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Quinolizidinone carboxylic acid selective M1 allosteric modulators: SAR in the piperidine series

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ABSTRACT

SAR study of the piperidine moiety in a series of quinolizidinone carboxylic acid M_1 positive allosteric modulators was examined. While the SAR was generally flat, compounds were identified with high CNS exposure to warrant additional in vivo evaluation.

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Cholinergic neurons serve critical functions in both the peripheral and central nervous systems (CNS). Acetylcholine serves as the key neurotransmitter in these systems, targeting both nicotinic and metabotropic (muscarinic) receptors. Muscarinic receptors are class A G-protein coupled receptors (GPCR) widely expressed in the CNS. There are five muscarinic subtypes, designated M_1 to M_5,[1,2] of which M_1 is most highly expressed in the hippocampus, striatum, and cortex,[3] suggesting a central role in memory and higher brain function.

During the course of Alzheimer's disease (AD) there is a progressive degeneration of cholinergic neurons in the basal forebrain leading to cognitive decline.[4] One potential tactic to treat the symptoms of AD is the direct activation of the M_1 receptor.[5] As a result, a number of non-selective M_1 agonists have shown potential to improve cognitive performance in AD patients, but were clinically limited due to cholinergic side effects thought to be mediated via activation of the other muscarinic sub-types thru the highly conserved orthosteric acetylcholine binding site.[6,7]

One approach to engender selectively for M_1 over the other subtypes is to target allosteric sites on M_1 that are less highly conserved than the orthosteric site.[8,9] Ma et al.[10] recently reported the quinolone carboxylic acid **1** as a selective positive allosteric modulator of the M_1 receptor (Fig. 1).[11] Attempts to improve the

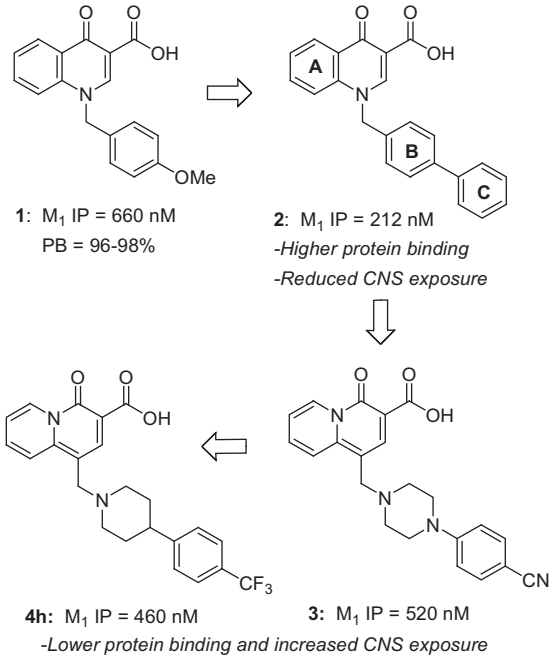

Figure 1. Quinolone and quinolizidinone leads.

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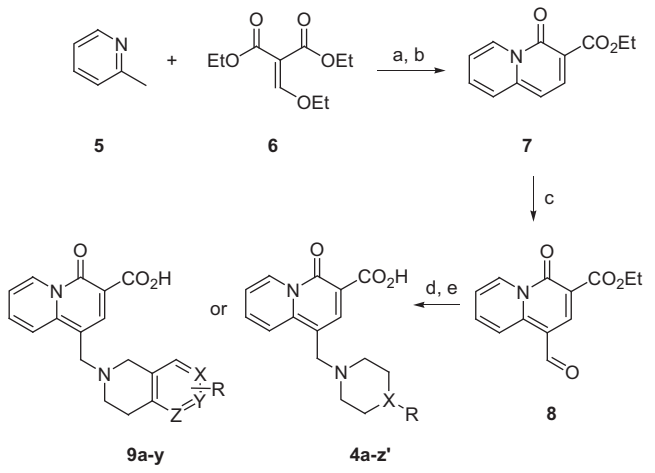
potency and physicochemical properties of **1** led to a number of advancements such as potent biphenyl **2**.[12] While these compounds were improved in terms of in vitro activity, higher plasma protein binding led to decreased CNS exposure impeding further in vivo evaluation.

We recently described the identification of quinolizidinone carboxylic acids as replacements for the quinolone ring system.[13] Inclusion of this motif allowed for the incorporation of basic amines such as piperazine **3** and piperidine **4** in lieu of a benzylic group, which led to dramatic improvement of physicochemical properties and free fraction, leading to enhanced CNS exposure. This communication describes efforts to optimize the potency and CNS exposure of piperidine containing M_1 positive allosteric modulators as represented by **4h**.

