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Heterocyclic fused pyridone carboxylic acid M_1 positive allosteric modulators

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ABSTRACT

The phenyl ring in a series of quinolone carboxylic acid M_1 positive allosteric modulators was replaced with a variety of heterocycles in order to reduce protein plasma binding and enhance CNS exposure.

Cholinergic neurons serve essential functions in both the peripheral and central nervous systems (CNS). Acetylcholine is the principal neurotransmitter targeting nicotinic and muscarinic metabotropic receptors. Muscarinic receptors are class A G-protein coupled receptors (GPCR) widely expressed in the CNS. There are five muscarinic sub-types, designated M_1–M_5,[1,2] of which M_1 is most highly expressed in the hippocampus, striatum, and cortex,[3] implying it may play a central role in memory and higher brain function.

A common observation in Alzheimer's disease (AD) is the progressive degeneration of cholinergic neurons in the basal forebrain leading to cognitive decline.[4] One approach to treat these cognitive symptoms of AD would be the direct activation of the M_1 receptor.[5] In this regard, a number of non-selective M_1 agonists have shown potential to improve cognitive performance in AD patients, but were clinically limited by cholinergic side effects thought to be the result of activation of other muscarinic sub-types via binding to the highly conserved orthosteric acetylcholine binding site.[6,7]

One conduit to stimulate selectively for M_1 over the other sub-types is to target allosteric sites on M_1 that are less highly conserved than the orthosteric site.[8,9] It was recently reported that quinolone carboxylic acid **1** is a selective positive allosteric modulator of the M_1 receptor with excellent selectivity for this sub-type.[10,11] Endeavors to improve the potency of **1** led to the identification of biaryl replacements for the *para*-methoxybenzyl group such as biphenyl **2** (Fig. 1).[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. Previous SAR efforts on the A-ring showed substitution was not tolerated, with the exception of fluorination at the 5- and 8-positions. This Letter, describes efforts to identify heterocyclic fused A-rings that would retain M_1 potency, reduce protein binding, and improve CNS exposure.

The chemistry used to prepare test compounds is shown in Scheme 1. The appropriate heterocyclic acid chloride **3** is treated with potassium 3-(ethoxy)-3-oxopropanoate and $MgCl_2$ to pro-

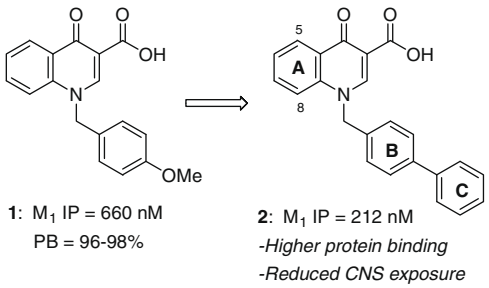

Figure 1.

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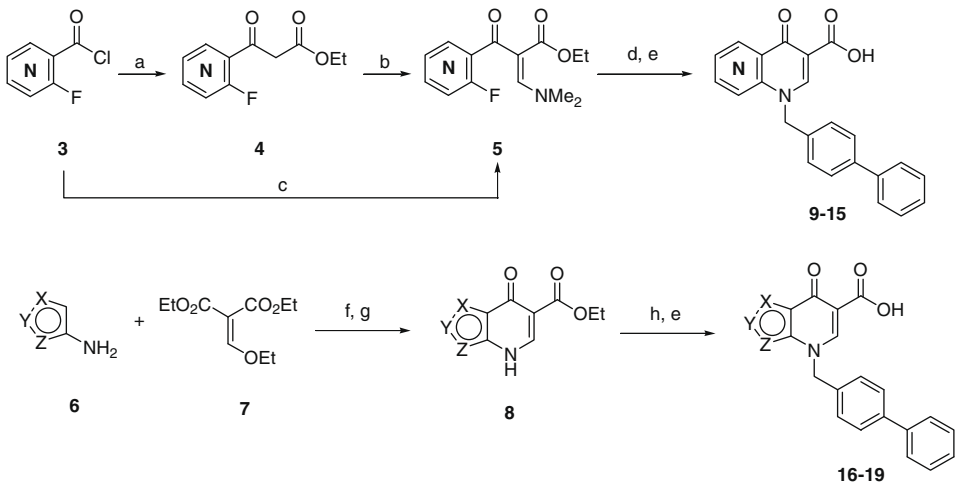


