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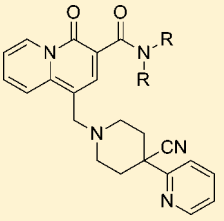
Identification of Amides as Carboxylic Acid Surrogates for Quinolizidinone-Based M_1 Positive Allosteric Modulators

Scott D. Kuduk,*[,†] Ronald K. Chang,[†] Thomas J. Greshock,[†] William J. Ray,[‡] Lei Ma,[‡] Marion Wittmann,[‡] Matthew A. Seager,[‡] Kenneth A. Koeplinger,[§] Charles D. Thompson,[§] George D. Hartman,[†] and Mark T. Bilodeau[†]

Departments of [†]Medicinal Chemistry, [‡]Alzheimer's Research, and [§]Drug Metabolism, Merck Research Laboratories, Sumneytown Pike, P.O. Box 4, West Point, Pennsylvania 19486, United States

Supporting Information

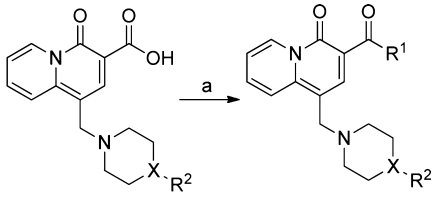
ABSTRACT: Selective activation of the M_1 muscarinic receptor via positive allosteric modulation represents an approach to treat the cognitive decline in patients with Alzheimer's disease. A series of amides were examined as a replacement for the carboxylic acid moiety in a class of quinolizidinone carboxylic acid M_1 muscarinic receptor positive allosteric modulators, and leading pyran **4o** and cyclohexane **5c** were found to possess good potency and in vivo efficacy.



KEYWORDS: *carboxylic acid surrogates, quinolizidinone, positive allosteric modulators*

Cholinergic neurons serve critical functions in both the peripheral and the central nervous systems (CNS). Acetylcholine is the key neurotransmitter in these neurons targeting 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–M_5,[1,2] of which M_1 is most highly expressed in the hippocampus, striatum, and cortex,[3] implying that it may play a central role in memory and higher brain function.

Scheme 1[a]



[a]Reagents and conditions: (a) BOP, amine, triethylamine, DMF.

One of the hallmarks of Alzheimer's disease (AD) is the progressive degeneration of cholinergic neurons in the basal forebrain leading to cognitive decline.[4] Accordingly, direct activation of the M_1 receptor represents an approach to treat the symptoms of AD.[5] In this regard, a number of nonselective M_1 agonists have shown potential to improve cognitive performance in AD patients but were clinically limited by cholinergic side effects thought to be due to activation of other muscarinic subtypes via binding to the highly conserved orthosteric acetylcholine binding site.[6,7]

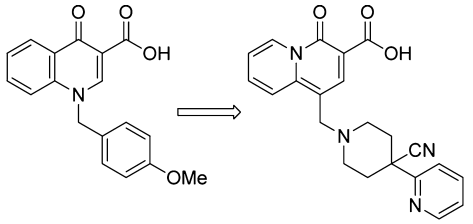


Figure 1.

One avenue to engender selectively for M_1 over the other muscarinic subtypes is to target allosteric sites on M_1 that are less highly conserved than the orthosteric site.[8,9] Quinolone carboxylic acid **1** (Figure 1) has been previously identified as a selective positive allosteric modulator of the M_1 receptor with exclusive selectivity for the M_1 subtype.[10] Recent efforts to improve the potency and CNS penetration of **1** led to a number of structural enhancements.[11−15] Further advancements were discovered by incorporation of a quinolizidinone ring system such as **2** in lieu of the quinolone, particularly in the area of improved free drug levels and in vivo activity.[16−18] However, the presence of the acid in this scaffold limited the extent of CNS exposure, and dominant clearance was by means of direct glucuronidation of the parent acid, complicating human pharmacokinetic predictions. Accordingly, it was of interest to see if noncarboxylic acid M_1 potentiators[19] could be identified from this acid scaffold. This communication describes efforts to

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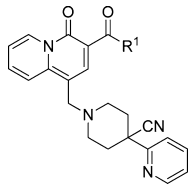
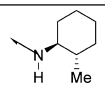
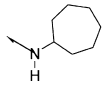
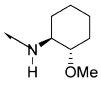
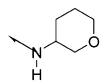
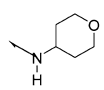
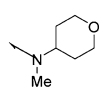
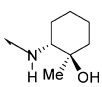
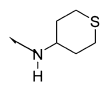
identify CNS penetrant quinolizidinone carboxylic amides as replacements for the acid motif.