The synthesis of requisite quinolizidinones is shown in Scheme 1. The chemistry commenced with lithiation of 2-picoline **5** with LDA followed by addition of ethoxymethylene diethylmalonate **6** and subsequent thermal cyclization in refluxing *o*-xylene to provide quinolizidinone **7**. Vilsmeier formylation at the 1-position provided the requisite aldehyde **8**. Reductive amination of **8** with the appropriate amines was followed by saponification of the ethyl ester to afford target N-linked quinolizidinone carboxylic acid piperidines **4a–z′**, and tetrahydroquinolines **9a–y**.

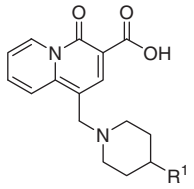
A library of amines was employed in the reductive amination protocol. The SAR data for a representative group of piperidine containing quinolizidinone carboxylic acids is shown in Table 1. Compound potencies were determined in the presence of an EC_{20} concentration of acetylcholine at human M_1 expressing CHO cells using calcium mobilization readout on a $FLIPR_{384}$ fluorometric imaging plate reader.

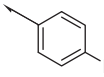
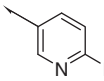
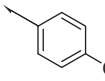
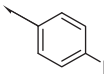
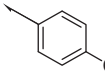
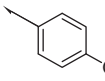
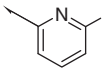
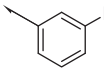
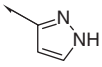
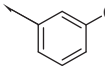
Phenyl piperidine **4a** was a modest potentiator with an M_1 IP = 1.3 μM.[14] Substitution at the *para* position of the phenyl ring (compounds **4b–i**) generally presented flat SAR with the aforementioned **4h** proving to be the most potent analog (M_1 IP = 460 nM). The position of the substituent on the ring also had little effect as movement of fluorine or chlorine to the *meta*- (**4k, 4m**) and *ortho*-positions (**4t**) did not provide any significant improvements. Replacement of phenyl with un-substituted (**4n**) or substituted pyridines (**4c, j, s**) provided similarly flat SAR. Diazines **4o–r** were a notable exception where a significant decrease was noted relative to the phenyl or pyridine counterparts. N-Linked azoles (**4u–y**) proved to be acceptable phenyl alternatives, with benzimidazole **4x** the best among the group. Moreover, the 2-substituted benzimidazole **4z** was the most potent piperidine identified



Scheme 1. Reagents and conditions: (a) LDA, THF, −80 to −20 °C; (b) *o*-xylene, reflux; (c) $POCl_3$, DMF, 0 to 20 °C; (d) $NaBH(OAc)_3$, DCE, AcOH, 4 Å molecular sieves; (e) NaOH, THF, EtOH, 50 °C.

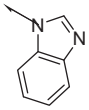
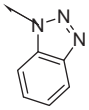
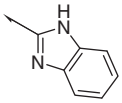
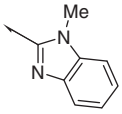
Table 1
M_1 FLIPR data for select piperidines



Compd	R^1	M_1 Pot IP (μM)[a]
4a		1.3
4b		1.7
4c		2.1
4d		0.77
4e		1.2
4f		1.3
4g		1.7
4h		**0.46**
4i		4.3
4j		2.2
4k		1.5
4l		1.5
4m		0.91
4n		0.95
4o		11.0
4p		4.3
4q		7.0
4r		13.0
4s		0.84
4t		1.7

(*continued on next page*)

Table 1 (*continued*)

Compd	R^1	M_1 Pot IP (μM)[a]
4u		0.78
4v		1.9
4w		1.7
4x		**0.55**
4y		6.0
4z		**0.12**
4z′		30.0

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

(M_1 IP = 120 nM), but capping the N–H with a methyl (**4z′**) led to a >200-fold decrease in activity.

In the quinolone carboxylic acid series, we have previously observed that the B- and C-rings of the biphenyl could be tied back, followed by C-ring excision to provide naphthyl **10** leading to an ~3-fold improvement in functional activity (Fig. 2).[15] In order to drive potency beyond what was observed in Table 1, this strategy was employed for piperidine **4a** leading to tetrahydroisoquinoline **9a**. While **9a** did not show an improvement over **4a**,[16] a series of analogs was examined to see if substitution would lead to improvements in this tetrahydroisoquinoline context. Results are shown in Table 2.

