MK-7622: A First-in-Class M_1 Positive Allosteric Modulator Development Candidate

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S Supporting Information

ABSTRACT: Identification of ligands that selectively activate the M_1 muscarinic signaling pathway has been sought for decades to treat a range of neurological and cognitive disorders. Herein, we describe the optimization efforts focused on addressing key physicochemical and safety properties, ultimately leading to the clinical candidate MK-7622, a highly selective positive allosteric modulator of the M_1 muscarinic receptor that has entered Phase II studies in patients with Alzheimer's disease.

KEYWORDS: M_1 , muscarinic, positive allosteric modulator, M_1 PAM, MK-7622

Alzheimer's disease (AD) involves the progressive and irreversible degeneration of cholinergic neurons in the basal forebrain, which serve critical functions in the central nervous systems (CNS) with respect to both memory and cognition, ultimately contributing to cognitive decline in patients.¹ Cholinergic signaling is mediated by acetylcholine (ACh), the endogenous ligand of both the nicotinic and muscarinic receptors; the latter are class A G-protein coupled receptors (GPCR) comprising five subtypes (M_1 through M_5).^{2,3} Of the subtypes, M_1 is most highly expressed in the hippocampal, striatal, and cortical areas crucial in memory and higher brain function.⁴ As a result, direct activation of the M_1 receptor has represented an attractive approach to treat the cognitive deficits associated with AD, and for more than two decades, the pharmaceutical industry has pursued this goal.⁵

Moderate cognitive improvement has been demonstrated with acetylcholine esterase inhibitors like donepezil (Aricept), which represent the current standard of care for AD patients, impacting both nicotinic and muscarinic signaling pathways. Direct activation of the muscarinic system with orthosteric agonists, such as xanomeline, provides evidence that muscarinic activation may improve cognitive performance in AD patients.⁶ However, xanomeline's clinical utility was ultimately limited by tolerability believed to arise from nonselective activation of the M_2 – M_5 muscarinic subtypes, a consequence of the ligand binding to the highly conserved muscarinic orthosteric binding site. Alternate strategies to achieve preferential activation of the M_1 receptor have also been pursued, including bitopic and allosteric agonists that interact at less conserved binding regions.^{7–9} Another strategy to pursue is the identification of a positive allosteric modulator (PAM) of the M_1 receptor, an approach that relies on the selective amplification of

endogenous ACh signaling tone with minimal intrinsic activity in the absence of ACh. An allosteric modulator approach was pursued at Merck Research Laboratories (MRL), and progress in this area has been reported.

Screening of the MRL sample collection provided lead BQCA (Figure 1, IP is defined as the inflection point of the

BQCA: M_1 IP = 0.66 μ M

PQCA: M_1 IP = 0.14 μ M

Figure 1. BQCA and PQCA M_1 positive allosteric modulators.

potentiation curve)¹⁰ and has been well-characterized.¹¹ Optimization efforts aimed toward improving physicochemical properties led to the identification of quinolinquinoline PQCA.^{12–14} While both BQCA and PQCA served as excellent tools to probe the pharmacological effects of allosteric modulation,¹⁵ there were a number of improvements required to render a suitable development compound. First, these carboxylic acid-containing modulator classes exhibited generally low central nervous system exposure in which a relationship

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was not observed between CNS exposure and P-gp efflux ratios (as measured by CSF/U_{plasma} ratios), suggesting the involvement of additional transport mechanisms. Furthermore, due to the propensity for acyl glucuronide formation and low confidence in predicting human pharmacokinetics, a suitable candidate could not be identified. While amide-based strategies that addressed pharmacokinetic and metabolic properties were pursued by MRL and others,^{16,17} these approaches enhanced P-gp efflux and hERG channel activity, which were ultimately limiting. This Letter summarizes key data and medicinal chemistry efforts, which culminated in the identification of MK-7622, a CNS penetrant, M_1 PAM suitable for clinical development.

