# (*R*)-3′-(3-Methylbenzo[*b*]thiophen-5-yl)spiro[1-azabicyclo[2,2,2]octane-3,5′-oxazolidin]-2′-one, a Novel and Potent α7 Nicotinic Acetylcholine Receptor Partial Agonist Displays Cognitive Enhancing Properties

Ryo Tatsumi,* Masakazu Fujio, Shin-ichi Takanashi, Atsushi Numata, Jiro Katayama, Hiroyuki Satoh, Yasuyuki Shiigi, Jun-ichi Maeda, Makoto Kuriyama, Takashi Horikawa, Takahiro Murozono, Kenji Hashimoto,† and Hiroshi Tanaka

*Pharmaceuticals Research Unit, Research & Development Division, Mitsubishi Pharma Corporation, 1000 Kamoshida-cho, Aoba-ku, Yokohama, Kanagawa 227-0033, Japan*

*Received March 4, 2006*

Recent studies have suggested that the α7 nicotinic acetylcholine receptors play important roles in learning and memory. Herein, we describe our research of the structure−activity relationships (SAR) in a series of (*S*)-spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin]-2′-ones bearing various bicyclic moieties to discover novel α7 receptor agonists. Through a number of SAR studies on the series, we have found out that inhibition of CYP 2D6 isozyme, which was a primary obstacle for the previously identified compound, was avoidable by the introduction of bicyclic moieties. Chemical optimization of the series led to the identification of a novel and potent α7 nicotinic acetylcholine receptor partial agonist **23**. This compound not only possessed high binding affinity ($K_i$ = 3 nmol/L) toward the α7 receptor but also showed agonistic activity even at a concentration of 0.1 μmol/L. In addition, compound **23** improved cognition in several rat models, which might suggest the potential of the α7 receptor partial agonist for the treatment of neurological disorders including cognitive dysfunction.

## Introduction

Nicotinic acetylcholine receptors (nAChRs) belong to the superfamily of ligand-gated ion channels possessing a pentameric structure.[1,2] These receptors are composed of multiple subunits, which have been divided into muscle-type (α1, β1, δ, γ, and ε) and neuronal (α2−α10 and β2−β4) subunits.[3] Neuronal nAChRs are abundantly expressed in various central nervous systems (CNS) regions. Among them, the α7 receptors are highly expressed in brain regions such as the hippocampus, thalamus, and the prefrontal cortex.[4] The α7 receptors, which form homooligomeric receptors, are distinguished by their high permeability to $Ca^{2+}$, their affinity for the antagonists α-bungarotoxin (α-BTX) and methyllycaconitine (MLA), and more rapid desensitization rates than those of the muscle type and heteromeric neuronal α/β nAChRs.[5−8] Presynaptically, α7 receptors modulate transmitter release; postsynaptically, they are excitatory and generate depolarizing currents; and perisynaptically, they provide a neuromodulatory function.[9] It is also suggested that the α7 receptor has been implicated in several important biological activities including memory, attention, and information processing.[10,11]

Schizophrenic patients have an impaired ability to process sensory information. Abnormality in the P50 auditory-evoked potential gating is an endophenotype associated with schizophrenia. Biochemical and genetic studies have suggested that the α7 receptor is involved in this sensory gating deficit.[12,13] Additional evidence indicates the involvement of the α7 receptor in schizophrenia. Intracerebroventricular injections of α-BTX, the α7 receptor antagonist, induced a hippocampal auditory gating deficit.[14] Furthermore, recent studies also indicate a correlation between the α7 receptor and several aspects of schizophrenia,[15] and postmortem brain tissue from schizophrenic patients displayed diminished numbers of α7 receptors labeled by [$^{125}$I] α-BTX in the hippocampus.[16] In view of these findings, pharmacological agents that selectively activate α7 nAChRs have been proposed as potential new strategies to treat several neurological and psychiatric disorders including cognitive dysfunction.[17,18]

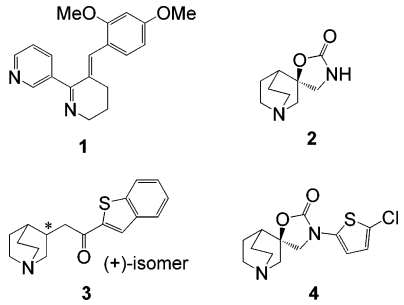



**Figure 1.** Structures of previously described α7 nicotinic acetylcholine receptor agonists and compounds identified in our research programs.

Several reports have been published and indicate the therapeutic potential of α7 nAChR ligands including 3-(2,4-dimethoxybenzylidene)-anabaseine (**1**) (GTS-21) and (*S*)-spiro-[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin]-2′-one (**2**) (AR-R-17779).[19−22] Interestingly, it also has been reported that a cognitive-enhancing property was observed in the rat social recognition test for **2**.[23] It supports the hypothesis that the α7 receptor agonists may represent new molecular targets for the treatment of several neurological and psychiatric disorders. Previous work performed in our laboratories led to the discovery of (+)-3-[2-(benzo[*b*]thiophen-2-yl)-2-oxoethyl]-1-azabicyclo-[2.2.2]octane (**3**)[24] and (*R*)-3′-(5-chlorothiophen-2-yl)spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin]-2′-one (**4**).[25] Compound **4** possesses a high affinity ($K_i$ = 9 nmol/L) toward the α7 receptor and showed promise as a potential candidate for further

* To whom correspondence should be addressed. Phone: +81-(0)45-963-4624. Fax: +81-(0)45-963-4211. E-mail: Tatsumi.Ryo@mg.m-pharma.co.jp.

† Present address: Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, 1-8-1 Inohana, Chuou-ku, Chiba 260-8670, Japan.

10.1021/jm060249c CCC: $33.50 
Published on Web 06/16/2006

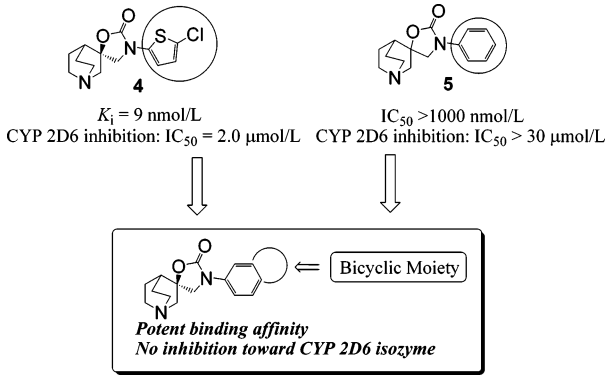
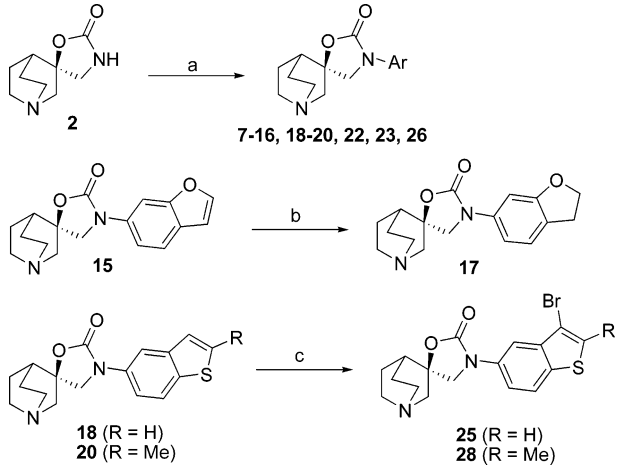



**Figure 2.** Synthetic strategy to discover a potent α7 agonist without CYP 2D6 inhibition.

**Scheme 1.** Synthesis of Oxazolidinone Derivatives with Various Bicyclic Moieties[a]




[a] Reagents: (a) Ar−Br, $K_2CO_3$, CuI, DMF. (b) 10% Pd−C, $H_2$, EtOH. (c) $Br_2$, AcOH, MeOH−$H_2O$.

development of its profiles. However, **4** also exhibited the potent cytochrome P450 2D6 isozyme (CYP 2D6) inhibition, which might cause the drug−drug interaction. The drug−drug interaction sometimes induces serious adverse reactions that should be avoided.

To avoid this problem, we envisioned the strategy shown in Figure 2. Although compound **4** bearing a thiophene ring possessed a potential for CYP 2D6 inhibition, no inhibition was observed in phenyl analogue **5**. In addition, our previous work demonstrated that fused bicyclic analogues (e.g., benzo[*b*]-thiophen-2-yl and 2-naphthyl as a bicyclic part in the previous article[24,25]) maintained affinity toward the α7 nAChR. These results prompted us to reduce CYP 2D6 inhibitory activity by changing the aromatic group into a fused bicyclic aromatic group. Herein, we describe the design, synthesis, and structure−activity relationships (SAR) of the series of compounds leading to the discovery of (*R*)-3′-(3-methylbenzo[*b*]thiophen-5-yl)spiro-[1-azabicyclo[2,2,2]-octane-3,5′-oxazolidin]-2′-one (**23**).

## Chemistry

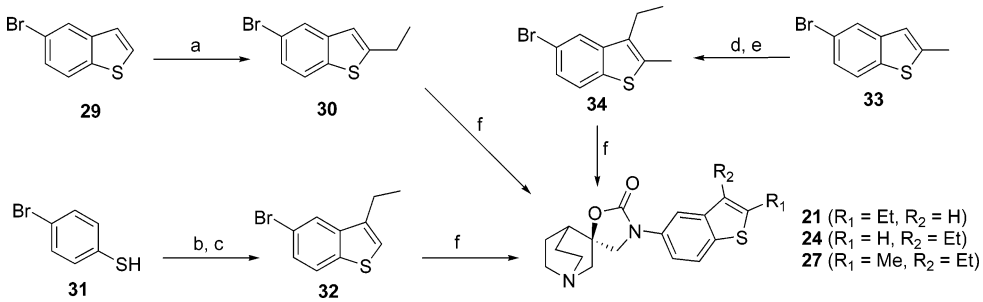
Most compounds were readily synthesized by the coupling of enantiomerically pure **2**, which was previously reported and established to obtain the enantiomerically pure **2**,[20] with halogenated compounds as shown in Scheme 1. In addition, our previous work revealed that the (*R*)-enantiomer was the eutomer for both α7 binding and agonistic properties. Therefore, we synthesized and evaluated (*R*)-enantiomers in this study. The coupling of **2** with various aryl bromides in the presence of potassium carbonate and copper (I) iodide gave compounds **7**−**16**, **18**−**20**, **22**, **23**, and **26** in moderate yields. Dihydrobenzo-[*b*]furan (**17**) was obtained by the hydrogenation of compound **15** with 10% palladium−carbon under a hydrogen atmosphere in good yield. The bromination of compounds **18** and **20** at the 3-position for benzo[*b*]thiophene ring was accomplished using bromine to give compounds **25** and **28**, respectively.

