ACS Medicinal Chemistry Letters

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Discovery of Novel *N*-Substituted Oxindoles as Selective M_1 and M_4 Muscarinic Acetylcholine Receptors Partial Agonists

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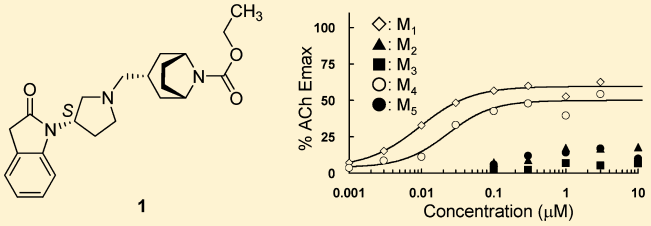
Supporting Information

ABSTRACT: Activation of the M_1 and M_4 muscarinic acetylcholine receptors is thought to play an important role in improving the symptoms of schizophrenia. However, discovery of selective agonists for these receptors has been a challenge, considering the high sequence homology and conservation of the orthosteric acetylcholine binding site among muscarinic acetylcholine receptor subtypes. We report in this study the discovery of novel *N*-substituted oxindoles as potent muscarinic acetylcholine receptor partial agonists selective for M_1 and M_4 over M_2, M_3, and M_5. Among these oxindoles, compound **1** showed high selectivity for the M_1 and M_4 receptors with remarkable penetration into the central nervous system. Compound **1** reversed methamphetamine- and apomorphine-induced psychosis-like behaviors with low potency to extrapyramidical and peripheral side effects.



KEYWORDS: *muscarinic acetylcholine receptor, selective agonist, partial agonist, schizophrenia, oxindole*

Schizophrenia is a complex psychiatric disorder characterized by three major symptoms: (i) positive (e.g., hallucinations, delusions, and excitement), (ii) negative (e.g., flattened affect, apathy, and social withdrawal), and (iii) cognitive (e.g., deficit in working memory, executive function, attentional processing, and memory).[1,2] Dopamine D_2 antagonists are currently used to treat schizophrenia, particularly its positive symptoms. However, we have an ongoing need for alternative approaches to the current clinical treatment of schizophrenia because of a dose limitation of D_2 antagonists by extrapyramidal side effects (EPS)[3] and nonresponders to D_2 antagonists.[4] Therefore, new antipsychotics with a new mechanism of action have been desired in the treatment of schizophrenia.

Many of the important central actions of acetylcholine (ACh) are mediated by muscarinic ACh receptors (mAChRs).[5] To date, five mAChR subtypes (M_1−M_5) have been identified and are considered to play important roles in the peripheral and central nervous systems (CNS).[6−8] M_1 and M_4 receptors are the most heavily expressed in CNS and represent attractive therapeutic targets for cognition, Alzheimer's disease, and schizophrenia.[9] In fact, clinical trials with xanomeline (Figure S1 in Supporting Information), an M_1 and M_4 preferring orthosteric agonist, demonstrated the efficacy of this agent as both an antipsychotic and a cognition-enhancing agent.[10,11] Data from mAChR-knockout mice have also suggested that selective M_1 agonists would be beneficial for psychosis and cognition,[12] whereas M_4 agonists would provide an antipsychotic effect for the treatment of schizophrenia.[13] This proposal is further supported by recent studies showing that M_4 receptors modulate the dynamics of cholinergic and dopaminergic neurotransmission and that loss of M_4 function results in a state of dopamine hyperfunction.[14] In addition, the M_1 agonistic activity of *N*-desmethyl clozapine, a major metabolite of clozapine, suggests that M_1 may also play an important role in the antipsychotic effects of clozapine.[15] Put together, these findings suggest that selective activators of M_1 and M_4 may provide a novel treatment strategy for schizophrenia patients.