To address these limitations, replacement of the carboxylic acid became a key focus of our medicinal chemistry efforts. The favorable CNS properties of BQCA are unique, as most carboxylic acids are peripherally restricted.¹⁸ The measured pK_a of BQCA-like carboxylic acids are higher (ranging between 6.8 and 7.4) than typical carboxylic acids, suggesting an intramolecular keto-hydrogen bond (Figure 2). Mimicry of this

Figure 2. Optimization of noncarboxylic acid M_1 PAMs.

motif was pursued through the introduction of a third ring fused to the bicyclic core. Bioisostere-like replacement of the intramolecular keto-hydrogen bond with removal (isoxazolinone 1) or retention (pyrazolone 2) of hydrogen bond donating ability proved unfruitful. Further substitution of the tricyclic ring system was required to establish activity, and the addition of an *N*-phenyl ring provided modest potency (3, M_1 IP = 8.9 μM). Introduction of an *ortho* substituent on the phenyl ring was critical for achieving potent M_1 activity. In the case of compound 4, an *ortho*-fluorine provided M_1 potency comparable to BQCA. Extensive structure–activity relationship (SAR) studies with pyrazolone-containing compounds revealed that exquisite potencies could be achieved (IP < 5 nM).¹⁹ However, increases in potency were orthogonal to desirable physicochemical properties (i.e., solubility), as compounds like 4 possess few rotatable bonds and high aromatic ring count.

This structure-based observation proved to be critical, as physicochemical properties were modulated by changes to the tricyclic core. Exploration of core replacements with the simplified *para*-methoxybenzyl moiety revealed that the fused

pyridinone 5 (Table 1) possessed weak activity and a dramatic increase in cLogP. Potency was restored with the introduction

Table 1. Properties of PAMs 4–9

compd	4	5	6	7	8	9
M_1 potency (IP, μM) ^a	0.41	3.3	0.82	0.052	0.22	0.015
cLogP ^b	3.4	6.5	5.5	4.0	4.3	3.6
LLE ^c	3.0	−1.0	0.56	3.3	2.3	4.2

^aValues represent the numerical average of at least two experiments. Interassay variability was $\pm 30\%$ (IP, nM), unless otherwise noted. ^bcLogP calculated with ACD version 12. ^cLLE was calculated as follows: $pIP - pLogD$, where $pIP = -\log_{10}(IP[M])$.

of the pyrimidinone 6, but the low sp^3 -character of these naphthylpyridinones also impaired physicochemical properties, resulting in only modest improvements in ligand-lipophilic efficiency (LLE).²⁰ Replacement of the fluorophenyl with *trans*-cyclohexanols, as observed with lactam 7, proved fruitful through the introduction of significant sp^3 -character with gains in potency and LLE. While improvements in physicochemical properties were achieved with lactams, P-gp efflux remained endemic and was not resolvable within this series.²¹ Interestingly, as the *trans*-cyclohexanol was introduced into the pyrimidinone scaffold 8, further improvements in LLE were realized (compared to 6) and P-gp efflux was attenuated. Further improvements in LLE were realized with introduction of the chloropyridine 9.

Having identified scaffold 9 as displaying low potential for P-gp efflux (BA/AB ratio = 1.1), further evaluation of the southern benzyl ring was undertaken, providing compounds 9–13 (Table 2). The substituent changes had little effect on compound potency with all compounds exhibiting good M_1 potentiation. Compounds 9–12 were evaluated for and exhibited good CNS penetration as determined by CSF/U_{plasma} ratios. Interestingly, as pyridine basicity was modulated in this class through electron withdrawing groups (9, 12, 13), a marked increase in LogD was observed. This was reflected in decreased solubility at pH 2, which was even more pronounced at physiologically relevant pH 7 at which nonbasic compounds exhibited no detectable solubility. More basic pyridines (i.e., 10, 11) had good pH 2 solubility with moderate solubility at pH 7. Additionally, nonbasic and unsubstituted pyridines inhibited

Table 2. Properties of PAMs 9–13

compound	R	M ₁ IP (nM) ^a	rat CSF/U _{plasma} ^b	HPLC LogD	LLE ^c	solubility pH 2, 7 (μM) ^d	hERG IC ₅₀ (μM)
10	H	42	0.5	2.4	5.0	181, 34	12
11	Me	19	0.5	2.6	5.1	191, 17	60
9	Cl	13	0.9	3.1	4.8	<2, <2	13
12	OMe	18	1.0	3.0	4.7	71, <3	6.4
13	SMe	21	ND	3.2	4.5	102, <2	7.5