The synthesis of the oxazolidinone derivatives bearing the commercially unavailable benzo[*b*]thiophene moiety is outlined in Scheme 2. 5-Bromobenzo[*b*]thiophene (**29**) was treated with LDA at −78 °C followed by the reaction with ethyl iodide to give the 5-bromo-2-ethylbenzo[*b*]thiophene (**30**) in good yield. Compound **32** was prepared by the alkylation of 4-bromobenzenethiol (**31**) in the presence of potassium carbonate followed by cyclization under acidic condition.[26] 5-Bromo-2-methylbenzo[*b*]thiophene (**33**) was acetylated with acetic anhydride in the presence of aluminum chloride and then reduced under Wolff−Kishner conditions to afford compound **34**. The coupling of resulting 5-bromobenzo[*b*]thiophene derivatives **30**, **32**, and **34** with **2** gave compounds **21**, **24**, and **27** in the same procedure as that mentioned above.

## Results and Discussions

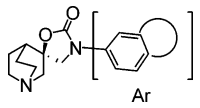
The compounds were evaluated for in vitro binding affinity toward the α7 nAChR and expressed as the $K_i$ value for the displacement of [$^{125}$I] α-BTX. The results are summarized in Table 1, together with the $IC_{50}$ values of CYP 2D6 inhibition. Previous efforts in our laboratories identified 5-halogenated thiophene analogues **4** and **6** with high binding affinity for the α7 receptor. As mentioned above, these compounds showed undesirable CYP 2D6 inhibition ($IC_{50}$ = 2.0 and 9.0 μmol/L, respectively). First we evaluated the compounds bearing a variety of bicyclic moieties (**7**−**18**). Overall, most compounds exhibited a reduced affinity toward the α7 receptor. In particular, compounds bearing the hydrophilic bicyclic moiety, such as compounds **8**, **11**, and **12**, diminished their binding affinity all

**Scheme 2.** Synthesis of Oxazolidinone Derivatives Bearing the Commercially Unavailable Benzo[*b*]thiophene Moiety[a]




[a] Reagents: (a) LDA, EtI, THF. (b) 1-bromobutan-2-one, $K_2CO_3$, DMF. (c) PPA, chlorobenzene. (d) $Ac_2O$, $AlCl_3$, $CH_2Cl_2$. (e) hydrazine, KOH, ethylene glycol. (f) **2**, $K_2CO_3$, CuI, DMF.

**Table 1.** Binding Affinities and CYP 2D6 Inhibitions of Oxazolidinone Derivatives with the Bicyclic Moiety



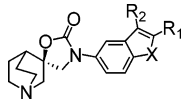

| compd. no. | Ar | α7[a] ($K_i$; nmol/L) | CYP 2D6[d] ($IC_{50}$; μmol/L) |
|---|---|---|---|
| **4** | 5-chlorothiophen-2-yl | 9[b] | 2.0 |
| **6** | 5-bromothiophen-2-yl | 4[b] | 9.0 |
| **7** | indan-5-yl | 200 | |
| **8** | quinolin-6-yl | >1000[c] | |
| **9** | 1,3-benzodioxol-5-yl | 37 | >30 |
| **10** | 2,3-dihydro-1,4-benzodioxin-6-yl | 175 | |
| **11** | 3-methyl-2-oxo-2,3-dihydro-1,3-benzoxazol-6-yl | >1000[c] | |
| **12** | 3-methyl-2-oxo-2,3-dihydro-1,3-benzothiazol-6-yl | >1000[c] | |
| **13** | 2-methylbenzothiazol-5-yl | 60 | |
| **14** | benzo[*b*]furan-5-yl | 19 | >30 |
| **15** | benzo[*b*]furan-6-yl | 50 | |
| **16** | 2,3-dihydrobenzo[*b*]furan-5-yl | 85 | |
| **17** | 2,3-dihydrobenzo[*b*]furan-6-yl | 410 | |
| **18** | benzo[*b*]thiophen-5-yl | 28 | >30 |

[a] α-Bungarotoxin binding. Tests were performed in two experiments in a way similar to that described in ref 25. Among the $K_i$ values in reproducibility runs, the assay showed less than 20% variability. [b] Previously reported in ref 25. [c] $IC_{50}$ value. [d] Data are shown as the mean of two experiments. A known inhibitor (quinidine) was used as the positive control.

together. However, compounds bearing the hydrophobic bicyclic moiety, such as compounds **14** and **18**, maintained their binding affinity. Although indan analogue **7** possessed a reduced binding affinity compared to that of compound **4**, it was still better than that of phenyl analogue **6**. There seems to be a trend that the α7 receptor allows bicyclic moieties containing one or two atoms such as oxygen or sulfur (compounds **9**, **14**−**18**). Of these compounds, a clear preference substitution of the 5-position over the 6-position of the benzo[*b*]furan ring (**14**−**17**) was seen, which demonstrates the predominance of the 5-position analogue to achieve α7 binding affinity. The introduction of the benzo[*b*]thiophen-5-yl group was also investigated and found to possess high affinity (**18**: $K_i$ = 28 nmol/L) comparable to that of compound **14** ($K_i$ = 19 nmol/L). Subsequently we also assessed the CYP 2D6 inhibition of a sample of compounds possessing high binding affinity for the α7 receptor. As has been suggested in the literature, it is desirable that the potential for drug−drug interactions of candidate compounds be minimized; compounds should not alter the metabolism of, or have their metabolism altered by, coadministered compounds.[27] As described earlier, compound **4** displayed potent CYP 2D6 inhibition, although no inhibition of CYP 1A2, 2C9, 2C19, and 3A4 isozymes was found (data not shown). Similarly, the 5-bromo derivative **6** also exhibited potent CYP 2D6 inhibition ($IC_{50}$ = 9.0 μmol/L). However, compounds **9**, **14**, and **18** possessing high affinities for the α7 receptor showed no inhibition of CYP 2D6 isozyme at the concentration of 30 μmol/L, supporting our original hypothesis.

The moderate consequence in α7 binding affinity and CYP 2D6 inhibition resulting from the introduction of a benzo[*b*]furan and a benzo[*b*]thiophene encouraged us to investigate the effect of a substituent on the ring system at the 2- and 3-position. The results are shown in Table 2. At first, we examined the effect of alkylation at the 2-position of benzo[*b*]furan and benzo[*b*]thiophene. Although 2-methylbenzo[*b*]furan analogue **19** reduced the binding affinity 2-fold relative to that of compound **14**, 2-methylbenzo[*b*]thiophene analogue **20** demonstrated 2-fold greater affinity than compound **18** (compounds **19** and **20**, $K_i$ = 38 and 16 nmol/L, respectively). Therefore, we focused on the modification of benzo[*b*]thiophene analogues because of their greater potential for α7 binding affinity. 2-Ethylbenzo[*b*]thiophene analogue **21** showed decreased binding affinity ($K_i$ = 135 nmol/L). This result suggests that there are some important steric factors to consider at the 2-position to achieve α7 binding affinity. Compound **22** displayed equal affinity for nonsubstituted compound **18** ($K_i$ = 34 nmol/L), suggesting that electronic factors play a marginal role in achieving α7 binding affinity.

**Table 2.** Binding Affinities and CYP 2D6 Inhibitions of Oxazolidinone Derivatives with Substituted Benzo[*b*]furan or Benzo[*b*]thiophene as the Bicyclic Aryl Moiety




| compd. no. | X | $R_1$ | $R_2$ | α7[a] ($K_i$; nmol/L) | CYP 2D6[b] ($IC_{50}$; μmol/L) |
|---|---|---|---|---|---|
| **14** | O | H | H | 19 | >30 |
| **18** | S | H | H | 28 | >30 |
| **19** | O | Me | H | 38 | >30 |
| **20** | S | Me | H | 16 | >30 |
| **21** | S | Et | H | 135 | >30 |
| **22** | S | Cl | H | 34 | >30 |
| **23** | S | H | Me | 3 | >30 |
| **24** | S | H | Et | 8 | 21 |
| **25** | S | H | Br | 4 | >30 |
| **26** | S | Me | Me | 4 | >30 |
| **27** | S | Me | Et | 15 | |
| **28** | S | Me | Br | 11 | |

[a] α-Bungarotoxin binding. Tests were performed in two experiments in a way similar to that described in ref 25. Among the $K_i$ values in reproducibility runs, the assay showed less than 20% variability. [b] Data are shown as the mean of two experiments. A known inhibitor (quinidine) was used as the positive control.

Next, we investigated the effect of a substituent at the 3-position on the benzo[*b*]thiophene ring. Employing the same method used to evaluate the 2-position, alkylation at the 3-position was examined. Interestingly, greatly enhanced affinities were observed in the 3-methyl and 3-ethyl derivatives (compounds **23** and **24**, $K_i$ = 3 and 8 nmol/L, respectively). Furthermore, it was also found that 3-bromo derivative **25** possessed equipotent binding affinity compared to that of alkylated compounds **23** and **24** ($K_i$ = 4 nmol/L). In addition, disubstituted benzo[*b*]thiophene derivatives were synthesized and evaluated for α7 binding affinities. The effect of substitution

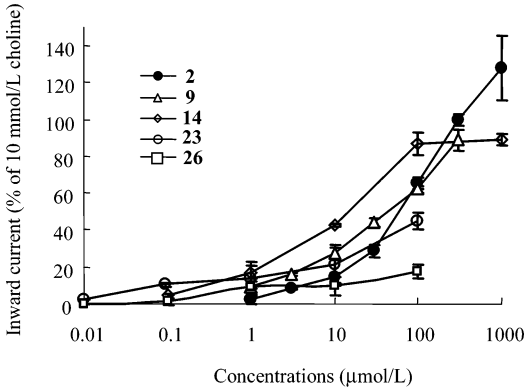



**Figure 3.** Agonistic activities of compounds **2**, **9**, **14**, **23**, and **26** on the α7 nicotinic receptor in cultured hippocampal neurons. Data are represented as described in ref 25.

at the 3-position was assessed, whereas the substituent at the 2-position was fixed as a methyl group (see compound **20**). As observed for compounds **26**−**28**, the introduction of methyl (**26**: $K_i$ = 4 nmol/L), ethyl (**27**: $K_i$ = 15 nmol/L), and bromo substituents (**28**: $K_i$ = 11 nmol/L) slightly enhanced or maintained the α7 binding affinities of 2-methyl derivative **20**. Overall, the introduction of a substituent to the 3-position in benzo[*b*]thiophene led to an enhancement of α7 binding affinity, although substitution at the 2-position resulted in maintained or reduced binding affinities. It was also found that disubstituted compounds **26**−**28** showed high or moderate affinities. In addition, a tolerance for substituents at the 3-position in the benzo[*b*]thiophene moiety was observed. This may explain the why α7 binding affinity differed from the 2-substituted series. Among these substituted benzo[*b*]thiophene derivatives **20**−**28**, 3-methyl benzo[*b*]thiophene derivatives **23** showed the highest affinity toward the α7 receptor. For compounds **19**−**26**, CYP 2D6 inhibition was not observed, except for compound **24** ($IC_{50}$ = 21 μmol/L). Furthermore, the most active compound **23** also showed no inhibition of CYP 1A2, 2C9, 2C19, and 3A4 isozymes (data not shown). These properties might negate the drug−drug interaction, which sometimes induces serious adverse reactions.

