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

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Recent advances in molecular biology suggest that neuronal nicotinic acetylcholine receptors play important roles in the central nervous system (CNS). Of these receptors, the α7 group has recently attracted interest for its CNS-related actions and is looked to as a potential new class of pharmacological targets for cognition, schizophrenia, sensory gating, and anxiety. In the course of a research program aimed at the discovery of α7 receptor agonists with high affinity, subtype selectivity, and good pharmacokinetic profile, we discovered (*R*)-3′-(5-chlorothiophen-2-yl)spiro-1-azabicyclo[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one (**25**). Compound **25** has potent binding affinity (K_i = 9 nmol/L) and good selectivity toward the other nicotinic subtypes (α4β2 and α1β2γδ) and has been found in pharmacokinetic evaluation to have good oral bioavailability and brain permeability.

Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) are pentameric cation-selective ion channels that are formed from various combinations of alpha (α2−α10) and beta (β2−β4) subunits.[1,2] In heterologous expression systems, most neuronal nAChRs are constructed from α and β subunits, and the combinations are associated with functional nAChRs.[3−6] The contributions of α5, α6, and β3 are less well understood.[7−10] On the other hand, only α7, α8, and α9 can form homo-oligomeric receptors that are inhibited by α-bungarotoxin (α-BTX).[11−14] The most prevalent subtypes are associated with the α7 and α4β2 subunits that are strongly expressed during brain development[15−17] and have been implicated in memory, attention, and information processing.[18,19]

Schizophrenia is a neuropsychiatric disorder that affects about 1% of the population worldwide and frequently demonstrates a strong hereditary component. The condition is characterized by positive symptoms (delusions, hallucinations), negative symptoms (apathy, anhedonia, avolition, amotivation), and neurocognitive dysfunction.[20] Abnormality in 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.[21] Moreover, intracerebroventricular injections of α-BTX, an α7 (and α8, α9) receptor antagonist, disrupt hippocampal auditory gating,[22] while recent studies also indicate a correlation between the α7 receptor and several aspects of schizophrenia,[23] and decreased levels of this receptor in the postmortem brains of schizophrenic patients have been reported.[24,25] The α7 nicotinic acetylcholine receptor agonists are therefore thought to be a potential pharmacological target for CNS disorders including neurocognitive dysfunction.

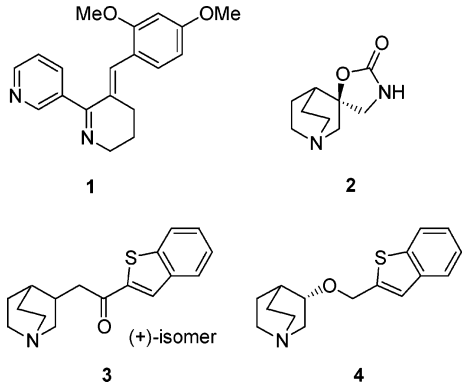

Figure 1. Structure of previously described α7 nicotinic acetylcholine receptor agonists and compounds identified in our laboratory.

Several α7 receptor agonists have been reported in the literature and show prospects in terms of therapeutic potential (Figure 1). For example, 3-(2,4-dimethoxybenzylidene)anabaseine (GTS-21, **1**) is a partial agonist of the α7 receptor.[26] It has been reported to be a "functionally" selective α7 receptor agonist, it but possesses a higher affinity for the α4β2 receptor, toward which it acts as an antagonist,[27] and fails to show a satisfactory pharmacokinetic (PK) profile in the areas of bioavailability and brain permeability. (−)-Spiro-1-azabicyclo[2.2.2]octane-3,5′-oxazolidin-2′-one (AR-R-17779, **2**), meanwhile, is the first subtype-selective agonist of the α7 receptor which demonstrates a high degree of selectivity for the α7 subtype of nAChRs.[28] Compound **2**, however, remains at the preclinical trial stage. Accordingly, there continues to be interest in new

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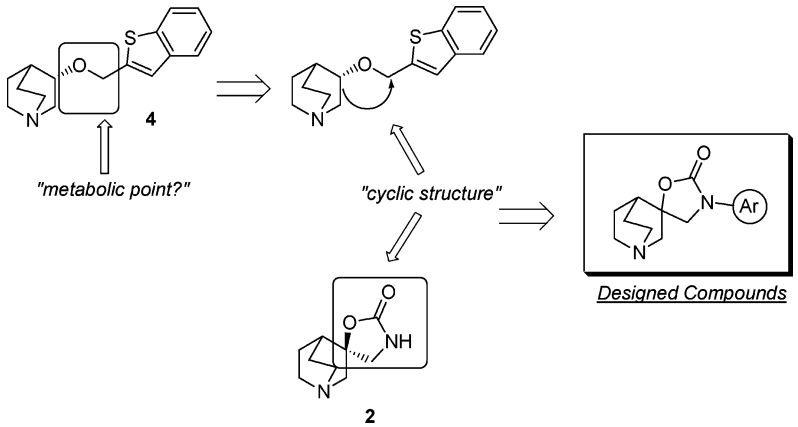


Figure 2. Synthetic strategy to improve the pharmacokinetic profile of α7 nicotinic acetylcholine receptor agonists.

compounds for clinical development. As previously established, (+)-3-[2-(benzo[*b*]thiophen-2-yl)-2-oxoethyl]-1-azabicyclo[2.2.2]octane (**3**) shows high affinity for the α7 receptor (K_i = 65 nmol/L).[29] Further modification led to a discovery of (*S*)-3-(benzo[*b*]thiophen-2-ylmethoxy)-1-azabicyclo[2.2.2]octane (**4**) (K_i = 13 nmol/L). Although compound **4** was not detected in plasma following oral administration (rat, 1 mg/kg), we estimated that this defect may be attributable to its ether part, which might constitute a metabolic point. Furthermore, it is commonly suggested that one of the key factors controlling bioavailability is molecular flexibility (the number of rotatable bonds). On the basis of the structure of compound **2** and this hypothesis, a strategy was devised to fix the ether part with a cyclic structure possessing potent binding affinity compared with compound **4** to improve pharmacokinetic profiles (Figure 2). We report here the design, synthesis, structure−activity relationships, and PK profiles of the series of compounds leading to the discovery of (*R*)-3′-(5-chlorothiophen-2-yl)spiro-1-azabicyclo[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one **25**, the first potent and selective agonist of the α7 nicotinic acetylcholine receptor to display good oral bioavailability and brain permeability.

