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Quinolizidinone Carboxylic Acids as CNS Penetrant, Selective M_1 Allosteric Muscarinic Receptor Modulators

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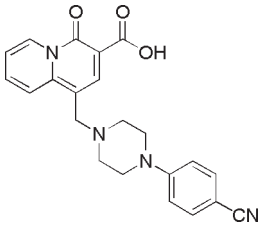
ABSTRACT Positive allosteric modulation of the M_1 muscarinic receptor represents an approach to treat the cognitive decline in patients with Alzheimer's disease. Replacement of a quinolone ring system in a quinolone carboxylic acid series of M_1 modulators with a quinolizidinone bearing a basic amine linkage led to a series of compounds with higher free fraction, enhanced CNS exposure, and improved efficacy in rodent in vivo models of cognition.

KEYWORDS Quinolizidinone, M_1 muscarinic receptor, allosteric modulator, cognition, Alzheimer's



One of the pathologies of Alzheimer's disease (AD) concerns the progressive degeneration of the neurons of the basal forebrain cholinergic system, contributing to the profound cognitive deficits manifest in AD.[1] Acetylcholinesterase inhibitors are currently the front line of treatment for AD symptoms and act by limiting the degradation of synaptic acetylcholine (ACh) levels to activate cholinergic receptors. Although these agents do offer cognitive benefit, the magnitude and duration of the effects are limited.

ACh activates both nicotinic (ligand-gated ion channels) and muscarinic (metabotropic) receptors, which belong to the class A family of G protein coupled receptors (GPCRs). There are five receptor subtypes in the muscarinic family (classed M_1 to M_5),[2,3] of which the M_1 receptor has been found to be highly expressed in the cortex and hippocampus areas that are critically involved in memory formation and are affected early in AD. As such, M_1 has been widely viewed as the subtype most likely modulating cognitive and memory effects in this region of the brain.[4] As a result, a number of nonselective or partially selective M_1 agonists such as xanomeline have been evaluated clinically, exhibiting efficacy in improving cognitive performance in AD patients.[5,6] However, adverse events (gastrointestinal distress, salivation, sweating), thought to be mediated by activation of other peripheral muscarinic receptors, have prevented their utility.

Efforts to identify compounds highly selective for the M_1 receptor subtype have failed due to the highly conserved nature of the orthosteric acetylcholine binding site. One avenue to address this is to target allosteric binding sites on the M_1 receptor that may be less highly conserved and provide ample specificity for M_1 over the M_2-M_5 subtypes.[7] As such, M_1 allosteric activators in the form of allosteric agonists[8] or positive allosteric modulators[9] could confer the high selectivity over their orthosteric counterparts.

In this regard, the quinolone carboxylic acid HTS lead **1**[10,11] and related analogues[12−14] have been reported as highly M_1 selective positive allosteric modulators (Figure 1). Compound **1** has modest M_1 receptor activity and brain penetration, high plasma protein binding, and low solubility in a neutral form. As a result, high plasma levels are required for in vivo efficacy. Attempts to address these issues via incorporation of basic amines extending off a biphenyl construct augmented some of these issues (Figure 1). However, this afforded compounds that were substrates for the human efflux pump P-glycoprotein (P-gp) leading to reduced CNS exposure.

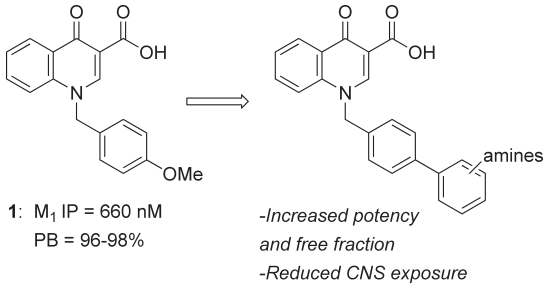


Figure 1

Accordingly, efforts were focused on the identification of an alternate scaffold for the quinolone carboxylic acid that would allow for improvement of these aforementioned

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issues.[15,16] Specifically, it was of interest to evaluate if a quinolizidinone ring system could replace the quinolone (Figure 2) and confer dissimilar properties. Ideally, this strategy would also allow for the incorporation of a basic amine at the benzylic position in the form of a piperidine or piperazine that could not be achieved in the quinolone context. Herein we report a series of novel brain penetrant quinolizidinone carboxylic acid M_1 positive allosteric modulators that address many of the concerns found in quinolone **1**, with improved in vivo efficacy and pharmacokinetic properties suitable for further development.

