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Discovery of *N*-sulfonyl-7-azaindoline derivatives as potent, orally available and selective M_4 muscarinic acetylcholine receptor agonists

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ABSTRACT

We designed and synthesized novel *N*-sulfonyl-7-azaindoline derivatives as selective M_4 muscarinic acetylcholine receptor agonists. Modification of the *N*-carbethoxy piperidine moiety of compound **2**, an M_4 muscarinic acetylcholine receptor (mAChR)-preferring agonist, led to compound **1**, a selective M_4 mAChR agonist. Compound **1** showed a highly selective M_4 mAChR agonistic activity with weak hERG inhibition in vitro. A pharmacokinetic study of compound **1** in vivo revealed good bioavailability and brain penetration in rats. Compound **1** reversed methamphetamine-induced locomotor hyperactivity in rats (1–10 mg/kg, po).

Psychosis is the key features in schizophrenia, and is currently treated with dopamine D_2 receptor antagonists.[1] However, there is an ongoing need for alternative approaches considering non-responders to D_2 receptor antagonists.[2] A pilot clinical trial with xanomeline (Fig. 1), an M_1 and M_4 muscarinic acetylcholine receptor (mAChR)-preferring agonist, has demonstrated the efficacy of this agent as both an antipsychotic and a cognition-enhancing agent in schizophrenic patients who had exhibited poor response with previous antipsychotic treatment.[3] While it has been widely accepted that M_1 mAChR is key subtype for cognitive function,[4] animal studies with M_1 or M_4 mAChR-knockout mice have suggested that M_4 mAChR, but not M_1 mAChR, predominantly mediates antipsychotic efficacy of xanomeline.[5] It is further supported by recent studies showing that M_4 mAChRs regulates dopaminergic neurotransmission in the nucleus accumbens that is the key brain region for psychosis.[4] Although the development of xanomeline has been halted by the peripheral side effects via M_3 mAChR activation,[6] M_1 and M_4 mAChR-dual agonist would be promising both for psychosis and cognitive deficits in schizophrenia. However, there is a large hurdle to achieve the optimal balance between M_1 and M_4 mAChR agonism for developing dual-agonists. It is essential because a recent clinical study have shown that even M_1 mAChR-selective agonism might induce some dose-related muscarinic side effects.[7] Furthermore, animal studies have shown that over-activation of M_1 mAChR might have a risk for seizure.[8] Alternative approach especially for psychosis is M_4 mAChR-selective agonist or positive allosteric modulators (PAM) such as LY2033298.[9] Brady and colleagues have identified, VU0152100 (Fig. 1), a brain-penetrant M_4 mAChR PAM, reversed amphetamine-induced locomotor hyperactivity without causing sedation in rats.[10] On the other hand, identifying agonists selective for M_4 mAChR has so far been challenging because of limited resources in the literature. Here, we describe the identification of novel *N*-sulfonyl-7-azaindoline derivatives as potent, orally available, and selective M_4 mAChR agonists (Fig. 1).

Among the compounds synthesized in our drug discovery program for M_1 and M_4 mAChRs selective agonists,[11] we picked up the *N*-sulfonyl-7-azaindoline compound **2** as an M_4 mAChR preferring agonist. A calcium mobilization assay[12] confirmed that compound **2** M_4 mAChR agonistic activity (88% at 0.3 μM) is more potent than its M_1 mAChR agonistic activity (20% at 0.3 μM) (Table 1). This finding indicates that the *N*-methansulfonyl-7-azaindoline scaffold increases M_4 mAChR preference. However, the selectivity of compound **2** for M_4 (EC_{50} = 93 nM, IA = 83%) versus M_1 mAChR (EC_{50} = 352 nM, IA = 59%) was not so high (Fig. 2).

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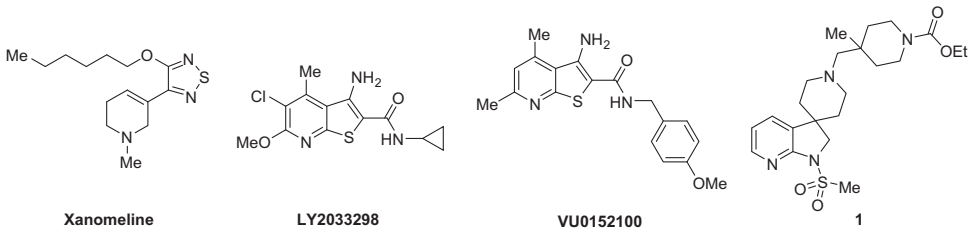
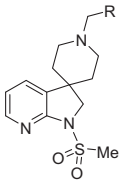
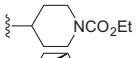
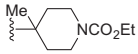
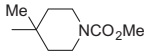
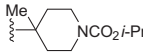
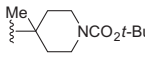


Figure 1. Structures of M_4 mAChR agonists and modulators.