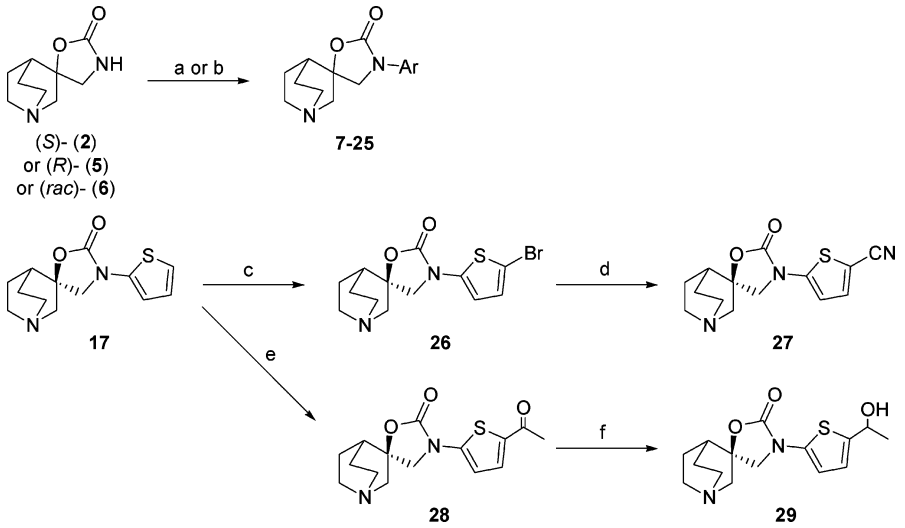
Chemistry

At the beginning, our synthetic strategy was based, as previously reported, on the preparation of compound **2** and (*R*)-**5**.[28] A previous study suggested that methylation at the nitrogen atom of the carbamate moiety of compound **2** attenuates the affinity for the α7 receptor. We estimated, however, from the structure of compound **4** that an aryl moiety would be permissible. As shown in Scheme 1, the coupling of **2** with various aryl halides in the presence of potassium carbonate and copper(I) iodide or using sodium hydride gave compounds **7**, **9**, **11**−**14**, and **16**−**25** in moderate yields. In a similar manner, compounds **8** and **10** were obtained from (*R*)-**5** and **15** from (*rac*)-**6**. Compound **27** was obtained by heating the bromide **26**, which was prepared by bromination of compound **17**, with $Zn(CN)_2$ and $Pd(PPh_3)_4$. Friedel−Crafts acylation of compound **17** in the presence of acetyl chloride and aluminum chloride afforded compound **28**. The resulting acetyl derivative **28** was subsequently treated with $NaBH_4$ to afford the alcohol derivative **29** as a single isomer (absolute configuration was not determined).

Results and Discussions

Previous efforts in our laboratories had identified two compounds, **3** and **4**, with high binding affinity (K_i = 65 and 13 nmol/L, respectively) for the α7 receptor, as determined by α-bungarotoxin binding inhibition. We first examined the effect of a spiro-carbamate bearing various bicyclic aromatic parts on binding affinity to the α7 receptor. The results are summarized in Table 1. The benzo[*b*]thiophene derivative **7**, which has the same aryl part as compound **4**, showed moderate affinity. In contrast, the (*S*)-isomer **8** had no affinity for the α7 receptor. Introduction of a β-naphthyl moiety, also known as an isostere of the benzothiophene part, increased the affinity of compounds **7** and **8**, again with lower affinity observed for compound **10** than compound **9**. A clear stereogenic preference can be seen for the (*R*) versus the (*S*) enantiomer of compounds **7**−**10**, which demonstrates the necessity for the (*R*)-configuration to achieve binding affinity. These observations were supported by previous finding for compound **2**.[28] (*R*)-Compounds were therefore assessed for further evaluation. Although moderate affinity was observed in compounds **7** and **9**, which have a benzo[*b*]thiophene and a β-naphthyl, respectively, as the bicyclic aromatic part, the conversion of the bicyclic part with quinoline (**11**), benzothiazole (**12**), benzoxazole (**13**), and benzofuran (**14**) led to reduction or loss of the affinity for the α7 receptor. Some monocyclic aromatic parts were also evaluated for α7 binding affinity to evaluate the influences of steric and electric factors. Compound **15**, which has a benzene ring as the aromatic part, showed no binding affinity. The replacement of the phenyl ring with a pyridine (**16**) led to a similar result. Interestingly, while the binding affinity was strongly enhanced compared to other monocyclic derivatives by the introduction of a thiophen-2-yl moiety (**17**, K_i = 80 nmol/L), the thiophen-3-yl derivative **18** had no binding affinity. This finding suggests that the presence of a heteroatom, such as nitrogen or oxygen, is not conducive to α7 binding affinity, which may be affected by some electronic interaction with the α7 receptor. Nevertheless, the phenyl derivative **15**, which possesses no heteroatom for the aryl moiety, showed no affinity. Moreover, potent affinity was observed in the thiophen-2-yl derivative **17**, despite the absence of affinity in the thiophen-3-yl derivative **18**. There may thus be unknown interactions with the α7 receptor.

Scheme 1. Synthesis of 1-Azabicyclo[2.2.2]octane Derivatives[a]



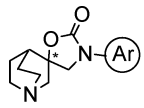
[a] Reagents: (a) Ar−Br, K_2CO_3, CuI, DMF. (b) Ar−Br (Cl), NaH, DMF. (c) NBS, DMF. (d) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF. (e) AcCl, $AlCl_3$, nitrobenzene. (f) $NaBH_4$, MeOH.

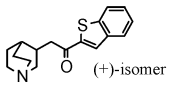
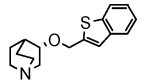
As the thiophen-2-yl derivative **17** showed higher affinity (K_i = 80 nmol/L) than other monocyclic derivatives, the effect on α7 binding affinity of a substituent on the thiophene ring was evaluated. Table 2 shows the results. Introduction of a methyl moiety at the 3- or 4-position of the thiophene ring resulted in loss of the affinity (**19** and **20**). Surprisingly, however, greatly enhanced affinity was observed in the 5-methyl thiophene derivative **21** (K_i = 12 nmol/L). The dimethyl thiophene derivative **22**, meanwhile, had no binding affinity. These results suggest that steric effect is important for binding affinity, as there may be a sterically restricted binding pocket for the receptor as observed with compounds **17**−**22**. A preference in relation to the position of the substituent can also be seen, with the 5-position being important for achieving binding affinity. Subsequently, various substituents at the 5-position of the thiophene ring were examined. The ethyl and isopropyl derivatives **23** and **24** had slightly enhanced affinity compared to compound **17**. To investigate the electronic effect on the thiophene ring, introduction of a halogen atom to the thiophene ring as an electron-withdrawing group was investigated and found to result in an enhancement of affinity to a level higher than that of the previously identified compound **4** (**25**, K_i = 9 nmol/L; **26**, K_i = 4 nmol/L). Substitution of the cyano group in compound **27** slightly reduced affinity relative to compound **17**, while the acetyl group in compound **28** reduced affinity 2-fold. A similar reduction of the affinity was observed in the alcohol derivative **29**. These findings suggest not only an electric but also a steric effect on binding to the α7 receptor. Nevertheless, the results indicate clearly that a sulfur atom is preferred as the component for the aromatic part to the other heteroatoms and that 5-position is sensitive for α7 binding affinity.

We selected compound **25** for further evaluation. To evaluate the agonistic activities of compounds **25**, **1**, **2**, and nicotine, we carried out electrophysiological measurement of the α7 receptor-mediated response using cultured hippocampal neurons, in which the efficacy was assessed by measurement of the relative inward current toward 10 mmol/L choline. Figure 3 shows the agonistic activities for these compounds. Nicotine, an endogenous ligand for the α7 receptor, and compound **2** were studied at 1, 3, 10, 30, 100, 300, and 1000 μmol/L, while compound **1** was studied at 1, 3, 10, 30, 100, and 300 μmol/L. Consistent with previous knowledge, the nicotine-induced relative inward current toward 10 mmol/L choline was dose-dependent. At the concentrations studied, compound **2** displayed greater potency than nicotine at all concentrations in stimulating the relative inward current. As previously reported, compound **1** showed a dose−response curve indicating a profile as a partial agonist with lower efficacy than compound **2**, which is known as a full agonist. Our selected compound **25** was studied at 0.1, 0.3, 1, 3, 10, 30, and 100 μmol/L. It was observed that compound **25** displayed a similar dose−response curve to that of compound **1**, also indicating it to be a partial agonist. It should be noted that compound **25** not only showed agonistic activity at 0.1 μmol/L but also displayed greater efficacy than compound **1** at all doses. Although it is unclear whether a full or partial agonist of the α7 receptor is more valid from the therapeutic viewpoint, the partial agonist compound **1** did, as previously reported,[30] exhibit effect on some cognition-measuring behaviors. Compound **25** would therefore also seem to have potential in the treatment of cognitive dysfunctions.

