Published in final edited form as:

Bioorg Med Chem Lett. 2012 August 1; 22(15): 5084–5088. doi:10.1016/j.bmcl.2012.05.109.

Discovery of *N*-(4-methoxy-7-methylbenzo[d]thiazol-2-yl)isonicatinamide, ML293, as a novel, selective and brain penetrant positive allosteric modulator of the muscarinic 4 (M₄) receptor

James M. Salovich^{a,c,d}, Paige N. Vinson^{a,c,d}, Douglas J. Sheffler^{a,c,d}, Atin Lamsal^{a,c,d}, Thomas J. Utley^{a,c,d}, Anna L. Blobaum^{a,c,d}, Thomas M. Bridges^{a,c,d}, Uyen Le^{a,c,d}, Carrie K. Jones^{a,c,d,e}, Michael R. Wood^{a,b,c,d}, J. Scott Daniels^{a,c,d}, P. Jeffrey Conn^{a,c,d}, Colleen M. Niswender^{a,c,d}, Craig W. Lindsley^{a,b,c,d}, and Corey R. Hopkins^{a,b,c,d,*}

^aDepartment of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

^bDepartment of Chemistry, Vanderbilt University, Nashville, TN 37232, USA

^cVanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University Medical Center, Nashville, TN 37232, US

^dVanderbilt Specialized Chemistry Center for Probe Development (MLPCN), Nashville, TN 37232, USA

^eU.S. Department of Veterans Affairs, Tennessee Valley Healthcare System, Nashville, TN 37232, USA

Abstract

Herein we describe the discovery and development of a novel class of M₄ positive allosteric modulators, culminating in the discovery of ML293. ML293 exhibited modest potency at the human M₄ receptor (EC₅₀ = 1.3 μM) and excellent efficacy as noted by the 14.6-fold leftward shift of the agonist concentration-response curve. ML293 was also selective versus the other muscarinic subtypes and displayed excellent *in vivo* PK properties in rat with low IV clearance (11.6 mL/min/kg) and excellent brain exposure (PO PBL, 10 mg/kg at 1 h, [Brain] = 10.3 μM, B:P = 0.85).

Keywords

Positive allosteric modulator; M₄; ML293; CNS penetration; Muscarinic receptor 4

The neurotransmitter acetylcholine (ACh) regulates a diverse set of physiological actions through the activation of two classes of cell-surface receptors. These receptors, the nicotinic ACh receptors (nAChRs)¹ are ACh-gated cation channels and the muscarinic ACh receptors (mAChRs) are G protein-coupled receptors (GPCRs).^{2,3} Both sets of receptors are involved in numerous physiological processes. Molecular cloning has identified five separate

© 2012 Elsevier Ltd. All rights reserved.

*Corresponding author. Tel: +1-615-936-6892. Fax: +1-615-936-4381. corey.r.hopkins@vanderbilt.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

subtypes of the mAChRs ($M_1 - M_5$) which are subclassified due to their coupling patterns to different G proteins.²⁻⁴ One class couples to G_q , activating phospholipase C ($M_{1,3,5}$), and the second class couples to $G_{i/o}$ ($M_{2,4}$), regulating cAMP levels and various ion channels.²⁻⁴ Two mAChRs are widely distributed in the CNS (M_1 and M_4) and have been shown in pre-clinical experiments to be potentially viable therapeutic targets for Alzheimer's disease and schizophrenia.⁵ In addition to pre-clinical validation, these mechanisms have been shown to be clinically effective utilizing the non-selective M_1/M_4 -preferring agonist xanomeline.⁶ Finally, significant work utilizing knockout mice has shown the importance of the role of M_1 and M_4 in cognition and psychosis, respectively.^{2, 4}

Unfortunately, due to high sequence homology and conservation of the ACh binding site among the mAChRs, development of selective orthosteric modulators has been difficult. Due to this, we and others have been investigating the use of allosteric modulators in order to overcome the homology of the orthosteric site and develop selective tools for these receptors. As part of the NIH-sponsored Molecular Libraries Probe Production Center Network, we have recently reported three selective M_4 positive allosteric modulator probe molecules (Figure 1). These molecules have been important tool compounds and have shown efficacy in pre-clinical *in vivo* animal models of schizophrenia; however, these compounds suffer from less than ideal pharmacokinetic properties.⁷

