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Discovery of a selective M₄ positive allosteric modulator based on the 3-amino-thieno[2,3-*b*]pyridine-2-carboxamide scaffold: development of ML253, a potent and brain penetrant compound that is active in a preclinical model of schizophrenia

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Abstract

Herein we report a next generation muscarinic receptor 4 (M₄) positive allosteric modulator (PAM), ML253 which exhibits nanomolar activity at both the human (EC₅₀ = 56 nM) and rat (EC₅₀ = 176 nM) receptors and excellent efficacy by the left-ward shift of the ACh concentration response curve (Fold Shift, human = 106; rat = 50). In addition, ML253 is selective against the four other muscarinic subtypes, displays excellent CNS exposure and is active in an amphetamine-induced hyperlocomotion assay.

Keywords

Muscarinic receptor 4; Positive allosteric modulator; Amphetamine induced hyperlocomotion; CNS; PAM

Schizophrenia (SZ) is a complex brain disorder that affects more than 1% of the total adult population worldwide. SZ is a devastating disease as it first presents itself in affected individuals early in their lives and they struggle with the disease throughout their productive years. The disease is characterized by three main conditions or symptoms: positive symptoms involving hallucinations, delusions and paranoia; negative symptoms involving

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social withdrawal and anhedonia; and cognitive dysfunction with lapses in attention and impairments in working memory.¹ Although there have been significant improvements in the pharmaceutical intervention for the treatment of SZ over the past 50 years, the current atypical antipsychotics still only treat the positive symptoms associated with the disease. In addition, due to the polypharmacology associated with these drugs (most exhibit their effectiveness through the inhibition of dopamine and/or serotonin receptors), there exist significant side effects that lead to compliance issues. As the dopamine hypothesis for the total treatment of SZ has fallen short, there remains significant research dedicated to the discovery of alternative treatment mechanisms.

One such mechanism that has been advanced over the past several years is the muscarinic hypothesis.² The muscarinic acetylcholine receptors (mAChR) are members of the family A 7 Transmembrane Spanning Receptors (7TMRs) and are further divided into five subtypes ($M_1 - M_5$).³ Of the receptors that have been identified, M_1 and M_4 have the highest distribution within the CNS and have been implicated as potential therapeutic targets for SZ.^{2, 4} Postmortem analyses of schizophrenia patients have shown a reduction in the level of muscarinic receptor binding in the frontal cortex.² Further work has shown that levels of M_1 and M_4 are decreased in the caudate and putamen^{2, 5}, hippocampus⁶ and the prefrontal cortex.⁷ These studies, along with the extensive research into knockout mice models⁸, have excited the research community into exploring selective activation of the M_1 and M_4 subtypes. Unfortunately, due to the high sequence homology of the orthosteric binding region of the mAChR subtypes, development of novel and selective M_1 and M_4 orthosteric agonists has been met with difficulty. However, we⁹ and others¹⁰, have recently disclosed efforts towards the development of novel positive allosteric modulators (PAMs) of select mAChRs, namely the M_4 subtype.

Initial efforts towards a selective M_4 PAM stemmed from a report of the human M_4 selective compound, LY2033298, from Eli Lilly.¹⁰ Unfortunately, this compound showed a significant bias towards the human receptor (65 nM), ~10-fold less active against the rat receptor (629 nM) – something required to study selective M_4 activation in preclinical models. Utilizing the thieno[2,3-*b*]pyridine scaffold and chemoinformatics and medicinal chemistry, we have reported two novel, selective and potent rat M_4 PAMs, **ML108** ($hM_4 = 620$ nM; $rM_4 = 230$ nM) and **ML173** ($hM_4 = 500$ nM; $rM_4 = 900$ nM) (Figure 1).^{9a-c} Although both **ML108** and **ML173** are important tool compounds, they still lacked several key features – sufficient M_4 potency, rat and human receptor potency correspondence, and CNS exposure – properties we deemed essential for a superior M_4 tool compound. The newest M_4 PAM compound from our laboratories, **ML293**, which represents a fundamentally novel chemical scaffold, addressed the *in vivo* PK and CNS exposure deficiencies; however, this compound was much less potent than its predecessors ($hM_4 = 1.3$ μ M).^{9d} Herein, we report the discovery of a novel M_4 PAM from the thieno[2,3-*b*]pyridine scaffold that overcomes the species bias and provides increased brain exposure.