To evaluate the structural requirements for α7 receptor agonistic activities, compounds **9**, **14**, **23**, and **26** were selected for further evaluation. An electrophysiological measurement of the α7 receptor-mediated response was performed using cultured hippocampal neurons, in which the efficacy was assessed by the measurement of the relative inward current toward 10 mmol/L of choline as described in our previous article.[25] Figure 3 shows the agonistic activities for these compounds. The parental compound **2** was studied at 1, 3, 10, 30, 100, 300, and 1000 μmol/L, whereas our compounds **9**, **14**, **23**, and **26** were studied from 0.01 to 1000 μmol/L. At the concentrations studied, compound **2** displayed great potency in stimulating the relative inward current. The dose−response curve shown for compound **2** indicated that it is a full agonist. However, our synthesized compounds, 1,3-benzodioxol derivative **9** and benzo[*b*]furan-5-yl derivative **14**, also showed full agonistic activities. The efficacies shown in compound **9** were equipotent comparable to that of compound **2**, whereas compound **14** showed more potent activities than compound **2** ranging from 1 to 100 μmol/L. Interestingly, compound **23**, bearing the 3-methylbenzo[*b*]-thiophen-5-yl group, produced a partial agonistic concentration−response curve, although compounds **9** and **14** showed full agonistic activities. We noted that agonistic activity was observed even at a concentration of 0.1 μmol/L in compound **23**. We also observed that compound **26** showed weak agonistic activity relative to that of compound **23**, although these compounds possess the same aryl moiety. These results suggest that agonistic efficacy toward the α7 receptor could be tuned by modification of the aromatic group. We selected compound **23**, which showed moderate partial agonism, for further evaluations from the evidence shown in our previous article.[25] In our previous study, compound **4**, which also showed moderate partial agonistic activity, indicated a significant cognition-enhancing property. According to these results, we speculated that α7 receptor-mediated cognitive improvement does not particularly depend on efficacy, and the diminutive agonistic activity evoked by partial agonists may be sufficient to lead to cognitive enhancement. Although compound **23** has a lower maximum efficacy, it has the advantage of being active at lower concentrations, which is more therapeutically useful.

**Table 3.** Receptor Binding Profile of Compound **23**

| binding site | $K_i$ (nmol/L) | binding site | $K_i$ (nmol/L) |
|---|---|---|---|
| α7nAChR | 3 | D1 | >10000[a] |
| α4β2nAChR | >10000[a] | D2 | >10000[a] |
| muscarinic | >10000[a] | D3 | >10000[a] |
| 5-$HT_{1A}$ | >10000[a] | D4 | >10000[a] |
| 5-$HT_2$ | >10000[a] | histamine H1 | >10000[a] |
| 5-$HT_3$ | 10 | histamine H3 | >10000[a] |
| adrenergic α1 | >10000[a] | AMPA | >10000[a] |
| adrenergic α2 | >10000[a] | NMDA PCP site | >10000[a] |
| adrenergic β | >10000[a] | NMDA glycine site | >10000[a] |

[a] $IC_{50}$ value.

Furthermore, the receptor binding profiles of compound **23** were determined in several receptors. The results are shown in Table 3. No significant binding of compound **23** was detected at α4β2 nAChRs or muscarinic receptors. Moreover, compound **23** did not affect other known receptors, although binding affinity toward the 5-$HT_3$ receptor was observed ($K_i$ = 10 nmol/L). Because the neuronal nicotinic α7 receptor and the 5-$HT_3$ receptor are ligand-gated ion channels with a homologous topological organization,[28] the affinity for the 5-$HT_3$ receptor observed in this assessment was readily predictable. Although there are several reports regarding the cross-reactivity of various α7 and 5-$HT_3$ receptor ligands,[29] compound **23** identified from our research program is no exception. For the functional assay, our preliminary examination showed that compound **23** inhibited the 5-HT-induced contraction of ileum in guinea pigs indicating that it acts as an antagonist at the 5-$HT_3$ receptor (data not shown). From common findings that antiemetic drugs acting on the 5-$HT_3$ receptor would not have severe side effects toward neurological function, we think that the effect of the 5-$HT_3$ receptor in compound **23** might be permissible for further development.

Next, pharmacokinetic (PK) studies of compound **23** were carried out in rats and monkeys. The results are summarized in Table 4. The AUC and $C_{max}$ values for compound **23** were approximately dose-dependent in rats and monkeys with a moderate PK profile. Although the rat $T_{1/2}$ values were not noted, compound **23** produced a longer duration of plasma concentration (mean $T_{1/2}$ = 4.2−6.0 h) after oral dosing in monkeys than in rats. In addition, in the monkey, the oral bioavailability (BA) of compound **23** at a dose of 10 mg/kg was therapeutically sufficient (BA = 82.8%). For brain permeability, compound **23** also exhibited moderate values in rats ($C_B/C_P$ = 10.2).

To assess the effect of compound **23** on cognitive enhancement, auditory sensory gating was investigated in a rat model. The outline of this auditory sensory gating had been already published in our previous article and other articles.[25,30,31]

**Table 4.** Pharmacokinetic Profiles of Compound **23** for Rats and Monkeys

| species | dose (mg/kg) | AUC[a,e] (ng∗h/mL) | $C_{max}$[b,e] (ng/mL) | $T_{max}$[e] (h) | $T_{1/2}$[e] (h) | CL[e] (L/h/kg) | Vss[e] (L/kg) | BA[c,e] (%) | brain permeability[d,e] ($C_B/C_P$) |
|---|---|---|---|---|---|---|---|---|---|
| rat | 3 (i.v.) | 605 ± 63 | | | 3.3 ± 1.4 | 5.00 ± 0.49 | 7.59 ± 4.75 | | |
| | 10 (p.o.) | 225 ± 107 | 120 ± 79 | 0.44 ± 0.38 | 3.0 ± 1.6 | | | 10.5 ± 5.9 | 10.2 ± 2.9 |
| | 30 (p.o.) | 1010 ± 360 | 618 ± 187 | 0.38 ± 0.14 | 1.7 ± 0.5 | | | 17.3 ± 6.2 | |
| monkey | 3 (i.v.) | 732 ± 144 | 288 ± 55 | 0.36 ± 0.24 | 1.9 ± 0.2 | 4.21 ± 0.86 | 10.7 ± 1.9 | | |
| | 1 (p.o.) | 63.0 ± 7.4 | 9.10 ± 2.50 | 2.0 ± 1.0 | 4.2 ± 1.5 | | | 25.8 ± 3.1 | |
| | 3 (p.o.) | 387 ± 257 | 75.5 ± 45.8 | 3.3 ± 1.2 | 5.4 ± 6.1 | | | 57.2 ± 47.1 | |
| | 10 (p.o.) | 2020 ± 210 | 384 ± 123 | 2.3 ± 1.5 | 6.0 ± 0.5 | | | 82.8 ± 8.7 | |

[a] Integrated area under plasma concentration vs time curve from time 0 to time infinity. [b] Maximum plasma concentration after oral dosing. [c] Oral bioavailability. [d] $C_B/C_P$ = concentration in brain/concentration in plasma. Rats ($N = 3$) were given a single dose (10 mg/kg, p.o.) of compound **23**. The $C_B/C_P$ values were calculated 30 min after the administration of compound **23**. [e] Data are shown as mean ± SD.

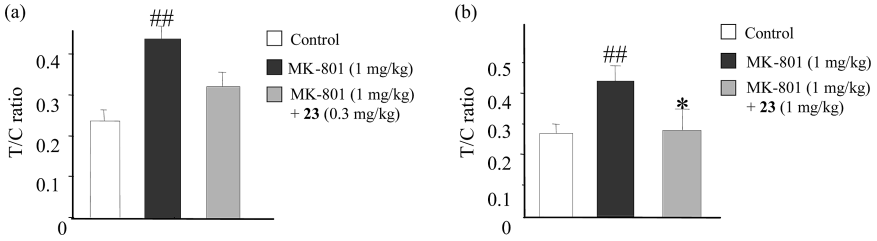



**Figure 4.** Effects of compound **23** on MK-801-induced sensory gating deficit in rats ($N = 5-6$) (*$p < 0.05$ vs MK-801, ##$p < 0.01$ vs control, paired *t*-test).

Because the atypical antipsychotic agent clozapine improved cognitive impairment from our previous findings (ref 25), the validity of this model is already established. The results are shown in Figure 4. The gating deficit was significantly induced by NMDA antagonist MK-801 in subcutaneous administration at a dose of 1 mg/kg. Compound **23** was assessed in subcutaneous administration at doses of 0.3 and 1 mg/kg. Although a dosing of 0.3 mg/kg did not show significant amelioration as shown in Figure 4a, compound **23** at a dose of 1 mg/kg improved the gating deficit with a significant difference ($p < 0.05$) as shown in Figure 4b. The results further support the hypothesis that the α7 receptor is crucially involved in the auditory sensory gating process, and the α7 receptor agonist might be a promising candidate for the treatment of cognitive dysfunction including auditory sensory gating deficit.

Microdialysis was used to measure the extracellular dopamine (DA) concentrations in the medial prefrontal cortex of male Wistar rats. It has been suggested that the deterioration of dopaminergic systems in the prefrontal cortex is responsible for the functional abnormalities of schizophrenia.[32] In support of this theory, clozapine, which has an atypical antipsychotic effect, showed significant DA release in the medial prefrontal cortex.[33] Consequently, it is readily assumed that useful antipsychotics accelerate DA release in the medial prefrontal cortex. The effects for compound **23** are shown in Figure 5. Compound **23** was orally administered at doses of 10 and 30 mg/kg, and DA release was measured every 20 min after dosing. It was observed that compound **23** at doses of 10 and 30 mg/kg rapidly and significantly increased the extracellular level of DA in the medial prefrontal cortex, although it was a transient effect. One possible explanation is that rapid $T_{max}$ (about 0.4 h) and short $T_{1/2}$ (1.7−3.0 h) values of compound **23** in rats (as shown in Table 4) caused the rapid onset and short duration of DA release. However, the involvement of the rapid desensitizing properties of α7 receptors is not ruled out at present.

Additional pharmacological evaluations in a rat model were performed in an 8-arm radial maze task to determine the properties of cognitive enhancement. In this model, we used scopolamine, which is the muscarinic acetylcholine antagonist, to cause the impairment of cognitive function. Figure 6 shows

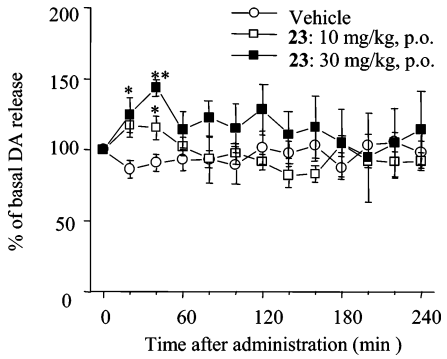



**Figure 5.** Effect of compound **23** on extracellular DA levels in the medial prefrontal cortex. Each symbol indicates the mean ± SEM of five experiments. Asterisks indicate significant changes relative to that of control values at the corresponding time point (*$p < 0.05$, **$p < 0.01$, Dunnett's multiple comparison test).