The preparation of requisite amides is shown in Scheme 1. Quinolizidinone carboxylic acids **2** and **3**, as well as related analogues, were prepared using previously described procedures.[17] Amide bond formation with the appropriate amine using benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) provided amides **4** and **5**.

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. A large number of amines were used in library format in Scheme 1 with either 4-cyanopiperidine (**2**)- or piperazine (**3**)-linked quinolizidinone carboxylic acids. Select examples are shown in Table 1. Replacement of the acid in

Table 1. M_1 FLIPR Data for Select Compounds[a]



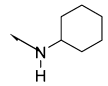
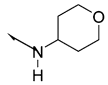
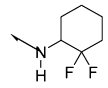
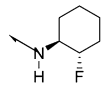
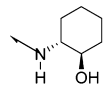
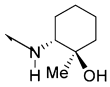
Compds	R^1	M_1 IP (nM)	Compds	R^1	M_1 IP (nM)	Compds	R^1	M_1 IP (nM)
2	OH	136	**4i**		2200	**4r**		190
4a	NH_2	1900	**4j**		980	**4s**		205
4b	$NHCH_3$	4300	**4k**		780	**4t**		147
4c		23000	**4l**		253	**4u**		177
4d		2400	**4m**		304	**4v**		381
4e		7800	**4n**		2600	**4w**		31
4f		4400	**4o**		338	**4x**		220
4g		1300	**4p**		4400	**4y**		93
4h		640	**4q**		344	**4z**		>20000

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

2 with NH_2 (**4a**) led to a >10-fold drop in potency, but at least some activity was preserved. Methylamine (**4b**) or pyrrolidine (**4c**) further decreased activity. Several aromatic amines were investigated as represented by **4d**−**h**, with 2-aminopyridine **4h** highlighting the most promising among the group.

While cyclopropyl amide **4i** was similarly equipotent to amide **4a**, cyclobutane **4j** was a submicromolar compound. Increasing

Table 2. Protein Binding Data and P-gp Data for Select Compounds

Compds	R^1	Papp[a]	MDR1[b]	MDR1a[b]	Rat PB	Human PB
2	OH	18	0.5	1.4	68.2	70.9
4l		28	1.6	2.0	95.6	93.4
4o		33	2.4	7.5	73.2	51.0
4s		29	1.7	3.4	96	89.5
4t		26	1.9	3.4	92.7	81.5
4w		29	11	21	86	52
4z		28	10	12	-	-

[a]Passive permeability (10^{-6} cm/s). [b]MDR1 (human) and MDR1a (rat) directional transport ratio (B to A)/(A to B). Values represent the average of three experiments, and the interassay variability was ±20%.

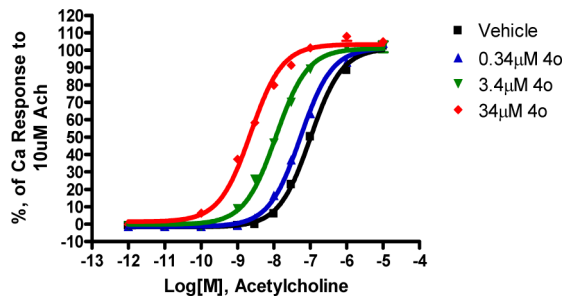


Figure 2.

the ring size (**4k**−**m**) led to an increase in functional activity, with cyclohexyl **4l** (M_1 IP = 253 nM) being the optimal fit. Insertion of a heteroatom such as oxygen into the cyclohexane ring in the form of a pyran was tolerated, with preference for the 4-position (**4o**) over the 3-position (**4n**), while the sulfide analogue (**4q**) was similarly active. N-Methylation of **4o** to tertiary amide **4p** lost >10-fold activity, highlighting the requirement for a secondary amide for M_1 activity.