The structure-activity relationship (SAR) plan for this project started with the addition of the 5-chloro substituent found in **LY2033298** into the core scaffold of **ML108**. The 4,6-dimethyl substitution in **ML108** was shown to alleviate some of the species bias seen in **LY2033298** and **ML173**. Both of these compounds employ an ether linkage at the 6-position of the thieno[2,3-*b*]pyridine scaffold and each of these compounds show significant bias towards the human receptor (weaker or inactive against the rat receptor). Incorporation of the 5-chloro substituent resulted in a much improved compound (**1**, 107 nM) against the human M_4 receptor when compared to **ML108** (~600 nM). This improvement in potency was also evident in both the 2,3-difluorobenzyl group (**ML173**-like) (**2**, 124 nM) and the cyclopropyl derivative (**LY2033298**-like) (**3**, 77 nM), both providing a 5-fold increase in potency. Substitution of the amide moiety proved to exhibit robust SAR and, unlike many

other PAM optimizations, many different groups were well tolerated. From the 4-methoxybenzyl group we next explored a variety of heteroarylmethyl moieties with much success. The pyridine-4-ylmethyl group (**4**, 56 nM) was a very potent compound, with the potency decreasing with different regioisomers (pyridine-3-ylmethyl, **7**, 208 nM; pyridine-2-ylmethyl, **9**, 356 nM). As the methylene group of the benzyl moiety can provide a site for metabolic activation, methyl substitution at that center was explored. However, these compounds were much less active than their unsubstituted partners (**5**, 600 nM; **8**, 669 nM; **10**, 1370 nM). Also, deletion of this spacer group was not tolerated (phenyl derivatives, **27–28**). Methyl substitution next to the pyridyl nitrogen was well tolerated (**6**, 61 nM). In addition, increasing the chain length from a methylene to an ethylene group also led to an erosion in potency (**11**, 128 nM; **12**, >30 μ M; **13**, 844 nM). Looking at other heterocycles (pyrazine, **14**; pyrimidine, **15** and **16**; pyridazine, **17**; thiazole, **24**) led to active compounds (<500 nM), but none were more active than the 4-pyridyl moiety.

The substitution around the benzylic group was well tolerated regardless of the position (**18–23**), with all compounds showing sub-500 nM potency. As with the cyclopropyl derivative (**3**, 77 nM), other cycloalkyl and branched alkyl groups were explored with success. Breaking the cyclopropyl group into the isopropyl group (**31**, 147 nM) or expanding the group to the cyclohexyl (**32**, 226 nM) led to a slight erosion in potency. Incorporation of a heteroatom in the cycloalkyl group was also tolerated, although the SAR showed a preference for the 5- and 6-membered groups. The 3-tetrahydrofuran (racemic, **29**, 52 nM) and 4-pyran (**33**, 289 nM) were potent compounds; however, the oxetane (**30**, 2010 nM) exhibited a drastic loss of activity. Finally, unlike the phenyl and benzylic compounds, addition of a methylene spacer in the cycloalkyl groups led to a loss of activity (**37**, 1410 nM).