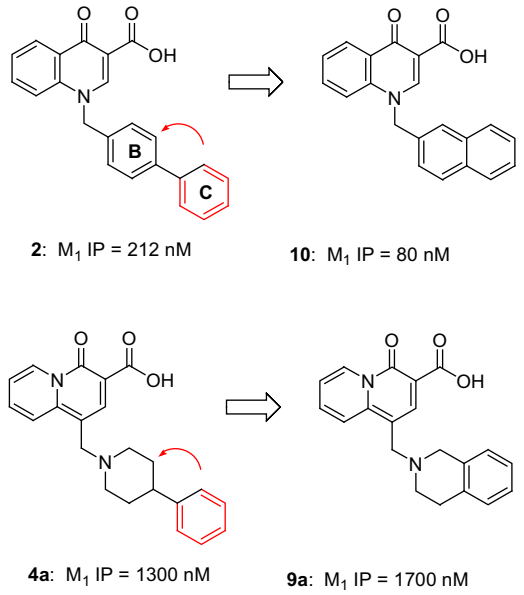
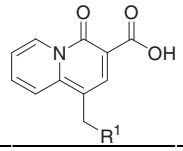
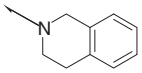
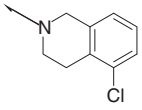
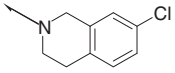
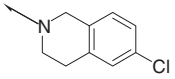
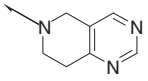
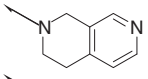
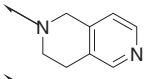
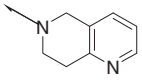
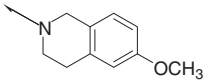
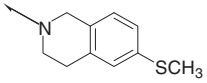
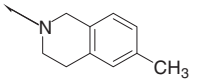
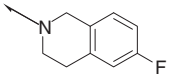
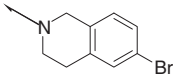
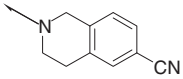
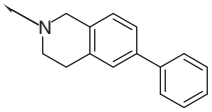
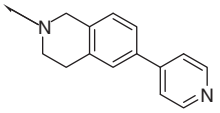


Figure 2. Design of tetrahydroisoquinolines.

Addition of a chlorine (**9b–d**) on the phenyl moiety of **9a** led to an improvement of functional activity with 6-chloro **9d** showing an ~5-fold increase (M_1 IP = 330 nM) relative to **9a**. Incorporation of heteroatoms provided equipotent (**9e–f**) or improved activity (**9g–h**) relative to the phenyl counterpart. A number of variations of **9d** were made substituting for the chlorine (**9i–p**). This position

Table 2
M_1 FLIPR data for select tetrahydroisoquinolines



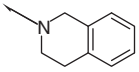
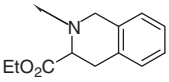
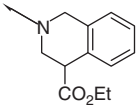
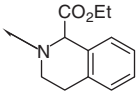
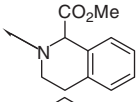
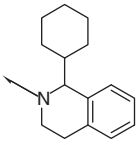
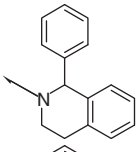
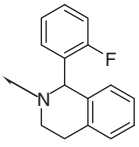
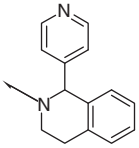
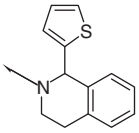
Compd	R^1	M_1 Pot IP (μM)[a]
9a		1.7
9b		0.85
9c		0.63
9d		0.33
9e		1.8
9f		1.7
9g		0.90
9h		0.83
9i		1.4
9j		>10
9k		1.1
9l		0.78
9m		0.63
9n		0.61
9o		0.56
9p		0.39

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

proved highly tolerant of substitution, with a flat SAR similar to that for the piperidines in Table 1, albeit with a slight preference for small, lipophilic groups such as fluorine (**9l**) or cyano (**9n**). Aromatics were also tolerated at this position of the tetrahydroisoquinoline, with 4-pyridyl **9p** showing very similar potency (M_1 IP = 390 nM) to chloro **9d**.