Table 1
SAR at the *N*-carbamoyl piperidine moiety of the *N*-sulfonyl-7-azaindoline derivatives



Compound	R	Agonistic activity (% effect)					hERG inhibition[12] (IC_{50}: μM)
		M_1 [a]	M_2 [a]	M_3 [a]	M_4 [a]	M_5 [b]	
2		20	12	7	88	3	1.90
3		NT	NT	NT	4	NT	NT
1		5	3	4	94	3	3.63
4		3	2	1	52	NT	17
5		NT	NT	NT	3	NT	2.75
6		NT	NT	NT	2	NT	NT

NT: Not tested.
[a] Maximum efficacy of each receptor subtype was defined as 100%. Concentration of the test compound was 0.3 μM.
[b] Concentration of the test compound was 10 μM.

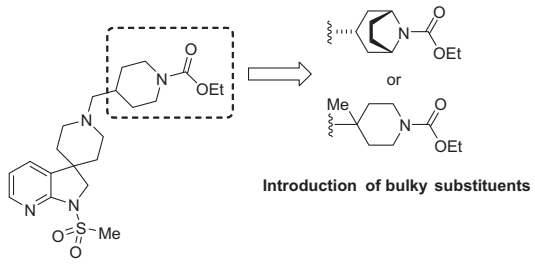


Figure 3. Strategy to increase selectivity for M_4 mAChR.

Table 2
PK and pharmacological profiles of compound **1**

Compound **1**	
PK profiles	
P-gp efflux ratio	0.9
BA (%)	49
Brain/plasma ratio	0.9
Pharmacological profiles	
M_4 EC_{50} (nM)	13
M_4 IA(%)	81
Binding assay	
α_{1D}R inhibition (%)	0 [a]
D_2R inhibition (%)	0 [a]
H_1R inhibition (%)	27 [a]

[a] Concentration of the test compound was 3 μM.

To further increase selectivity for the M_4 mAChR, we introduced into compound **2** another moiety that decreases M_1 mAChR agonistic activity. We focused on the *N*-carbethoxy piperidine moiety (Fig. 3) because Lindsley and co-workers reported that a tropane unit partializes M_1 mAChR agonistic activity.[13]

The structure–activity relationships (SARs) of the prepared *N*-sulfonyl-7-azaindoline derivatives are summarized in Table 1. Replacement of the piperidine by a tropane depleted M_4 mAChR agonistic activity (compound **3**). Assuming that a tropane unit is too large to maintain M_4 mAChR agonistic activity, we designed compound **1** with a methyl group at the 4-position of the *N*-carbethoxypiperidine. As expected, compound **1** decreased M_1 mAChR agonistic activity, while maintaining M_4 mAChR agonistic activity. A further SAR study of the carbethoxy moiety revealed that relatively small alkyl group can maintain M_4 mAChR agonistic activity

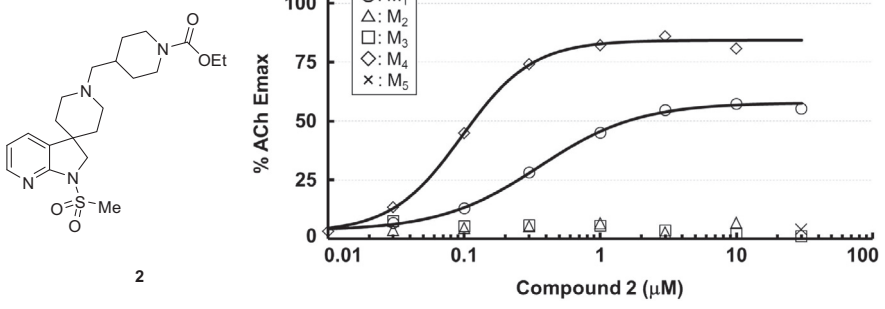


Figure 2. Functional activity of compound **2**. Agonistic activity on M_{1-5} mAChRs. The maximum efficacy of each subtype of ACh was defined as 100%.