The chemistry employed to access target molecules examined in this communication is shown in Scheme 1. Lithiation of 2-picoline with *n*-butyllithium is followed by addition of ethoxymethylene diethylmalonate **3** and subsequent cyclization in refluxing *o*-xylene to provide quinolizidinone **4**.[17] Vilsmeier formylation at the 1-position afforded the requisite aldehyde **5**. Addition of the appropriate Grignard reagents to **5**, which were subsequently hydrolyzed using NaOH in THF led to acids **9c**,**d**. Oxidation using IBX followed by hydrolysis yielded ketone **9b**. Alternatively, deoxygentation of **6c**,**d** produces **9c**,**d** after ester hydrolysis. Intermediate **9d** (R = 4-chlorophenyl) underwent Suzuki cross-coupling with the appropriate boronic acid to result in **9e**−**g**. Lastly, reduction amination of **5** with the appropriate amines followed by saponification of the ethyl ester afforded target *N*-linked quinolizidinone carboxylic acids **10a**−**i**.

Carbon linked homologues of the quinolone carboxylic were evaluated first as shown in Table 1. Modifications

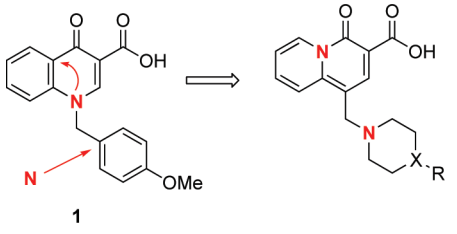


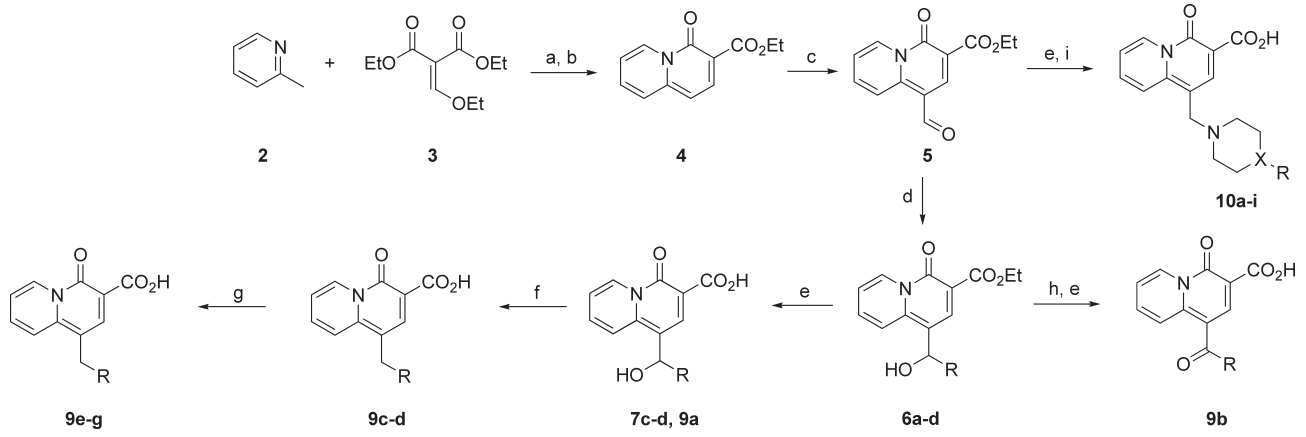
Figure 2

containing a hydroxyl group (**9a**) or ketone (**9b**) at the benzylic position were inactive. The benzyl analogue **9c** gave an M_1 IP = 0.92 μM, while potency was enhanced with the *p*-chloro (**9d**; M_1 IP = 200 nM) and *p*-phenyl (**9e**; M_1 IP = 110 nM). However, all of these compounds were fully bound to plasma proteins. Efforts to enhance free fraction via incorporation of aryl pyridines[12] (**9f**,**g**) in lieu of the biphenyl yielded potent M_1 potentiators, but had negligible reduction on protein binding.

Quinolizidinone carboxylic acids that are nitrogen linked at the benzylic position were evaluated as shown in Table 2. Having a simple piperidine (**10a**) in place gave an M_1 IP = 7.6 μM, but addition of a 4-phenyl group (**10b**) increased activity ~6-fold. Ring sizes other than a 6-membered ring were not tolerated as highlighted by phenyl pyrrolidine **10c**. Incorporation of a piperazine in lieu of the