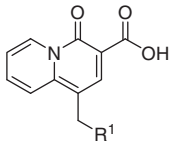
In addition to substitution of the phenyl sector of **9a**, a scan of the tetrahydroisoquinoline alkyl skeleton of **9a** was also conducted as shown in Table 3. Ethyl esters **9q–s** showed the 3-position was completely intolerant of substitution, while the 1-position led to significant increase in activity (M_1 IP = 340 nM). Additional substitutions were subsequently investigated at the 1-position (**9u–y**). Aliphatic groups were inactive as shown by cyclohexyl **9u**, but more favorable results were obtained with aromatic groups. Phenyl **9v** and thiophene **9y** gave similar data, but *ortho*-fluro phenyl **9w** showed a marked improvement. Moreover, 4-pyridyl **9x** proved to be the most potent compound reported here, with an M_1 IP = 53 nM.

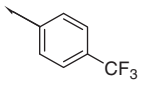
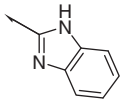
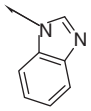
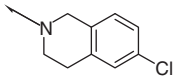
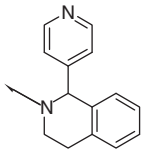
Table 3
M_1 FLIPR data for select tetrahydroisquinolines

Compd	R^1	M_1 Pot IP (μM)[a]
9a		1.7
9q		>100
9r		3.6
9s		0.34
9t		1.7
9u		>100
9v		0.56
9w		0.15
9x		0.053
9y		0.6

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

Table 4
Protein binding data for select analogs



Compd'	R^1	Rat PB (%)	Human PB (%)	Log *D*
4g		89	91	2.97
4x		76	79	0.92
4z		77	82	nd
9d		96	98	2.37
9x		94	96	nd

Select compounds from Tables 1–3 were evaluated for their human and rat plasma protein binding (Table 4). The piperidines gave high free fractions, particularly for the two benzimidazole regioisomers **4x,z** with ~20% free in each species. The tetrahydroisoquinolines **9d**,**x** were more highly bound, but still have between 2% and 6% free fraction. In addition, the log *D*'s were all in a desirable range, similar to what was observed for piperazines such as **3**.

Based on the acceptable levels of protein binding, the compounds in Table 4 were evaluated further for their CNS exposure potential. Accordingly, they were evaluated for passive permeability and potential as substrates for the CNS efflux transporter P-gp, as well as their CNS exposure in rat utilizing oral dosing at 10 mpk (Table 5).

All compounds except for benzimidazoles **4x** and **4z** gave acceptable permeability (>15). With the exception of **9x**, all other compounds were not P-gp substrates with good efflux ratios (<2.5). The 4-trifluoromethylphenyl piperidine gave a very high CSF/U_{plamsa} ratio of 0.7 and a surprisingly high brain to plasma (B/P) ratio of 6. Benzimidazole **4z** showed poor oral absorption and undetectable CNS levels consistent with the low passive permeability.[17] Chloro-tetrahydroisoquinoline **9d** presented higher plasma levels than piperidine **4g**, with a similar CSF/U_{plamsa} ratio. Lastly, the pyridyl-tetrahydroisoquinoline **9x** provided the best plasma levels among the group, but CNS ratios were lower, consistent with **9x** being a substrate for P-gp.

Based on their overall profiles[18] and high CSF levels, piperidine **4g** and tetrahydroisoquinoline **9d** were examined in a contextual fear conditioning model of episodic memory using B6SJL mice. Compounds were tested at 3, 10, and 30 mpk, but no significant reversal of scopolamine deficit was observed at the doses examined (data not shown). High plasma levels were obtained at 30 mpk with both **4g** (31 μM) and **9d** (16 μM) suggesting the lack of efficacy in this model was not due to insufficient drug levels.[19] This lack of activity in this mouse model[20] with the piperidines was particularly surprising as the related piperazine analog **3**, which had similar in vitro activity (M_1 IP = 520 nM) and brain

Table 5
Permeability, P-gp, and bioanalysis of plasma, brain, and CSF levels for selected compounds

Compd	P_{app} [a]	MDR1 [b]	MDR1a [b]	Plasma concn[c] (nM)	Brain concn[c] (nM)	CSF concn[c] (nM)	B/P	CSF/U_{plamsa}[d]
4g	35	0.9	0.9	915	5779	28	6.0	0.70
4x	<5	—	—	—	—	—	—	—
4z	6.6	0.4	1.9	132	0	0	—	—
9d	42	1.4	2.9	4245	3692	86	0.86	0.63
9x	27	2.8	9.7	9777	1345	230	0.14	0.37

[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 4.