Scheme 1. Reagents: (a) Potassium 3-(ethoxy)-3-oxopropanoate, TEA, $MgCl_2$, CH_3CN; (b) dimethylformamide diethylacetal, neat, 50 °C; (c) dimethylamino ethyl acrylate, TEA, toluene, 70 °C; (d) 1-(biphenyl-4-yl)methanamine, K_3PO_4, DMA, 50 °C; (e) 1 N NaOH, THF, EtOH; (f) 130 °C, neat; (g) diphenylether, 230 °C; (h) 4-(bromomethyl)biphenyl, DMF, K_2CO_3.

duce beta keto ester **4**. Subsequent treatment with dimethylformamide diethylacetal afforded **5**.[13] Alternatively, **3** may be converted directly to **5** in a single pot process using dimethylamino ethyl acrylate and TEA. Treatment of **5** with 1-(biphenyl-4-yl)methanamine followed by ester hydrolysis afforded **9–15**.

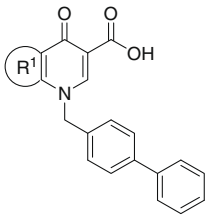
A second approach initiated with the condensation of the appropriate heterocyclic amine **6** with diethyl ethoxymethylene malonate **7**, and subsequent thermal cyclization afforded **8**.[14] Alkylation of **8** with 4-(bromomethyl)biphenyl followed by ester hydrolysis provided **16–19**. Analogous compounds were made by similar methods using modifications previously described.[15]

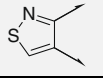
Compound potencies were determined in the presence of an EC_{20} concentration of acetylcholine at human M_1 expressing CHO cells using calcium mobilization readout on a $FLIPR_{384}$ fluorometric imaging plate reader and are presented as the inflection point (IP).[16] The percent max is the response of compound + EC_{20} acetylcholine relative to maximal acetylcholine effect. Plasma protein binding was determined using the equilibrium dialysis method in the presence of rat and human serum.

Data for A-ring modified compounds is shown in Table 1. Incorporation of a pyridine led to variable effects compared to **2**, in which the 6-pyridyl **10** exhibited a complete loss of activity and the 5-pyridyl **9** an ~7-fold decrease in activity. The 7- and 8-pyridyl analogs (**11–12**) lost functional activity; the latter did not exhibit any benefit in terms of reduced protein binding. Diazines **13–15** were even less active with only pyrazine **13** having potency under 1 μM. Thiophenes **16–17** exhibited good M_1 functional activity, with **16a** representing the most potent replacement (M_1 IP = 150 nM) with similar protein binding relative to the phenyl in **2**. However, further insertion of a nitrogen into these scaffolds in the form of thiadiazole **18** or isothiazole **19** lost an additional ~10-fold activity.

In light of the relatively good potency observed for the 7- and 8-pyridyl isomers, and previous experience with *N*-heterocyclic C-ring quinolone carboxylic acids,[17] hybrid analogs of **11a** and **12a** were evaluated as shown in Table 2. Replacement of the C-ring phenyl with a pyrazole did not improve potency (**11b–12b**), but a 3-methylpyrazole was better, particularly in the context of 7-aza isomer **11c** (M_1 IP = 250 nM). This compound also had lower free fraction relative to 8-aza isomer **12c**, although a very significant species difference was observed. The related 5-methyl analogs 11d–**12d** were substantially less potent, and imidazoles **11e–12e** were also less active than had been anticipated from experience with *N*-aryl derivatives in the quinolone carboxylic acid series.