Despite the promising results shown by xanomeline in clinical trials of Alzheimer's disease and schizophrenia, there are concerns over its peripheral side effects, especially gastrointestinal (GI) distress and syncope, which may ultimately limit the therapeutic potential of this agent. Administration of xanomeline resulted in vomiting in more than 40% of patients taking part in a clinical trial, terminating further development of this compound. High blood pressure-derived syncope was another undesirable effect observed with xanomeline.[16] These adverse effects are thought to be primarily due to activation of the peripheral M_3 mAChR (see Table S1 in the Supporting Information) and other off-targets. Identification of a highly selective M_1 and M_4 agonist is challenging, because of the high sequence homology and conservation of the orthosteric ACh binding site among the mAChR subtypes.[17] The discovery of AC-42 led us to identify selective allosteric ligands to M_1 mAChR.[18] Christopoulos and co-workers reported that M_4

Received: November 1, 2012
Accepted: January 25, 2013
Published: January 27, 2013

mAChR also has an allosteric binding site by using M_4 mAChR positive allosteric modulator (PAM) LY2033298.[19] To date, M_1 mAChR allosteric agonists, M_1 PAMs, and M_4 mAChR PAMs have been reported (Figure S1 in the Supporting Information).[18] Especially, the reports of centrally active modulators TBPB,[20] VU0357017[21] (M_1 agonist), VU0456940[22] (M_1 PAM), and VU0152100[23,24] (M_4 PAM) indicate that selective activations of central M_1 and M_4 mAChRs are an attractive strategy for the treatment of CNS diseases. However, PAMs are not suitable for dual activation of M_1 and M_4 mAChRs due to their high selectivity. As such, the development of agents that are selective for M_1 and M_4 mAChRs with druggable safety profiles has until now been a challenge.

Here, we describe a novel M_1 and M_4 selective partial agonist (**1**) that reverses methamphetamine-induced hyperlocomotion and improves apomorphine-induced climbing behavior and sensorimotor gating deficits. At doses providing antipsychotic effects, compound **1** does not produce significant GI distress, cardiovascular side effects, and EPS. These important findings suggest that it is possible to overcome major hurdles to the clinical development of mAChR agonists.

In our search for selective M_1 and M_4 agonists, the hit compound **2** was identified as a potential candidate. However, this compound showed weak M_4 agonistic activity. To overcome this problem, we replaced a dimethyl olefin unit in compound **2** by the *N*-carbethoxy piperidine unit known as a pharmacophore of M_4 agonists (Figure 1). The obtained lead

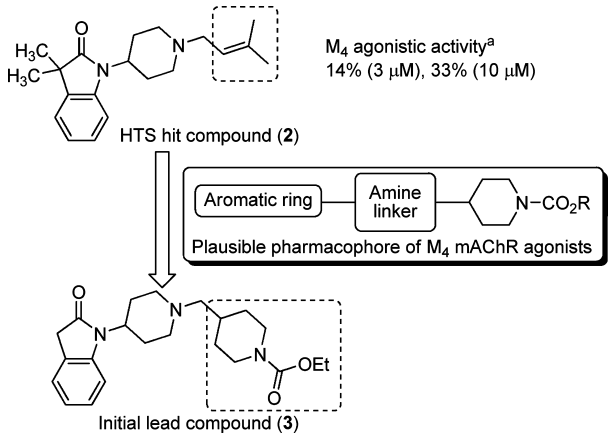
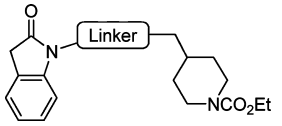


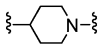
Figure 1. Strategy to enhance hit compound **2** M_4 agonistic activity. [a]M_4 mAChR agonistic activity was evaluated by a calcium mobilization assay.

compound **3** showed moderate M_4 agonistic activity (33% at 3 μM) with good selectivity for M_1 and M_4 over M_2, M_3, and M_5 (Table 1). To potentiate the M_4 agonistic activity of compound **3**, we modified the central amine linker. Replacement of the piperidine linker of compound **3** by an azetidine linker (**4**) resulted in moderate M_4 agonistic activity (46% at 3 μM). On the other hand, the use of an azepane linker (**5**) significantly decreased M_4 agonistic activity. Finally, the pyrrolidine linker was found to be the most potent linker, giving the *S*-substituted compound **7**, which was more potent than *R*-substituted compound **6**. Compound **7** was therefore selected for further optimization.

