments in the present study has shown Ki values in typical and atypical antipsychotics, which were comparable to those in the previous reports (e.g., Kroeze et al., 2003). The present study reveals that lurasidone has potent affinity for dopamine D_2 and 5-HT_2A receptors, however, unlike most second generation antipsychotics, completely lacks binding affinity for histamine H_1 and muscarinic receptors. Olanzapine, risperidone and clozapine have higher affinity for the histamine H_1 receptor, and olanzapine and clozapine have significant binding affinity for the muscarinic receptor. Histamine H_1 receptor blockade has been implicated as a key mechanism in weight gain induced by second generation antipsychotics (Kroeze et al., 2003). Muscarinic M_1 receptor blockade is associated with cognitive deficits (Terry et al., 2007), which may be especially severe in vulnerable populations such as the elderly. Furthermore, lurasidone also has weak binding affinity for the 5-HT_2C receptor which has been implicated in weight gain caused by atypical antipsychotics (Reynolds et al., 2006). Results from recent clinical trials have confirmed that lurasidone has minimal effects on weight (Meyer et al., 2009).

Lurasidone possesses potent 5-HT_7 and 5-HT_1A receptor binding affinities. Functional activity studies suggest that lurasidone acts as an antagonist at 5-HT_7 receptors and a partial agonist at 5-HT_1A receptors. 5-HT_7 receptors are highly expressed in the hippocampus, which is involved in learning and memory, and selective 5-HT_7 antagonists such as SB-269970 improved reference memory in animals (Gasbarri et al., 2008). Indeed, we previously demonstrated that lurasidone markedly reversed MK-801-induced memory dysfunction in the rat passive avoidance model (Ishiyama et al., 2007), Morris water maze test and radial maze test (Enomoto et al., 2008). Since the efficacy of lurasidone for
procognitive effects in these tests appears greater than the other antipsychotics, it is possible that lurasidone may have procognitive effects in patients with schizophrenia. Contrary to the above speculation, there are other studies suggesting that the function of 5-HT7 receptor is essential in contextual fear learning (Roberts et al., 2004) or allocentric spatial memory information processing (Sarkisyan and Hedlund, 2009) in mice. Although these studies demonstrated that 5-HT7 receptor gene knockout or 5-HT7 receptor antagonist action has potential to impair some cognitive function in mice, the evidence does not exclude the possibility that 5-HT7 receptor antagonist action becomes to be beneficial for cognitive function that is originally impaired by the disease or by drug such as MK-801, as previously reported (Meneses, 2004). Furthermore, lurasidone has binding activities not only for 5-HT7 receptor but also for 5-HT1A and noradrenaline α2C receptors, which have also been implicated as potentially relevant to cognitive function (Meltzer et al., 2003; Björklund M et al., 2000). Clinical studies are currently underway to characterize the capacity of lurasidone to enhance cognition in patients with schizophrenia.

The present study has performed a series of behavioral pharmacological experiments that is well-established to investigate on in vivo D2 or 5-HT2A receptor blocking activities. All of antipsychotics such as haloperidol, risperidone, olanzapine, and clozapine investigated in this study were found to be effective in some or all of these tests with ED50 values that were comparable to those as previously shown (e.g., Moore et al., 1992, Rigdon et al., 1996). Lurasidone potently inhibited the dopamine D2 receptor-mediated behaviors, i.e., MAP-induced hyperactivity in rats, and APO-induced stereotyped behavior in rats and climbing behavior in mice, with ED50 values of about 2 to 5 mg/kg. The potency of these inhibitory actions of lurasidone ranged from equivalence to that of olanzapine to one-tenth that of haloperidol. In addition, in the CAR test in rats, considered to be a good model for predicting clinical antipsychotic efficacy (Arnt, 1982), lurasidone selectively inhibited the CAR at a dose at which no inhibitory effects were seen on the escape response. These findings suggest that lurasidone could improve the symptoms of schizophrenia, especially the positive symptoms, through blocking dopamine D2 receptors, similar to marketed antipsychotics.
The other cardinal characteristic of second generation antipsychotic drugs is antagonist activity at 5-HT\textsubscript{2A} receptors. Lurasidone showed potent binding affinity for 5-HT\textsubscript{2A} receptors, and inhibited the 5-HT\textsubscript{2A} receptor-mediated behavior at doses equivalent to those required for antidopaminergic actions. In behavioral studies, lurasidone showed the inhibitory effects of 5-hydroxytryptophan-induced wet dog shake and 5-methoxytryptamine-induced head twitch with ED\textsubscript{50} values of 2.4 and 3.4 mg/kg, respectively (data on file). These findings indicate that lurasidone has antiserotonergic activity via blockade of 5-HT\textsubscript{2A} receptors.