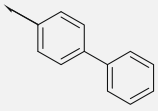
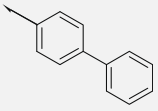
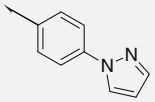
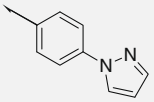
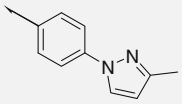
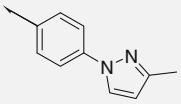
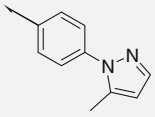
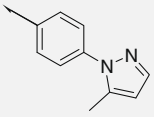
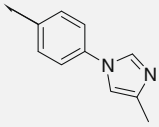
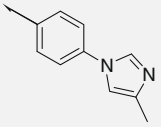
Table 1
M_1 FLIPR and protein binding data for select compounds



Compd	R^1	M_1 Pot IP[a] (μM)	% Max	Rat PB (%)	Human PB (%)
2		0.21	92	98.8	99.5
9		1.38	92	n.d.	n.d.
10		>100	4	n.d.	n.d.
11a		0.43	62	n.d.	n.d.
12a		0.58	56	99.7	99.2
13		0.78	96	97.7	97.7
14		87.2	48	n.d.	n.d.
15		5.95	34	n.d.	n.d.
16a		0.15	90	99.2	98.7
17		0.37	78	99.6	99.3
18		3.50	69	n.d.	n.d.
19		3.35	76	n.d.	n.d.

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

Table 2
M_1 FLIPR and protein binding data for select compounds

Compd	R^1	M_1 Pot IP[a] (μM)	Rat PB	Human PB	Compd	R^1	M_1 Pot IP[a] (μM)	Rat PB	Human PB
11a		0.43	—	—	**12a**		0.58	99.7	99.2
11b		1.14	—	—	**12b**		0.81	95.2	93.9
11c		0.25	98.9	87.5	**12c**		0.56	100	98.8
11d		13.0	—	—	**12d**		17.9	—	—
11e		1.03	—	—	**12e**		0.34	94.8	76.7

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

Due to the improved potency of thiophene **16a**, a number of both N- and C-linked C-ring heterocycles were evaluated and select examples are shown in Table 3. Pyridine C-rings (**16b**,**c**) and N-linked heterocycles did not confer any potency improvements relative to **16a**, although pyrazole **16d** and imidazole **16e** did have very high free fractions. The C-linked pyrazole **16g** had similar activity relative to **16a**, but species dependent plasma protein binding was also noted.

Previous SAR in the quinolone series identified that a pyridine in the B-ring was an acceptable replacement that could lower protein binding. Accordingly, analogs **16h–n** incorporated a 2-pyridyl B-ring with a variety of different C-rings. Amongst them, *meta*-aniline **16h** (M_1 IP = 38 nM) and C-linked pyrazole **16n** (M_1 IP = 340 nM), looked most promising from a potency and free fraction standpoint. Bi-pyridyls (**16i–l**) were all less potent than their arylpyridine counterparts as were other C-ring heterocycles such as thiophene (**16m**).

Select compounds with good potency and free fractions were evaluated for P-glycoprotein transport and brain exposure in rat. P-glycoprotein (P-gp) is an ATP-driven efflux transporter at the blood brain barrier (BBB), responsible for the efflux of a number of xenobiotic substances from the brain. Accordingly, P-gp efflux potential for human (MDR1) and rat (MDR1a), as well as passive permeability, were evaluated to triage potential candidates.

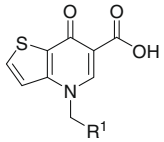
As can be seen in Table 4, 4 out of 5 analogs examined had subadequate permeability (<15) and appeared to be substrates for P-gp, with the exception of thiophene **16d**. These poor properties were consistent with low CNS exposure in rat for 7-pyridyl **11c** and thiophene **16n**. Imidazole **16e** exhibited particularly low permeability (P_{app} <2) presumably due to the strong basicity of the imidazole ring. Passive permeability of aniline **16h** was the highest of the group, but was a substrate for P-gp. Thus incorporation of heteroatoms in the A-ring tended toward compounds with decreased permeability and increased P-gp efflux. However, thiophene **16d** bearing an N-linked pyrazole, was an exception with a CSF/U_{plasma} ratio of 0.21.