piperidine afforded **10d**, which further improved M_1 potentiation and also gave very high rat and human free fractions (>25%). Ketopiperazine **10e** gave similar M_1 potency compared to **10d**, and led to further reductions in plasma protein binding. Attempts to incorporate a pyridine as exemplified by **10f** led to reduction in M_1 functional activity. Substitution on the phenyl ring was tolerated, with the best results observed for groups at the *para* position. For example, bromide **10g** was the most potent potentiator in this series with an M_1 IP = 132 nM and a reasonable free fraction in rat (~4%). A trifluoromethyl group (**10h**) resulted in a similar free fraction, with a ~2-fold loss in M_1 potency. A cyano group provided a reasonably potent potentiator (**10i**; M_1 IP = 520 nM), but had an improved plasma free fraction relative to **10g**,**h**.

As P-glycoprotein (P-gp) is considered the dominant efflux transporter at the blood brain barrier (BBB) responsible for the efflux of a number of xenobiotic substances from the CNS, select compounds from Table 2 were evaluated for their passive permeability properties (P_{app}) and susceptibility as P-gp substrates in order to further evaluate their CNS exposure potential. As can be seen from Table 3, most of the compounds possessed good passive permeability (P_{app}) and were not substrates for human (MDR1) or rat (MDR1a) P-gp

Scheme 1[a]



[a] Reagents: (a) LDA, THF, −80 to −20 °C; (b) *o*-xylene, reflux; (c) $POCl_3$, DMF, 0 to 20 °C; (d) RMgBr, THF−CH_2Cl_2, 0 to 25 °C; (e) NaOH, THF, EtOH, 50 °C; (f) TFA, Et_3SiH, CH_2Cl_2; (g) $ArB(OH)_2$, $Pd(t\text{-}Bu_3P)_2$, Cs_2CO_3, THF, 100 °C; (h) IBX, CH_3CN, 75 °C; (i) $NaBH(OAc)_3$, DCE, AcOH, 4 Å molecular sieves.

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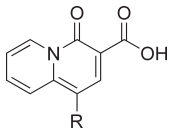
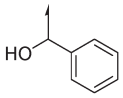
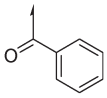
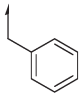
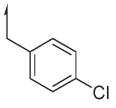
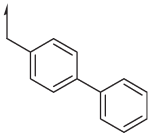
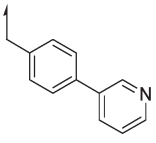
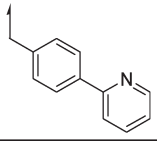
Table 1