Xanomeline did not induce EPS in clinical trials, indicating that single use of M_4 mAChR agonists does not produce EPS. However, Fink-Jensen and co-workers reported that D_2

Table 1. Modification of the Central Amine Linker of Compound 3



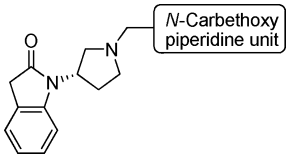
Compound	Linker	% effect[a] M_1	M_2	M_3	M_4	M_5
xanomeline		145	34	85	83	33
3		88	6	2	33	6
4		65	2	3	46	3
5		7	4	2	4	3
6		88	27	8	86	4
7		83	30	7	124	5

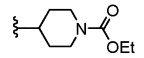
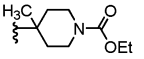
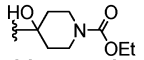
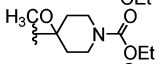
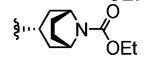
[a]The maximum efficacy of each subtype of ACh was defined as 100%. The concentration of the test compound was 3 μM.

antagonists (haloperidol and risperidone)-induced catalepsy decreased in M_4 knockout mice.[25] This report indicates the possibility that a combination of an M_4 mAChR agonist and a D_2 antagonist may lower the threshold of catalepsy. In fact, additional administration of xanomeline (30 mg/kg, sc) worsened haloperidol-induced catalepsy in rats (see Figure S2 in the Supporting Information). On the basis of the importance of D_2 antagonism in the treatment of schizophrenia, our efforts aimed at the discovery of M_4 mAChR agonists that do not worsen EPS when used in combination with D_2 antagonists. To minimize EPS risk for our M_4 agonists, while maintaining antipsychotic activity, we considered lowering compounds intrinsic activity (IA) without significantly lowering their EC_{50} values. The use of M_4 partial agonists can be supported by the findings that sabcomeline, a partial M_4 agonist (M_4 EC_{50}, 5.7 nM; IA, 28%), reverses methamphetamine-induced hyperlocomotion in rats (see Table S1 in the Supporting Information). The idea that the bulky hetero ring of sabcomeline represents an essential structural feature of this agent led us to modify the critical *N*-carbethoxy piperidine unit in our compounds to a substituted *N*-carbethoxy piperidine unit (Table 2). The report of M_1 agonists with carbethoxy tropane unit also supported our working hypothesis that introduction of substituents to *N*-carbethoxy piperidine unit was allowed to M_1 and M_4 mAChR agonistic activities.[26] Introduction of a methyl group at the 4-position of the piperidine did not lower IA (**8**). On the other hand, introduction of hydrophilic substituents at the same position significantly lowered both EC_{50} and IA (**9** and **10**). However, compound **1** with an *exo* tropane structure showed 42% IA with low EC_{50} (29 nM). This final modification achieved the desired M_4 partial agonistic activity with good selectivity for M_1 and M_4 mAChR over M_2, M_3, and M_5 (see Figure S3 in the Supporting Information). Increasing the concentration of compound **1** from 0.001 to 10 μM partially activated the M_1 (EC_{50}, 12 nM; IA, 60%) and M_4 mAChRs. On the other hand, compound **1** showed very weak or negligible activation of M_2, M_3, and M_5 even at the highest dose tested (30 μM). Compound **1** selectivity was next evaluated in the radioligand-binding panel assay, which consists of a broad range of G protein-coupled receptors, ion channels, enzymes, and transporters. Compound

dx.doi.org/10.1021/ml300372f | ACS Med. Chem. Lett. 2013, 4, 244−248

Table 2. Structure and Activity Relationship at the *N*-Carbethoxy Piperidine Unit