The first SAR library kept the left-hand benzothiazole portion constant and looked at a variety of amide analogs (Table 1). These compounds were synthesized by utilizing the commercially available 4-methoxy-7-methylbenzo[d]thiazol-2-amine and coupling with an appropriate acid chloride under basic conditions. The original molecule, **1**, reconfirmed and exhibited micromolar potency ($EC_{50} = 1.8 \mu M$). Addition of a methyl group, **18**, led to an inactive compound; in addition, replacement of the dioxine moiety with a saturated pyran was deleterious to activity (**16** and **17**). Replacement with other heteroaryl groups proved more fruitful with the 2-furyl group (**4**, $1.3 \mu M$) showing a slight improvement in activity. In addition, 3-pyridyl (**20**, $2.33 \mu M$), 4-pyridyl (**21**, $1.3 \mu M$) and 2-pyrazine (**23**, $1.1 \mu M$) modifications were all well tolerated. However, these analogs showed significant differences in efficacy (%ACh Max) with the 4-pyridyl analog, **20**, proving to be the most efficacious (65% vs. <40% for **20** and **23**). Replacement of the heterocyclic moiety with alkyl, cycloalkyl or aromatic groups led to inactive compounds.

Due to these shortcomings, we were still interested in the discovery of novel M_4 tool compounds that offer improved *in vivo* PK properties. To this end, we performed a screen at Vanderbilt which revealed a novel structural class of compounds as potential M_4 positive allosteric modulators (Figure 2, **1**). A small library was generated around this molecule and from this effort there were many compounds that potentiated a submaximal (EC_{20}) concentration of ACh (Figure 2). Based on these promising initial results, a lead optimization campaign was initiated in our laboratories.

Next, we turned our attention to amide replacements – an exercise which proved to be less informative and highlights the shallow nature of allosteric modulator SAR (Table 2).⁹ Moving from the 4-pyridyl amide to the 4-pyridyl urea (**29**, inactive) led to a complete loss of activity. In fact, all ureas that were analyzed were inactive, with the lone exception being **35**, which was weak in terms of both potency ($7.1 \mu M$) and efficacy (36%). Other amide replacements (sulfonamide/reverse amides) were not tolerated, resulting in either inactive or weakly active compounds.

Lastly, we investigated replacements of the benzothiazole moiety (Table 3). Removal of both the 4-methoxy and 7-methyl groups were not tolerated (**50**). In addition, replacement of the benzothiazole with thiazolo[5,4-*b*]pyridine groups also resulted in inactive compounds

(**51** – **55**). However, deletion of the 7-methyl group (while maintaining the 4-methoxy) was tolerated, resulting in compound **56** (2.8 μ M).

We determined that compound **21** possessed the appropriate balance between *in vitro* potency and efficacy, thus, this compound was further evaluated in our muscarinic selectivity panel as well as in a fold-shift assay (an evaluation of a compound's ability to produce a left-ward shift of the ACh curve). Compound **21** was examined at the other four muscarinic subtypes ($M_{1,2,3,5}$) and was inactive against each of these receptors at a concentration up to 30 μ M. In our fold-shift assay (at a constant concentration, 30 μ M), **21** displayed a robust left-ward shift of the ACh curve (14-fold) showing a potentiation of the ACh potency (Figure 3B). Based on the hM_4 potency, fold shift and selectivity profile, **21** was declared an MLPCN probe molecule and redesignated ML293.¹⁰

ML293 was next profiled in a number of Tier 1 *in vitro* pharmacokinetic assays (Table 4). ML293 was assessed using an intrinsic clearance assay (CL_{INT}) in hepatic microsomes to evaluate the oxidative metabolism potential. This assay also provides an *in vitro* prediction for eventual assessment of clearance values in *in vivo* PK studies (CL_{HEP}). ML293 was predicted to display moderate clearance in both human and rat microsomes (14.9 and 48.5 mL/min/kg). In addition to intrinsic clearance, we determined the plasma protein binding (human and rat equilibrium dialysis studies) of ML293. ML293 showed high plasma protein binding in human plasma (1.0%, unbound); however, in rat plasma, ML293 showed a more favorable free fraction (3.2%, unbound). Lastly, ML293 was evaluated in a rat brain homogenate binding study to predict the fraction of unbound compound in brain. ML293 showed higher brain homogenate binding profile versus the plasma binding (BHB, 0.9% unbound vs. 3.2% unbound, respectively).