Besides measurement of the α7 binding affinity and agonistic activity of compound **25**, additional pharmacological evaluations were required to determine its selectivity compared to that of other receptors to establish relative benefit in the treatment of chronic neuropsychiatric disorders such as neurocognitive dysfunction. Binding affinities were therefore determined for the other nicotinic subtypes (α4β2 and α1β2γδ). These are displayed in Table 3. For comparison, compounds **1** and **2** were also examined. None of the compounds showed affinity for the α1β2γδ receptor, but compound **1** possessed strong affinity for the α4β2 receptor, greater than that for the α7 receptor. It was observed that

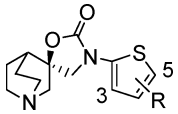
Table 1. Binding Affinities of Oxazolidinone Derivatives with Various Aryl Moieties



compd. no.	Ar	config.	α7 (K_i; nmol/ L)[a)]
3	 (+)-isomer		65
4			13
7	benzothiophen-2-yl	*R*	75
8	benzothiophen-2-yl	*S*	>1000[b)]
9	2-naphthyl	*R*	31
10	2-naphthyl	*S*	205
11	quinolin-2-yl	*R*	450
12	benzothiazol-2-yl	*R*	430
13	benzoxazol-2-yl	*R*	>1000[b)]
14	benzofuran-2-yl	*R*	370
15	phenyl	*rac*	>1000[b)]
16	2-pyridyl	*R*	>1000[b)]
17	thiophen-2-yl	*R*	80
18	thiophen-3-yl	*R*	>1000[b)]
1			220
2			340

[a] α-Bungarotoxin binding. Tests were performed in two experiments. Among K_i values in reproducibility runs, the assay showed less than 20% variability. [b] IC_{50} value.

Table 2. Binding Affinities of (*R*)-Oxazolidinone Derivatives with Various Substituents on the Thiophene Ring



compd no.	R	α7[a] (K_i; nmol/L)
17		80
19	3-methyl	>1000[b]
20	4-methyl	>1000[b]
21	5-methyl	12
22	4,5-dimethyl	>1000[b]
23	5-ethyl	21
24	5-isopropyl	65
25	5-chloro	9
26	5-bromo	4
27	5-cyano	85
28	5-acetyl	190
29	5-1′-hydroxyethyl	145

[a] α-Bungarotoxin binding. Tests were performed in two experiments. Among K_i values in reproducibility runs, the assay showed less than 20% variability. [b] IC_{50} value.

compounds **25** and **2** showed good selectivity toward the other nicotinic subtypes, with compound **25** displaying 200-fold selectivity.

Subsequently, we selected compound **25** for further evaluation of pharmacokinetic profiles in rats, as shown in Table 4. Compound **25** was examined at doses of 1 and 10 mg/kg (po), while compounds **1** and **2** were administered only at the dose of 10 mg/kg (po). Compounds **1** and **2** showed AUC_{0-24} values of 453 ± 44 and 5390 ± 660 ng h/mL, respectively. The value for compound **1** was only twice the AUC value for compound **25** at 1 mg/kg (po). In turn, the value at 10 mg/kg (po)

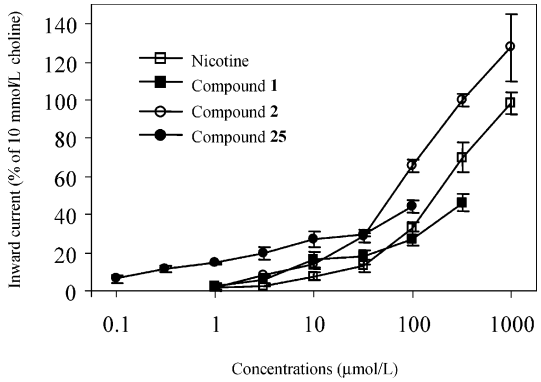


Figure 3. Agonistic activities of nicotine and compounds **1**, **2**, and **25** on α7 nicotinic receptor in cultured hippocampal neurons. Data are represented as mean ± SEM ($N = 2-4$).

Table 3. Selectivity of Compounds **25**, **1**, and **2** Relative to Other Nicotinic Subtypes

	binding affinity (K_i; nmol/L)[a] of nicotinic subtypes		
compd no.	α7[b]	α4β2[c]	α1β2γδ[d]
25	9	1800	>10000[e]
1	220	84	>10000[e]
2	340	>10000[e]	>10000[e]

[a] Tests were performed in two experiments. Among K_i values in reproducibility runs, the assay showed less than 20% variability. [b] α-Bungarotoxin binding. [c] Cytisine binding. [d] α-Bungarotoxin binding. [e] IC_{50} value.

for compound **25** (4640 ± 570 ng h/mL) was approximately comparable with the value for compound **2**. Although the rat oral peak plasma levels were not remarkable, it was notable that compound **25** produced longer duration of plasma concentration (mean $T_{1/2} = 3.13 \pm 1.37$ h, iv) than compounds **1** and **2**. The oral bioavailability (BA) of compounds **25** and **2** was very satisfactory in both cases. Most importantly, although compounds **1** and **2** showed low or moderate brain permeability (C_B/C_P = <0.3 and 0.76 ± 0.23, respectively), compound **25** was found to have a high brain permeability (C_B/C_P = 59 ± 9.8), which is a desirable profile for a drug to treat CNS (central nervous system) disorders. Compound **25** is thus a compound with high oral bioavailability and brain penetration.

Finally, to assess the effect of compound **25** on cognitive enhancement, auditory sensory gating was investigated in a rat model. Auditory sensory gating, a measurement of the ability to depress the evoked response to the second of two auditory stimuli (conditioning−testing paradigm expressed as *T*/*C* ratio), is impaired in humans with schizophrenia, and involvement of α7 receptor dysfunction in the deficit has been suggested.[31,32] In our research programs, we have used the known NMDA antagonist MK-801 in a rat model of auditory gating dysfunction.[33] In this model, the typical antipsychotic haloperidol had no improving effect on auditory gating dysfunction (data not shown), but the atypical antipsychotic clozapine showed significant amelioration, as shown in Figure 4a. In patients with schizophrenia, cognitive dysfunction is not affected by medication with typical antipsychotics, but as previously reported, atypical antipsychotics such as clozapine alleviate the deficit.[34,35] An MK-801-induced auditory sensory gating model was therefore used to assess the ability of the α7 agonist to correct cognitive dysfunction.

Table 4. Pharmacokinetic Profiles of Compounds **25**, **1**, and **2** in Rats ($N = 4$)'

compd no.	po dose (mg/kg)	AUC_{0-24h}[a,f] (ng h/mL)	C_{max}[b,f] (ng/ mL)	$T_{1/2}$[c,f] (h)	BA[d] (%)	brain permeability[e,f] (C_B/C_P)
25	1	224 ± 25	56 ± 17		52	
	10	4640 ± 570	997 ± 87	3.13 ± 1.37	108	59 ± 9.8
1	10	453 ± 44	138 ± 39	0.47 ± 0.08	14	<0.3
2	10	5390 ± 660	1580 ± 320	0.75 ± 0.14	103	0.76 ± 0.23

[a] Estimated area under plasma concentration time curve after oral dosing. [b] Maximum plasma concentration after oral dosing. [c] Half-life at 1 mg/kg, iv. [d] Oral bioavailability. [e] C_B/C_P = concentration in brain/concentration in plasma. Rats ($N = 3$) were given a single dose (10 mg/kg, po) of either compound **25** and compound **1** or a single dose (1 mg/kg, sc) of compound **2**. The C_B/C_P values were calculated 2 h after administration for compounds **25** and **1** and 1 h after for compound **2**. [f] Data are shown as mean ± SD.