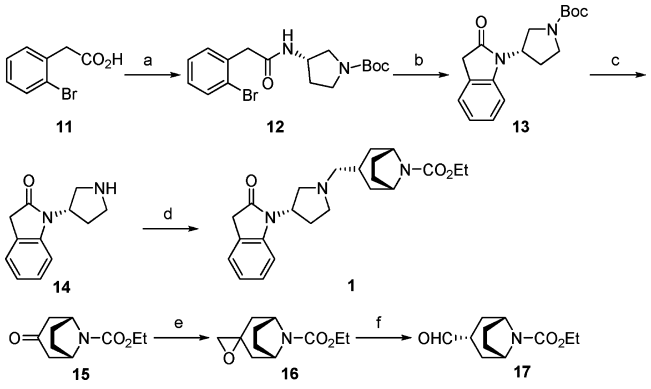
Compound	*N*-Carbethoxy piperidine unit	EC_{50} (nM) / IA (%) M_4	% effect[a] M_1	M_2	M_3	M_5	hERG IC_{50} (μM)
7		79% at 30 nM	83	30	7	5	0.8
8		15 / 65	49	3	2	4	3.5
9		320 / 45	57	12	31	3	3.9
10		13% at 3 μM	NT	NT	NT	NT	NT
1		29 / 42	63	25	7	17	2.4

[a]The maximum efficacy of each subtype of ACh was defined as 100%. The concentration of the test compound was 3 μM. NT, not tested.

1 (3 μM) produced less than 30% inhibitions against all targets in the panel, except for sigma receptor (62% inhibition at 3 μM; see Table S2 in the Supporting Information). Furthermore, compound **1** (3 μM) did not show any agonist and antagonist activities in the calcium mobilization assays using the cells transiently expressing dopamine and serotonin receptors (see Table S3 in the Supporting Information). Compound **1** improved human ether-a-go-go related gene (hERG) channel inhibition (IC_{50}, 2.4 μM) over compound **7** (IC_{50}, 0.8 μM). Interestingly, compound **1** (1 mg/kg, sc) showed good CNS penetration (brain/blood ratio = 2.0) in rat pharmacokinetic study. These encouraging results made compound **1** an excellent candidate for *in vivo* animal studies.

Compound **1** was synthesized as shown in Scheme 1. The *N*-pyrrolidinyl oxindole **14** was prepared by amide formation of 2-bromophenylacetic acid **11** and (3*S*)-1-Boc-3-aminopyrrolidine followed by palladium coupling.[27] Deprotection of the Boc group followed by reductive amination with aldehyde **17** afforded compound **1**. The aldehyde **17** was prepared by epoxidation of the *N*-carbethoxy nortropinone **15** followed by formation of a formyl group using Lewis acid.

Scheme 1[a]



[a]Reagents and conditions: (a) (3*S*)-1-Boc-3-aminopyrrolidine, WSC, HOBt, DIPEA, CH_2Cl_2, room temperature. (b) $Pd(OAc)_2$, X-Phos, $PhB(OH)_2$, K_2CO_3, *t*-BuOH, reflux. (c) TFA, CH_2Cl_2, room temperature. (d) Compound **17**, $NaBH(OAc)_3$, CH_2Cl_2, room temperature. (e) Me_3SOI, KO*t*Bu, THF, reflux. (f) $BF_3{\cdot}OEt_2$, THF, room temperature.