Having profiled these compounds in the human M₄ receptor assay, we next chose the most promising compounds (**3** and **4**) for further profiling in our rat M₄ receptor assay, along with Tier 1 *in vitro* DMPK assays and into efficacy analyses. The cyclopropyl, **3**, and 4-pyridylbenzyl, **4**, were evaluated against the rat M₄ receptor and both compounds were potent (**3**, 147 nM; **4**, 176 nM), although each were ~2-fold less potent against the rat receptor compared to the human receptor. To further develop PAM molecules, we next assessed the ability of the compounds to enhance the potency of acetylcholine (ACh) as measured by the magnitude of the left-ward shift of the ACh agonist CRC. Both of these compounds robustly left-shifted the ACh CRC with values of >100 fold-shift against the human M₄ receptor (**3**, 224 FS; **4**, 106 FS). When evaluated at the rat receptor, the compounds also showed an exceptional ability to left-shift ACh with values ~50-fold (**3**, 54 FS; **4**, 50 FS). The robust fold shifts of both the rat and human receptor responses for these compounds are some of the largest observed for family A GPCR PAMs. Further evaluation of **3** and **4** in a battery of *in vitro* PK parameters showed these compounds are predicted to have high clearance in the rat (CL_{HEP}). However, both compounds possess reasonable free fraction in rat (~2% free in rat equilibrium dialysis plasma protein binding studies). At this stage, both **3** and **4** appeared to be promising tool compounds for the study of M₄ in the CNS.

Prior to *in vivo* evaluation, the compounds were profiled for their selectivity against the other four mAChR subtypes. Both compounds were inactive against the M₃ and M₅ receptors. However, **3** showed PAM activity at both M₁ and M₂ (~900 nM and ~600 nM, respectively against both the human and rat receptors). In contrast, **4** was inactive (>30 μ M) against all four of the mAChR subtypes that were profiled. Next, **4** was evaluated using Ricerca Biosciences Lead Profiling Screen which is a radioligand binding panel of 68 7TMRs, ion channels and transporters.¹¹ Compound **4** was found to bind in only 2 of the 68 assays conducted (inhibition of radio ligand binding >50% at 10 μ M) – 64% inhibition of rat

GABA_A and 74% inhibition of human dopamine transporter (DAT), although these binding values are likely insignificant compared to **4**'s M₄ potency. Based on the potency of **4** at the rat and human M₄ receptor, the robust efficacy and the very favorable overall selectivity profile, **4** was declared an MLPCN probe molecule and designated as **ML253**.^{12, 13, 14}

The *in vitro* PK data predicted that ML253 would be a high clearance compound *in vivo* and the IV clearance experiment confirmed this prediction. In this experiment (1 mg/kg, IV, rat), ML253 showed an above hepatic blood flow clearance value of 103.5 mL/min/kg with a short half-life ($t_{1/2}$ = 17.5 min). Although this molecule possesses sub-optimal properties for oral dosing, there exist several alternative dosing options for pre-clinical evaluations in animal models. To test this, we evaluated ML253 for CNS exposure in a PBL (Plasma:Brain Level) experiment after intraperitoneal injection (i. p.). The plasma and brain levels of ML253 were evaluated over a six hour time course with measurements at 15 and 30 min., followed by 1, 3 and 6 hour time points. ML253 had a brain:plasma ratio of 0.88 and significant brain levels (3410 hr-ng/mL). Due to the cumulative positive *in vitro* and *in vivo* properties, we evaluated ML253 in a pre-clinical antipsychotic animal model – amphetamine-induced hyperlocomotion – a model that is sensitive to known antipsychotic agents. In this assay, ML253 reversed amphetamine-induced hyperlocomotion in a dose-dependent fashion following a 30 min pretreatment interval and assessment after 90 mins.^{9b} ML253 showed a modest reduction of 26% at a dose of 10 mg/kg; however, at the three highest doses (30, 56.6 and 100 mg/kg), the compound showed significant reductions of 47.9%, 53.7% and 57.3%, respectively (Table 4).