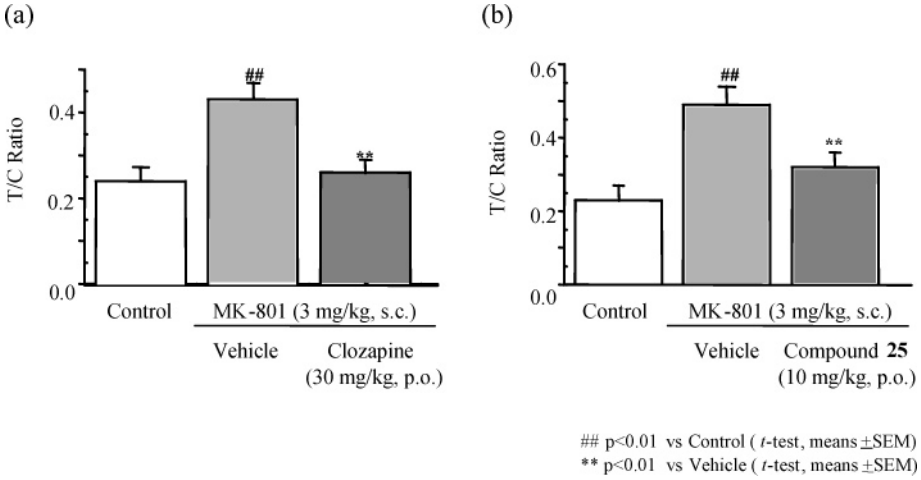


Figure 4. Effects of clozapine (30 mg/kg, po) and compound **25** (10 mg/kg, po) on MK-801-induced sensory gating deficit in rats ($N = 6$).

Figure 4b shows the effect of compound **25**, which was found to reduce MK-801-induced auditory gating deficit.Significant differences were observed in oral administration at doses of 10 and 30 mg/kg (data not shown for 30 mg/kg). This finding suggests that the α7 agonists have the potential to improve sensory gating deficit, and indeed, it is reported that nicotine improves sensory gating deficit in patients with schizophrenia.[31] Furthermore, nicotine's improving effect on sensory gating deficit is inhibited by the known α7 antagonist α-bungarotoxin.[36] It was concluded that nicotine's effect may be caused by stimuli acting on the α7 receptors.

Conclusion

A series of substituted-spiro-1-azabicyclo[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-ones was synthesized and evaluated for α7 binding affinity. Preliminary analysis of structure−activity relationships using various aryl moieties identified the thiophene derivative **25** as having potent binding affinity (K_i = 9 nmol/L) and agonistic activity. The agonistic activity profile of compound **25** is different from that of compound **2**, which is known as the first selective agonist of the α7 receptors. It also showed satisfactory selectivity for the other nicotinic subtypes (α4β2 and α1β2γδ). Furthermore, pharmacokinetic screening demonstrated that compound **25** had a PK profile improved relative to the earlier compounds and also more favorable than that of compounds **1** and **2**, which thus supports our original hypothesis. In an MK-801-induced sensory gating model, compound **25** had an improving effect on cognitive dysfunction. Compound **25** thus shows promise as a potential candidate for further development as a novel, potent, and selective agonist of the α7 nicotinic acetylcholine receptor with good oral bioavailability and brain permeability.

Experimental Section

Chemistry.

Melting points were determined in open capillary tubes with a Buchi 530 apparatus and are uncorrected. All ^{1}H NMR spectrum were recorded on a JEOL GSX-270 spectrometer (270 MHz) and a JEOL GSX-400 spectrometer (400 MHz), using tetramethylsilane as 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), 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). Abbreviations for solvents used are DMSO, dimethyl sulfoxide; DMF, dimethylformamide; IPE, diisopropyl ether; AcOEt, ethyl acetate; IPA, isopropyl alcohol. All yields were unoptimized.

(*R*)-3′-(Benzo[*b*]thiophen-2-yl)spiro-1-azabicyclo[2.2.2]-octane-3,5′-[1′,3′]oxazolidin-2′-one Hydrochloride (7). A mixture of (*S*)-spiro-1-azabicyclo[2,2,2]octane-3,5′-oxazolidin-2′-one (**2**) (1.0 g, 5.49 mmol), 2-bromobenzo[*b*]thiophene (2.6 g, 12.20 mmol), CuI (0.1 g, 0.53 mmol), and K_2CO_3 (0.69 g, 4.99 mmol) was heated at 130 °C for 12 h. The reaction mixture was cooled and diluted with chloroform and purified by silica gel column chromatography (eluent, $CHCl_3$:MeOH = 10:1) to give compound **7**, which was then converted to a HCl salt (0.40 g, 21%) to give pale yellow crystals: mp >270 °C; ^{1}H NMR (DMSO-d_6) δ 1.85−2.10 (m, 4H), 3.19−3.23 (m, 3H), 3.24−3.40 (m, 2H), 3.68 (dd, J = 15 Hz, 22 Hz, 2H), 4.19 (d, J = 10 Hz, 1H), 4.40 (d, J = 10 Hz, 1H), 6.81 (s, 1H), 7.25 (t, J = 7 Hz, 1H), 7.34 (t, J = 7 Hz, 1H), 7.71 (d, J = 8 Hz, 1H), 7.87 (d, J = 8 Hz, 1H), 10.64 (brs, 1H). Anal. ($C_{17}H_{18}N_2O_2S$·HCl) C, H, N.

(*S*)-3′-(Benzo[*b*]thiophen-2-yl)spiro-1-azabicyclo[2.2.2]-octane-3,5′-[1′,3′]oxazolidin-2′-one Hydrochloride (8) This compound was prepared from (*R*)-spiro-1-azabicyclo[2,2,2]-octane-3,5′-oxazolidin-2′-one (**5**) (0.9 g, 4.94 mmol) and 2-bromobenzo[*b*]thiophene (2.7 g, 12.67 mmol) using the same procedure as described in the preparation of compound **7** and then converted to a HCl salt (1.03 g, 59%) to give pale yellow crystals: mp >270 °C; ^{1}H NMR (DMSO-d_6) δ 1.85−2.10 (m, 4H), 3.19−3.23 (m, 3H), 3.24−3.40 (m, 2H), 3.68 (dd, J = 15 Hz, 22 Hz, 2H), 4.19 (d, J = 10 Hz, 1H), 4.40 (d, J = 10 Hz 1H), 6.81 (s, 1H), 7.25 (t, J = 7 Hz, 1H), 7.34 (t, J = 7 Hz, 1H), 7.71(d, J = 8 Hz, 1H), 7.87 (d, J = 8 Hz, 1H), 10.64 (brs, 1H). Anal. ($C_{17}H_{18}N_2O_2S$·HCl) C, H, N.