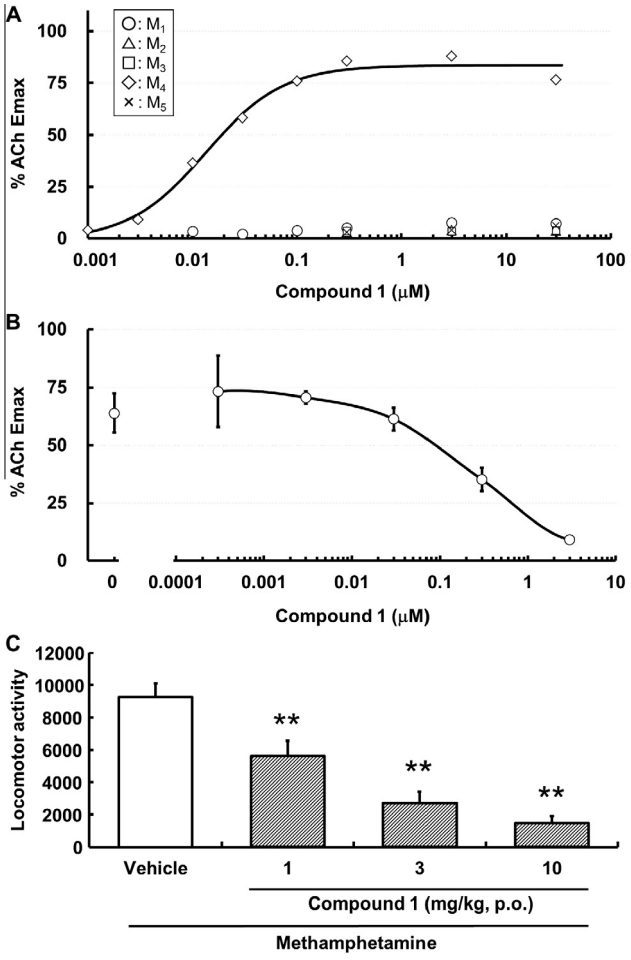


Figure 4. Functional activity of compound **1**. (A) Agonistic activity on M_{1-5} mAChRs. The maximum efficacy of each subtype of ACh was defined as 100%. (B) Antagonism for ACh-stimulated activity on M_1 mAChR. M_1 mAChR was expressed in CHO cells. Both compound **1** and ACh (0.01 μM) were added at the same time. The maximum efficacy of ACh (0.3 μM) was defined as 100%. (C) Effects on methamphetamine-induced hyperactivity in rats. Compound **1** or vehicle was administered into rats 60 min before injection with methamphetamine (0.1 mg/kg, ip). Locomotor activity was measured for 80 min from 10 min after the methamphetamine injection. Values correspond to the mean ± SEM ($n = 6$). $^{**}P$ <0.01 versus vehicle/methamphetamine-treated group (Dunnett test).

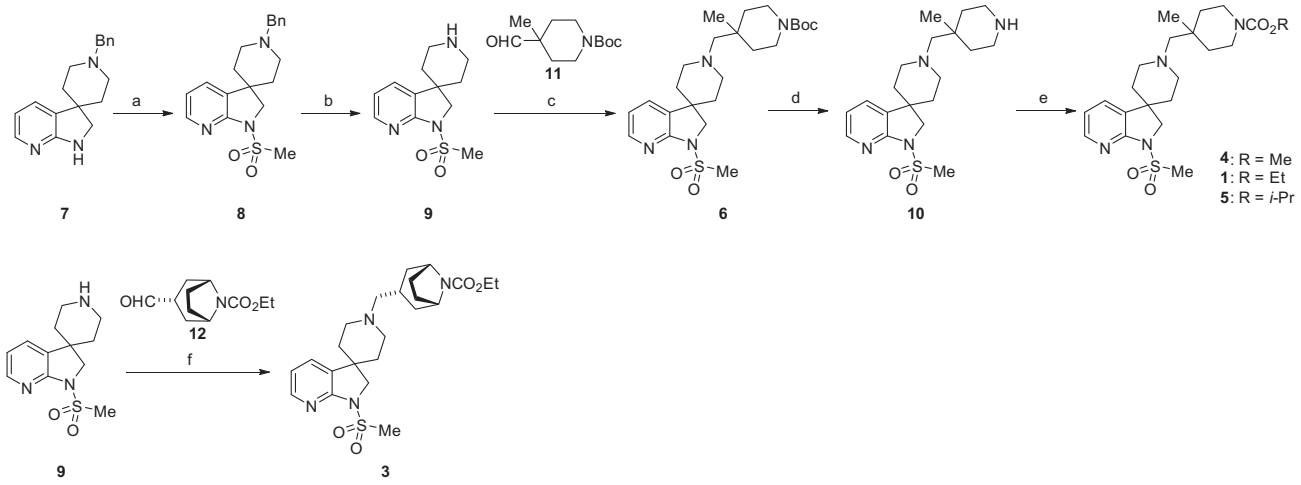
(compound **4**), while bulky alkyl groups diminish M_4 mAChR agonistic activity (compounds **5** and **6**). Considering that compounds **1** showed only micromolar inhibition of human Ether-Go-Go-Related-Gene (hERG), we selected this compound for further evaluation as a potent M_4 mAChR-selective agonist.

