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Identification of a methoxynaphthalene scaffold as a core replacement in quinolizidinone amide M_1 positive allosteric modulators

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ABSTRACT

A series of methoxynaphthalene amides were prepared and evaluated as alternatives to quinolizidinone amide M_1 positive allosteric modulators. A methoxy group was optimal for M_1 activity and addressed key P-gp issues present in the aforementioned quinolizidinone amide series.

Neurons expressing cholinergic receptors operate critical functions in both the peripheral and central nervous systems (CNS). The key neurotransmitter in these systems, acetylcholine, targets both nicotinic and metabotropic (muscarinic) receptors. The muscarinic receptors are members of class A G-protein coupled receptors (GPCR) that are extensively expressed in the CNS. Within the family, there are five muscarinic subtypes, designated M_1 to M_5.[1,2] The M_1 receptor is most highly expressed in brain regions of the hippocampus, striatum, and cortex,[3] signifying a fundamental role in memory and cognition.

As Alzheimer's disease (AD) advances, there is a gradual degeneration of these cholinergic neurons in the basal forebrain leading to increasing cognitive deficits.[4] An approach to address the symptoms of AD is via the direct stimulation of the M_1 receptor.[5]Toward this end, a number of non-selective M_1 agonists have previously shown promising clinical results on cognitive performance in patients with AD. However, none of these could advance beyond proof-of-concept studies as a result of untoward cholinergic side effects thought to be mediated via pan activation of other muscarinic sub-types as a consequence of the highly conserved orthosteric acetylcholine binding site.[6,7]

In order to preferentially activate the M_1 receptor over the other muscarinic sub-types, the strategy chosen was to target an allosteric site on M_1 that would be expected to be less well conserved than the orthosteric site.[8,9] Toward this end, lead quinolone carboxylic acid BQCA[10] was identified via screening of the MRL sample repository as a highly selective positive allosteric modulator (PAM) of the M_1 receptor and has been the subject of extensive lead optimization.[11] For example, we have described the evolution of BQCA into quinolizidinone carboxylic acids such as **1** as potent, selective, and CNS penetrant M_1 positive allosteric modulators.[12,13] Further analog work in this quinolizidinone context allowed further advancement identifying replacement of the carboxylic acid with amides such as **2**,[14] which were highly potent in functional assays, but were substrates for the CNS efflux transporter P-glycoprotein (P-gp). This communication describes efforts to identify an alternate core template for the quinolizidinone nucleus that would be bereft of P-gp mediated transport (Fig. 1).

One of the key features targeted in the selection of a core replacement would be the ability to form a putative intra-molecular hydrogen bond between the amidic N–H and an acceptor on the template.[15] This was a requirement for M_1 functional activity

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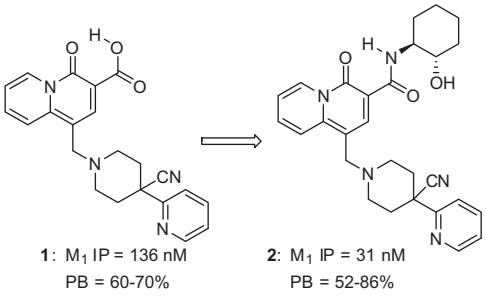


Figure 1.

during the quinolizidinone carboxamide SAR analysis as both tertiary amides and esters lose substantial activity. During the course of SAR efforts on HTS lead BQCA **3**, it was found that the quinolone ring system could be replaced with a methoxynaphthalene, in the context of the carboxylic acid, at the expense of ~5-6-fold loss of functional potency.[16] It was proposed to investigate the methoxynaphthalene in the framework of amides, to see if the methoxy group could serve to mimic the proposed hydrogen bond motif thought to be necessary for M_1 activity (Fig. 2).

The synthesis of requisite compounds is shown in Scheme 1. Starting with commercially available methyl 1-methoxy-2-napthoate, bromination provided intermediate **8**. Suzuki cross-coupling followed by hydrolysis afforded acid **9**. Amide formation with (1*S*,2*S*)-2-aminocyclohexanol was implemented using Bop reagent followed by oxidative cleavage of the olefin produced aldehyde **10**. Reductive amination of **10** delivered target amines **5a**–**c**. Alternatively, bromide **8** underwent Negishi coupling with the appropriate zinc reagent followed by cleavage of the methyl ester and methyl ether to provide acid **11**. Aforementioned Bop coupling of **11** was followed by alkylation with the appropriate alkyl halide to lead to targets **6a**–**f**. Lastly, the chloropyridine present in **6a** was further functionalized to prepare analogs **6g**–**n**.