In addition to the above evidence indicating antipsychotic mechanisms including dopamine D\textsubscript{2} and serotonin 5-HT\textsubscript{2A} blocking actions \textit{in vivo}, we have previously shown that lurasidone exerted the effects to improve cognitive deficits induced by an N-methyl-d-aspartate (NMDA) receptor antagonist MK-801 in rats (Ishiyama et al., 2007, Enomoto et al., 2008). Such evidence may imply that lurasidone also has the potential to treat symptoms in schizophrenia through targeting the pathophysiology such as NMDA receptor hypofunction. Regardless the mechanisms, randomized, double-blind, placebo-controlled studies (Nakamura et al., 2009; Meyer et al., 2009) have provided the consistent results that lurasidone had significant efficacy in treating both the positive and negative symptoms of chronic schizophrenia.

In the present study, we have investigated the antidepressant-like effect of lurasidone by using olfactory bulbectomy model. The previous studies using preclinical animal models to detect antidepressant-like effects in atypical antipsychotics have been challenging, while atypical antipsychotics are known to be clinically useful for treating symptoms in patients with major depression or bipolar depression. Indeed, a previous report using learned helplessness paradigm (Ballard et al., 2007) demonstrated that haloperidol, risperidone, olanzapine, and aripiprazole aggravated depressive behavior, whereas quetiapine showed no effect. As a result, it has been proposed that the learned helplessness paradigm may be a model to evaluate antipsychotic-induced dysphoria but not to evaluate the antidepressant effects in antipsychotics. Other study (Bourin et al., 2009) also showed that single treatment with aripiprazole was not effective to reduce immobility in the forced swimming test, although
co-administration of SSRI with aripiprazole was found to be effective. Therefore, in this study, rather than using the above paradigms, we have tried to use olfactory bulbectomy as another useful animal model to explore antidepressant-like effects, and found that lurasidone demonstrated the similar effects like an antidepressant imipramine. Although the present study suggests that lurasidone has an antidepressant-like property, the study is only explorative and further preclinical and clinical studies are apparently needed to confirm if lurasidone is truly useful for treating depressive symptoms in patients with psychiatric conditions.

In addition to the antidepressant-like potential, the present study also demonstrated that lurasidone possessed anxiolytic potential in Vogel’s conflict test and the social interaction test. The mechanism underlying the positive results in these behavioral paradigms is unclear. One possibility is lurasidone’s potent inhibitory effect on the 5-HT7 receptor. Recently, 5-HT7 antagonists have received attention as a new target for antidepressant and anxiolytic drugs (Hedlund and Sutcliffe, 2004). 5-HT7 knockout mice exhibit antidepressant-like behavior in the forced swim test and the tail suspension test (Hedlund et al., 2005). Furthermore, selective 5-HT7 antagonists such as SB-269970 show antidepressant- and anxiolytic-like actions, e.g., decrease of immobility time in the tail suspension test, the forced swim test and the Vogel conflict test (Wesolowska et al., 2006). Since the 5-HT7 receptor is highly expressed in the hippocampus and amygdala, which are involved in anxious states (Hedlund and Sutcliffe, 2004), lurasidone might produce its anxiolytic-like actions by modulating the neural activity in these regions via blockade of 5-HT7 receptors. Biochemical studies have shown that the 5-HT7 receptor is involved in 5-HT release in the dorsal raphe which is important in the indirect control of serotonergic neurons via the GABA<sub>A</sub> receptor (Roberts et al., 2004). Since detailed mechanisms of the anxiolytic- and antidepressant-like actions of 5-HT7 antagonists are still unclear, further investigation is needed.