In conclusion, we report a potent and selective positive allosteric modulator of the muscarinic M₄ receptor, ML253. ML253 is more potent at both the rat and human M₄ receptors than previously reported tool compounds – overcoming the species bias that has been seen prior in the LY2033298 and ML173 compounds which is attributed to the 6-ether moiety. In addition, ML253 is inactive against the other four AChR subtypes (>30 μ M, >800-fold selectivity) as well as against 66 of the 68 receptors evaluated in the Ricerca Lead Profiling screen. Although ML253 does not possess an ideal *in vivo* PK profile for oral dosing, it does exhibit excellent CNS exposure after IP dosing, allowing for its evaluation in appropriate animal models. Lastly, ML253 showed a dose-dependent reversal of amphetamine-induced hyperlocomotion, a standard animal model to evaluate compounds for their antipsychotic potential. ML253 is a probe molecule as part of the NIH's Roadmap Initiative and is freely available through the network.

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References and Notes

1. Andreasen NC. Brain Res. Rev. 2000; 31:106. [PubMed: 10719138]
2. Raedler TJ, Bymaster FP, Tandon R, Copolov D, Dean B. Mol. Psychiatry. 2007; 12:232. [PubMed: 17146471]
3. Wess J. Crit. Rev. Neurobiol. 1996; 10:69. [PubMed: 8853955]
4. Dencker D, Thomsen M, Wörtwein G, Weikop P, Cui Y, Jeon J, Wess J, Fink-Jensen A. ACS Chem. Neurosci. 2012; 3:80. [PubMed: 22389751]
5. Dean B, Crook JM, Opeskin K, Hill C, Keks N, Copolov DL. Mol. Psychiatry. 1996; 1:54. [PubMed: 9118315]

6. Crook JM, Tomaskovic-Crook E, Copolov DL, Dean B. *Biol. Psychiatry*. 2000; 48:381. [PubMed: 10978721]
7. Crook JM, Tomaskovic-Crook E, Copolov DL, Dean B. *Am. J. Psychiatry*. 2001; 158:918. [PubMed: 11384900]
- 8(a). Bymaster FP, McKinzie DL, Felder CC, Wess J. *Neurochem. Res.* 2003; 28:437. [PubMed: 12675128] (b) Wess J. *Annu. Rev. Pharmacol. Toxicol.* 2004; 44:423. [PubMed: 14744253] (c) Wess J, Eglen RM, Gautam D. *Nature Rev. Drug. Dis.* 2007; 6:721.
- 9(a). Shirey JK, Xiang Z, Orton D, Brady AE, Johnson KA, Williams R, Ayala JE, Rodriguez AL, Wess J, Weaver D, Niswender CM, Conn PJ. *Nature Chem. Biol.* 2008; 4:42. [PubMed: 18059262] (b) Brady AE, Jones CK, Bridges TM, Kennedy JP, Thompson AD, Heiman JU, Breninger ML, Gentry PR, Yin H, Jadhav SB, Shirey JK, Conn PJ, Lindsley CW. *J. Pharmacol. Exp. Ther.* 2008; 327:941. [PubMed: 18772318] (c) Kennedy JP, Bridges TM, Gentry PR, Brogan JT, Kane AS, Jones CK, Brady AE, Shirey JK, Conn PJ, Lindsley CW. *Chem Med. Chem.* 2009; 4:1600. [PubMed: 19705385] (d) Salovich JM, Vinson PN, Sheffler DJ, Lamsal A, Utley TJ, Blobaum AL, Bridges TM, Le U, Jones CK, Wood MR, Daniels JS, Conn PJ, Niswender CM, Lindsley CW, Hopkins CR. *Bioorg. Med. Chem. Lett.* 2012; 22:5084. [PubMed: 22738637]
10. Chan WY, McKinzie DL, Bose S, Mitchell SN, Witkin JM, Thompson RC, Christopolous A.; Lazareno, S. Birdsall NJM, Bymaster FP, Felder CC. *Proc. Nat. Acad. Sci.* 2008; 105:10978. [PubMed: 18678919]
11. www.ricerca.com
12. 4, VU0448088 (ML253) has been declared a probe via the Molecular Libraries Probe Production Centers Network (MLPCN) and is freely available through the network, see: <http://mli.nih.gov/mli/>
13. 3-amino-5-chloro-4,6-dimethyl-N-(pyridinyl-4-methyl)thieno[2,3-b]pyridine-2-carboxamide, 4, ML253: To a 20 mL microwave vial fitted with a magnetic stir bar was added 2,5-dichloro-4,6-dimethylnicotinonitrile 1 (1.0 g, 5.0 mmol) and MeOH (10 mL). Methyl thioglycolate (490 μ L, 5.5 mmol) was added followed by the addition of a 1 M solution of NaOH (aq, 10 mL, 10 mmol). The microwave vial was sealed and the solution was heated to 125 °C for 30 min. The reaction was cooled to room temperature and the solution was diluted with MeOH (75 mL). The solution was concentrated in vacuo to yield sodium 3-amino-5-chloro-4,6-dimethylthieno[2,3-b]pyridine-2-carboxylate as a powder (870 mg, 68%). The solids were of sufficient purity to use without further purification. To a 20 mL vial fitted with a stir bar and a setum cap, was added sodium 3-amino-5-chloro-4,6-dimethylthieno[2,3-b]pyridine-2-carboxylate 2 (130 mg, 0.50 mmol) and the vial was purged with argon. DMF (3 mL) was added followed by triethylamine (210 μ L, 1.50 mmol). 2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium (HATU, 190 mg, 0.50 mmol) was added to the solution and stirred at ambient temperature for 10 min. 4-aminomethylpyridine (55 μ L, 0.54 mmol) was added and the solution was stirred for an additional hour. The mixture was diluted with deionized water (10 mL) and a precipitate formed. The solids were filtered and dried in a vacuum oven at 50 °C for 24 h. Recrystallization from ethanol provided the target compound (81 mg, 51% yield).
- 14(a). For information on the Vanderbilt Specialized Chemistry Center, see: <http://www.mc.vanderbilt.edu/centers/mlpcn/index.html>; <http://mli.nih.gov/mli/>