^aValues represent the numerical average of at least two experiments. Interassay variability was $\pm 30\%$ (IP, nM), unless otherwise noted. ^bDetermined in Sprague–Dawley rats following oral dose of 10 mg/kg in 90% PEG 400. Interanimal variability was less than 30% for all values. Ratios determined by rat CSF/(plasma concentration $\times$ fraction unbound in plasma). ^cLLE was calculated as follows: pIP – pHPLCLogD where pIP = $-\log_{10}(\text{IP}[\text{M}])$. ^dKinetic solubility measured at noted pH.

Scheme 1. Synthetic Scheme^a

^aReagents: (a) *tert*-butoxybis(dimethylamino)methane, toluene 120 °C (96%). (b) KMnO₄, K₂CO₃, *tert*-BuOH, H₂O (61%). (c) HCl, MeOH, 90 °C (88%). (d) i. Pd/C, H₂, MeOH, THF. ii. Br₂, dioxane, CCl₄ (95%). (e) LiOH, THF, H₂O (94%). (f) (1*H*-1,2,3-Benzotriazol-1-yl)oxy[tris(dimethylamino)]phosphonium hexafluorophosphate, (1*S*,2*S*)-2-aminocyclohexanol, DMF, TEA (73%). (g) DMF-DMA, 80 °C (78%). (h) Pd(PPh₃)₄, (2-chloro-5-pyridyl)methylzinc chloride, THF, (80%). (i) *trans*-*N*¹,*N*²-dimethylcyclohexane-1,2-diamine, CuI, MeOH, 160 °C (41%). (j) MeZnCl, Pd(PPh₃)₄, THF, 90 °C (95%). (k) MeOH, *trans*-*N*¹,*N*²-dimethylcyclohexane-1,2-diamine, Cs₂CO₃, CuI, 160 °C (10%). (l) MeSNa, DMF, 120 °C (84%).

hERG. 2-Methylpyridine **11** represented the optimized balance of properties with respect to hERG inhibition, P-gp efflux ($P_{\text{app}} = 33 \times 10^{-6}$ cm/s; MDR1 BA/AB ratio = 1.6), solubility profile, and LLE.

The synthetic approach for preparing pyrimidinones **9**–**13** is shown in Scheme 1. Methyl naphthalene **14** was treated with Bredereck's reagent to form intermediate **15**. Oxidative cleavage and ester formation afforded methyl ester **16**. Nitro reduction and subsequent bromination afforded **17**. Upon hydrolysis, HATU-mediated coupling of acid **18** with (*S,S*)-*trans*-1-hydroxy-2-amino-cyclohexane provided amide **19**, which was cyclized with DMF-DMA to form benzoquinazoline **20**. Negishi-type cross coupling of **20** with (2-chloro-5-pyridyl)-methylzinc chloride afforded chloropyridine **9**, which was used for the synthesis of compounds **10**–**13** via standard trans-formations.

Further profiling revealed that methylpyridine **11** exhibited the lowest intrinsic clearance in human microsomes ($\text{Cl}_{\text{int}} = 15$ mL/min/kg) among compounds examined from Table 2 and

was selected for advanced characterization. Pharmacokinetic studies with **11** were conducted in rats, dogs, and monkeys, and the parameters obtained are summarized in Table 3. Plasma clearances were low to moderate with good oral bioavailability. Terminal half-lives were moderate, ranging from 1.3 to 8.7 h. M₁ PAM **11** is eliminated primarily via oxidative metabolism and in vitro–in vivo correlation analysis utilizing the “well stirred” model of hepatic clearance was used to predict in vivo

Table 3. Rat, Dog, and Rhesus Pharmacokinetics of **11**

	F (%)	t _{1/2} (h)	Cl (mL/min/kg)	Vdss (L/kg)
rat ^a	56	1.3	18.9	0.94
dog ^b	76	8.7	4.5	2.4
Rhesus ^c	40	1.8	7.6	1.2

^aSprague–Dawley rats ($n = 3$). Oral dose 10 mg/kg, IV dose = 2 mg/kg. ^bMongrel dogs ($n = 2$). Oral dose 3 mg/kg, IV dose = 1 mg/kg. ^cRhesus monkeys ($n = 2$). Oral dose 1 mg/kg, IV dose = 0.5 mg/kg. Interanimal variability was less than 20% for all values.

clearances from microsomal stability data.²² Based on this analysis, **11** was predicted to exhibit low to moderate total clearance in human within the range of 0.7–2.3 mL/min/kg, supportive of once-daily dosing.