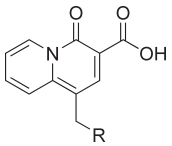


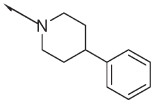
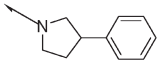
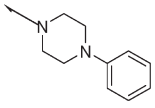
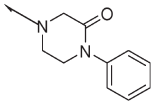
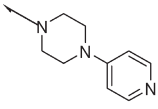
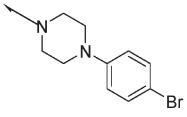
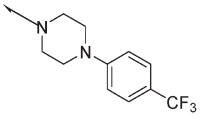
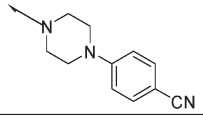
Compds	R[1]	M_1 Pot IP (μM)[a]	% Max	Rat PB	Human PB
9a		>100	-	-	-
9b		>100	-	-	-
9c		0.92	81	100	100
9d		0.20	93	100	100
9e		0.11	86	insol	insol
9f		0.075	74	98.5	99.7
9g		0.061	78	100	100

[a] Values represent the numerical average of at least two experiments. Interassay variability was ±30% (IP, nM), unless otherwise noted.

with efflux ratios less than 2. A notable exception was ketopiperazine **10f**, which had very poor P_{app} leading to very low brain and CSF levels despite having a very high rat free fraction (~48%). Bromide **10g** provided the highest plasma level and brain levels when dosed orally at 10 mpk. Trifluoromethyl piperazine **10h** provided a similar CSF/U_{plasma} ratio compared to **10g**, but with a moderately higher B/P ratio (~2.7). The highest CSF levels were observed with nitrile **10i**, which presented a CSF/U_{plasma} ratio of 0.42, albeit with a lower B/P ratio. The higher total brain levels of **10g**−**h** could be attributed to the increased lipophilicity of the bromo and trifluoromethyl groups relative to the nitrile (log D = 1.4) moiety in **10i**, which may lead to higher nonspecific brain tissue binding.[18] In addition, this greater degree of lipophilicity may be responsible for off-target activity at the hERG cardiac channel for **10g** (IC_{50} = 2.1 μM) and **10h** (IC_{50} = 0.1 μM), compared to **10i** (IC_{50} = 7.4 μM). Based

Table 2



Compds	R	M_1 Pot IP (μM)[a]	% Max	Rat PB	Human PB
10a		7.6	87	-	-
10b		1.3	58	-	-
10c		>100	-	-	-
10d		0.93	52	63.1	74.0
10e		1.10	89	38.0	52.1
10f		1.5	86	-	-
10g		0.13	77	96.1	93.5
10h		0.27	67	96.6	95.1
10i		0.52	94	93.8	86.6

[a] Values represent the numerical average of at least two experiments. Interassay variability was ±30% (IP, nM), unless otherwise noted.

Table 3. Permeability, P-gp, and Bioanalysis of Plasma, Brain, and CSF Levels in Rat for Selected Compounds

				concn (nM)[c]				
compds	P_{app}[a]	MDR1[b]	MDR1a[b]	plasma	brain	csf	brain/plasma	CSF/U_{plasma}[d]
10f	< 5			2071	59	16	0.03	0.02
10g	16	0.1	0.6	3646	4581	70	1.3	0.32
10h	32	1.4	1.3	1138	3085	14	2.7	0.25
10i	22	0.7	1.9	1495	248	85	0.17	0.42

[a] Passive permeability (10^{-6} cm/s). [b] MDR1 directional transport ratio (B to A)/(A to B). Values represent the average of three experiments, and interassay variability was ±20%. [c] Sprague−Dawley rats. Oral dose 10 mg/kg in 0.5% methocel. Interanimal variability was less than 20% for all values. [d] Determined using rat plasma protein binding from table 2.

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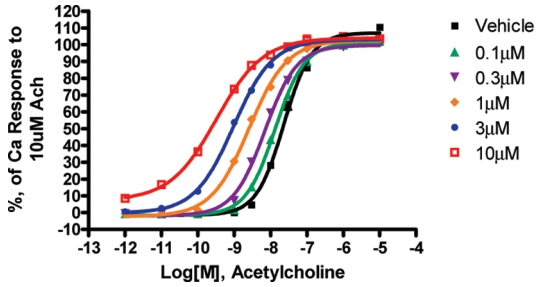

Figure 3. Compound **10i** potentiates the acetylcholine dose response curve for the M_1 receptor.