As a follow-up to the *in vitro* studies, a standard rat IV PK experiment was performed to determine the *in vivo* clearance of ML293. In this experiment (1 mg/kg, IV), ML293 exhibited low clearance ($CL_p = 11.5$ mL/min/kg) with a modest half-life ($t_{1/2} = 57$ mins) and significant plasma levels ($AUC = 1445$ hr $\cdot$ ng/mL, 4.8 μ M). These values are in contrast to the previously reported M_4 probe molecules (Figure 1), as each of those molecules exhibit high clearance in rat when assessed using IV PK experiments ($CL_p > 100$ mL/min/kg).^{7b,11} Lastly, we evaluated the CNS exposure of ML293 in a PBL (Plasma:Brain level) experiment after a single dose and at a single time point. ML293 was dosed orally at 10 mg/kg and after 1 hour the Brain:Plasma (B/P) ratio was 0.85 with absolute brain levels of 3093 ng/g (~10 μ M). In addition, in this experiment we assessed the concentrations in the hepatic portal vein as well as plasma. The levels found in the HPV and plasma can be used as another assessment of the extent of oxidative metabolism, as a ratio at or close to 1:1 indicates a low level of metabolism; ML293 exhibits a roughly 0.8 ratio in these studies. Based on these experiments, ML293 is a potent and selective M_4 PAM with favorable pharmacokinetics that demonstrates significant CNS penetration following oral administration.

In conclusion, we have discovered an additional M_4 tool compound (ML293) with superior *in vivo* PK properties compared to previously published molecules. ML293 represents a novel chemical scaffold as other reported M_4 PAMs have been derived from a common 3-amino-N-(aryl)-4,6-disubstitutedthieno[2,3-b]pyridine-2-carboxamide moiety, and have all suffered from similar poor *in vivo* PK properties. ML293 is a potent and selective small molecule M_4 positive allosteric modulator that significantly leftward shifts the ACh curve (14.9 Fold Shift). In addition, ML293, in contrast to the previously reported compounds, displays low clearance when evaluated in an *in vivo* rat IV clearance assay and is highly brain penetrant (B:P = 0.85, [brain] = ~10 μ M).

Acknowledgments

The authors would like to thank Tammy Santomango, Frank Byers, and Ryan Morrison for technical assistance with the PK experiments and Nathan Kett and Sichen Chang for the purification of compounds. Vanderbilt is a member of the MLPCN and houses the Vanderbilt Specialized Chemistry Center for Accelerated Probe Development (U54MH084659).