The initial SAR data for a representative group of methoxynaphthalene amides that possess different groups at the 4-position of the naphthalene ring is shown in Table 1. Compound potencies were determined in the presence of an EC_{20} concentration of

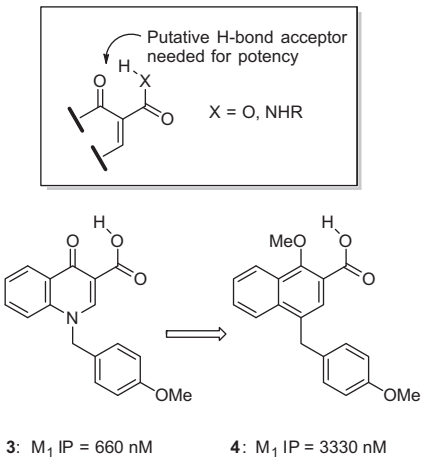
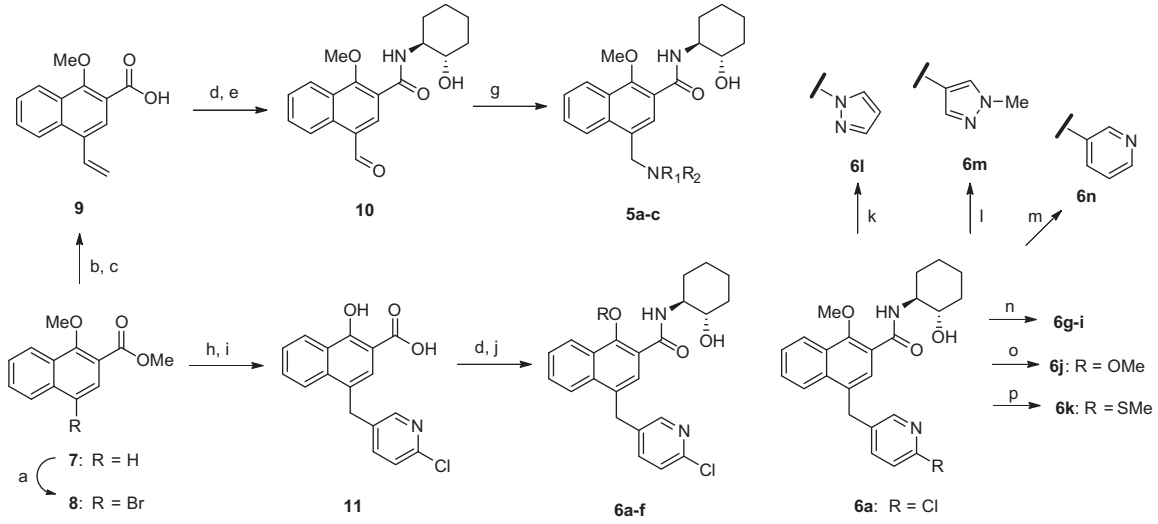


Figure 2.

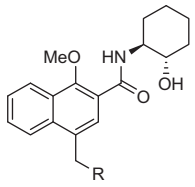
acetylcholine at human M_1 expressing CHO cells using calcium mobilization readout on a $FLIPR_{384}$ fluorometric imaging plate reader. The percent max represents the maximum potentiated EC_{20} response generated. Both N- and C-linked analogs were examined. The N-linked analog (**5a**) of amide **2** lost ~10-fold in functional potency, but the piperazines **5b** and **5c** were substantially less active. C-linked analog **6a** possessed good potency for M_1, and the chloropyridine was selected to keep in place for additional SAR work on the naphthalene scaffold. All compounds possessed relatively similar percent max.