(*R*)-3′-(2-Naphthyl)spiro-1-azabicyclo[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one (9). This compound was prepared as pale yellow crystals (1.2 g, 71%) from (*S*)-spiro-1-azabicyclo-[2,2,2]octane-3,5′-oxazolidin-2′-one (**2**) (1.0 g, 5.49 mmol) and 2-bromonaphthalene (2.6 g, 12.56 mmol) using the same procedure as described in the preparation of compound **7**: mp 193−195 °C; ^{1}H NMR ($CDCl_3$) δ 1.51−1.90 (m, 3H), 2.10−2.25 (m, 2H), 2.76−3.08 (m, 4H), 3.04 (d, J = 15 Hz, 1H), 3.39 (d, J = 15 Hz, 1H), 3.92 (d, J = 9 Hz, 1H), 4.23 (d, J = 9 Hz, 1H), 7.43−7.51 (m, 2H), 7.70 (d, J = 2 Hz, 1H), 7.78−7.87 (m, 3H), 7.99 (dd, J = 2 Hz, 9 Hz, 1H). Anal. ($C_{19}H_{20}N_2O_2$) C, H, N.

(*S*)-3′-(2-Naphthyl)spiro-1-azabicyclo[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one (10). This compound was prepared as pale yellow crystals (0.75 g, 49%) from (*R*)-spiro-1-azabicyclo-[2,2,2]octane-3,5′-oxazolidin-2′-one (**5**) (0.9 g, 4.94 mmol) and 2-bromonaphthalene (2.6 g, 12.56 mmol) using the same procedure as described in the preparation of compound **7**: mp 193−194 °C; ^{1}H NMR ($CDCl_3$) δ 1.51−1.90 (m, 3H), 2.10−2.25 (m, 2H), 2.76−3.08 (m, 4H), 3.04 (d, J = 15 Hz, 1H), 3.39 (d, J = 15 Hz, 1H), 3.92 (d, J = 9 Hz, 1H), 4.23 (d, J = 9 Hz, 1H), 7.43−7.51 (m, 2H), 7.70 (d, J = 2 Hz, 1H), 7.78−7.87 (m, 3H), 7.99 (dd, J = 2 Hz, 9 Hz, 1H). Anal. ($C_{19}H_{20}N_2O_2$) C, H, N.

(*R*)-3′-(Quinolin-2-yl)spiro-1-azabicyclo[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one (11). A solution of (*S*)-spiro-1-azabicyclo[2,2,2]octane-3,5′-oxazolidin-2′-one (**2**) (0.55 g, 3.02 mmol) and NaH (60%, 132 mg, 9.17 mmol) was stirred in DMF (5 mL) at room temperature for 0.5 h. After addition of 2-chloroquinoline (0.55 g, 3.36 mmol) at room temperature, the reaction mixture was stirred at 100 °C for 1.5 h. Water was added to the resulting reaction mixture, which was then extracted with $CHCl_3$. The combined $CHCl_3$ solution was washed with brine, dried over anhydrous magnesium sulfate, and evaporated to afford the residue, which was crystallized from AcOEt/IPE and washed with IPE to give compound **11** (0.14 g, 15%) as pale yellow crystals: mp 170−172 °C; ^{1}H NMR ($CDCl_3$) δ 1.49−1.57 (m, 1H), 1.72−1.82 (m, 2H), 2.13−2.20 (m, 2H), 2.79−3.02 (m, 4H), 3.07 (dd, J = 2 Hz, 15 Hz, 1H), 3.36 (d, J = 15 Hz, 1H), 4.15 (d, J = 11 Hz, 1H), 4.55 (d, J = 11 Hz, 1H), 7.46 (dt, J = 1 Hz, 8 Hz, 1H), 7.67 (dt, J = 1 Hz, 7 Hz, 1H), 7.78 (d, J = 8 Hz, 1H), 7.88 (d, J = 9 Hz, 1H), 8.14 (d, J = 9 Hz, 1H), 8.44 (d, J = 9 Hz, 1H). Anal. ($C_{18}H_{19}N_3O_2$·0.2H_2O) C, H, N.

(*R*)-3′-(Benzothiazol-2-yl)spiro-1-azabicyclo[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one (12). This compound was prepared as colorless crystals (0.17 g, 18%) from (*S*)-spiro-1-azabicyclo[2,2,2]octane-3,5′-oxazolidin-2′-one (**2**) (0.55 g, 3.02 mmol) and 2-chlorobenzothiazole (0.56 g, 3.30 mmol) using the same procedure as described in the preparation of compound **11**: mp 195−197 °C; ^{1}H NMR ($CDCl_3$) δ 1.52−1.59 (m, 1H), 1.70−1.77 (m, 2H), 2.11−2.15 (m, 2H), 2.79−2.98 (m, 4H), 3.05 (d, J = 15 Hz, 1H), 3.37 (d, J = 15 Hz, 1H), 4.13 (d, J = 11 Hz, 1H), 4.53 (d, J = 10 Hz, 1H), 7.32 (t, J = 7 Hz, 1H), 7.44 (dt, J = 1 Hz, 8 Hz, 1H), 7.80 (d, J = 8 Hz, 2H). Anal. ($C_{16}H_{17}N_3O_2S$) C, H, N.

(*R*)-3′-(Benzoxazol-2-yl)spiro-1-azabicyclo[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one (13). This compound was prepared as colorless crystals (0.12 g, 13%) from (*S*)-spiro-1-azabicyclo[2,2,2]octane-3,5′-oxazolidin-2′-one (**2**) (0.55 g, 3.02 mmol) and 2-chlorobenzoxazole (0.51 g, 3.32 mmol) using the same procedure as described in the preparation of compound **11**: mp 201−203 °C; ^{1}H NMR ($CDCl_3$) δ 1.51−1.81 (m, 3H), 2.10−2.15 (m, 2H), 2.76−2.94 (m, 4H), 3.04 (d, J = 15 Hz, 1H), 3.36 (d, J = 15 Hz, 1H), 4.05 (d, J = 10 Hz, 1H), 4.41 (d, J = 10 Hz, 1H), 7.25−7.35 (m, 2H), 7.54 (d, J = 8 Hz, 1H), 7.59 (d, J = 9 Hz, 1H). Anal. ($C_{16}H_{17}N_3O_3$·0.1H_2O) C, H, N.

(*R*)-3′-(Benzo[*b*]furan-2-yl)spiro-1-azabicyclo[2.2.2]-octane-3,5′-[1′,3′]oxazolidin-2′-one (14). This compound was prepared as brown crystals (6.0 mg, 7%) from (*S*)-spiro-1-azabicyclo[2,2,2]octane-3,5′-oxazolidin-2′-one (**2**) (0.54 g, 2.96 mmol) and 2-bromobenzo[*b*]furan (1.50 g, 7.61 mmol) using the same procedure as described in the preparation of compound **7**: mp 96−98 °C; ^{1}H NMR ($CDCl_3$) δ 1.60−1.92 (m, 3H), 2.15−2.30 (m, 2H), 2.90−3.14 (m, 4H), 3.15−3.32 (m, 1H), 3.40−3.53 (m, 1H), 3.97−4.06 (m, 1H), 4.35 (d, J = 10 Hz, 1H), 6.67 (s, 1H), 7.16−7.32 (m, 2H), 7.37 (d, J = 7 Hz, 1H), 7.49 (d, J = 7 Hz, 1H). Anal. ($C_{17}H_{18}N_2O_3$·0.2H_2O) C, H, N.

3′-Phenylspiro-1-azabicyclo[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one (15). This compound was prepared as brown crystals (506 mg, 36%) from (*rac*)-spiro-1-azabicyclo[2,2,2]-octane-3,5′-oxazolidin-2′-one (**6**) (1.00 g, 5.49 mmol) and bromobenzene (1.96 g, 12.5 mmol) using the same procedure as described in the preparation of compound **7**: mp 153−154 °C; ^{1}H NMR ($CDCl_3$) δ 1.49−1.80 (m, 3H), 2.08−2.21 (m, 2H), 2.76−3.06 (m, 5H), 3.36 (dd, J = 2 Hz, 15 Hz, 1H), 3.79 (d, J = 9 Hz, 1H), 4.11(d, J = 10 Hz, 1H), 7.15 (t, J = 6 Hz, 1H), 7.39 (t, J = 8 Hz, 2H), 7.54 (d, J = 9 Hz, 2H). Anal. ($C_{15}H_{18}N_2O_2$·0.2H_2O) C, H, N.