Next, we determined the pharmacokinetic (PK) of compound **1** both in vitro and in vivo (Table 2). Compound **1** was not a substrate of p-glycoprotein (P-gp). As for PK in vivo, compound **1** showed high bioavailability (BA, 49%, 1 mg/kg, po) in rats with good brain penetration (brain/plasma ratio: 0.9). These findings indicate that compound **1** is a druggable candidate.

Finally, we evaluated compound **1** pharmacological potential. In a radioligand-binding panel assay, compound **1** had weak affinity to human dopamine D_2, histamine H_1 and adrenaline α_{1D} receptors (Table 2). In addition, compound **1** showed a highly selective M_4 mAChR agonistic activity (EC_{50} = 13 nM, IA = 81%) with no activation of the M_{1-3} and M_5 mAChRs (Fig. 4A). In addition, compound **1** competitively antagonizes the M_1 mAChR functional response to ACh (Fig. 4B). These results indicate that introduction of a methyl group at the 4-position of the *N*-carbethoxypiperidine led to a completely abolished M_1 mAChR agonistic activity. Next, we evaluated the antipsychotic potency of compound **1** in rats. Oral administration of compound **1** reversed methamphetamine-induced hyperlocomotion in dose dependent manner (1–10 mg/kg, Fig. 4C).[12] As far as we know, this is the first agonist highly selective for M_4 mAChR which reverses psychosis-like behavior in rodents. Compound **1** is a potent, orally available, brain-penetrant and highly selective M_4 mAChR agonist with a druggable safety profile. Although further investigation is necessary, the results of this study demonstrate that M_4 mAChR-selective agonist compound **1** is a promising candidate for the treatment of schizophrenia. Furthermore, it would be useful as a pharmacological tool for investigating the physiological role of M_4 mAChR in vivo.

The 3-spiro-7-azaindoline derivatives **2–6** were synthesized as shown in Scheme 1. Compound **7**[14] was converted to the sulfonamide **8**. Deprotection of the benzyl group of **8** by catalytic reduction afforded compound **9**. Reductive amination with the *N*-Boc-4-formylpiperidine **11** and aldehyde **12** afforded compounds **3** and **6**. Boc deprotection followed by introduction of substituents at the final step using appropriate carbamoyl chloride afforded compounds **1**, **4** and **5**.[15]

In summary, we have identified *N*-sulfonyl-7-azaindoline derivatives as selective M_4 mAChR agonists. Modification of the



Scheme 1. Reagents and conditions: (a) methanesulfonyl chloride, *N*,*N*-diisopropylethylamine, CH_2Cl_2, rt, 17 h, 78%; (b) 10% Pd/C, HCO_2NH_4, MeOH, reflux, 12 h, 79%; (c) *tert*-butyl 4-formyl-4-methylpiperidine-1-carboxylate **11**, Ti(O-*i*Pr)$_4$, $NaBH(OAc)_3$, CH_2Cl_2, reflux, 19 h, quant.; (d) trifluoroacetic acid, CH_2Cl_2, rt, 17 h, 87%; (e) methyl chloroformate, ethyl chloroformate, or isopropyl chloroformate, *N*,*N*-diisopropylethylamine, CH_2Cl_2, rt, 1.5 h, 78%; (f) **12**, $NaBH(OAc)_3$, CH_2Cl_2, rt, 19 h, quant.

N-carbethoxy piperidine moiety of compound **2** led to the discovery of compound **1**, which showed selective M_4 mAChRs agonistic activity, high oral bioavailability, and good brain penetration in rats. In addition, compound **1** reversed methamphetamine-induced psychosis-like behavior in rats. These findings indicate that compound **1** is a promising antipsychotic candidate with a new mechanism of action.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.04.083.

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15. Analytical data of compound **1** is described in Supporting information.