Figure 1.

Previously reported M₄ PAM probes (**LY2033298**, **ML108**, **ML173**) from Eli Lilly and Vanderbilt University based on the thieno[2,3-*b*]pyridine scaffold and the novel benzo[*d*]thiazole scaffold (**ML293**).

Table 1SAR Analysis for human M₄ positive allosteric modulators.

Entry	R	pEC ₅₀ ± SEM ^{a,b}	Human M4 ^{9d} EC50 (nM)	%ACh _{max} ± SEM
1		6.97 ± 0.04	107	83.6 ± 3.2
2		6.91 ± 0.05	124	79.5 ± 4.4
3		7.11 ± 0.32	77	94.0 ± 2.5
4		7.25 ± 0.05	56	94.1 ± 1.8
5		6.22 ± 0.01	600	64.1 ± 2.4
6		7.21 ± 0.05	61	75.8 ± 5.8

Entry	R	pEC ₅₀ ± SEM ^{a,b}	Human M4 ^{9d} EC50 (nM)	%ACh _{max} ± SEM
7		6.68 ± 0.13	208	84.9 ± 4.7
8		6.17 ± 0.08	669	57.3 ± 5.5
9		6.45 ± 0.06	356	74.1 ± 8.1
10		5.86 ± 0.07	1370	51.1 ± 4.0
11		6.89 ± 0.09	128	74.5 ± 8.0
12		< 4.52	>30,000	
13		6.07 ± 0.06	844	61.7 ± 9.7
14		6.53 ± 0.06	294	83.1 ± 5.3

Entry	R	pEC ₅₀ ± SEM ^{a,b}	Human M4 ^{9d} EC50 (nM)	%ACh _{max} ± SEM
15		6.57 ± 0.06	271	78.5 ± 7.0
16		6.09 ± 0.05	810	51.0 ± 1.8
17		6.49 ± 0.08	326	74.5 ± 7.7
18		7.09 ± 0.08	80	71.7 ± 3.9
19		6.97 ± 0.05	108	81.0 ± 4.5
20		6.81 ± 0.28	160	42.0 ± 1.7
21		6.81 ± 0.07	160	32.4 ± 0.8