(*R*)-3′-(2-Pyridyl)spiro-1-azabicyclo[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one (16). This compound was prepared as colorless crystals (23.6 mg, 3%) from (*S*)-spiro-1-azabicyclo-[2,2,2]octane-3,5′-oxazolidin-2′-one (**2**) (0.54 g, 2.96 mmol) and 2-bromopyridine (0.32 mL, 3.30 mmol) using the same procedure as described in the preparation of compound **11**: mp 124−125 °C; ^{1}H NMR ($CDCl_3$) δ 1.50−1.65 (m, 1H), 1.66−1.84 (m, 2H), 2.08−2.16 (m, 2H), 2.78−3.12 (m, 5H), 3.31−3.45 (m, 1H), 3.94 (d, J = 11 Hz, 1H), 4.38 (d, J = 11 Hz, 1H), 7.03 (t, J = 6 Hz, 1H), 7.70 (t, J = 8 Hz, 1H), 8.20 (t, J = 9 Hz, 1H), 8.30 (t, J = 3 Hz, 1H). Anal. ($C_{14}H_{17}N_3O_2$) C, H, N.

(*R*)-3′-(2-Thienyl)spiro-1-azabicyclo[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one Hydrochloride (17). This compound (3.3 g, 76%) was prepared from (*S*)-spiro-1-azabicyclo[2,2,2]-octane-3,5′-oxazolidin-2′-one (**2**) (3.0 g, 16.5 mmol) and 2-bromothiophene (6.7 g, 41.1 mmol) using the same procedure as described in the preparation of compound **5**. Part of compound **17** was then converted to a HCl salt to give colorless crystals: mp >270 °C; ^{1}H NMR (DMSO-d_6) δ 1.78−1.93 (m, 3H), 2.02−2.12 (m, 1H), 2.42 (brs, 1H), 3.12−3.21 (m, 3H), 3.25−3.36 (m, 1H), 3.59 (d, J = 15 Hz, 1H), 3.66 (dd, J = 2 Hz, 15 Hz, 1H), 4.10 (d, J = 9 Hz, 1H), 4.27 (d, J = 10 Hz, 1H), 6.56 (dd, J = 1 Hz, 4 Hz, 1H), 6.92 (dd, J = 1 Hz, 4 Hz, 1H), 7.10 (dd, J = 1 Hz, 5 Hz, 1H), 10.72 (brs, 1H). Anal. ($C_{13}H_{16}N_2O_2S$·HCl) C, H, N.

(*R*)-3′-(3-Thienyl)spiro-1-azabicyclo[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one hydrochloride (18). This compound was prepared from (*S*)-spiro-1-azabicyclo[2,2,2]octane-3,5′-oxazolidin-2′-one (**2**) (0.54 g, 2.96 mmol) and 3-bromothiophene (1.2 g, 7.36 mmol) using the same procedure as described in the preparation of compound **7** and was then converted to a HCl salt (0.31 g, 35%) to give brown crystals: mp >270 °C; ^{1}H NMR (DMSO-d_6) δ 1.80−1.98 (m, 3H), 2.05−2.13 (m, 1H), 2.42 (brs, 1H), 3.14−3.25 (m, 3H), 3.26−3.40 (m, 1H), 3.64 (dd, J = 4 Hz, 20 Hz, 2H), 4.07 (d, J = 10 Hz, 1H), 4.26 (d, J = 10 Hz, 1H), 7.19 (dd, J = 1 Hz, 3 Hz, 1H), 7.42 (dd, J = 1 Hz, 5 Hz, 1H), 7.61 (dd, J = 3 Hz, 5 Hz, 1H), 10.57 (brs, 1H). Anal. ($C_{13}H_{16}N_2O_2S$·HCl·0.1H_2O) C, H, N.

(*R*)-3′-(3-Methylthiophen-2-yl)spiro-1-azabicyclo[2.2.2]-octane-3,5′-[1′,3′]oxazolidin-2′-one Hydrochloride (19). This compound was prepared from (*S*)-spiro-1-azabicyclo[2,2,2]-octane-3,5′-oxazolidin-2′-one (**2**) (0.54 g, 2.96 mmol) and 2-bromo-3-methylthiophene (1.33 g, 7.50 mmol) using the same procedure as described in the preparation of compound **7** and was then converted to a HCl salt (48.5 mg, 5%) to give brown crystals: mp 269−271 °C; ^{1}H NMR (DMSO-d_6) δ 1.75−1.90 (m, 3H), 2.00−2.20 (m, 1H), 2.06 (s, 3H), 2.42 (brs, 1H), 3.10−

3.60 (m, 4H), 3.50−3.70 (m, 2H), 3.95 (d, J = 9 Hz, 1H), 4.02 (d, J = 9 Hz, 1H), 6.84 (d, J = 5 Hz, 1H), 7.33 (d, J = 5 Hz, 1H), 10.78 (brs, 1H). Anal. ($C_{14}H_{18}N_2O_2S$·HCl·0.5H_2O) C, H, N.

(*R*)-3′-(4-Methylthiophen-2-yl)spiro-1-azabicyclo[2.2.2]-octane-3,5′-[1′,3′]oxazolidin-2′-one Hydrochloride (20). This compound was prepared from (*S*)-spiro-1-azabicyclo[2,2,2]-octane-3,5′-oxazolidin-2′-one (**2**) (1.1 g, 6.04 mmol) and a mixture of 2-bromo-4-methylthiophene and 2-bromo-3-methylthiophene (2.6 g, ratio = 3:2), which was obtained by a previously reported method,[37] using the same procedure as described in the preparation of compound **7** and was then converted to a HCl salt (0.76 g, 39%) to give pale gray crystals: mp >270 °C; ^{1}H NMR (DMSO-d_6) δ 1.77−1.93 (m, 3H), 2.00−2.10 (m, 1H), 2.15 (s, 3H), 2.40 (brs, 1H), 3.08−3.43 (m, 4H), 3.57 (d, J = 14 Hz, 1H), 3.65 (d, J = 16 Hz, 1H), 4.07 (d, J = 10 Hz, 1H), 4.24 (d, J = 10 Hz, 1H), 6.40 (s, 1H), 6.68 (dd, J = 1 Hz, 2 Hz, 1H), 11.11 (brs, 1H). Anal. ($C_{14}H_{18}N_2O_2S$·HCl·0.25H_2O) C, H, N.

(*R*)-3′-(5-Methylthiophen-2-yl)spiro-1-azabicyclo[2.2.2]-octane-3,5′-[1′,3′]oxazolidin-2′-one Hydrochloride (21). This compound was prepared from (*S*)-spiro-1-azabicyclo[2,2,2]-octane-3,5′-oxazolidin-2′-one (**2**) (0.54 g, 2.96 mmol) and 2-bromo-5-methylthiophene (1.3 g, 7.34 mmol) using the same procedure as described in the preparation of compound **7** and was then converted to a HCl salt (0.35 g, 37%) to give brown crystals: mp >270 °C; ^{1}H NMR (DMSO-d_6) δ 1.80−1.94 (m, 3H), 2.04−2.13 (m, 1H), 2.37 (s, 3H), 2.43 (brs, 1H), 3.14−3.24 (m, 3H), 3.28−3.40 (m, 1H), 3.64 (dd, J = 15 Hz, 20 Hz, 2H), 4.05 (d, J = 10 Hz, 1H), 4.24 (d, J = 10 Hz, 1H), 6.36 (d, J = 3 Hz, 1H), 6.61 (d, J = 3 Hz, 1H), 10.70 (brs, 1H). Anal. ($C_{14}H_{18}N_2O_2S$·HCl·0.25H_2O) C, H, N.