Another possible mechanism is agonist activity at the 5-HT<sub>1A</sub> receptor, which is well-known to possess anxiolytic- and antidepressant-like activity in rodents and humans (Millan,
2000). Previous studies (e.g., Santarelli et al., 2003) have also suggested that serotonin 5-HT$_{1A}$ receptor activation mediates the effects of selective serotonin reuptake inhibitor (SSRI) on the hippocampus neurogenesis, which is considered to underlie the anxiolytic and antidepressant-like effects of chronic administration with SSRI. As mentioned above, lurasidone is a partial agonist at the 5-HT$_{1A}$ receptor with an Emax value of 33% of 5-HT. Thus, it is possible that partial activation of 5-HT$_{1A}$ receptor directly by lurasidone induces acute symptomatic or neuroplasticity effects to lead to the anxiolytic and antidepressant-like effects. Finally, the $\alpha_{2C}$ receptor has also reported to be involved in depression (Sallinen et al., 2007), and may contribute to the effects of lurasidone in animal models of depression.

EPS continues to be a clinically important problem in the treatment of schizophrenia and is an important cause of reduced patient compliance with therapy (Barnes et al., 1998). In the present study, lurasidone did not induce EPS in various animal behavioral tests at doses up to 1000 mg/kg. In contrast, haloperidol and risperidone induced EPS at relatively low doses. Furthermore, compared to various other antipsychotic drugs, lurasidone showed a markedly higher safety ratio, calculated as the potency ratio of ED$_{50}$ values for dopamine D$_2$ blocking activity relative to EPS induction. This suggests that lurasidone may have a low risk for EPS.

The precise mechanism for the minimal EPS liability of lurasidone in rodents is not clear. Since 5-HT$_{2A}$ blocking activity might reduce EPS induced by antipsychotics (Bersani et al., 1990; Ohno et al., 1994), one possibility is that the 5-HT$_{2A}$ receptor blocking action of lurasidone is implicated. However, the 5-HT$_{2A}$ receptor blocking activity of lurasidone is less potent than that of risperidone or olanzapine, whereas the cataleptogenic activities of these drugs are much higher than that of lurasidone, suggesting that other mechanisms are also involved. It is also possible that 5-HT$_{1A}$ receptor activity may contribute to the low EPS risk. Studies have clearly demonstrated that 5-HT$_{1A}$ receptor activation attenuate EPS induced by dopamine D$_2$ blockade (Millan, 2000).

In addition to the low risk of EPS, the CNS depressive effects of lurasidone, including potentiation of anesthesia, muscle relaxation, ptosis and inhibition of motor coordination,
were much weaker than those of the other antipsychotics tested. These behavioral changes are presumably mediated by blocking of histamine H₁ receptor or α₁ receptor (Skibell et al., 2007), and these results are in agreement with in vitro receptor binding profiles that lurasidone has relatively low affinity for noradrenergic α₁ receptor or negligible affinity for histamine H₁ receptor. These findings suggested that lurasidone would have fewer risks of cardiovascular side effects such as orthostatic hypotension and CNS depressant side effects such as sedation or somnolence. In addition, lurasidone showed only a negligible action in inhibiting oxotremorine-induced tremor in mice (ED₅₀ >1000 mg/kg), indicating that lurasidone does not induce anticholinergic side effects such as amnesia or dry mouth.

In summary, the present preclinical results demonstrate that lurasidone has pharmacological characteristics of a novel second generation antipsychotic agent with anxiolytic- and antidepressant-like activity, and a more favorable safety profile compared to several existing antipsychotics. In behavioral tests, lurasidone produces potent antipsychotic effects, with few EPS and CNS depressive side effects, and minimal α₁ blocking and muscarinic acetylcholine blocking actions. Furthermore, its in vitro binding profile suggests that lurasidone will have a lower propensity for weight gain and metabolic dysfunction, while having the potential to improve cognitive deficits associated with schizophrenia.
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Disclosure/Conflict of interest

All authors were full-time employees of Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan, when this study was conducted.
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Footnotes

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Portions of these data were presented: Ishibashi T et al. (2002) Receptor Binding Characteristics of SM-13496, a novel atypical antipsychotic agent. *Annual Meeting of the Society for Neuroscience*; 2002 Nov 3-6; Orlando FL, Society for Neuroscience, Washington, DC; and cited in a review paper (Meyer et al., 2009).