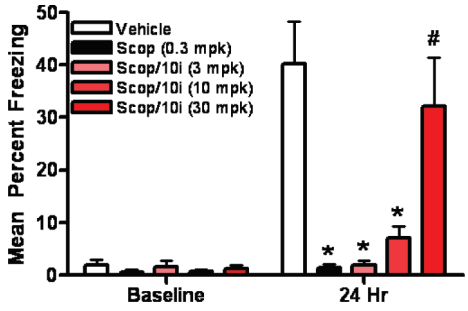

Figure 4. Compound **10i** in the mouse model of fear conditioning.

on the lower hERG affinity and high CSF levels, **10i** was chosen for further evaluation.

The effect of **10i** was evaluated on the affinity of acetylcholine for the M_1 receptor in a functional assay utilizing calcium mobilization as a readout. In CHO cells expressing the human M_1 receptor, increasing concentrations of compound **10i** from 0.1 to 10 uM potentiate the effect of acetylcholine leading to a leftward shift in the acetylcholine M_1 dose–response curve (Figure 3). In the absence of acetylcholine, there was no direct activation (agonism) of M_1 by compound **10i** up to the highest dose tested (10 uM). In addition, no agonist or antagonist activity is observed up to 100 μM on M_2, M_3, or M_4 receptors with or without acetylcholine present.

Potentiator **10i** was evaluated in a mouse contextual fear conditioning model of memory (Figure 4). In this experiment, the nonselective muscarinic antagonist scopolamine was administered to block formation of the association of a novel environment with an aversive stimulus (a foot shock). Animals that learn to associate the novel context with the adverse stimulus will respond with a freezing response when they are brought back into the same context 24 h later. Quinolizidinone **10i** was codosed at 3, 10, and 30 mpk (ip) with scopolamine on the training day, and produced no baseline (training day) effects. Mice codosed with **10i** showed a reversal of scopolamine deficits in a dose dependent manner in terms of freezing behavior compared to vehicle treated animals. The maximal effect noted at 30 mpk corresponded to 7.4 μM plasma levels for **10i**. By way of comparison, quinolone **1** required ~33 μM for reversal of scopolamine deficit in this model.

The pharmacokinetic properties of **10i** were evaluated in rat and dog (Table 4). Adequate bioavailability was observed

Table 4. Rat and Dog Pharmacokinetic Data for **10i**[a]

rat			dog		
F (%)	$t_{1/2}$ (h)	Cl	F (%)	$t_{1/2}$ (h)	Cl
23	6.4	10.8	33	2.5	13

[a] F% oral bioavailability, half-life is represented in hours, Cl in mL/min/kg. Sprague–Dawley rats (n = 3). Oral dose = 10 mg/kg in methocel, iv dose = 2 mg/kg in DMSO. Interanimal variability was less than 20%.

in rat (F = 23%) with a good half-life (6.4 h) and relatively low clearance (10.8 mL/min/kg). The dog clearance was higher with a shorter half-life. Overall, **10i** possessed suitable pharmacokinetic properties further highlighting the quinolizidinone as a suitable replacement for the quinolone carboxylic acid.

In summary, a quinolizidinone ring system has been identified as an advanced replacement for the quinolone core in a series of carboxylic acid M_1 positive allosteric modulators. The quinolizidinone scaffold allowed for incorporation of a basic amine in the form of a piperazine ring, which markedly improved the plasma free fraction. Quinolizidinone **10i** exemplified this leading to a potentiator with markedly improved performance in the mouse contextual fear conditioning model. Moreover, **10i** retains suitable pharmacokinetic and physicochemical properties for additional study to determine potential for clinical evaluation.

SUPPORTING INFORMATION AVAILABLE

Representative assay and experimental procedures and data for test compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

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