References and notes

1. Dani JA, Bertrand D. *Annu. Rev. Pharmacol. Toxicol.* 2007; 47:699. [PubMed: 17009926]
2. Wess J, Eglén RM, Gautam D. *Nature Rev. Drug. Dis.* 2007; 6:721.
3. 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]
4. Wess J. *Annu. Rev. Pharmacol. Toxicol.* 2004; 44:423. [PubMed: 14744253]
5. (a) Bymaster FP, McKinzie DL, Felder CC, Wess J. *Neurochem. Res.* 2003; 28:437. [PubMed: 12675128] (b) Raedler TJ, Bymaster FP, Tandon R, Copolov D, Dean B. *Mol. Psychiatry.* 2007; 12:232. [PubMed: 17146471] (c) Felder CC, Bymaster FP, Ward J, DeLapp N. *J. Med. Chem.* 2000; 43:4333. [PubMed: 11087557]
6. Shekhar, A.; Potter, WZ.; Lienemann, J.; Sundblad, K.; Lightfoot, J.; Herrera, J.; Unverzagt, F.; Bymaster, FP.; Felder, C. 40th Annual Meeting of American College of Neuropsychopharmacology; Hawaii. 2001. (b) Bodick NC, Offen WW, Levey AI, Cutler NR, Gauthier SG, Satlin A, Shannon HE, Tollefson GD, Rasmussen K, Bymaster FP, Hurley DJ, Potter WZ, Paul SM. *Arch. Neurol.* 1997; 54:465. [PubMed: 9109749]
7. (a) Brady AE, Jones CK, Bridges TM, Kennedy JP, Thompson AD, Heiman JU, Breininger ML, Gentry PR, Yin H, Jadhav SB, Shirey JK, Conn PJ, Lindsley CW. *J. Pharmacol. Exp. Ther.* 2008;327.(b) 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]
8. **Human muscarinic acetylcholine receptor cell line creation and culture.** Human M₁ (hM₁) cDNA in pcDNA3.1(+) was purchased from www.cdna.org and stably transfected into Chinese hamster ovary (CHO K1) cells purchased from the ATCC (www.atcc.org). CHO cells stably expressing hM₂, hM₃, and hM₅ were generously provided by A. Levey (Emory University, Atlanta, GA). hM₄ cDNA in pcDNA3.1 (+) was purchased from www.cdna.org and stably transfected into CHOK1 cells. To make stable hM₂ and hM₄ cell lines for use in calcium mobilization assays, cell lines were cotransfected with the chimeric G protein G_qi5 (Conklin et al., 1993). All transfections utilized Lipofectamine 2000. hM₁, hM₃, and hM₅ CHO cells were cultured in Ham's F-12; 10% heat-inactivated fetal bovine serum (FBS), 20mM HEPES, and 500 µg/mL G418 (Mediatech, Inc., Herndon, VA). hM₂-G_qi5 and hM₄-G_qi5 were grown in the same medium supplemented with 500 µg/mL hygromycin B. All cell culture reagents were purchased from Invitrogen Corp. (Carlsbad, CA) unless otherwise noted. **Calcium Mobilization Assays** - Potency determinations. Assays were performed within the Vanderbilt Center for Neuroscience Drug Discovery's Screening Center. CHO cell lines expressing muscarinic acetylcholine receptors were plated (15,000 cells/20 µl/well) in black-walled, clear-bottomed, TC treated, 384 well plates (Greiner Bio-One, Monroe, North Carolina) in Ham's F-12, 10% FBS, 20 mM HEPES. The cells were grown overnight at 37 °C in the presence of 5% CO₂. The following day, plated cells had their medium exchanged to Assay Buffer (Hank's balanced salt solution, 20 mM HEPES and 2.5 mM Probenecid (Sigma-Aldrich, St. Louis, MO)) using an ELX405 microplate washer (BioTek), leaving 20 µL/well, followed by addition of with 20 µL of 4.5 µM Fluo-4, AM (Invitrogen, Carlsbad, CA) prepared as a 2.3 mM stock in DMSO and mixed in a 1:1 ratio with 10% (w/v) pluronic acid F-127 and diluted in Assay Buffer for 45 minutes at 37 °C. The dye was then exchanged to Assay Buffer using an ELX405, leaving 20 µL/well and the plates were incubated at room temperature for 10 min prior to assay. Test compounds were transferred to daughter plates using an Echo acoustic plate reformatter (Labcyte, Sunnyvale, CA) and then diluted into Assay Buffer to generate a 2× stock in 0.6% DMSO (0.3% final). Acetylcholine (ACh) EC₂₀ and EC₈₀ were prepared at a 5X stock solution in assay buffer prior to addition to assay plates. Calcium mobilization was measured at 37 °C using a Functional Drug Screening System 6000 or 7000 (FDSS6000 or FDSS7000, Hamamatsu, Japan) kinetic plate reader according to the following protocol. Cells were preincubated with test compound (or vehicle) for