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Current Affiliation (MN): Faculty of Pharmaceutical Sciences, Setsunan University, Osaka, Japan
Legend of figures

**Fig. 1** Chemical structure of lurasidone.

**Fig. 2** Functional activity of lurasidone for dopamine D\(_{2L}\), 5-HT\(_7\) and 5-HT\(_{1A}\) receptors. **A.** Antagonism of dopamine (3 μM)-stimulated \(^{35}\)S-GTP\(_{\gamma}\)S binding at human dopamine D\(_{2L}\) receptor. \(^{35}\)S-GTP\(_{\gamma}\)S binding is expressed as a percentage of stimulation of dopamine. **B.** Antagonism of 5-HT (100 nM)-stimulated cAMP accumulation in the CHO/h5-HT\(_7\) cells. Result is expressed as a percentage of stimulation of 5-HT. **C.** Agonism of lurasidone at human 5-HT\(_{1A}\) receptors. Results is expressed as a percentage of stimulation of 5-HT at 10 μM. Each point shown indicates the mean of three to four independent experiments, and error bar indicates standard error of the mean (SEM).

**Fig. 3** Effect of lurasidone and other antipsychotics on dopamine turnover in rats: comparison in the frontal cortex and striatum. The ratios of DOPAC, a dopamine metabolite, to dopamine (DA) after administration of lurasidone, haloperidol and clozapine are represented as the points (open circles - frontal cortex, and closed circles - striatum) with vertical bars (mean ± SEM of 6 rats). **P<0.01; significantly different from vehicle control (Dunnett’s test). **P<0.05, **P<0.01; significantly different from the value in the striatum (Student’s t-test).

**Fig. 4** Anxiolytic-like activities of lurasidone in A) Vogel conflict test, B) Social interaction test. **A.** Effect on number of shocks in Vogel’s test. Each column shows mean ± SEM of 11-22 rats. **B.** Effect on social interaction in Lister hooded rats. Each column represents mean ± SEM of 10 rats. *P<0.05, **P<0.01; significantly different from vehicle group (Dunnett’s test).

**Fig. 5** Effect of lurasidone on olfactory bulbectomy-induced hyperactivity. Repeated treatment of imipramine (10 mg/kg/day, p.o., 1 week) significantly reduced olfactory bulbectomy-induced hyperactivity, whereas this treatment did not affect the activity in sham-operated rats (A). Similar efficacy was seen in the repeated treatment of lurasidone (3 mg/kg/day, p.o., 2 weeks; (B)). Each column represents mean ± SEM of 7-14 rats. **P<0.01, ###P<0.001; significantly different from vehicle treatment in sham-operated rat (Student’s t-test). **P<0.01, ###P<0.001; significantly different from vehicle treatment in olfactory
bulbectomy rat (Student's t-test).
### Table 1 Conditions for receptor binding assays