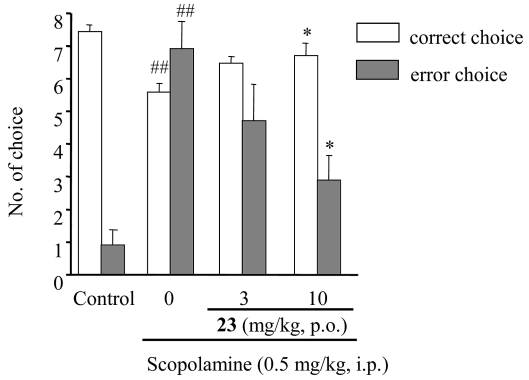



**Figure 6.** Effect of compound **23** on the scopolamine-induced spatial cognitive impairment in an 8-arm radial maze. (*$p < 0.05$ vs scopolamine, ##$p < 0.01$ vs control, Dunnett's multiple comparison test).

the results of this model. Scopolamine (0.5 mg/kg, i.p.) significantly decreased the correct choices and increased the number of errors. A single administration of compound **23** (3 and 10 mg/kg, p.o.) dose-dependently reversed the scopolamine-induced impairment of cognitive performance. At a dose of 10

mg/kg of compound **23**, significant ameliorations were observed in the number of correct and incorrect choices. These results suggest that compound **23** has therapeutic potential for the treatment of cognitive dysfunction, at least that caused by cholinergic dysfunction.

## Conclusion

In summary, a series of substituted-spiro[1-azabicyclo[2.2.2]-octane-3,5′-oxazolidin]-2′-ones was synthesized and evaluated for their α7 binding affinity. A preliminary analysis of structure−activity relationships using various bicyclic moieties identified compound **23** bearing the 3-methylbenzo[*b*]thiophen-5-yl group. It showed high binding affinity ($K_i$ = 3 nmol/L) and agonistic activity even at a concentration of 0.1 μmol/L with partial agonism. For the inhibition of cytochrome P450 including 2D6 isozyme, no inhibition was observed in compound **23**, which supported our original strategy. A pharmacokinetic assessment indicated moderate profiles of compound **23** in rats and monkeys. Studies in rat models of cognitive impairment indicated a promising effect of compound **23**. We conclude that the novel α7 nicotinic acetylcholine receptor agonist, compound **23**, is a promising drug candidate for the treatment of cognitive impairment associated with neurological disorders.

## Experimental Section

**Chemistry.** Melting points were determined in open capillary tubes with a Buchi 530 apparatus and are uncorrected. All $^1$H NMR spectra were recorded on a JEOL GSX-270 spectrometer (270 MHz) and a JEOL GSX-400 spectrometer (400 MHz), using tetramethylsilane as the internal standard. The following NMR abbreviations are used: br (broad), brs (broad singlet), s (singlet), d (doublet), dd (double doublet), dt (double triplet), t (triplet), q (quartet), and m (multiplet). Elemental analysis was performed on a Yanamoto CHN CORDER MT-6. Analytical results for all compounds were within ±0.4% of the theoretical values unless otherwise noted. Column chromatography was carried out on silica gel PSQ-100B (Fuji Silicia). Thin-layer chromatography (TLC) was performed using plates precoated with silica gel 60 F-254 (Merck). The abbreviations for solvents and reagents used are DMSO, dimethyl sulfoxide; DMF, *N*,*N*-dimethylformamide; IPE, diisopropyl ether; AcOEt, ethyl acetate; LDA, lithium diisopropylamide; THF, tertahydrofuran; and PPA, polyphosphoric acid. All yields were nonoptimized. The key intermediate **2** was synthesized according to published procedures.[20]

**(*R*)-3′-(Indan-5-yl)spiro[-1-azabicyclo[2.2.2]octane-3,5′-oxazolidin]-2′-one HCl (7).** A mixture of (*S*)-spiro[1-azabicyclo[2,2,2]-octane-3,5′-oxazolidin]-2′-one (**2**) (547 mg, 2.9 mmol), 5-bromoindan (1.2 g, 6.0 mmol), CuI (54 mg, 0.3 mmol), and $K_2CO_3$ (414 mg, 3.0 mmol) was heated at 130 °C. The resultant mixture was diluted with $CHCl_3$, washed with 10% $K_2CO_3$ aqueous solution, and dried over $K_2CO_3$. The organic solution was evaporated and purified by silica gel column chromatography (eluent: $CHCl_3$/MeOH = 4:1) to give compound **7**, which was then converted to the HCl salt (38 mg, 4%) as pale brown crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) δ 1.84−1.99 (m, 4H), 2.01−2.08 (m, 4H), 2.80−2.83 (m, 4H), 3.18−3.33 (m, 3H), 3.61−3.62 (m, 2H), 4.06−4.09 (m, 1H), 4.22−4.24 (m, 1H), 7.23 (d, *J* = 8 Hz, 1H), 7.29 (d, *J* = 8 Hz, 1H), 7.40 (s, 1H), 10.32 (brs, 1H). Anal. ($C_{18}H_{22}N_2O_2$·HCl·0.5$H_2O$), C, H, N.

**(*R*)-3′-(Quinolin-6-yl)spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin]-2′-one 3HCl (8).** This compound was prepared from compound **2** (550 mg, 3.0 mmol) and 6-bromoquinoline (1.6 g, 7.5 mmol) using the same procedure described for the preparation of compound **7** to yield compound **8**, which was then converted to the HCl salt (589 mg, 46%) as pale yellow crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) δ 1.76−1.96 (m, 3H), 2.05−2.15 (m, 1H), 2.45 (m, 1H), 3.22−3.39 (m, 4H), 3.60−3.75 (m, 2H), 4.28 (d, *J* = 10 Hz, 1H), 4.46 (d, *J* = 10 Hz, 1H), 7.89 (m, 1H), 8.16 (s, 1H), 8.33 (d, *J* = 9 Hz, 1H), 8.42 (d, *J* = 9 Hz, 1H), 8.84 (d, *J* = 9 Hz, 1H), 9.08 (d, *J* = 4 Hz, 1H), 10.93 (brs, 1H). Anal. ($C_{18}H_{19}N_3O_2$·3HCl·0.5$H_2O$), C, H, N.

**(*R*)-3′-(1,3-Benzodioxol-5-yl)spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin]-2′-one HCl (9).** This compound was prepared from compound **2** (550 mg, 3.0 mmol) and 5-bromobenzodioxol (1.5 g, 7.5 mmol) using the same procedure described for the preparation of compound **7** to yield compound **9**, which was then converted to the HCl salt (375 mg, 36%) as pale yellow crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) δ 1.70−1.88 (m, 3H), 2.04−2.06 (m, 1H), 2.36 (m, 1H), 3.02−3.40 (m, 4H), 3.58 (dd, *J* = 14 Hz, 25 Hz, 2H), 4.03 (d, *J* = 10 Hz, 1H), 4.18 (d, *J* = 9 Hz, 1H), 6.00 (s, 2H), 6.85 (dd, *J* = 3 Hz, 9 Hz, 1H), 6.93 (d, *J* = 8 Hz, 1H), 7.24 (d, *J* = 2 Hz, 1H), 10.74 (brs, 1H). Anal. ($C_{16}H_{18}N_2O_4$·HCl·0.25$H_2O$), C, H, N.

**(*R*)-3′-(2,3-Dihydro-1,4-benzodioxin-6-yl)spiro[1-azabicyclo-[2.2.2]octane-3,5′-oxazolidin]-2′-one HCl (10).** This compound was prepared from compound **2** (540 mg, 3.0 mmol) and 5-bromo-2,3-dihydro-1,4-benzodioxine (1.6 g, 7.5 mmol) using the same procedure described for the preparation of compound **7** to yield compound **10**, which was then converted to the HCl salt (17 mg, 2%) as brown crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) δ 1.75−1.92 (m, 3H), 2.04−2.10 (m, 1H), 2.37 (m, 1H), 3.10−3.38 (m, 4H), 3.61 (dd, *J* = 14 Hz, 22 Hz, 2H), 4.04 (d, *J* = 9 Hz, 1H), 4.18−4.28 (m, 5H), 6.88 (d, *J* = 9 Hz, 1H), 6.98 (dd, *J* = 3 Hz, 9 Hz, 1H), 7.09 (d, *J* = 3 Hz, 1H), 10.68 (brs, 1H). Anal. ($C_{17}H_{20}N_2O_4$·HCl·0.5$H_2O$), C, H, N.

**(*R*)-3′-(3-Methyl-2-oxo-2,3-dihydro-1,3-benzoxazol-6-yl)spiro-[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin]-2′-one HCl (11).** This compound was prepared from compound **2** (456 mg, 2.5 mmol) and 6-bromo-3-methyl-1,3-benzoxazol-2(3*H*)-one (1.5 g, 6.6 mmol) using the same procedure described for the preparation of compound **7** to yield compound **11**, which was then converted to the HCl salt (138 mg, 14%) as colorless crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) δ 1.80−1.85 (m, 3H), 1.90−1.93 (m, 1H), 3.19−3.20 (m, 3H), 3.34 (s, 3H), 3.58−3.64 (m, 4H), 4.10 (d, *J* = 8 Hz, 1H), 4.29 (d, *J* = 12 Hz, 1H), 7.29−7.34 (m, 2H), 7.63 (s, 1H). Anal. ($C_{17}H_{19}N_3O_4$·HCl·$H_2O$), C, H, N.

**(*R*)-3′-(3-Methyl-2-oxo-2,3-dihydro-1,3-benzothiazol-6-yl)-spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin]-2′-one HCl (12).** This compound was prepared from compound **2** (540 mg, 3.0 mmol) and 6-bromo-3-methyl-1,3-benzothiazol-2(3*H*)-one (1.7 g, 6.8 mmol) using the same procedure described for the preparation of compound **7** to yield compound **12**, which was then converted to the HCl salt (607 mg, 49%) as pale brown crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) δ 1.77−1.95 (m, 3H), 2.05−2.10 (m, 1H), 2.42 (m, 1H), 3.13−3.39 (m, 4H), 3.40 (s, 3H), 3.63 (dd, *J* = 14 Hz, 26 Hz, 2H), 4.12 (d, *J* = 10 Hz, 1H), 4.29 (d, *J* = 10 Hz, 1H), 7.37 (d, *J* = 9 Hz, 1H), 7.55 (dd, *J* = 2 Hz, 9 Hz, 1H), 7.88 (d, *J* = 2 Hz, 1H), 10.79 (brs, 1H). Anal. ($C_{17}H_{19}N_3O_3S$·HCl·0.5$H_2O$·0.5EtOH), C, H, N.

**(*R*)-3′-(2-Methylbenzothiazol-5-yl)spiro[1-azabicyclo[2.2.2]-octane-3,5′-oxazolidin]-2′-one 2HCl (13).** This compound was prepared from compound **2** (911 mg, 5.0 mmol) and 5-bromo-2-methylbenzothiazole (2.3 g, 10.0 mmol) using the same procedure described for the preparation of compound **7** to yield compound **13**, which was then converted to the HCl salt (193 mg, 12%) as yellow crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) δ 1.84−1.95 (m, 4H), 2.10−2.22 (m, 1H), 2.66 (s, 3H), 3.18−3.20 (m, 3H), 3.29−3.31 (m, 1H), 3.59−3.70 (m, 2H), 4.20 (d, *J* = 8 Hz, 1H), 4.37 (d, *J* = 12 Hz, 1H), 7.68 (d, *J* = 8 Hz, 1H), 8.03−8.05 (m, 2H). Anal. ($C_{17}H_{19}N_3O_2S$·2HCl·2$H_2O$), C, H, N.