Entry	R	pEC ₅₀ ± SEM ^{a,b}	Human M4 ^{9d} EC50 (nM)	%ACh _{max} ± SEM
22		6.98 ± 0.10	104	46.8 ± 5.0
23		6.75 ± 0.14	180	33.2 ± 4.6
24		6.60 ± 0.07	249	65.1 ± 4.9
25		5.76 ± 0.27	1750	33.6 ± 2.3
26		<4.52	>30,000	
27		<5.00	>10,000	30.7 ± 7.3
28		<4.52	>30,000	
29		7.28 ± 0.07	52	92.2 ± 2.3

Entry	R	pEC ₅₀ ± SEM ^{a,b}	Human M4 ^{9d} EC50 (nM)	%ACh _{max} ± SEM
30		5.70 ± 0.24	2010	69.9 ± 9.7
31		6.83 ± 0.06	147	77.3 ± 5.0
32		6.65 ± 0.03	226	41.4 ± 5.9
33		6.54 ± 0.08	289	78.6 ± 6.2
34		6.51 ± 0.05	307	68.3 ± 6.3
35		6.38 ± 0.04	418	85.7 ± 7.3
36		6.03 ± 0.07	926	38.0 ± 5.4
37		5.85 ± 0.08	1410	69.7 ± 10.8

^a pEC₅₀ values of <5.00 showed potentiation but the potency cannot be determined due to no plateau of the CRC curve; pEC₅₀ values of <4.52 showed no activity up to the 30 μM concentration test compound.

^b Data represent the mean values from at least 3 experiments [AID 58842].

Table 2Rat M₄ potency, Fold Shift and *In vitro* PK profiling.^{9d}

Entry	rM ₄ pEC ₅₀ ± SEM ^a	rM ₄ EC ₅₀ (nM) ^b	hM ₄ ACh fold-shift ^a	rM ₄ ACh fold-shift ^a
3	6.83 ± 0.09	147	224 ± 82	54.1 ± 3.1
4	6.76 ± 0.05	176	106 ± 18	49.6 ± 3.9

	hCL _{HEP} (mL/min/kg)	rCL _{HEP}	rPPB (%fu)	hPPB (% fu)
3	13.9	51.0	6.4	1.1
4	18.8	55.2	1.7	2.6

^aLeftward shift of an ACh CRC in the presence of 10 μM compound relative to ACh CRC control [AID 588751].

Table 3Muscarinic selectivity of lead compounds **3** and **4**.^{9d}

Entry	Muscarinic Selectivity (EC ₅₀ , μ M)									
	hM ₁	rM ₁	hM ₂	rM ₂	hM ₃	rM ₃	hM ₅	rM ₅	hM ₅	rM ₅
	(% ACh Max)									
3	0.97 (33)	0.94 (26)	0.55 (64)	0.64 (58)	>30	>30	>30	>30	>30	>30
4	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30

Table 4

In vitro and in vivo properties of ML253.

MW	346.8
tPSA (Å)	80.9
cLogP	2.19
rM ₄ EC ₅₀ (nM)	176
rM ₄ FS	49.6

PK parameters (rat)**IV dose (1 mg/kg) ^a**

Cl (mL/min/kg)	103.5
t _{1/2} (min)	17.5
MRT (min)	27.5
V _{ss} (L/kg)	2.6
AUC-IV (hr·ng/mL)	175

PBL (i. p., 10 mg/kg, 0 – 6 h), hr·ng/mL or g

Plasma Systemic	3884
Brain	3410
B/P	0.88

% Reversal of amphetamine-induced hyperlocomotion (10% Tween 80, i.p.)

3 mg/kg	18.7 ± 8.3%
10 mg/kg	25.7 ± 11.5%
30mg/kg	47.9 ± 7.8% *
56.6 mg/kg	53.7 ± 13.1% **
100 mg/kg	7.3 ± 4.4% **

* p = <0.01, Dunnet's test