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Preparation</th>
<th>$^3$H-Ligand (nM)</th>
<th>Displacer (µM)</th>
<th>Incubation temperature and time</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine D$_2$</td>
<td>Rat Striatum</td>
<td>$^3$H-spiperone (0.5nM)</td>
<td>Spiperone (10µM)</td>
<td>37°C, 10 min</td>
<td>Hirose et al., 1990</td>
</tr>
<tr>
<td>5-HT$_{1A}$</td>
<td>Rat Hippocampus</td>
<td>$^3$H-8-OH-DPAT (0.15nM)</td>
<td>8-OH-DPAT (1µM)</td>
<td>25°C, 30 min</td>
<td>Kato et al., 1990</td>
</tr>
<tr>
<td>5-HT$_{2A}$</td>
<td>Rat Cortex</td>
<td>$^3$H-ketanserin (1nM)</td>
<td>Ketanserin (10µM)</td>
<td>37°C, 30 min</td>
<td>Hirose et al., 1990</td>
</tr>
<tr>
<td>5-HT$_7$</td>
<td>Human recombinant</td>
<td>$^3$H-5-CT (1nM)</td>
<td>5-HT (1µM)</td>
<td>r.t., 2 hr</td>
<td>To et al., 1995</td>
</tr>
<tr>
<td>Noradrenaline α$_1$</td>
<td>Rat Cortex</td>
<td>$^3$H-prazosin (0.5nM)</td>
<td>Prazosin (1µM)</td>
<td>25°C, 30min</td>
<td>Kato et al., 1990</td>
</tr>
<tr>
<td>Noradrenaline α$_{2A}$</td>
<td>Human recombinant</td>
<td>$^3$H-MK-912 (0.7nM)</td>
<td>WB4101 (10µM)</td>
<td>27°C, 60min</td>
<td>Uhlén et al., 1998</td>
</tr>
<tr>
<td>Noradrenaline α$_{2C}$</td>
<td>Human recombinant</td>
<td>$^3$H-MK-912 (0.2nM)</td>
<td>WB4101 (10µM)</td>
<td>27°C, 60min</td>
<td>Uhlén et al., 1998</td>
</tr>
<tr>
<td>Histamine H$_1$</td>
<td>Guinea pig Whole brain</td>
<td>$^3$H-Pyrilamine (0.4nM)</td>
<td>Triprolidine (2µM)</td>
<td>25°C, 40 min</td>
<td>Chang et al., 1979</td>
</tr>
<tr>
<td>Muscarinic</td>
<td>Rat Cortex</td>
<td>$^3$H-QNB (0.15nM)</td>
<td>Oxotremorine (100µM)</td>
<td>25°C, 60 min</td>
<td>Yamamura et al., 1974</td>
</tr>
</tbody>
</table>

r.t.; room temperature, 5-CT; 5-carboxamidotryptamine, MK-912; (2S,12bS)-1',3'-Dimethylspiro[1,3,4,5',6,6',7,12b-octahydro-2H-benzo[b]furo[2,3-a]quinolizine-2,4'-pyrimidin]-2'-one, QNB; Quinuclidinyl benzilate
Table 2 Comparison of receptor binding profiles between lurasidone and other antipsychotic agents