**(*R*)-3′-(Benzo[*b*]furan-5-yl)spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin]-2′-one HCl (14).** This compound was prepared from compound **2** (730 mg, 4.0 mmol) and 5-bromobenzo[*b*]furan (2.0 g, 10.0 mmol) using the same procedure described for the preparation of compound **7** to yield compound **14**, which was then converted to the HCl salt (754 mg, 56%) as pale yellow crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) δ 1.76−1.92 (m, 3H), 2.05−2.15 (m, 1H), 2.40−2.45 (m, 1H), 3.10−3.35 (m, 4H), 3.56−3.70 (m, 2H), 4.17 (d, *J* = 9 Hz, 1H), 4.31 (d, *J* = 10 Hz, 1H), 6.99 (t,

$J$ = 2 Hz, 1H), 7.53 (dd, $J$ = 2 Hz, 9 Hz, 1H), 7.64 (d, $J$ = 9 Hz, 1H), 7.78 (d, $J$ = 2 Hz, 1H), 8.02 (d, $J$ = 2 Hz, 1H), 11.02 (brs, 1H). Anal. ($C_{17}H_{18}N_2O_3$·HCl), C, H, N.

**(*R*)-3′-(Benzo[*b*]furan-6-yl)spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin]-2′-one HCl (15).** This compound was prepared from compound **2** (360 mg, 2.0 mmol) and 6-bromo-benzo[*b*]furan (900 mg, 4.6 mmol) using the same procedure described for the preparation of compound **7** to yield compound **15**, which was then converted to the HCl salt (23 mg, 3%) as colorless crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.80−1.95 (m, 3H), 2.05−2.17 (m, 1H), 2.41−2.43 (m, 1H), 3.15−3.40 (m, 4H), 3.60−3.70 (m, 2H), 4.17 (d, $J$ = 10 Hz, 1H), 4.34 (d, $J$ = 10 Hz, 1H), 6.94 (t, $J$ = 1 Hz, 1H), 7.49 (dd, $J$ = 2 Hz, 8 Hz, 1H), 7.67 (d, $J$ = 9 Hz, 1H), 7.81 (s, 1H), 7.98 (d, $J$ = 2 Hz, 1H), 10.97 (s, 1H). Anal. ($C_{17}H_{18}N_2O_3$·HCl·0.5$H_2O$), C, H, N.

**(*R*)-3′-(2,3-Dihydrobenzo[*b*]furan-5-yl)spiro[1-azabicyclo[2.2.2]-octane-3,5′-oxazolidin]-2′-one HCl (16).** This compound was prepared from compound **2** (547 mg, 3.0 mmol) and 5-bromo-2,3-dihydrobenzo[*b*]furan (1.2 g, 6.0 mmol) using the same procedure described for the preparation of compound **7** to yield compound **16**, which was then converted to the HCl salt (489 mg, 48%) as brown crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.82−1.88 (m, 4H), 2.06−2.09 (m, 1H), 3.15−3.20 (m, 6H), 3.59−3.62 (m, 2H), 4.02−4.04 (m, 1H), 4.18−4.20 (m, 1H), 4.50−4.54 (m, 2H), 6.78 (d, $J$ = 8 Hz, 1H), 7.18 (dd, $J$ = 4 Hz, 8 Hz, 1H), 7.44(s, 1H), 10.19 (brs, 1H). Anal. ($C_{17}H_{20}N_2O_3$·HCl·0.25$H_2O$), C, H, N.

**(*R*)-3′-(2,3-Dihydrobenzo[*b*]furan-6-yl)spiro[1-azabicyclo[2.2.2]-octane-3,5′-oxazolidin]-2′-one HCl (17).** A solution of compound **15** (1.4 g, 4.2 mmol) in methanol (100 mL) was hydrogenated in the presence of 10%-Pd/C (700 mg) at 60 °C under 80 atm. After the reaction mixture was filtered with Celite, the solvent was removed under reduced pressure, and the resultant product was washed with ethanol to give compound **17** (1.0 g, 71%) as pale yellow crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.75−1.93 (m, 3H), 2.00−2.10 (m, 1H), 2.35−2.40 (m, 1H), 3.10−3.40 (m, 6H), 3.50−3.62 (m, 2H), 4.06 (d, $J$ = 9 Hz, 1H), 4.20 (d, $J$ = 9 Hz, 1H), 4.53 (t, $J$ = 8 Hz, 2H), 6.94 (d, $J$ = 7 Hz, 1H), 7.04 (s, 1H), 7.22 (d, $J$ = 8 Hz, 1H), 10.97 (brs, 1H). Anal. ($C_{17}H_{20}N_2O_3$·HCl·0.2$H_2O$), C, H, N.

**(*R*)-3′-(Benzo[*b*]thiophen-5-yl)spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin]-2′-one HCl (18).** This compound was prepared from compound **2** (550 mg, 3.0 mmol) and 5-bromobenzo[*b*]-thiophene (1.6 g, 7.5 mmol) using the same procedure described for the preparation of compound **7** to yield compound **18**, which was then converted to the HCl salt (610 mg, 58%) as pale brown crystals; mp >280 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.83−2.00 (m, 3H), 2.06−2.15 (m, 1H), 2.44 (brs, 1H), 3.16−3.36 (m, 4H), 3.65 (dd, $J$ = 14 Hz, 33 Hz, 2H), 4.20 (d, $J$ = 9 Hz, 1H), 4.35 (d, $J$ = 9 Hz, 1H), 7.46 (d, $J$ = 5 Hz, 1H), 7.67 (dd, $J$ = 3 Hz, 9 Hz, 1H), 7.82 (d, $J$ = 5 Hz, 1H), 7.99 (d, $J$ = 2 Hz, 1H), 8.03 (d, $J$ = 9 Hz, 1H), 10.93 (brs, 1H). Anal. ($C_{17}H_{18}N_2O_2S$·HCl·0.1$H_2O$), C, H, N.

**(*R*)-3′-(2-Methylbenzo[*b*]furan-5-yl)spiro[1-azabicyclo[2.2.2]-octane-3,5′-oxazolidin]-2′-one HCl (19).** This compound was prepared from compound **2** (910 mg, 5.0 mmol) and 5-bromo-2-methylbenzo[*b*]furan (2.6 g, 12.5 mmol) using the same procedure described for the preparation of compound **7** to yield compound **19**, which was then converted to the HCl salt (1.1 g, 63%) as pale yellow crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.76−1.93 (m, 3H), 2.05−2.15 (m, 1H), 2.44 (s, 3H), 2.35−2.40 (m, 1H), 3.18−3.40 (m, 4H), 3.58−3.70 (m, 2H), 4.16 (d, $J$ = 9 Hz, 1H), 4.30 (d, $J$ = 9 Hz, 1H), 6.61 (d, $J$ = 1 Hz, 1H), 7.42 (dd, $J$ = 2 Hz, 9 Hz, 1H), 7.52 (d, $J$ = 9 Hz, 1H), 7.66 (d, $J$ = 2 Hz, 1H), 10.64 (brs, 1H). Anal. ($C_{18}H_{20}N_2O_3$·HCl), C, H, N.

**(*R*)-3′-(2-Methylbenzo[*b*]thiophen-5-yl)spiro[1-azabicyclo-[2.2.2]octane-3,5′-oxazolidin]-2′-one HCl (20).** This compound was prepared from compound **2** (1.1 g, 6.2 mmol) and 5-bromo-2-methylbenzo[*b*]thiophene (3.5 g, 15.4 mmol) using the same procedure described for the preparation of compound **7** to yield compound **20**, which was then converted to the HCl salt (1.21 g, 53%) as pale yellow crystals; mp >300 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.78−1.94 (m, 3H), 2.05−2.15 (m, 1H), 2.41 (m, 1H), 2.53 (s, 3H), 3.10−3.35 (m, 4H), 3.56−3.62 (m, 2H), 4.14 (d, $J$ = 9 Hz, 1H), 4.31 (d, $J$ = 9 Hz, 1H), 7.11 (s, 1H), 7.53 (dd, $J$ = 2 Hz, 9 Hz, 1H), 7.80 (d, $J$ = 2 Hz, 1H), 7.86 (d, $J$ = 9 Hz, 1H), 10.45 (brs, 1H). Anal. ($C_{18}H_{20}N_2O_2S$·HCl), C, H, N.

**5-Bromo-2-ethylbenzo[*b*]thiophene (30).** Under a $N_2$ atmosphere and at −78 °C, to a stirred solution of 5-bromobenzo[*b*]-thiophene **29** (2.1 g, 9.9 mmol) in THF (25 mL) was added dropwise a solution of LDA (5.5 mL, 11.0 mmol, 2.0 M in heptane/THF/ethylbenzene). After stirring for 1 h at 0 °C, the reaction mixture was cooled to −78 °C. Ethyl iodide was added dropwise, and the reaction mixture was allowed to warm up to room temperature and stirred for 1 h, and then quenched with water. The mixture was extracted with $CHCl_3$, and the combined $CHCl_3$ phases were dried over $MgSO_4$. The organic solution was evaporated in vacuo and purified by silica gel column chromatography (eluent: hexane) to give compound **30** (2.2 g, 90%) as colorless crystals; mp 59−60C. $^1$H NMR ($CDCl_3$) $\delta$ 1.37 (t, $J$ = 8 Hz, 3H), 2.93 (q, $J$ = 8 Hz, 2H), 6.93 (s, 1H), 7.33 (dd, $J$ = 2 Hz, 8 Hz, 1H), 7.60 (d, $J$ = 8 Hz, 1H), 7.79 (d, $J$ = 1 Hz, 1H). Anal. ($C_{10}H_9BrS$), C, H, N.

**(*R*)-3′-(2-Ethylbenzo[*b*]thiophen-5-yl)spiro[1-azabicyclo[2.2.2]-octane-3,5′-oxazolidin]-2′-one HCl (21).** This compound was prepared from compound **2** (400 mg, 2.2 mmol) and 5-bromo-2-ethylbenzo[*b*]thiophene (**30**) (620 mg, 2.6 mmol) using the same procedure described for the preparation of compound **7** to yield compound **21**, which was then converted to the HCl salt (85 mg, 10%) as colorless crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.30 (t, $J$ = 8 Hz, 3H), 1.85−1.94 (m, 4H), 2.10 (m, 1H), 2.88−2.92 (m, 2H), 3.29−3.21 (m, 4H), 3.64−3.66 (m, 2H), 4.15 (d, $J$ = 12 Hz, 1H), 4.32 (d, $J$ = 8 Hz, 1H), 7.15 (s, 1H), 7.55 (d, $J$ = 8 Hz, 1H), 7.84 (s, 1H), 7.89 (d, $J$ = 8 Hz, 1H). Anal. ($C_{19}H_{22}N_2O_2S$·HCl), C, H, N.

