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N-Heterocyclic derived M_1 positive allosteric modulators

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ABSTRACT

Replacement of a phenyl ring with N-linked heterocycles in a series of quinolone carboxylic acid M_1 positive allosteric modulators was investigated. In particular, a pyrazole derivative exhibited improvements in potency, free fraction, and CNS exposure.

Neurotransmission via cholinergic pathways plays a critical role in both the peripheral and central nervous systems (CNS). In these systems, acetylcholine is the key neurotransmitter targeting nicotinic and metabotropic (muscarinic) receptors. Muscarinic receptors are class A G-protein coupled receptors (GPCR) with 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 it may play a central role in memory and higher brain function.

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

A potential strategy to generate selectively for M_1 over the other sub-types is to identify 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] Recent efforts to improve the potency of **1** led to the identification of biphenyl replacements such as **2** for the *para*-methoxybenzyl group.[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. This Letter describes efforts to replace the phenyl C-ring via N-linked heterocycles in order to improve the potency, free fraction, and CNS exposure for this class of M_1 allosteric modulators.

The chemistry employed to prepare the requisite test compounds is shown in Scheme 1. The quinolone esters **3a–c** were pre-

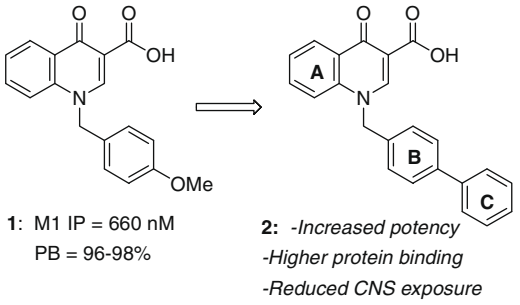

Figure 1.

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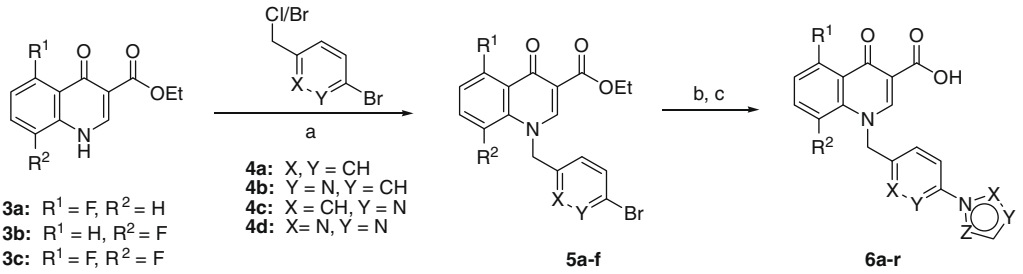
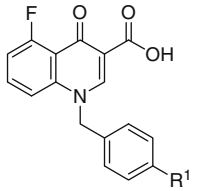


Scheme 1. Reagents and conditions: (a) K_2CO_3, KI, DMF, rt to 50 °C. (b) Amine, Cs_2CO_3, CuI, phenanthroline, DMSO, 120 °C. (c) LiOH, dioxane.

pared via a Gould–Jacobs cyclization[13] as previously described. Alkylation of **3a–c** with the appropriate halide **4a–d** afforded **5a–f**. Subsequent copper iodide mediated N-arylation of the resultant bromide with the appropriate N–H containing heterocycles followed by ester hydrolysis afforded analogs **6a–r**.

The SAR data for select analogs 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. Plasma protein binding was determined using the equilibrium dialysis method in the presence of rat and human serum.