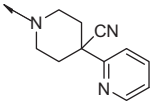
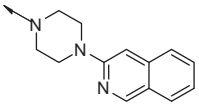
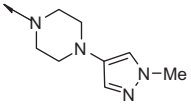
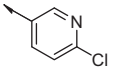
To test the hypothesis that the methoxy group was a potential hydrogen bond acceptor, a number of alternative groups were examined (Table 2). The des-methyl compound, naphthol **6b**, lost ~35 fold relative to methoxy. Increasing the steric size of the group to ethyl (**6c**, M_1 IP = 980 nM) and allyl (**6d**, M_1 IP = 1100 nM) also reduced potency while acetate **6e** was completely inactive. To match the steric size without the potential for hydrogen bonding, ethyl analog **6f** was made and was found to be a very weak M_1 PAM with an IP = 7400 nM. While this SAR does not prove the existence and bioactive role of a hydrogen bond between the amidic



Scheme 1. (a) Br_2, AcOH, (b) vinylpotassium fluoroborate, K_2CO_3, $PdCl_2$(dppf), THF, H_2O, 80 °C, (c) NaOH, THF, H_2O, (d) (1*S*,2*S*)-2-aminocyclohexanol, Bop reagent, TEA, DCM, (e) OsO_4, NMO, THF, acetone, H_2O, (f) Amine, AcOH, $NaBH(OAc)_3$, DCE, (g) $NaBH(OAc)_3$, CH_2Cl_2, (h) $Pd(PtBu_3)_2$, ((6-chloropyridin-3-yl)methyl)zinc(II) chloride, THF, 100 °C, (i) 33% HBr, AcOH, (j) Alkylhalide, K_2CO_3, DMF, (k) pyrazole, CuI, *trans*-*N*,*N*-dimethylcyclohexane-1,2-diamine, Cs_2CO_3, DMSO, 120 °C, (l) (1-methyl-1*H*-pyrazol-4-yl)boronic acid, $Pd(PtBu_3)_2$, THF, 100 °C, (m) 3-pyridine boronic acid, $Pd(PtBu_3)_2$, THF, 100 °C, (n) alkylB$(OH)_2$, $Pd(PtBu_3)_2$, Cs_2CO_3, THF, 100 °C, (o) CuI, MeOH, 160°C, (p) NaSMe, DMSO, 80°C.

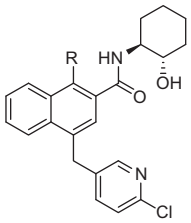
Table 1
M_1 FLIPR and protein binding data for select compounds



Compd	R^1	M_1 Pot IP[a] (nM)	% Max
2	—	31	62
5a		303	62
5b		4000	57
5c		3900	83
6a		260	74

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

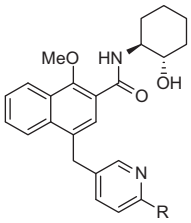
Table 2
M_1 FLIPR and protein binding data for select compounds

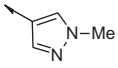


Compd	R^1	M_1 Pot IP[a] (nM)	% Max
6a	OMe	260	74
6b	OH	9100	35
6c	OEt	980	37
6d	OAllyl	1100	68
6e	OAc	>10,000	18
6f	Et	7400	47

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

Table 3
M_1 FLIPR and protein binding data for select compounds



Compd	R^1	M_1 Pot IP (nM)[a]	% Max	Rat PPB
6a	Cl	260	74	90.0
6g	Me	121	93	98.4
6h	Vinyl	74	88	Nd
6i	Et	190	73	Nd
6j	OMe	140	85	Nd
6k	SMe	170	76	Nd
6l		87	83	98.6
6m		17	77	99.9
6n		24	73	99.4

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

NH and the methoxy group, it is clear that having said functionality in place is important with this core.

Having identified the methoxy as the preferred group, additional SAR was examined at the 4-position on the naphthalene ring using chloride **6a** as the starting point. The chloro group could be replaced with a variety of alkyl substituents (**6g**–**n**) that increased potency with the methyl **6g** (M_1 IP = 121 nM) and vinyl **6h** (M_1 IP = 74 nM) standing out. Similarly, methyl ether (**6j**; M_1 IP = 140 nM) and thiomethyl (**6k**, M_1 IP = 170) were also well tolerated. However, the most substantial gains in modulator potency were obtained with the inclusion of heterocycles (**6l**–**n**). Pyrazoles **6l** and **6m** were excellent alternatives to a smaller substituent, especially the C-linked methyl pyrazole **6m** that gave an M_1 IP = 17 nM. Lastly, a simple 3-pyridyl ring (**6n**) afforded potency very similar to pyrazole **6m**, but both compounds are very highly protein bound in rat relative to the smaller groups.