Substitution on the cyclohexane ring identified the 2-position in the trans configuration as the most promising. The 2-methyl derivative **4r** gave an M_1 IP = 190 nM, and similar results were also noted with *gem*-difluoro **4s**. The 2-fluoro derivative is noteworthy as it was similarly equipotent to carboxylic acid **2**. Thiomethyl ether **4u** was similar to methyl **4r**, although methyl ether **4v** was less preferred. Moreover, hydroxyl **4w**, with the (1*S*,2*S*) configuration, was the most potent among the group (M_1 IP = 31 nM), ∼4-fold better than acid **2**. The associated (1*R*,2*R*) enantiomer **4x** was ∼7-fold less active. Interestingly, while addition of a methyl at the 2-position of **4w** in the form of tertiary alcohol **4y** was tolerated, addition of the methyl at the 1-position (**4z**) led to a complete loss of all M_1 activity. It is worth pointing out that select compounds were examined in functional assays at other muscarinic subtypes and showed no activity at M_2, M_3, or M_4

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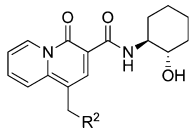
receptors, highlighting that quinolizidinone amides maintain selectivity for M_1.

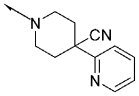
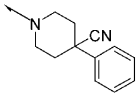
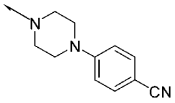
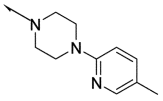
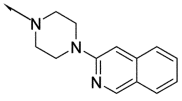
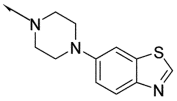
Having identified a number of potent amides, select compounds were examined to see if they were substrates for the multidrug resistant (MDR) efflux transporter P-glycoprotein (P-gp). In addition, plasma protein binding was determined using the equilibrium dialysis method in the presence of rat and human serum (Table 2).

Cyclohexane **4l** was not a P-gp substrate and exhibited good passive permeability with protein binding in the range of 93−95%. The corresponding pyran analogue **4o** increased efflux and was a substrate for rat P-gp (7.5) but showed markedly enhanced free fraction (27 and 49% in rat and human, respectively). Substitution at the 2-position of the cyclohexane with fluorine (**4s**,**t**) maintained good permeability and P-gp profiles with improved free fraction relative to cyclohexyl **4l**. The most potent potentiator alcohol **4w** unfortunately was a substrate for P-gp, as was the tertiary alcohol analogue **4z**.

It was thought that the P-gp efflux of highly potent alcohol **4w** could be modulated by finding a different amine in place of the 4-cyano-4-(2-pyridyl)piperidine. Replacement of the pyridyl with a phenyl (**4w′**) led to a modest reduction in M_1 potency and free fraction but substantially reduced P-gp efflux. However, this change also led to an increase in human ether-à-go-go-related gene (hERG) potassium channel binding

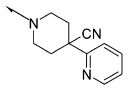
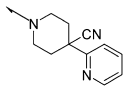
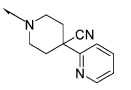
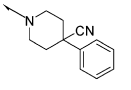
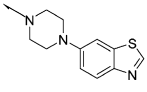
Table 3. M_1 FLIPR, Protein Binding, P-gp, and hERG Data for Select Compounds



Compds	R^2	M_1 IP (nM)[a]	Rat PB	Human PB	Papp[b]	MDR1[c]	MDR1a[c]	hERG[d]
4w		31	86	52	29	11	21	16.4
4w'		45	96.9	85.2	32	2.3	3.3	5.1
5a		244	93.9	90.5	30	1.1	4.7	0.39
5b		174	71.3	60.1	23	18	15	11.5
5c		110	96.8	91.3	29	1.5	1.7	2.0
5d		22	94.9	91.8	37	1.5	3.4	13.1

[a]Values represent the numerical average of at least two experiments. The interassay variability was ±30% (IP, nM), unless otherwise noted. [b]Passive permeability (10^{-6} cm/s) in Madin Darby canine kidney (MDCK) cells. [c]MDR1 directional transport ratio (B to A)/(A to B). Values represent the average of three experiments, and the interassay variability was ±20%. [d]Values represent the numerical average of at least two experiments. The interassay variability was ±20% (IC_{50}, μM).