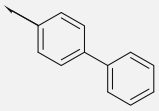
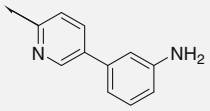
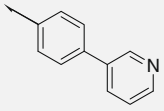
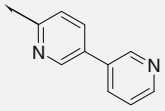
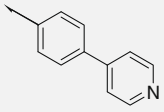
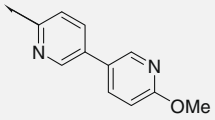
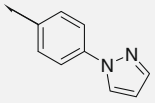
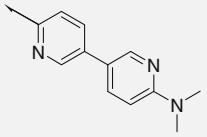
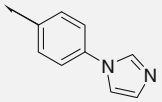
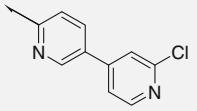
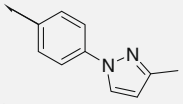
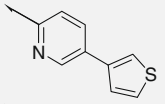
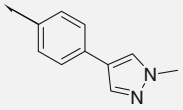
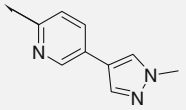
Based on the high CSF levels of **16d**, this compound was examined in a mouse contextual fear conditioning model of episodic memory, but had no effect at the doses examined (data not shown). This was surprising as the homologous quinolone analog worked in this model,[16] and **16d** also exhibited good pharmacokinetic properties.[18]

It was found that **16d** was a mouse P-gp substrate with a large efflux ratio of 18. To confirm P-gp affected CNS exposure, CF-1 wild type and P-gp knock out mice were treated with **16d**, and plasma and CSF were taken after 30 min. The wild type mice gave a low CSF/U_{plasma} ratio of ~0.05, while the mice lacking P-gp possessed three fold higher CSF/U_{plasma} ratio of ~0.16, approaching that observed in rat.[19,20] Consequently, P-gp may contribute to the lack of efficacy observed with **16d** in the mouse CFC model.

In summary, the SAR for A-ring heterocycles in place of the phenyl ring in quinolone carboxylic acid based M_1 positive allosteric modulators was examined. Thiophene and aza versions at the 7- and 8-position were tolerated, while diazines were not. Incorporation of many of the A-ring heterocycles negatively impacted the passive permeability and conferred increased P-gp susceptibility. Moreover, for thiophene **16d**, P-gp efflux in mouse likely contributed to a lack of in vivo efficacy. Evaluation of non-aromatic A-rings in lieu of the phenyl is the subject of the following paper.

Table 3
M_1 FLIPR and protein binding data for select compounds



Compd	R^1	M_1 Pot IP[a] (μM)	Rat PB	Human PB	Compd	R^1	M_1 Pot IP[a] (μM)	Rat PB	Human PB
16a		0.15	99.2	98.7	**16h**		0.038	90.1	88.3
16b		0.57	—	—	**16i**		1.05	—	—
16c		0.60	—	—	**16j**		2.0	—	—
16d		0.52	86.8	86.0	**16k**		0.5	—	—
16e		0.24	74.1	80.1	**16l**		0.81	—	—
16f		0.51	—	—	**16m**		1.66	—	—
16g		0.15	99.1	93.4	**16n**		0.34	90.6	72.1

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

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

Compd	Papp[a]	MDR1[b]	MDR1a[b]	Plasma Conc.[c] (nM)	Brain Conc.[c] (nM)	CSF Conc.[c] (nM)	Brain/plasma	CSF/U_{plasma}[d]
11c	9.0	2.4	5.3	78511	1201	24	0.15	0.03
16d	31	1.9	5.0	4240	316	123	0.07	0.21
16e	<2	—	—	—	—	—	—	—
16h	12	7.5	18.6	—	—	—	—	—
16n	5.6	4.3	7.4	9415	159	42	0.02	0.05

[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] CSF to unbound plasma ratio determined using rat plasma protein binding from Tables 2 and 3.

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