Since quinolizidinone amides such as **2** were P-gp substrates, naphthalene derivatives were evaluated for P-gp efflux potential in human (MDR1) and rat (MDR1a) expressing cell lines, as well as passive permeability, and select examples for CNS exposure in rat. As can be seen in Table 4, all naphthalene amides examined had good permeability (>15 cm/s) and were not substrates for P-gp with low efflux ratios (ER); notably N-linked naphthalene amide **5a** differentiates from the direct comparator quinolizidinone amide **2**. One exception was N-linked analog **6l** that was a

Table 4
Permeability, P-gp, and bioanalysis of plasma, brain, and CSF levels for selected compounds

Compd	P_{app}[a]	MDR1[b]	MDR1a[b]	Plasma Conc.[c] (nM)	Brain Conc.[c] (nM)	CSF Conc.[c] (nM)	*B*/*P*	CSF/U_{plasma}[d]
2	29	11.1	21.1	—	—	—	—	—
5a	31	1.7	2.3	—	—	—	—	—
6a	32	1.3	1.9	2060	420	5	0.22	<0.1
6g	35	1.6	2.1	6020	1200	91	0.21	0.9
6l	31	1.7	3.2	2100	370	8.7	0.17	0.34
6m	27	1.1	1.4	29,000	1600	—	0.06	—
6n	28	0.9	1.5	4900	860	11	0.2	0.38

[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 Tables 2 and 3.

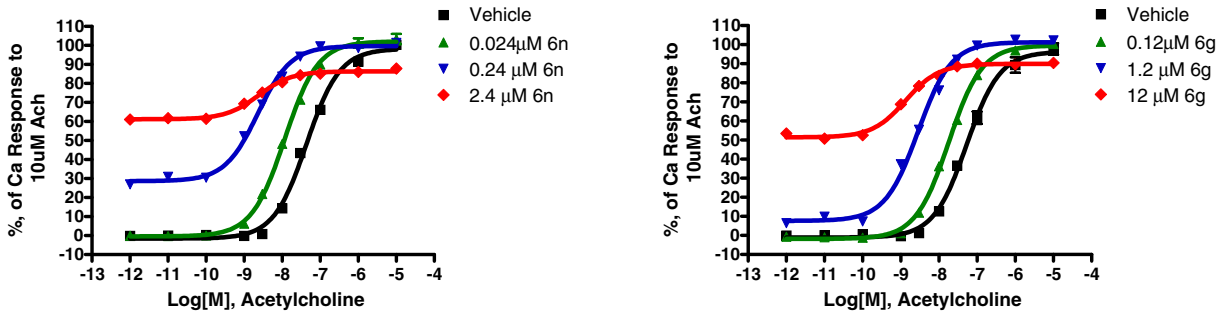


Figure 3. Fold potentiation curves for **6n** and **6g**.

substrate for rodent P-gp (ER > 2.5). In terms of central exposure, methyl analog **6g** gave a CSF/U_{plasma} ratio of 0.9, substantially higher than Cl analog **6a**.[17] N-linked pyrazole **6l** was somewhat lower, which could be a reflection of being a rat P-gp substrate. While the highly potent pyrazole analog **6m** did not show any detectable CSF exposure, pyridine **6n** was measurable with a CSF/U_{plasma} ratio of 0.38.

Finally, to confirm that the new methoxynaphthalenes behave similarly with respect to allosteric modulator **2**, the effects of **6n** and **6g** on the affinity of acetylcholine for the M_1 receptor in a functional assay utilizing calcium mobilization as the readout was evaluated. In CHO cells expressing the human M_1 receptor, increasing concentrations of **6n** and **6g** from their respective IP's, and 10 and 100× over, potentiates the effect of acetylcholine leading to a leftward shift in the acetylcholine M_1 dose-response curves (Fig. 3).

In summary, a series of methoxynaphthalene amides were prepared and evaluated as alternatives to quinolizidinone amide M_1 positive allosteric modulators. The methoxy group was optimal for M_1 activity and addressed the key P-gp issues present in the aforementioned quinolizidinone amide series. Accordingly, additional analog work is ongoing to leverage this and further optimize methoxynaphthalene analogs for improvements for additional characterization.

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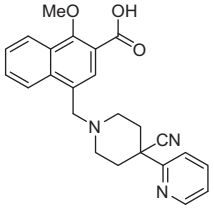
16. N-linked methoxynaphthalene carboxylic acids such as **12** below were also made and retained similar potency to C-linked acid **4**.



12: M_1 IP = 5400 nM

17. With respect to methyl analog **6g** exhibiting a higher CSF ratio than Cl analog **6a**, the high-throughput solubility of **6g** (90 μM at pH 7) was much better than Cl **6a** (insoluble at pH 7) which may help explain the difference.