Table 4. Bioanalysis of Plasma, Brain, and CSF Levels in Rat for Selected Compounds

Compds	R^1	R^2	Plasma Conc. (nM)[a]	Brain Conc. (nM)[a]	CSF Conc. (nM)[a]	Brain/ Plasma	CSF/U_{plasma}[b]
2	OH		1480	306	135	0.23	0.30
4t			1120	280	27	0.28	0.33
4o			2650	559	215	0.21	0.30
4w'			1610	896	29	0.52	0.58
5d			375	77	4	0.19	0.18

[a]Sprague−Dawley rats. Oral dose 10 mg/kg in 0.5% methocel. The interanimal variability was less than 20% for all values. [b]Determined using rat plasma protein binding from Tables 2 and 3.

(IC_{50} = 5.1 μM), a ~3-fold increase over pyridyl **4w**. Previously in the quinolizidinone carboxylic acid series, aryl piperazine derivatives were identified as potent and CNS penetrant.[16] The 4-cyanophenylpiperazine **5a** was reasonably potent (M_1 IP = 244 nM), with good free fraction, and was not a substrate for P-gp. Unfortunately, hERG binding (IC_{50} = 0.39 μM) was an issue. Incorporation of a methylpyridine (**5b**) for the cyanophenyl decreased the hERG binding but markedly increased P-gp efflux. Isoquinoline **5c** alleviated the P-gp issues observed with **5b** but did so at the expense of higher protein binding. Lastly, benzothioazole **5d** represented the most optimized potentiator among the group in terms M_1 potency, free fraction, P-gp profile, and hERG binding.

Selected compounds were examined for CNS exposure in rat as shown in Table 4. Brain, plasma, and cerebrospinal fluid (CSF) levels were measured after 2 h following a 10 mg/kg oral dose. Data for carboxylic acid **2** are shown for comparison.

Fluorocyclohexane **4t** gave a CSF/U_{plamsa} ratio of 0.33, which was similar to that observed with acid **2**, although absolute CSF levels were lower. Pyran **4o** gave robust plasma (2.6 μM) and CSF (215 nM) levels, with a comparable CSF/U_{plamsa} ratio of 0.30. The hydroxycyclohexane **4w′** possessed similar CSF levels to **4t** and had the highest total brain levels of the compounds tested. The high CSF level of **4o** was believed to be driven by the large free fraction due to the pyran (27%), while all other amides had greater than 90% protein binding. Interestingly, benzothiazole **5d** gave very low drug levels in all matrices examined, which was surprising since **5d** is not a P-gp substrate, with excellent permeability (P_{app} = 37) and free fraction similar to other compounds.

On the basis of the significant CSF levels of pyran **4o**, additional studies were conducted to further investigate the properties of amide derived modulators. Fold potentiation with a fixed concentration of modulator **4o** was evaluated on the M_1 dose response with acetylcholine as the agonist. As can be seen from Figure 2, with increasing concentration of potentiator, a left shift was observed up to 52-fold at 34 μM in the acetylcholine dose response, showing that **4o** is a potent positive allosteric modulator of the human M_1 receptor.

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To examine the in vivo properties, amide **4o** was evaluated in a mouse contextual fear conditioning (CFC) assay, which serves as a model of episodic memory (Figure 3). In this study, mice were treated with scopolamine before introduction to a novel environment to block a new association. Mice dosed by intraperitoneal injection with **4o** exhibited a significant reversal at 10 and 30 mg/kg, as compared to mice treated with scopolamine alone. The corresponding plasma levels at 10 mg/kg were 2.4 μM. By way of comparison, the analogous carboxylic acid **2** showed significant reversal at ∼1 μM plasma levels, which is consistent with greater in vitro potency than **4o** in the FLIPR assay. In addition, isoquinoline **5c** was also evaluated in mouse CFC and provided full reversal at a very similar plasma level (2.5 μM) to pyran **4o**. Accordingly, it appears that amides such as **4o** and **5c** behave similarly in this rodent cognition model relative to the parent quinolizidinone carboxylic acid M_1 positive allosteric modulators.[20]

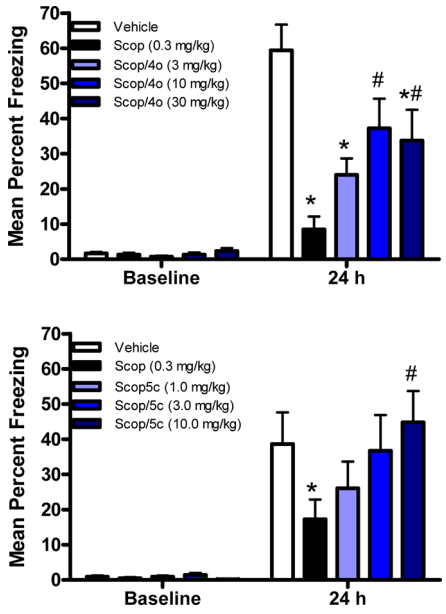

Figure 3. Evaluation of **4o** and **5c** in the mouse CFC model.