(*R*)-3′-(4,5-Dimethylthiophen-2-yl)spiro-1-azabicyclo-[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one Hydrochloride (22). This compound was prepared from (*S*)-spiro-1-azabicyclo-[2,2,2]octane-3,5′-oxazolidin-2′-one (**2**) (0.54 g, 2.96 mmol) and 2-bromo-4,5-dimethylthiophene (1.8 g, 9.42 mmol) using the same procedure as described in the preparation of compound **7** and was then converted to a HCl salt (0.16 g, 16%) to give brown crystals: mp >270 °C; ^{1}H NMR (DMSO-d_6) δ 1.74−1.93 (m, 3H), 1.96−2.11 (m, 1H), 2.03 (s, 3H), 2.22 (s, 3H), 2.39 (brs, 1H), 3.12−3.23 (m, 3H), 3.25−3.39 (m, 1H), 3.62 (dd, J = 15 Hz, 20 Hz, 2H), 4.02 (d, J = 9 Hz, 1H), 4.19 (d, J = 10 Hz, 1H), 6.27 (s, 1H), 10.72 (brs, 1H). Anal. ($C_{15}H_{20}N_2O_2S$·HCl·0.1H_2O) C, H, N.

(*R*)-3′-(5-Ethylthiophen-2-yl)spiro-1-azabicyclo[2.2.2]-octane-3,5′-[1′,3′]oxazolidin-2′-one Hydrochloride (23). This compound was prepared from (*S*)-spiro-1-azabicyclo[2,2,2]-octane-3,5′-oxazolidin-2′-one (**2**) (0.54 g, 2.96 mmol) and 2-bromo-5-ethylthiophene (1.43 g, 7.48 mmol) using the same procedure as described in the preparation of compound **7** and was then converted to a HCl salt (0.19 g, 20%) to give pale yellow crystals: mp >270 °C; ^{1}H NMR (DMSO-d_6) δ 1.19 (t, J = 8 Hz, 3H), 1.77−1.95 (m, 3H), 2.01−2.12 (m, 1H), 2.41 (brs, 1H), 2.72 (q, J = 8 Hz, 2H), 3.10−3.22 (m, 3H), 3.25−3.37 (m, 1H), 3.62 (dd, J = 15 Hz, 22 Hz, 2H), 4.04 (d, J = 9 Hz, 1H), 4.22 (d, J = 9 Hz, 1H), 6.36 (d, J = 4 Hz, 1H), 6.62 (d, J = 3 Hz, 1H), 10.73 (brs, 1H). Anal. ($C_{15}H_{20}N_2O_2S$·HCl) C, H, N.

(*R*)-3′-(5-Isopropylthiophen-2-yl)spiro-1-azabicyclo-[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one Hydrochloride (24). This compound was prepared from (*S*)-spiro-1-azabicyclo-[2,2,2]octane-3,5′-oxazolidin-2′-one (**2**) (0.54 g, 2.96 mmol) and 2-bromo-5-isopropylthiophene (1.54 g, 7.51 mmol) using the same procedure as described in the preparation of compound **7** and was then converted to a HCl salt (29.8 mg, 3%) to give yellow crystals: mp >270 °C; ^{1}H NMR (DMSO-d_6) δ 1.23 (d, J = 7 Hz, 6H), 1.75−1.95 (m, 3H), 2.00−2.10 (m, 1H), 2.40 (brs, 1H), 3.00−3.25 (m, 3H), 3.26−3.41 (m, 2H), 3.56−3.70 (m, 2H), 4.04 (d, J = 10 Hz, 1H), 4.22 (d, J = 9 Hz, 1H), 6.36 (d, J = 4 Hz, 1H), 6.63 (d, J = 4 Hz, 1H), 10.67 (brs, 1H). Anal. ($C_{16}H_{22}N_2O_2S$·HCl) C, H, N.

(*R*)-3′-(5-Chlorothiophen-2-yl)spiro-1-azabicyclo[2.2.2]-octane-3,5′-[1′,3′]oxazolidin-2′-one Hydrochloride (25). This compound was prepared from (*S*)-spiro-1-azabicyclo[2,2,2]-octane-3,5′-oxazolidin-2′-one (**2**) (0.54 g, 2.96 mmol) and 2-bromo-5-chlorothiophene (1.48 g, 7.49 mmol) using the same procedure as described in the preparation of compound **7** and was then converted to a HCl salt (0.16 g, 16%) to give pale yellow crystals: mp >270 °C; ^{1}H NMR (DMSO-d_6) δ 1.78−1.94 (m, 3H), 2.00−2.10 (m, 1H), 2.44 (brs, 1H), 3.10−3.24 (m, 3H), 3.25−3.36 (m, 1H), 3.62 (dd, J = 15 Hz, 25 Hz, 2H), 4.04 (d, J = 10 Hz, 1H), 4.26 (d, J = 10 Hz, 1H), 6.37 (d, J = 4 Hz, 1H), 6.97 (d, J = 4 Hz, 1H), 10.91 (brs, 1H). Anal. ($C_{13}H_{15}ClN_2O_2S$·HCl) C, H, N.

(*R*)-3′-(5-Bromothiophen-2-yl)spiro-1-azabicyclo[2.2.2]-octane-3,5′-[1′,3′]oxazolidin-2′-one (26). (*R*)-3′-(2-Thienyl)-spiro-1-azabicyclo[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one (**17**) (1.3 g, 4.92 mmol) was dissolved in DMF (20 mL) and treated with *N*-bromosuccinimide (NBS) (0.87 g, 4.89 mmol). The mixture was stirred at 80 °C for 3 h. Most of the DMF was then removed under reduced pressure. The resultant mixture was diluted with $CHCl_3$, washed with 10% aqueous K_2CO_3 solution, and dried over K_2CO_3. The organic solution was then evaporated and purified by silica gel column chromatography (eluent, $CHCl_3$:MeOH = 50:1) and the obtained crystals were recrystallized from EtOH to give compound **26** (0.86 g, 51%) as colorless crystals: mp 180−182 °C; ^{1}H NMR ($CDCl_3$) δ 1.50−1.71 (m, 2H), 1.73−1.83 (m, 1H), 2.10−2.19 (m, 2H), 2.81−3.00 (m, 4H), 3.07 (d, J = 15 Hz, 1H), 3.37 (d, J = 15 Hz, 1H), 3.74 (d, J = 9 Hz, 1H), 4.05 (d, J = 10 Hz, 1H), 6.17 (d, J = 4 Hz, 1H), 6.83 (d, J = 4 Hz, 1H). Anal. ($C_{13}H_{15}$-BrN_2O_2S) C, H, N.