Next, the antipsychotic effects of compound **1** were evaluated in rodents. Subcutaneous administration of compound **1** (1, 3, and 10 mg/kg) significantly reversed methamphetamine-induced hyperlocomotion in rats (Figure 2A). Furthermore, coadministration of subeffective doses of risperidone (0.6 mg/

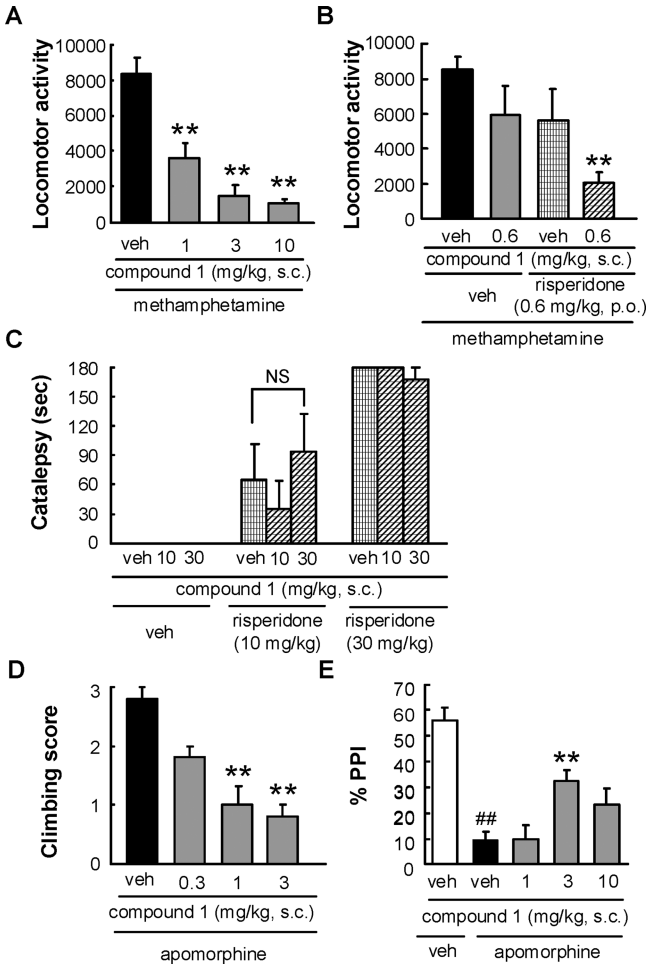


Figure 2. Effects of coumpound **1** and risperidone on methamphetamine-induced hyperactivity (A and B), catalepsy (C), apomorphine-induced climbing (D), and PPI deficits (E) in rats and mice. (A and B) Compound **1** or vehicle was injected into rats 30 min before injection with methamphetamine (0.1 mg/kg, ip). Risperidone or vehicle was administered 60 min before the injection with methamphetamine. Locomotor activity was measured for 80 min from 10 min after the methamphetamine injection. Values correspond to the mean ± SEM ($n = 6$). Veh, vehicle; $^{**}P < 0.01$ vs vehicle/methamphetamine-treated group (Dunnett test). (C) Risperidone or vehicle and compound **1** or vehicle were administered 60 min before the catalepsy tests in rats ($n = 6$). NS, not significant (Dunnett test). (D) Compound **1** or vehicle was first given to mice, and 30 min later, apomorphine (1 mg/kg, sc) was injected. The climbing behavior was observed for 20 min from 10 min after the injection with apomorphine ($n = 5$). $^{**}P < 0.01$ vs vehicle/apomorphine-treated group (Dunnett test). (E) Compound **1** or vehicle was first injected to rats, and 15 min later, apomorphine (0.5 mg/kg, sc) or vehicle was injected. The PPI test was preformed 15 min after the injection with apomorphine or vehicle ($n = 8$). $^{\#\#}P < 0.01$ vs vehicle/vehicle-treated group (unpaired *t* test). $^{**}P < 0.01$ vs vehicle/apomorphine-treated group (Dunnett test).