The equilibrium solubility of **11** was tested in various buffer solutions (pH 2–10), biorelevant media, and vehicles used for in vivo toxicology studies. Compound solubility was pH-sensitive, as observed in the kinetic solubility in Table 2; at acidic pH (<pH 4), solubility was observed to be between 6 and 9 mg/mL, and at pH > 6, solubility decreased; solubility was <0.001 mg/mL at pH 8 and 10. A crystalline fumarate salt was identified for development with a solubility of 2.3 mg/mL in simulated gastric fluid and 0.04 mg/mL in fasted state simulated intestinal fluid.

Advanced pharmacological profiling was conducted with M₁ PAM **11**. Selectivity over M₂–M₄ muscarinic subtypes was evaluated in CHO cells overexpressing the human receptor subtypes and neither potentiation nor agonism of the M₂–M₄ receptors was observed. This high selectivity for M₁ potentiation is consistent with the properties of the progenitor (BQCA) of **11** and the totality of the molecules prepared during the course of the optimization efforts. In addition, M₁ PAM **11** possessed similar M₁ potentiation across species: rat (M₁ IP = 14 nM), mouse (M₁ IP = 6.7 nM), dog (M₁ IP = 1.4 nM), and rhesus (M₁ IP = 3.8 nM) receptors. M₁ PAM **11** was found to have no significant findings in an ancillary pharmacology profile screen of 140 targets (CEREP panel) with only two hits having less than 5 μ M potency (lipoxygenase 5-LO IC₅₀ = 4.4 μ M, phosphodiesterase PDE₄ IC₅₀ = 2.1 μ M). Lastly, **11** proved to be a weak competitive reversible inhibitor of CYP2C9 (IC₅₀ = 13.3 μ M). The potential for cytochrome P450 induction was characterized by treatment of human hepatocytes with **11** (0.1–10 μ M), and no observed induction of CYP3A4 mRNA or activity was observed.

M₁ PAM **11** was further characterized regarding receptor allostery (Figure 3) and its ability to modulate the response of

Figure 3. Receptor allostery profile of **11** measured by Ca²⁺ flux in human M₁ overexpressing CHO cells.

ACh (12 pM to 1.0 μ M) to varying doses of allosteric modulator (22 pM to 22 μ M).²³ The responses were normalized to the ACh response at 1000 μ M, and these results indicate that **11** is a highly cooperative modulator (α = 338) with a 0.948 μ M affinity for the unbound receptor (KB) with a τ B of 1.067.

The in vivo properties of M₁ PAM **11** were evaluated in a mouse contextual fear conditioning (CFC) assay, which is dependent on hippocampal function (Figure 4).²⁴ In this study,

Figure 4. In vivo activity of **11**.

mice were treated with scopolamine before introduction to a novel environment to block a new association. Mice dosed by intraperitoneal injection with **11** exhibited a significant reversal at 3 mg/kg, compared to mice treated with scopolamine alone. The corresponding plasma levels were 2.4 μ M, and the estimated free brain levels at this dose were ~24 nM, within ~3-fold range of the M₁ inflection point in mouse.

In summary, quinolone carboxylic acid HTS lead BQCA was evolved into noncarboxylic acid modulators (i.e., **5**), which were optimized for physicochemical and safety properties into benzoisoquinolinone **11**. M₁ PAM **11** is an M₁-selective modulator with favorable preclinical pharmacokinetic and pharmacodynamic properties. On the basis of these properties and the potential for a good human ADMET profile, **11** (MK-7622) was selected for clinical development and has entered Phase II human proof-of-concept studies for the treatment of cognitive dysfunction in patients with AD. The results of these studies will be disclosed in due course.

■ ASSOCIATED CONTENT

§ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.8b00095.

Experimental procedures of selected compounds and spectra (PDF)

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