**(*R*)-3′-(2-Chlorobenzo[*b*]thiophen-5-yl)spiro[1-azabicyclo-[2.2.2]octane-3,5′-oxazolidin]-2′-one HCl (22).** This compound was prepared from compound **2** (910 mg, 5.0 mmol) and 5-bromo-2-chlorobenzo[*b*]thiophene (2.0 g, 8.0 mmol) using the same procedure described for the preparation of compound **7** to yield compound **22**, which was then converted to the HCl salt (278 mg, 14%) as pale yellow crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.76−1.98 (m, 3H), 2.05−2.15 (m, 1H), 2.45 (s, 1H), 3.17−3.40 (m, 4H), 3.58−3.67 (m, 2H), 4.17 (d, $J$ = 9 Hz, 1H), 4.35 (d, $J$ = 9 Hz, 1H), 7.58 (s, 1H), 7.66 (dd, $J$ = 2 Hz, 9 Hz, 1H), 7.95 (d, $J$ = 2 Hz, 1H), 7.99 (d, $J$ = 9 Hz, 1H), 10.41 (brs, 1H). Anal. ($C_{17}H_{17}ClN_2O_2S$·HCl), C, H, N.

**(*R*)-3′-(3-Methylbenzo[*b*]thiophen-5-yl)spiro[1-azabicyclo-[2.2.2]octane-3,5′-oxazolidin]-2′-one HCl (23).** This compound was prepared from compound **2** (4.00 g, 22.0 mmol) and 5-bromo-3-methylbenzo[*b*]thiophene (12.0 g, 52.9 mmol) using the same procedure described for the preparation of compound **7** to yield compound **23**, which was then converted to the HCl salt (3.5 g, 44%) as colorless crystals; mp 272−274 °C. ee > 99.9%. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.85−1.96 (m, 3H), 1.90−1.94 (m, 1H), 2.31 (s, 3H), 3.14−3.44 (m, 5H), 3.66−3.67 (m, 2H), 4.20 (d, $J$ = 8 Hz, 1H), 4.39 (d, $J$ = 8 Hz, 1H), 7.46 (s, 1H), 7.71 (d, $J$ = 8 Hz, 1H), 7.79 (s, 1H), 7.98 (d, $J$ = 8 Hz, 1H). Anal. ($C_{18}H_{20}N_2O_2S$·HCl), C, H, N.

**5-Bromo-3-ethylbenzo[*b*]thiophene (32).** To a solution of 4-bromobenzenethiol **31** (6.2 g, 33.0 mmol) and 1-bromobutan-2-one (5.0 g, 33.0 mmol) in DMF (150 mL) was added $K_2CO_3$ (5.4 g, 39.0 mmol) at 0 °C. After stirring for 2 h at room temperature, the reaction mixture was added to water and extracted with AcOEt. The combined organic phases were dried over $MgSO_4$ and evaporated in vacuo to give the crude intermediate. The obtained intermediate was heated at reflux with PPA (24 g) in chlorobenzene (200 mL) for 3 days. The resultant mixture was diluted with $CHCl_3$, washed with water, and dried over $MgSO_4$. The organic solution was evaporated and purified by silica gel column chromatography (eluent: hexane) to give compound **32** (6.8 g, 85%) as a colorless oil. $^1$H NMR ($CDCl_3$) $\delta$ 1.36 (t, $J$ = 8 Hz, 3H), 2.82 (q, $J$ = 8 Hz, 2H), 7.12 (s, 1H), 7.43 (dd, $J$ = 2 Hz, 8 Hz, 1H), 7.70 (d, $J$ = 8 Hz, 1H), 7.87 (d, $J$ = 2 Hz, 1H). Anal. ($C_{10}H_9BrS$), C, H, N.

**(*R*)-3′-(3-Ethylbenzo[*b*]thiophen-5-yl)spiro[1-azabicyclo[2.2.2]-octane-3,5′-oxazolidin]-2′-one HCl (24).** This compound was prepared from compound **2** (1.0 g, 5.5 mmol) and 5-bromo-3-ethylbenzo[*b*]thiophene (**32**) (2.7 g, 11.0 mmol) using the same procedure described for the preparation of compound **7** to yield compound **24**, which was then converted to the HCl salt (1.3 g, 60%) as colorless crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.29 (t, $J$ = 6 Hz, 3H), 1.82−1.87 (m, 3H), 1.94−1.96 (m, 1H), 2.79−2.81 (m, 2H), 3.19−3.32 (m, 5H), 3.61−3.71 (m, 2H), 4.21 (d, $J$ = 8 Hz, 1H), 4.38 (d, $J$ = 8 Hz, 1H), 7.46 (s, 1H), 7.69 (d, $J$ = 8 Hz, 1H), 7.84 (s, 1H), 7.99 (d, $J$ = 8 Hz, 1H). Anal. ($C_{19}H_{22}N_2O_2S$·HCl), C, H, N.

**(*R*)-3′-(3-Bromobenzo[*b*]thiophen-5-yl)spiro[1-azabicyclo[2.2.2]-octane-3,5′-oxazolidin]-2′-one HCl (25).** To a solution of compound **18** (4.5 g, 12.8 mmol) in AcOH (50 mL), MeOH (150 mL), and water (30 mL) was added bromine (1.3 mL, 25.8 mmol) at 0 °C. After stirring at room temperature overnight, the reaction mixture was added to a 10% $Na_2SO_3$ aqueous solution. The resultant mixture was extracted with $CHCl_3$ and dried over $K_2CO_3$. The organic solution was evaporated and purified by silica gel column chromatography (eluent: $CHCl_3$/MeOH = 20:1) to give compound **25**, which was then converted to the HCl salt (922 mg, 18%) as pale yellow crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.77−2.05 (m, 3 H), 2.06−2.15 (m, 1H), 2.45 (s, 1 H), 3.18−3.32 (m, 4 H), 3.63−3.71 (m, 2 H), 4.23 (d, $J$ = 9 Hz, 1 H), 4.40 (d, $J$ = 9 Hz, 1 H), 7.77 (dd, $J$ = 2 Hz, 9 Hz, 1 H), 7.87 (d, $J$ = 2 Hz, 1 H), 8.08 (s, 1 H), 8.12 (d, $J$ = 9 Hz, 1 H), 10.42 (brs, 1 H). Anal. ($C_{17}H_{17}BrN_2O_2S$·HCl·0.5$H_2O$), C, H, N.

**(*R*)-3′-(2,3-Dimethylbenzo[*b*]thiophen-5-yl)spiro[1-azabicyclo-[2.2.2]octane-3,5′-oxazolidin]-2′-one HCl (26).** This compound was prepared from compound **2** (490 mg, 2.69 mmol) and 5-bromo-2,3-dimethylbenzo[*b*]thiophene (1.3 g, 5.39 mmol) using the same procedure described for the preparation of compound **7** to yield compound **26**, which was then converted to the HCl salt (337 mg, 33%) as pale yellow crystals; mp >280 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.80−2.05 (m, 3 H), 2.05−2.10 (m, 1 H), 2.27 (s, 3 H), 2.40−2.50 (m, 1 H), 2.47 (s, 3 H), 3.15−3.30 (m, 4 H), 3.66 (dd, $J$ = 14 Hz, 21 Hz, 2 H), 4.21 (d, $J$ = 10 Hz, 1H), 4.38 (d, $J$ = 10 Hz, 1 H), 7.62 (d, $J$ = 9 Hz, 1 H), 7.71 (s, 1 H), 7.88 (d, $J$ = 9 Hz, 1 H), 10.67 (brs, 1 H). Anal. ($C_{19}H_{22}N_2O_2S$·HCl), C, H, N.

**5-Bromo-3-ethyl-2-methylbenzo[*b*]thiophene (34).** To a solution of 5-bromo-2-methylbenzo[*b*]thiophene (**33**) (8.0 g, 35.2 mmol) and acetic anhydride (4.3 g, 42.3 mmol) in $CH_2Cl_2$ (120 mL) was added $AlCl_3$ (11.3 g, 85.0 mmol) at 0 °C. After stirring for 2 h at room temperature, the reaction mixture was added to a 1 N HCl aqueous solution and extracted with $CHCl_3$. The combined organic phases were washed with water and brine and then dried over $MgSO_4$. The organic solution was evaporated in vacuo and purified by silica gel column chromatography (eluent: hexane/AcOEt = 100:1−5:1) to give the crude intermediate. The mixture of the obtained intermediate, ethylene glycol (50 mL), and hydrazine monohydrate (2.8 g, 55.8 mmol) was stirred for 1 h at 80 °C, and then the reaction mixture was added to KOH (2.1 g, 37.1 mmol). After stirring for 6 h at 190 °C, the reaction mixture was allowed to reach room temperature and extracted with AcOEt. The organic solution was washed with water and brine and dried over $MgSO_4$. After evaporation, the crude product was purified by silica gel column chromatography (eluent: hexane) to give compound **34** (2.7 g, 35%) as a pale yellow oil.: $^1$H NMR ($CDCl_3$) $\delta$ 1.19 (t, $J$ = 8 Hz, 3H), 2.49 (s, 3H), 2.75 (q, $J$ = 8 Hz, 2H), 7.34 (dd, $J$ = 2 Hz, 9 Hz, 1H), 7.59 (d, $J$ = 9 Hz, 1H), 7.73 (d, $J$ = 2 Hz, 1H). Anal. ($C_{11}H_{11}BrS$), C, H, N.

**(*R*)-3′-(3-Ethyl-2-methylbenzo[*b*]thiophen-5-yl)spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin]-2′-one HCl (27).** This compound was prepared from compound **2** (892 mg, 4.90 mmol) and 5-bromo-3-ethyl-2-methylbenzo[*b*]thiophene (**34**) (2.5 g, 9.80 mmol) using the same procedure described for the preparation of compound **7** to yield compound **27**, which was then converted to the HCl salt (414 mg, 21%) as pale yellow crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.14 (t, $J$ = 8 Hz, 3 H), 1.80−2.05 (m, 3 H), 2.05−2.15 (m, 1 H), 2.40−2.50 (m, 1 H), 2.62 (s, 3 H), 2.76 (q, $J$ = 8 Hz, 2 H), 3.10−3.30 (m, 4 H), 3.67 (brs, 2 H), 4.18 (d, $J$ = 10 Hz, 1 H), 4.37 (d, $J$ = 10 Hz, 1 H), 7.58 (dd, $J$ = 9 Hz, $J$ = 2 Hz, 1 H), 7.73 (d, $J$ = 2 Hz, 1 H), 7.86 (d, $J$ = 9 Hz, 1 H), 10.80 (brs, 1 H). Anal. ($C_{20}H_{24}N_2O_2S$·HCl·0.25$H_2O$), C, H, N.