dx.doi.org/10.1021/ml300372f | ACS Med. Chem. Lett. 2013, 4, 244−248

kg, po) and compound **1** (0.6 mg/kg, sc) significantly improved hyperlocomotion in rats (Figure 2B). On the other hand, compound **1** given alone at 10 and 30 mg/kg (sc) did not induce catalepsy (Figure 2C). Moreover, compound **1** did not significantly worsen risperidone (10 mg/kg, po)-induced catalepsy in rats (10 mg/kg, po, Figure 2C). However, further investigation is necessary to decide the EPS potency of the combination of M_4 mAChR partial agonists and D_2 antagonists. In addition, administration of compound **1** reversed apomorphine-induced climbing behavior in mice (Figure 2D) and impairment of prepulse inhibition (PPI) in rats (Figure 2E), which are claimed to have both face and construct validity vis a vis schizophrenia symptomology.[28]

Next, we evaluated the cholinergic side effects of compound **1**. Administration of xanomeline (10 and 30 mg/kg, sc) induced significant salivation in mice (Figure S5 in the Supporting Information). On the other hand, no significant increase in salivation was seen following treatment with compound **1** (10 and 30 mg/kg, sc). In addition, tissue assays showed that compound **1** (3 μM) induced only weak contraction of guinea pig ileum as compared to sabcomeline and xanomeline (Table S4 in the Supporting Information). These findings confirm high selectivity of compound **1** for M_1 and M_4 over the M_3 mAChR *ex vivo* and *in vivo*. In addition, administration of compound **1** (100 mg/kg, sc) did not induce seizure, a well-known side effect of mAChR agonists, such as pilocarpine, in mice (Table S5 in the Supporting Information).[29]

Finally, we evaluated the cardiac effects of xanomeline and compound **1** in telemetered cynomolgus monkey. Administration of xanomeline at 1 and 3 mg/kg increased mean blood pressure (MBP) and prolonged QRS duration and QTc interval (see Figure S6 and Tables S6 and S8 in the Supporting Information). On the other hand, single subcutaneous administration of compound **1** at 1.5 and 6 mg/kg did not elevate MBP and showed no effect on QRS duration or QTc interval. Both xanomeline and compound **1** increased heart rate. Xanomeline also induced vomiting and drowsiness, although these side effects were not observed with compound **1**. Salivation and miosis were observed with both xanomeline and compound **1**. Toxicokinetic studies in monkey supported the plasma concentrations of compound **1** as high enough to reverse methamphetamine-induced hyperlocomotion in rats (Table S6 and S7 in the Supporting Information). These findings indicate that the potential cardiac toxicity and GI distress of compound **1** are lower than those of xanomeline.

In summary, we have found compound **1** as a structurally novel dual M_1 and M_4 mAChRs selective partial agonist. Compound **1** reversed the psychosis-like behavior and showed low potency to EPS. In addition, compound **1** can, additively with risperidone, reverse methamphetamine-induced hyperlocomotion in rats. Moreover, GI distress and cardiac toxicity of compound **1** were less than those of xanomeline. These findings support that compound **1** is suitable for further drug development.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures for pharmacological experiments and synthetic procedures and characterization data for the compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

T.S. conceived and directed medicinal chemistry. T.E. directed pharmacology. K.T., Y.T., Y.K., Y.U., K.T., and A.S. synthesized compounds, and T.S. wrote the manuscript. T.E., H.M., T.N., and M.S. performed mAChR pharmacology. A.K. contributed to PK studies. Y.U. and A.K. performed safety studies.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Naohiro Nishimura and Chie Matsuba for HRMS and Dr. Norio Fujiwara and Yoshiaki Ochi for useful discussions.

■ ABBREVIATIONS

EPS, extrapyramidal side effects; ACh, acetylcholine; mAChR, muscarinic acetylcholine receptor; CNS, central nervous system; GI, gastrointestinal; PAM, positive allosteric modulator; Boc, *t*-butoxy carbonyl; IA, intrinsic activity; hERG, human ether-a-go-go related gene; WSC, water-soluble carbodiimide; HOBt, 1-hydroxybenzotriazole; DIPEA, *N*,*N*-diisopropylethylamine; *t*-BuOH, tertially butanol; TFA, trifluoroacetic acid; THF, tetrahydrofuran; PPI, prepulse inhibition; MBP, mean blood pressure

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