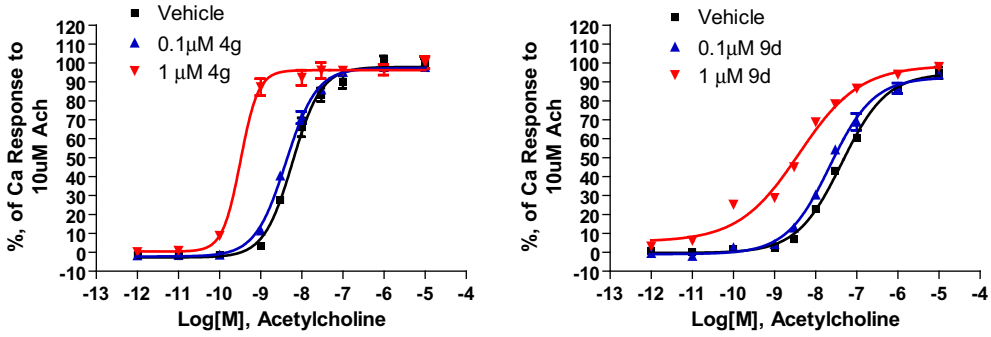


Figure 3. Fold potentiation curves for **4g** and **9d**.

exposure (CSF:U_{plasma} = 0.42), worked in this model at 7.4 μM plasma.[13]

To confirm that **4g** and **9d** were behaving similarly with respect to allosteric modulator **3**, their effects on the affinity of acetylcholine for the M_1 receptor in a functional assay utilizing calcium mobilization as the readout were evaluated. In CHO cells expressing the human M_1 receptor, increasing concentrations of both **4g** and **9d** from 0.1 to 1 μM potentiates the effect of acetylcholine leading to a leftward shift in the acetylcholine M_1 dose–response curves (Fig. 3). Thus it appears the lack of efficacy observed in the mouse CFC assay is not due to inability to potentiate the acetylcholine dose response.[21]

In summary, a series of piperidine and tetrahydroisoquinoline quinolizidinone carboxylic acids were prepared and evaluated. The SAR was thoroughly investigated and found that substitution on the piperidine was generally flat. Potentiators **4g** and **9d** were identified as having reasonable potency and both showed high CNS exposure. However, unlike the case for piperazine derived quinolizidione modulators such as **3**, these piperidine containing analogs did not show efficacy in a mouse model of episodic memory. Additional analog work along with more advanced in vitro studies is ongoing to explain this lack of activity.

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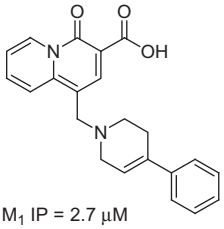
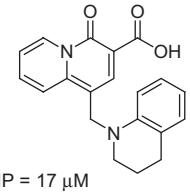
14. The tetrahydropyridine variant of **4a** was ~2 fold less potent than the saturated variant and was not investigated further.



M_1 IP = 2.7 μM

15. Kuduk, S.D.; Di Marco, C.N.; Cofre, V.; Pitts, D.R.; Ray, W.J.; Ma, L.; Wittmann, M.; Seager, M.; Koeplinger, KA..; Thompson, C.D.; Hartman, G.D.; Bilodeau, M.T. *Bioorg. Med. Chem. Lett.* **2010**, *19*, accepted.
16. The tetrahydroquinoline variant of 9a was ~10 fold less potent than the isoquinoline.



M_1 IP = 17 μM

17. It is worth noting that although **4z** does not appear to be a P-gp substrate, those values are not likely to reflect the true efflux ratio due the low passive permeability of the molecule in the parent cell line.
18. Compound **9x** was removed from further consideration due to its high P-gp efflux ratio and as it had significant binding to the hERG cardiac channel (<1 μM).
19. To confirm CNS exposure in mouse and that P-gp was not playing a role in CNS disposition in this species, CF-1 wild type and P-gp knock out mice were treated with **4g**, and plasma and CSF were taken after 30 minutes. The wild type mice gave a CSF/U_{plasma} ratio of ~0.37, and mice lacking P-gp gave a similar CSF/U_{plasma} ratio of ~0.42, showing that P-gp efflux in mouse was not a factor.
20. Potentiator **4g** was active at rat (M_1 IP = 760 nM) receptors, within twofold of human (M_1 IP = 460 nM).
21. Potentiators **4g** and **9d** were also evaluated for activity in the β-arrestin pathway, which recruits different signaling proteins than the Gαs-coupled Ca^{2+} release measured by the FLIPR assay, in the presence and absence of an EC15 concentration of ACh. Both compounds perform comparably in both FLIPR and β-arrestin assays relative to **3**, indicating that possible different signaling pathways are not responsible for the lack of in vivo activity.