**(*R*)-3′-(3-Bromo-2-methylbenzo[*b*]thiophen-5-yl)spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin]-2′-one HBr (28).** This compound was prepared from compound **20** (600 mg, 1.64 mmol) using the same procedure described for the preparation of compound **25** to yield compound **28**, which was then converted to the HBr salt (203 mg, 25%) as pale yellow crystals; mp >270 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.80−2.05 (m, 3 H), 2.05−2.20 (m, 1 H), 2.40−2.50 (m, 1 H), 2.62 (s, 3 H), 3.15−3.25 (m, 3 H), 3.25−3.40 (m, 1 H), 3.69 (dd, $J$ = 21 Hz, 15 Hz, 2 H), 4.22 (d, $J$ = 10 Hz, 1 H), 4.32 (d, $J$ = 10 Hz, 1 H), 7.83 (dd, $J$ = 9 Hz, 2 Hz, 1 H), 8.11 (d, $J$ = 9 Hz, 1 H), 8.66 (d, $J$ = 2 Hz, 1 H), 9.01 (s, 1 H), 10.30 (brs, 1 H). Anal. ($C_{18}H_{19}BrN_2O_2S$·HBr·0.25$H_2O$), C, H, N.

**Determination of Optical Purity.** Chiral HPLC was carried out using a Daicel OD-RH with a flow rate of 1.0 mL/min and detection at 254 nm with a mobile phase consisting of 0.1 mol/L of $KPF_6$/$CH_3CN$ (6:4) for compound **23** to indicate >99% ee. The retention times for compound **23** and its enantiomer were 8.53 and 10.29 min, respectively.

**Receptor Binding Assay. α7 nACh Receptor Affinity.** [$^{125}$I] α-Bungarotoxin binding to membranes prepared from rat hippocampi was performed using a modification of the method ussed by Briggs et al.[34] Hippocampi excised from Wistar rats were homogenized in 15 volumes of 0.32 mol/L of sucrose and centrifuged at 1000*g* for 10 min. The supernatant was centrifuged at 20 000*g* for 20 min and the resulting pellet suspended in pure water and centrifuged at 8000*g* for 20 min. This supernatant was centrifuged at 40 000*g* for 20 min and the pellet washed with pure water again. The final pellet was resuspended in a buffer solution (118 mmol/L NaCl, 4.8 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 1.2 mmol/L $MgCl_2$ and 20 mmol/L Na-HEPES, pH 7.5) and used for the binding assay. The suspended membranes were incubated with [$^{125}$I] α-bungarotoxin (Amersham) and test compounds at 37 °C for 3 h. Bound radioactivity was counted after rapid vacuum filtration on a GF/B glass filter. Nonspecific binding was determined using 100 $\mu$M (−)-nicotine (Research Biochemicals).

**α4β2 nACh Receptor Affinity.** [$^3$H]-Cytisine binding to membranes prepared from rat cerebral cortex was performed using a modification of the method by Briggs et al.[34] Cerebral cortices excised from Wistar rats were homogenized in 15 volumes of 0.32 mol/L of sucrose and centrifuged at 1000*g* for 10 min. The supernatant was centrifuged at 20 000*g* for 20 min and the resulting pellet suspended in pure water and centrifuged at 8000*g* for 20 min. This supernatant was centrifuged at 40 000*g* for 20 min and the pellet washed with pure water again. The final pellet was resuspended in a buffer solution (120 mmol/L NaCl, 2.5 mmol/L KCl, 1 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$ and 50 mmol/L Tris-HCl, pH 7.4) and used for the binding assay. The suspended membranes were incubated with [$^3$H]-cytisine (NEN Life Science Products) and test compounds at 4 °C for 75 min. After rapid vacuum filtration on a GF/B glass filter, the radioactivity was quantified by liquid scintillation spectroscopy. Nonspecific binding was determined using 100 $\mu$M (−)-nicotine (Research Biochemicals).

**α7 Nicotinic Acetylcholine Receptor Functional Assay.** Intrinsic activities of test compounds for the α7 nicotinic receptor were investigated by the electrophysiological measurement of α7 nicotinic receptor-mediated inward current in cultured hippocampal neurons. Hippocampal neuronal cultures were prepared according to a procedure described previously and maintained in a serum-free meduim.[35] The membrane currents were measured at a holding potential of −60 to −70 mV by whole-cell patch recording. The composition of the external solution was as follows. NaCl: 135 mmol/L; KCl: 2 mmol/L; $MgCl_2$: 1 mmol/L; $CaCl_2$: 5 mmol/L; D-glucose: 10 mmol/L; and HEPES: 12 mmol/L. During the recordings, 0.3 $\mu$mol/L oftetrodotoxin, 10 $\mu$mol/L of bicuculline, 1 $\mu$mol/L of atropine, and 0.01 $\mu$mol/L of dihydro-$\beta$-erythroidine were added to the external solution to block action potential generation, $GABA_A$-receptor mediated miniature IPSCs, muscarinic

receptor-mediated responses, and α4β2 nAChR-mediated responses, respectively. The composition of the internal solution was as follows. CsCl: 120 mmol/L; EGTA: 10 mmol/L; MgATP: 5 mmol/L; HEPES: 10 mmol/L; and diTris phosphocreatine 14 mmol/L. The pHs of the external solution and the internal solution were adjusted with Tris-base to 7.4 and 7.2, respectively. Application of choline (10 mmol/L), a full agonist at the α7 nicotinic receptor, induced a rapidly desensitizing inward current in over 50% of the neurons tested ($N = 200$). The choline-induced rapidly desensitizing current was blocked by 1 nmol/L of methyllycaconitine, an antagonist at the α7 nicotinic receptor, suggesting that the choline-induced inward current is mediated by this receptor. In all neurons tested, the inward currents induced by the test compounds were compared with that induced by 10 mmol/L of choline.

**Effects of Compound 23 on Paired Auditory-Evoked Potential Parameters.** This examination was performed using a procedure similar to that previously reported.[25,30] Rats (SLC, male Wistar) weighing 250−300 g were equipped with a skull-screw electrode for the recording of auditory-evoked potentials, which was permanently placed on the brain surface at the vertex (4.0 mm posterior to bregma on midline) under pentobarbital anesthesia (50 mg/kg, i.p.). Reference electrodes were placed on the dura, 3.0 mm anterior to the bregma on either side of the midline. Three screws and dental acrylic cement were applied to secure the electrodes to the skull surface. After 1-week of recovery from surgery, the rats were connected to the recording electronics via a cable attached to the headpiece and a commutator on top of the recording chamber. The rats were allowed free movement within the recording chamber. A speaker emitted the auditory stimuli at a sound level of 90 dB as measured by a sound meter (Neuropack 2, Nihon Kohden). These auditory stimuli consisted of paired clicks (condition and test clicks) of 0.5-ms duration, 0.5 s apart, at 15 s intervals. Normally, 30 trials in duplicate were accumulated for each recording to determine the T/C ratio (for N40). The following compounds were used for the study: MK-801 (1 mg/kg, s.c.) and compound **23** (0.3 and 1 mg/kg, s.c.). To avoid drug-induced alterations in sensitivity, a minimum of 1 week was left between compound testing sessions. MK-801 was administered after the measurement of the T/C ratio for the control. The increase in the T/C ratio, indicating MK-801-induced gating deficit, was measured 23 h later. Thirty minutes later, compound **23** was administered and the T/C ratio measured 30 min later.

**Effect of Compound 23 on Extracellular DA Levels in the Medial Prefrontal Cortex.** Male Wister rats (SLC) weighing 165−250 g were used in this examination. Compound **23** was dissolved in 0.5% hydroxypropylmethylcellulose aqueous solution and was injected (p.o.) in a volume of 0.2 mL per 100 g of body mass. The rats were anesthetized with pentobarbital (40 mg/kg, i.p.), and a concentric dialysis probe was inserted at the level of the medial prefrontal cortex (AP: +3.0 mm, L: +1.0 mm, V: −2.5 mm from bregma) according to the Paxinos atlas.[36] Experiments were performed 2 days after probe implantation. Ringer's solution (NaCl: 142 mmol/L, KCl: 4.0 mmol/L, $CaCl_2$: 1.2 mmol/L, $MgCl_2$: 1.1 mmol/L, 5.0 mmol/L sodium phosphate buffer (pH 7.0)) was pumped through the dialysis probe at a constant rate of 2 μL/min. Samples of dialysate were collected every 20 min and immediately analyzed for DA by high-performance liquid chromatography with coulometric detection (BMA-300 and BMA-100, Eicom Co.). The average DA concentration in the last three samples before compound **23** treatment was taken as 100%, and all posttreatment values were expressed relative to that of the basal value. At the end of each experiment, the placement of the probe was verified histlogically.

**Effect of Compound 23 on the Scopolamine-Induced Spatial Cognitive Impairment in 8-Arm Radial Maze.** This examination was performed using a procedure similar to that previously reported.[37] The assessment was conducted on an 8-arm radial maze (Neuroscience Co.). It was elevated 50 cm from the floor. The maze consisted of a central platform, 24 cm in diameter with eight arms extending radially. Each arm was 50 cm in length, 10 cm in width, and 50 cm in height with transparent plastic side walls. Food cups for the reinforcers were located near the end of each arm. The maze was located in a room containing many extra-maze visual cues. At first, the test rats (SLC, male Wister weighing 250−350 g) were habituated to the apparatus for a 10-min period and were allowed to move freely within the maze and to get pellets. Habituation was carried out for 2 days before the training period. For each training session, the rat was placed in a circular plastic ring on the central platform of the maze. Then, the ring was lifted, and the rat was allowed to move freely in the maze. The session continued until the rat entered all eight arms or 10 min had elapsed. The performance of the rat was assessed by two parameters: the number of correct choices of the first 8 arms chosen and the number of errors, which was defined as choosing an arm that had already been visited. If the rat reached the criterion of more than 7 correct choices and less than 1 error in 3 successive sessions, scopolamine (0.5 mg/kg, i.p.) was administrated to the rat 30 min prior to the test. Compound **23** (3 and 10 mg/kg, p.o.) was also administrated before the test. Test performance was assessed by the above two parameters.

**Acknowledgment.** We thank Dr. Masafumi Arita for useful suggestions and encouragement.

**Supporting Information Available:** Table of elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Anand, R.; Conroy, W. G.; Schoepfer, R.; Whiting, P.; Lindstrom, J. Neuronal nicotinic acetylcholine receptors expressed in Xenopus oocytes have a pentameric quaternary structure. *J. Biol. Chem.* **1991**, *266*, 11192−11198.

(2) Cooper, E.; Couturier, S.; Ballivet, M. Pentameric structure and subunit stoichiometry of a neuronal acetylcholine receptor. *Nature* **1991**, *350*, 235−238.

(3) Lukas, R. J.; Changeux, J. P.; Le Novere, N.; Albuquerque, E. X.; Balfour, D. J.; Berg, D. K.; Bertrand, D.; Chiappinelli, V. A.; Clarke, P. B.; Collins, A. C.; Dani, J. A.; Grady, S. R.; Kellar, K. J.; Lindstrom, J. M.; Marks, M. J.; Quik, M.; Taylor, P. W.; Wonnacott, S. International union of pharmacology. XX. Current status of the nomenclature for nicotinic acetylcholine receptors and their subunits. *Pharmacol. Rev.* **1999**, *51*, 397−401.

(4) Picciotto, M. R.; Caldarone, B. J.; Brunzell, D. H.; Zachariou, V.; Stevens, T. R.; King, S. L. Neuronal nicotinic acetylcholine receptor subunit knockout mice: physiological and behavioral phenotypes and possible clinical implications. *Pharmacol. Ther.* **2001**, *92*, 89−108.