144 seconds prior to the addition of an EC₂₀ concentration of the agonist, ACh. 86 seconds after this addition, an EC₈₀ concentration of ACh was added. Control wells also received a maximal ACh concentration (1 mM) for eventual response normalization. The signal amplitude was first normalized to baseline and then as a percentage of the maximal response to ACh. Microsoft XLfit (IDBS, Bridgewater, NJ) was utilized for curve fitting and EC₅₀ value determination using a four point logistical equation. **Calcium Mobilization Assays – Fold-Shift Assays.** Assays were performed within the Vanderbilt Center for Neuroscience Drug Discovery's Screening Center. CHO cell lines expressing muscarinic acetylcholine receptors were plated (15,000 cells/20 μ L/well) in black-walled, clear-bottomed, TC treated, 384 well plates (Greiner Bio-One, Monroe, North Carolina) in Ham's F-12, 10% FBS, 20 mM HEPES. The cells were grown overnight at 37 °C in the presence of 5% CO₂. The following day, plated cells had their medium exchanged to Assay Buffer (Hank's balanced salt solution, 20 mM HEPES and 2.5 mM Probenecid (Sigma-Aldrich, St. Louis, MO)) using an ELX405 microplate washer (BioTek), leaving 20 μ L/well, followed by addition of with 20 μ L of 4.5 μ M Fluo-4, AM (Invitrogen, Carlsbad, CA) prepared as a 2.3 mM stock in DMSO and mixed in a 1:1 ratio with 10% (w/v) pluronic acid F-127 and diluted in Assay Buffer for 45 minutes at 37 °C. The dye was then exchanged to Assay Buffer using an ELX405, leaving 20 μ L/well and the plates were incubated at room temperature for 10 min prior to assay. Test compounds were prepared in Assay Buffer to generate a 2 $\times$ stock in 0.6% DMSO (0.3% final). Acetylcholine (ACh) concentration responses were prepared at a 5X stock solution in assay buffer prior to addition to assay plates. Calcium mobilization was measured at 37 °C using a Functional Drug Screening System 6000 or 7000 (FDSS6000 or FDSS7000, Hamamatsu, Japan) kinetic plate reader according to the following protocol. Cells were preincubated with test compound (or vehicle) for 144 seconds prior to the addition of a concentration response of the agonist ACh and the fluorescence was monitored for a total of 5 min. The signal amplitude was first normalized to baseline and then as a percentage of the maximal response to acetylcholine. Microsoft XLfit (IDBS, Bridgewater, NJ) was utilized for curve fitting and EC₅₀ value determination using a four point logistical equation. Fold-Shift values were calculated by dividing the ACh EC₅₀ in the presence of 30 μ M compound by the ACh EC₅₀ in the presence of vehicle.

9. (a) Engers DW, Niswender CM, Weaver CD, Jadhav S, Menon UN, Zamorano R, Conn PJ, Lindsley CW, Hopkins CR. *J Med Chem.* 2009;52.(b) Williams R, Johnson KA, Gentry PR, Niswender CM, Weaver CD, Conn PJ, Lindsley CW, Hopkins CR. *Bioorg Med Chem Lett.* 2009;19.(c) Zhao Z, Wisnoski DD, O'Brien JA, Lemaire W, Williams DL Jr, Jacobsen MA, Wittman M, Ha SN, Schaffhauser H, Sur C, Pettibone DJ, Duggan ME, Conn PJ, Hartmann GD, Lindsley CW. *Bioorg. Med. Chem. Lett.* 2007;17.
10. VU0409524 (ML293) 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/>.
11. Lewis LM, Bridges TM, Niswender CM, Weaver CD, Lindsley CW. Bookshelf ID: NBK50687. 2010
12. ***N*-(4-methoxy-7-methylbenzo[d]thiazol-2-yl)isonicotinamide, ML293:** To a stirred solution of 4-methoxy-7-methylbenzo[d]thiazol-2-amine (0.050 g, 0.26 mmol) and DIEA (0.112 mL, 0.64 mmol) in DCM (1.3 mL) at 0 °C was added isonicotinoyl chloride hydrochloride (0.050 g, 0.28 mmol). The reaction was allowed to warm to room temperature and stir overnight. The reaction was concentrated under vacuum and the residue purified by reverse-phase HPLC to give 55 mg (71%) of the pure product. ¹H NMR (400 MHz, DMSO-d₆): δ 8.82 (d, J = 6.0 Hz, 2H), 8.02 (d, J = 6.1 Hz, 2H), 7.11 (d, J = 8.1 Hz, 1H), 6.95 (d, J = 8.1 Hz, 1H), 3.90 (s, 3H), 2.45 (s, 3H). LC/MS: R_T = 0.60 min., *m/z* = 300 [M + H]⁺.

Figure 1.
Previously reported M₄ probe molecules ML108, ML173, ML253.

Figure 2.

Initial ACh EC₂₀ triage screen of M₄ analogs. Assay was performed at 10 μ M using hM₄-G_qi5-CHO cells; activity was monitored using an intracellular calcium mobilization assay. Values represent a single screening experiment performed in triplicate; numbered compounds were then reassessed using full concentration-response curves.

Figure 3.

A. Concentration-response curve of ML293 in human M_4 cells in the presence of an EC_{20} concentration of ACh. B. ACh fold-shift assay for ML293 at a 30 μ M concentration. Data represent three independent determinations performed in triplicate.