(*R*)-3′-(5-Cyanothiophen-2-yl)spiro-1-azabicyclo[2.2.2]-octane-3,5′-[1′,3′]oxazolidin-2′-one (27). (*R*)-3′-(5-Bromothiophen-2-yl)spiro-1-azabicyclo[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one (**26**) (0.6 g, 1.75 mmol) was dissolved in DMF (10 mL), after which $Zn(CN)_2$ (0.2 g, 1.70 mmol) and the catalyst $Pd(PPh_3)_4$ (1.0 g, 0.87 mmol) were added. The reaction mixture was heated at 100 °C for 1 h, cooled to ambient temperature, and subjected to addition of 5% $Na_2S_2O_3$ aqueous solution, 10% K_2CO_3 aqueous solution, and AcOEt. The unsoluble solids were filtered off, and the solution was washed with H_2O and brine. The organic solution was then dried over Na_2SO_4, evaporated, and purified by silica gel column chromatography (eluent, $CHCl_3$:MeOH = 10:1). The obtained crystals were recrystallized from EtOH to give compound **27** (96.9 mg, 19%) as colorless crystals: mp 230−232 °C; ^{1}H NMR ($CDCl_3$) δ 1.50−1.67 (m, 2H), 1.70−1.82 (m, 1H), 2.08−2.17 (m, 2H), 2.76−2.97 (m, 4H), 3.02 (d, J = 15 Hz, 1H), 3.37 (d, J = 15 Hz, 1H), 3.80 (d, J = 9 Hz, 1H), 4.15 (d, J = 9 Hz, 1H), 6.38 (d, J = 4 Hz, 1H), 7.44 (d, J = 4 Hz, 1H). Anal. ($C_{14}H_{15}N_3O_2S$) C, H, N.

(*R*)-3′-(5-Acetylthiophen-2-yl)spiro-1-azabicyclo[2.2.2]-octane-3,5′-[1′,3′]oxazolidin-2′-one (28). Acetyl chloride (0.3 mL, 4.22 mmol) was added to a solution of (*R*)-3′-(2-Thienyl)spiro-1-azabicyclo[2.2.2]octane-3,5′-[1′,3′]oxazolidin-2′-one (**17**) (1.0 g, 3.78 mmol) in nitrobenzene (10 mL). The mixture was cooled to 0 °C, and aluminum chloride (1.1 g, 8.25 mmol) was added in small portions. The reaction mixture was stirred at 85 °C for 4 h and added to an ice−water solution. After addition of 10% K_2CO_3 aqueous solution and $CHCl_3$, the resultant mixture was filtered and the solvent extracted with $CHCl_3$ and dried over K_2CO_3. The organic solution was then evaporated and purified by silica gel column chromatography (eluent, $CHCl_3$:MeOH = 10:1), and the obtained crystals were washed with IPE to give compound **28** (0.62 g, 54%) as colorless crystals: mp >270 °C; ^{1}H NMR ($CDCl_3$) δ 1.47−1.81 (m, 3H), 2.06−2.17 (m, 2H), 2.50 (s, 3H), 2.73−2.93 (m, 4H), 3.00 (d, J = 15 Hz, 1H), 3.35 (d, J = 13 Hz, 1H), 3.77 (d, J = 9 Hz, 1H), 4.12 (d, J = 9 Hz, 1H), 6.58 (d, J = 4 Hz, 1H), 7.52 (d, J = 4 Hz, 1H). Anal. ($C_{15}H_{18}N_2O_3S$) C, H, N.

3′-[5-(1-Hydroxyethyl)thiophen-2-yl]spiro-1-azabicyclo-[2.2.2]octane-3,5′-[1′ ,3′]oxazolidin-2′-one (29). To a solution of (*R*)-3′-(5-Acetylthiophen-2-yl)spiro-1-azabicyclo[2.2.2]-octane-3,5′-[1′,3′]oxazolidin-2′-one (**28**) (0.34 g, 1.10 mmol) in MeOH (10 mL) was added $NaBH_4$ (127 mg, 3.30 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 5 h. The resultant mixture was evaporated and was subjected to addition of 10% K_2CO_3 aqueous solution, extracted

with $CHCl_3$, and dried over K_2CO_3. The organic solution was then evaporated and recrystallized from IPA/IPE to give compound **29** (230.9 mg, 68%) as colorless crystals: mp 207−209 °C; ^{1}H NMR ($CDCl_3$) δ 1.42−2.25 (m, 9H), 2.70−3.15 (m, 5H), 3.36 (d, J = 15 Hz, 1H), 3.76 (d, J = 9 Hz, 1H), 4.07 (d, J = 9 Hz, 1H), 5.00−5.07 (m. 1H), 6.35 (d, J = 4 Hz, 1H), 6.75 (d, J = 4 Hz, 1H). Anal. ($C_{15}H_{20}N_2O_3S\cdot H_2O$) C, H, N.

Receptor Binding Assay.

α7 nACh Receptor Affinity. [^{125}I]-α-Bungarotoxin binding to membranes prepared from rat hippocampi was determined using a modification of the method of Briggs et al.[38] Hippocampi excised from Wistar rats were homogenized in 15 volumes of 0.32 M 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 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 20 mM Na−HEPES, pH 7.5) and used for 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 μM (−)-nicotine (Research Biochemicals).

α4β2 nACh Receptor Affinity. [^{3}H]-Cytisine binding to membranes prepared from rat cerebral cortex was determined using a modification of the method of Briggs et al.[38] Cerebral cortex excised from Wistar rats was homogenized in 15 volumes of 0.32 M 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 mM NaCl, 2.5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 50 mM 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 μM (−)-nicotine (Research Biochemicals).

Muscle-type (α1β2γδ) nACh Receptor Affinity. [^{125}I]-α-Bungarotoxin binding to membranes prepared from BC3H1 cells, a muscle cell line, was determined in order to investigate the affinity to muscle-type nAChRs. BC3H1 cells were purchased from American Type Culture Collection. BC3H1 cells were cultured in Dulbecco's modified Eagle's minimal essential medium (DMEM) supplemented with 4 mM l-glutamine and 20% fetal bovine serum. Confluent BC3H1 cells were harvested, homogenized in a buffer solution (118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$ and 2 0mM Na−HEPES, pH 7.5), and centrifuged at 40 000*g* for 20 min. The resulting pellet was resuspended in a buffer solution and centrifuged at 40 000*g* for 20 min. The final pellet was resuspended in a buffer solution (118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 20 mM 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 μM α-bungarotoxin (Sigma).

α7 Nicotinic Acetylcholine Receptor Functional Assay. Intrinsic activities of test compounds for the α7 nicotinic receptor were investigated by 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.[39] The membrane currents were measured at a holding potential of −60 to −70 mV by whole-cell patch recording. The composition (mmol/L) of the external solution was as follows: NaCl, 135; KCl, 2; $MgCl_2$, 1; $CaCl_2$, 5; D-glucose, 10; and HEPES, 12. During the recordings, 0.3 μmol/L tetrodotoxin, 10 μmol/L bicuculline, 1 μmol/L atropine, and 0.01 μmol/L dihydro-*b*-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 (mmol/L) of the internal solution was as follows: CsCl, 120; EGTA, 10; MgATP, 5; HEPES, 10; and diTris phosphocreatine, 14. 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 methylcaconitine, 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 choline.

Effects of Compound 25 on Paired Auditory-Evoked Potential Parameters. This examination was performed using a procedure similar to that previously reported.[31] Rats (SLC 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 the bregma on the midline) under pentobarbital anesthesia (50 mg/kg, intraperitoneally). 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 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 rat was 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 (Neuropack2, 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 (3 mg/kg, sc), clozapine (30 mg/kg, po), and compound **25** (10 mg/kg, po). To avoid drug-induced alterations in sensitivity, a minimum of 1 week was left between the compound testing sessions. MK-801 was administered after measurement of the *T*/*C* ratio for control. The increase in the *T*/*C* ratio, indicating MK-801-induced gating deficit, was measured 22 h later. Thirty minutes later, each compound was administered and the *T*/*C* ratio was measured 30−60 min later.

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

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