(5) Bertrand, D.; Galzi, J. L.; Devillers-Thiely, A.; Bertrand, S.; Changeux, J. P. Mutations at two distinct sites within the channel domain M2 alter calcium permeability of neuronal α7 nicotinic receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6971−6975.

(6) Couturier, S.; Bertrand, D.; Matter, J. M.; Hernandez, M. C.; Bertrand, S.; Millar, N.; Valera, S.; Barkas, T.; Ballivet, M. A neuronal nicotinic acetylcholine receptor subunit (α7) is developmentally regulated and forms a homo-oligomeric channel blocked by α-BTX. *Neuron* **1990**, *5*, 847−856.

(7) Schoepfer, R.; Conroy, W.; Whiting, P.; Gore, M.; Lindstrom, J. Brain α-bungarotoxin binding protein cDNAs and mAbs reveal subtypes of this branch of the ligand-gated ion channel gene superfamily. *Neuron* **1990**, *5*, 35−48.

(8) Séguéla, P.; Wadiche, J.; Dineley-Miller, K.; Dani, J. A.; Patrick, J. W. Molecular cloning, functional properties, and distribution of rat brain α7: a nicotinic cation channel highly permeable to calcium. *J. Neurosci.* **1993**, *13*, 596−604.

(9) Berg, D. K.; Conroy, W. G. Nicotinic alpha 7 receptors: synaptic options and downstream signaling in neurons. *J. Neurobiol.* **2002**, *53*, 512−523.

(10) Picciotto, M. R.; Caldarone, B. J.; King, S. L.; Zachariou, V. Nicotinic receptors in the brain. Links between molecular biology and behavior. *Neuropsychopharmacology* **2000**, *22*, 451−465.

(11) Rezvani, A. H.; Levin, E. D. Cognitive effects of nicotine. *Biol. Psychiatry* **2001**, *49*, 258−267.

(12) Raux, G.; Bonnet-Brilhault, F.; Louchart, S.; Houy, E.; Gantier, R.; Levillain, D.; Allio, G.; Haouzir, S.; Petit, M.; Martinez, M.; Frebourg, T.; Thibaut, F.; Campion, D. The −2 bp deletion in exon 6 of the '*alpha 7-like*' nicotinic receptor subunit gene is a risk factor for the P50 sensory gating deficit. *Mol. Psychiatry* **2002**, *7*, 1006−1011.

(13) Freedman, R.; Olincy, A.; Ross, R. G.; Waldo, M. C.; Stevens, K. E.; Adler, L. E.; Leonard, S. The genetics of sensory gating deficits in schizophrenia. *Curr. Psychiatry Rep.* **2003**, *5*, 155−161.
(14) Luntz-Leybman, V.; Bickford, P. C.; Freedman, R. Cholinergic gating of response to auditory stimuli in rat hippocampus. *Brain Res.* **1992**, *587*, 130−136.
(15) Guan, Z.; Zhang, X.; Blennow, K.; Nordberg, A. Decreased protein level of nicotinic receptor α7 subunit in the frontal cortex from schizophrenic brain. *Neuroreport* **1999**, *10*, 1779−1782.
(16) Freedman, R.; Hall, M.; Adler, L. E.; Leonald, S. Evidence in postmortem brain tissue for decreased numbers of hippocampal nicotinic receptors in schizophrenia. *Biol. Psychiatry* **1995**, *38*, 22−33.
(17) Levin, E. D. Nicotinic receptor subtypes and cognitive function. *J. Neurobiol.* **2002**, *53*, 633−640.
(18) Martin, L. F.; Kem, W. R.; Freedman, R. Alpha-7 nicotinic receptor agonists: potential new candidates for the treatment of schizophrenia. *Psychopharmacology (Berlin)* **2004**, *174*, 54−64.
(19) Nanri, M.; Miyake, H.; Murakami, Y.; Matsumoto, K.; Watanabe, H. GTS-21, a nicotinic agonist, attenuates multiple infarctions and cognitive deficits caused by permanent occlusion of bilateral common carotid arteries in rats. *Jpn. J. Pharmacol.* **1998**, *78*, 463−469.
(20) Mullen, G.; Napier, J.; Balestra, M.; DeCory, T.; Hale, G.; Macor, J.; Mack, R.; Loch, III J.; Wu, E.; Kover, A.; Verhoest, P.; Sampognaro, A.; Phillips, E.; Zhu, Y.; Murray, R.; Griffith, R.; Blosser, J.; Gurley, D.; Machulskis, A.; Zongrone, J.; Rosen, A.; Gordon, J. (−)-Spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin-2′-one], a conformationally restricted analogue of acetylcholine, is a highly selective full agonist at the α7 nicotinic acetylcholine receptor. *J. Med. Chem.* **2000**, *43*, 4045−4050.
(21) Hashimoto, K.; Koike, K.; Shimizu, E.; Iyo, M. α7 Nicotinic receptor agonists as potential therapeutic drugs for schizophrenia. *Curr. Med. Chem.* **2005**, *5*, 171−184.
(22) Hajos, M.; Hurst, R. S.; Hoffmann, W. E.; Krause, M.; Wall, T. M.; Higdon, N. R.; Gropp,i V. E. The selective α7 nicotinic acetylcholine receptor agonist PNU-282987 [N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide hydrochloride] enhances GABAergic synaptic activity in brain slices and restores auditory gating deficits in anesthetized rats. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 1213−1222.
(23) Van Kampen, M.; Selbach, K.; Schneider, R.; Schiegel, E.; Boess, F.; Schreiber, R. AR−R-17779 improves social recognition in rats by activation of nicotinic alpha7 receptors. *Psychopharmacology* **2004**, *172*, 375−383.
(24) Tatsumi, R.; Seio, K.; Fujio, M.; Katayama, J.; Horikawa, T.; Hashimoto, K.; Tanaka, H. (+)-3-[2-(Benzo[*b*]thiophen-2-yl)-2-oxoethyl]-1-azabicyclo[2.2.2]-octane as potent agonists for the α7 nicotinic acetylcholine receptor. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3781−3784.
(25) Tatsumi, R.; Fujio, M.; Satoh, H.; Katayama, J.; Takanashi, S.; Hashimoto, K.; Tanaka, H. Discovery of the α7 nicotinic acetylcholine receptor agonists. (*R*)-3′-(5-Chlorothiophen-2-yl)spiro-1-azabicyclo-[2.2.2]octane-3,5′-[1′,3′]-oxazolidin-2′-one as a novel, potent, selective and orally bioavailable ligand. *J. Med. Chem.* **2005**, *48*, 2678−2686.
(26) Ple, P. A.; Marnett, L. J. Synthesis of substituted benzo[*b*]thiophenes by acid-catalyzed cyclization of thiophenylacetals and ketones. *J. Heterocycl. Chem.* **1988**, *25*, 1271.
(27) (a) Bjornsson, T. D.; Callaghan, J. T.; Einolf, H. J.; Fischer, V.; Gan, L.; Grimm, S.; Kao, J.; King, S. P.; Miwa, G.; Ni, L.; Kumar, G.; McLeod, J.; Obach, S. R.; Roberts, S.; Roe, A.; Shah, A.; Snikeris, F.; Sullivan, J. T.; Tweedie, D.; Vega, J. M.; Walsh, J.; Wrighton, S. A. The conduct of in vitro and in vivo drug-drug interaction studies: A PhRMA perspective. *J. Clin. Pharmacol.* **2003**, *43*, 443−469. (b) Newton, D. J.; Wang, R. W.; Lu, A. Y. Cytochrome P450 inhibitors. Evaluation of specificities in the in vitro metabolism of therapeutic agents by human liver microsomes. *Drug Metab. Dispos.* **1995**, *23*, 154−158. (c) Lu, A. Y. H.; Wang, R. W.; Lin, J. H. Cytochrome P450 in vitro reaction phenotyping: A reevaluation of approaches used for P450 isoform identification. *Drug Metab. Dispos.* **2003**, *31*, 345−350.
(28) Eisele, J. L.; Bertrand, S.; Galzi, J. L.; Devillers-Thiery, A.; Changeux, J. P.; Bertrand, D. Chimaeric nicotinic−serotonergic receptor combines distinct ligand binding and channel specificities. *Nature* **1993**, *366*, 479−483.
(29) Broad, L. M.; Felthouse, C.; Zwart, R.; McPhie, G. I.; Pearson, K. H.; Craig, P. J.; Wallace, L.; Broadmore, R. J.; Boot, J. R.; Keenan, M.; Baker, S. R.; Sher, E. PSAB-OFP, a selective α7 nicotinic receptor agonist, is also a potent agonist of the 5-HT3 receptor. *Eur. J. Pharmacol.* **2002**, *452*, 137−144.
(30) Stevens, K. E.; Johnson, R. G.; Rose, G. M. Rats reared in social isolation show schizophrenia-like changes in auditory gating. *Pharmacol. Biochem. Behav.* **1997**, *58*, 1031−1036.
(31) Adler, L. E.; Hoffer, L. J.; Griffith, J.; Waldo, M. C.; Freedman, R. Normalization by nicotine of deficient auditory sensory gating in the relatives of schizophrenics. *Biol. Psychiatry*. **1992**. *32*, 607−616.
(32) Weinberger, D. R. Implications of normal brain development for the pathogenesis of schizophrenia. *Arch. Gen. Psychiatry* **1987**, *44*, 660−669.
(33) Moghaddam, B.; Bunney, B. S. Acute effects of typical and atypical antipsychotic drugs on the release of dopamine from prefrontal cortex, nucleus accumbens, and striatum of the rat: an in vivo microdialysis study. *J. Neurochem.* **1990**, *54*, 1755−1760.
(34) Briggs, C. A.; Anderson, D. J.; Brioni, J. D.; Buccafusco, J. J.; Buckley, M. J.; Campbell, J. E.; Decker, M. W.; Donnelly-Roberts, D.; Elliott, R. L.; Gopalakrishnan, M.; Holladay, M. W.; Hui, Y. H.; Jackson, W. J.; Kim, D. J.; Marsh, K. C.; O'Neill, A.; Prendergast, M. A.; Ryther, K. B.; Sullivan, J. P.; Arneric, S. P. Functional characterization of the novel neuronal nicotinic acetylcholine receptor ligand GTS-21 in vitro and in vivo. *Pharmacol. Biochem. Behav.* **1997**, *57*, 231−241.
(35) Brewer, G. J.; Torricelli, J. R.; Evege, E. K.; Price, P. J. Optimized survial of hippocampal neurons in B-27-supplemented neurobasal, a new serum-free medium combination. *J. Neurosci. Res.* **1993**, *35*, 567−576.
(36) Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*; Academic Press: New York, 1982.
(37) Egawa, T.; Mishima, K.; Matsumoto, Y.; Iwasaki, K.; Iwasaki, K.; Fujiwara, M. Rolipram and its optical isomers, phosphodiesterase 4 inhibitors, attenuated the scopolamine-induced impairments of learning and memory in rats. *Jpn. J. Pharmacol.* **1997**, *75*, 275−281.

JM060249C