Table 1

SAR around the eastern amide moiety.⁸

Entry	R	hM ₄ pEC ₅₀ ± SEM	hM ₄ EC ₅₀ (μM)	%ACh _{max} ± SEM
1		5.74 ± 0.03	1.8	55.7 ± 4.6
2		<5 *	>10	52.5 ± 3.1
3		Inactive		
4		5.87 ± 0.02	1.3	51.8 ± 2.6
5		<5	>10	43.7 ± 2.9
6	Me	Inactive		
7		ND **	ND	Weak
8		Inactive		
9		<5	>10	61.5 ± 3.1
10		<5	>10	43.2 ± 2.7
11		Inactive		
12		<5	>10	66.5 ± 2.2
13		Inactive		
14		<5	>10	52.2 ± 4.6
15		<5	>10	45.0 ± 1.8
16		<5	>10	64.1 ± 1.4
17		<5	>10	44.0 ± 2.4
18		Inactive

Entry	R	hM ₄ pEC ₅₀ ± SEM	hM ₄ EC ₅₀ (μM)	%ACh _{max} ± SEM
19		Inactive		
20		5.63 ± 0.04	2.3	37.6 ± 3.1
21		5.89 ± 0.01	1.3	65.2 ± 2.7
22		Inactive		
23		5.95 ± 0.09	1.1	38.2 ± 1.3
24		Inactive		
25		Inactive		
26		Inactive		
27		Inactive		
28		Inactive		

For all tables, means are determined from at least 3 experiments performed in triplicate on separate days.

* Compounds that did not exhibit a plateau in their concentration- response were given a potency value of >10 μM and therefore no error was calculated for this parameter.

*** ND: not determined. This compound showed weak (low %ACh_{max}) potentiation in two runs and did not meet criteria for PAM categorization in the third run.

Table 2

Amide replacements.⁸

Entry	R	hM ₄ pEC ₅₀ ± SEM	hM ₄ EC ₅₀ (μM)	%ACh _{max} ± SEM
29		Inactive		
30		Inactive		
31		<5 **	>10	39.3 ± 3.0
32		Inactive		
33		<5 **	>10	43.5 ± 2.3
34		Inactive		
35		5.15 ± 0.15 *	7.1	36.5 ± 2.3
36		Inactive		
37		Inactive		
38		Inactive		
39		Inactive		
40		Inactive		
41		Inactive		
42		Inactive

Entry	R	hM ₄ pEC ₅₀ ± SEM	hM ₄ EC ₅₀ (μM)	%ACh _{max} ± SEM
43		Inactive		
44		ND ***	ND	Weak
45		Inactive		
46		Inactive		
47		5.11 ± 0.11 *	7.7	35.6 ± 2.0
48		ND ***	ND	Weak
49		ND ***	ND	Weak

* Average of two low potency estimates (10 μM) and one potency measure.

** Compounds that did not exhibit a plateau in their concentration- response were given a potency value of >10 μM and therefore no error was calculated for this parameter.

*** ND: Not determined. These compounds showed weak (low %ACh_{max}) potentiation and did not meet criteria for PAM categorization in one or two of the three runs.

Table 3Benzothiazole replacements.⁸

Entry	R	hM ₄ pEC ₅₀ ± SEM	hM ₄ EC ₅₀ (μM)	%ACh _{max} ± SEM
50		Inactive		
51		Inactive		
52		Inactive		
53		Inactive		
54		Inactive		
55		Inactive		
56		5.55 ± 0.04	2.8	36.5 ± 1.3

For all tables, means are determined from at least 3 experiments performed in triplicate on separate days.

Table 4

In vitro and *in vivo* properties of ML293.¹²

21, ML293 (VU0409524)

MW	299.35
tPSA (Å)	64.1
cLogP	1.76
hM ₄ EC ₅₀ (μM)	1.30
hM ₄ FS	14.9 ± 1.5
<i>Intrinsic Clearance (mL/min/kg)</i>	
Human CL _{HEP}	14.9
Rat CL _{HEP}	48.5
<i>Plasma Protein Binding (PPB)</i>	
human (%fu)	1.0
rat (%fu)	3.2
BHB (rat, %fu)	0.9
<i>PK parameters (rat)</i>	
IV dose (1 mg/kg) ^a	
Cl (mL/min/kg)	11.5
t _{1/2} (min)	57
MRT (min)	85
V _{ss} (L/kg)	0.97
AUC-IV (ng/mL)	1445
PBL (p.o., 10 mg/kg @ 1 h), ng/mL or g	
Plasma Systemic	3740
HPV	3070
Brain	3093
B/P	0.85