Previously, amides of lead quinolone carboxylic acid **1** have been examined with little success. As can be seen in Figure 4, similar amides made in both the quinolone and the quinolizidinone gave disparate results. For example, while cyclohexyl and tolyl were accommodated in the quinolizidinone context for both **4l** and **4d**, the quinolone congeners **6a** and **6b** lost greater than 20-fold potency in the FLIPR assay by way of comparison. Although the exact reason why amides are viable in combination with the quinolizidinone nucleus and not the quinolone is unclear, it serves as another example in which apparently seemingly minor core modifications lead to distinct SAR in allosteric modulator lead optimization.

In summary, amides of quinolizidinone carboxylic acids were found to be potent and CNS penetrant alternatives as selective M_1 positive allosteric modulators. Amides derived from (1*S*,2*S*)-2-aminocyclohexanol were found to be markedly the most potent variants but were substrates for the P-gp efflux transporter. Isoquinoline **5c** was an exception, which provided efficacy in a mouse contextual fear model of episodic memory but possessed low micromolar hERG channel inhibition. Pyran **4o** showed minimal inhibition of hERG (IC_{50} > 15 μM), was reasonably potent with high CSF levels in rat, and also provided good in vivo activity in mouse CFC. Further optimization of these amides and employment of this strategy in other scaffolds is ongoing.

■ ASSOCIATED CONTENT

Supporting Information

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.

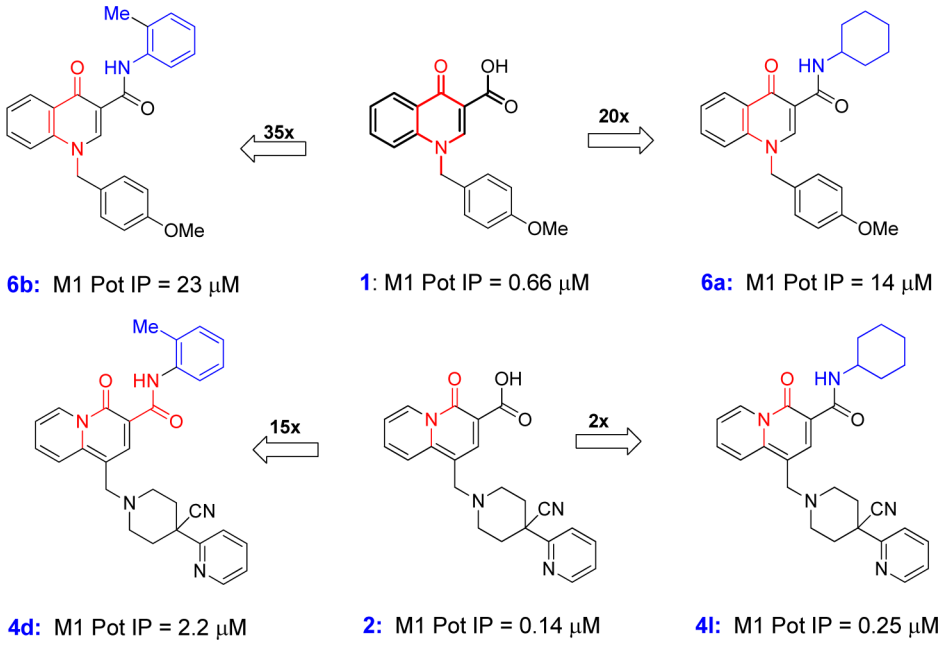

Figure 4.

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AUTHOR INFORMATION

Corresponding Author

*Tel: 215-652-5147. Fax: 215-652-3971. E-mail: scott_d_kuduk@merck.com.

Notes

The authors declare no competing financial interest.

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(20) In addition, **4o** exhibited good preclinical PK properties (dog Cl = 2.8 mL/min/kg, $t_{1/2}$ = 11 h).