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Binding Affinity, $K_i$ (nM)$^a$</th>
<th>Lurasidone</th>
<th>Risperidone</th>
<th>Olanzapine</th>
<th>Clozapine</th>
<th>Haloperidol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine $D_2$</td>
<td></td>
<td>1.68 ± 0.09</td>
<td>2.91 ± 0.16</td>
<td>14.4 ± 3.2</td>
<td>108 ± 27</td>
<td>3.28 ± 0.42</td>
</tr>
<tr>
<td>5-HT$_{1A}$</td>
<td></td>
<td>6.75 ± 0.97 (4.0)</td>
<td>262 ± 21 (90)</td>
<td>&gt;1000$^b$ (&gt;69)</td>
<td>123 ± 5 (1.1)</td>
<td>&gt;1000$^b$ (&gt;300)</td>
</tr>
<tr>
<td>5-HT$_{2A}$</td>
<td></td>
<td>2.03 ± 0.46 (1.2)</td>
<td>0.205 ± 0.066 (0.070)</td>
<td>5.78 ± 0.89 (0.40)</td>
<td>9.17 ± 1.46 (0.085)</td>
<td>84.7 ± 13.1 (26)</td>
</tr>
<tr>
<td>5-HT$_7$</td>
<td></td>
<td>0.495 ± 0.090 (0.29)</td>
<td>2.72 ± 0.42 (0.93)</td>
<td>n.t.</td>
<td>42.2 ± 12.0 (0.39)</td>
<td>&gt;1000$^b$ (&gt;300)</td>
</tr>
<tr>
<td>Noradrenaline $\alpha_1$</td>
<td></td>
<td>47.9 ± 7.8 (29)</td>
<td>1.42 ± 0.09 (0.49)</td>
<td>22.1 ± 7.7 (1.5)</td>
<td>17.5 ± 5.0 (0.16)</td>
<td>17.9 ± 1.5 (5.5)</td>
</tr>
<tr>
<td>Noradrenaline $\alpha_{2A}$</td>
<td></td>
<td>40.7 ± 7.7 (24)</td>
<td>13.7 ± 1.1 (4.7)</td>
<td>n.t.</td>
<td>147 ± 14 (1.4)</td>
<td>&gt;1000$^b$ (&gt;300)</td>
</tr>
<tr>
<td>Noradrenaline $\alpha_{2C}$</td>
<td></td>
<td>10.8 ± 0.64 (6.4)</td>
<td>11.0 ± 1.4 (3.8)</td>
<td>n.t.</td>
<td>15.6 ± 2.0 (0.14)</td>
<td>&gt;1000$^b$ (&gt;300)</td>
</tr>
<tr>
<td>Histamine $H_1$</td>
<td></td>
<td>&gt;1000$^b$ (&gt;590)</td>
<td>3.46 ± 0.17 (1.2)</td>
<td>3.83 ± 0.52 (0.27)</td>
<td>2.02 ± 0.20 (0.019)</td>
<td>330 ± 22 (100)</td>
</tr>
<tr>
<td>Muscarinic</td>
<td></td>
<td>&gt;1000$^b$ (&gt;590)</td>
<td>&gt;1000$^b$ (&gt;340)</td>
<td>7.6 ± 1.3 (0.53)</td>
<td>4.9 ± 2.0 (0.045)</td>
<td>&gt;1000$^b$ (&gt;300)</td>
</tr>
</tbody>
</table>

Values are means ± SEM of three or more separate experiments. n.t.; not tested.

$^a$ Each number in the parentheses is indicated as relative potency ratio of $K_i$ value of receptors to that of dopamine $D_2$ receptor.

$^b$ IC$_{50}$ value
**Table 3** Antipsychotic actions of lurasidone and other antipsychotics

<table>
<thead>
<tr>
<th>Drugs</th>
<th>MAP-induced hyperactivity in rats</th>
<th>APO-induced stereotyped behavior in rats</th>
<th>APO-induced climbing behavior in mice</th>
<th>Conditioned avoidance response in rats</th>
<th>TRY-induced clonic seizure</th>
<th>pCAMP-induced hyperthermia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lurasidone</td>
<td>2.3 (0.89–6.1)</td>
<td>5.0 (3.6–6.9)</td>
<td>4.1 (2.0–8.4)</td>
<td>6.3 (3.4–12)</td>
<td>5.6 (3.4–9.3)</td>
<td>3.0 (1.5–5.8)</td>
</tr>
<tr>
<td>Risperidone</td>
<td>1.8 (0.86–3.6)</td>
<td>11 (6.2–18)</td>
<td>0.14 (0.047–0.40)</td>
<td>1.5 (0.89–2.4)</td>
<td>0.16 (0.044–0.62)</td>
<td>0.098 (0.039–0.25)</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>3.3 (1.5–7.3)</td>
<td>5.1 (2.6–10)</td>
<td>1.1 (0.35–3.2)</td>
<td>n.t.</td>
<td>1.4 (0.59–3.3)</td>
<td>0.62 (0.31–1.2)</td>
</tr>
<tr>
<td>Clozapine</td>
<td>65 (29–140)</td>
<td>290 (170–480)</td>
<td>9.5 (3.8–24)</td>
<td>38 (17–83)</td>
<td>5.1 (2.6–10)</td>
<td>5.0 (2.7–9.5)</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>0.88 (0.42–1.8)</td>
<td>1.7 (1.2–2.5)</td>
<td>0.44 (0.20–1.0)</td>
<td>0.89 (0.48–1.7)</td>
<td>14 (6.8–27)</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>

ED\textsubscript{50} values and 95% confidence limits in parenthesis were obtained 1 hr after drug administration. n.t.; not tested.
Table 4 EPS liability of lurasidone and other antipsychotics

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Catalysis test in rats</th>
<th>Paw test in rats</th>
<th>Catalysis test in mice</th>
<th>Pole test in mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED_{50} (mg/kg)</td>
<td>MED (mg/kg)</td>
<td>ED_{50} (mg/kg)</td>
<td>MED (mg/kg)</td>
</tr>
<tr>
<td></td>
<td>Ratio^a</td>
<td>Ratio^a</td>
<td>Ratio^a</td>
<td>Ratio^a</td>
</tr>
<tr>
<td>Lurasidone</td>
<td>&gt;1000</td>
<td>&gt;430</td>
<td>&gt;1000</td>
<td>&gt;430</td>
</tr>
<tr>
<td>Risperidone</td>
<td>20 (9.3–43)</td>
<td>11</td>
<td>30</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.85 (0.44–1.6)</td>
<td></td>
<td>6.1</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>28 (11–74)</td>
<td>8.5</td>
<td>30</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.6</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Clozapine</td>
<td>&gt;300</td>
<td>&gt;4.6</td>
<td>300</td>
<td>&gt;30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.6</td>
<td></td>
<td>&gt;3.1</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>12 (6.8–23)</td>
<td>14</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.0 (1.1–3.5)</td>
</tr>
</tbody>
</table>

ED_{50} values and 95% confidence limits in parenthesis or MED values were obtained 1 hr after drug administration.

^a Ratio is calculated as the potency ratio of EPS measure to that of D_2 antagonism (i.e., inhibition of MAP hyperactivity in rats and of APO climbing behavior in mice).
### Table 5 CNS depressive actions of lurasidone and other antipsychotics in mice

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Potentiation of hexobarbital-induced anesthesia</th>
<th>Muscle relaxation</th>
<th>Impairment of motor coordination</th>
<th>Ptosis in mice</th>
<th>Inhibition of oxotremoline-induced tremor in mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lurasidone</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>250 (150–430)</td>
<td>ca. 1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Risperidone</td>
<td>1.5 (0.67–3.3)</td>
<td>11 (6.8–19)</td>
<td>1.8 (1.1–3.0)</td>
<td>0.68 (0.31–24)</td>
<td>n.t.</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>8.3 (3.8–18)</td>
<td>11 (6.8–19)</td>
<td>5.2 (2.9–9.3)</td>
<td>9.2 (5.5–15)</td>
<td>4.9 (3.0–7.8)</td>
</tr>
<tr>
<td>Clozapine</td>
<td>8.2 (4.9–14)</td>
<td>32 (20–50)</td>
<td>8.7 (4.7–16)</td>
<td>n.t.</td>
<td>n.t.</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>11 (5.8–20)</td>
<td>33 (26–43)</td>
<td>2.7 (1.4–5.2)</td>
<td>6.3 (3.5–11)</td>
<td>23 (10–52)</td>
</tr>
</tbody>
</table>

**ED\textsubscript{50}** values and 95% confidence limits in parenthesis were obtained 1 hr before drug administration.

n.t.; not tested

\(^a\) Data quoted from Hirose (1990)
Figure 1
Figure 2

A. % of dopamine (3μM) response vs. Lurasidone (nM).

B. % of 5-HT (100 nM) response vs. Lurasidone (nM).

C. % of maximal 5-HT response vs. Lurasidone (nM).
Figure 3

![Figure 3](image-url)

**Lurasidone**

**Clozapine**

**Haloperidol**

DOPAC/DA (% of control) vs. Dose (mg/kg) for Lurasidone, Clozapine, and Haloperidol.
**Figure 4**

A) No. of shocks received (3 min) vs. Lurasidone (mg/kg, p.o.)

B) Social interaction (seconds) vs. Lurasidone (mg/kg, p.o.)
Figure 5

A

B

average number of line crosses (number / 5 min)

Vehicle Imipramine Vehicle Imipramine
Sham-ope rat OB rat

Vehicle Lurasidone Vehicle Lurasidone
Sham-ope rat OB rat

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