Table 1
M_1 Potentiation, rat and human protein binding for compounds **6a–r**



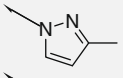
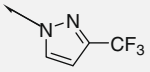
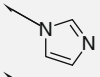
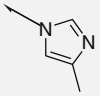
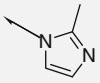
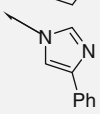
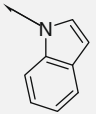
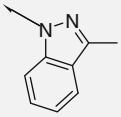
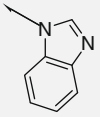
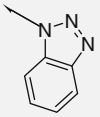
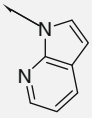
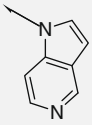
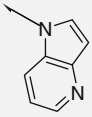
Compd	R^1	M_1 Pot IP[a] (nM)	Rat PB	Human PB
6a		181	99.5	99.7
6b		94	89.8	88.4
6c		36	92.3	94.3
6d		2900	nd	nd
6e		290	nd	nd
6f		110	88.1	83.8
6g		140	89.8	88.9
6h		920	nd	nd
6i		39	99.1	98.8
6j		250	nd	nd

Table 1 (*continued*)

Compd	R^1	M_1 Pot IP[a] (nM)	Rat PB	Human PB
6k		620	nd	nd
6l		96	96.1	91.6
6m		228	nd	nd
6n		90	98.8	99.0
6o		160	98.2	96.9
6p		150	96.1	93.0
6q		2500	nd	nd
6r		500	77.6	88.4

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

Pyrrole derivative **6a** provided enhanced M_1 potency relative to **1**, but was very highly protein bound. A very nice result was noted with pyrazole **6b** however, which gave an M_1 IP = 94 nM and a high free fraction (~10%) in rat and human plasma. Further SAR on the pyrazole indicated a strong preference for the 3-position (**6c**) over the 5-position (**6d**) with a methyl group. Larger groups were tolerated, but electron withdrawing ones tended to decrease potency as exemplified by **6e**. Imidazole **6f** was not as good an M_1 potentiator (M_1 IP = 110 nM) as the pyrazole, but did provide a substantial free fraction. The SAR on the imidazole showed a phenyl group (**6i**) was the most active, but lost all benefits in terms of decreased protein binding.

A number of benzo-fused heterocycles were also examined (**6j–p**). Among them, benzimidole **6l** provided a nice balance of M_1 activity (IP = 96 nM) and free fraction (~9% in human). Aza-indoles 6n–**p** were all more potent than the parent indole **6j** (M_1 IP = 250 nM), but the protein binding was greatly affected by the relative position of nitrogen atom. Lactam **6q** and oxazolidinone **6r** were less potent than the other simple heterocycles, although substantial free fraction was observed with **6r**.

In order to further evaluate compounds for their CNS exposure potential, key compounds were evaluated for their passive permeability (Table 2). In addition, since P-gp is considered the major efflux transporter at the blood–brain barrier (BBB) responsible for the efflux of a number of xenobiotic substances from the CNS, compounds were examined for their potential as substrates for this transporter.

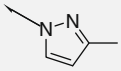
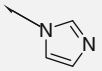
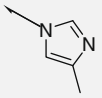
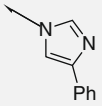
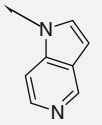
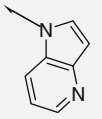
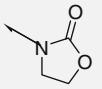
Pyrazole **6b** was not a substrate for human P-gp (2.0), but was for rat (5.1). In fact, this was a trend observed for all of these N-heterocyclic derivatives which presented higher rat efflux ratios relative to human. The 3-methyl pyrazole **6c** had improved permeability, but higher efflux. Interestingly, imidazoles **6f–g** had poor permeability, but this could be modulated by incorporating a highly lipophilic phenyl group (**6i**). Azaindole **6o** was a P-gp substrate with poor permeability, but regioisomer **6p**, improved these properties, with the exception of rat P-gp. Oxazolidinone **6r** was also a substrate with poor Papp.

All compounds in Tables 1 and 2 possessed a fluorine at the 5-position on the A-ring. Variable effects have been noted on potency, permeability, P-gp, and CNS availability depending on both the number of fluorines and their relative positions on the A-ring.[14] Accordingly, the 5,8-di-fluoro (**7**) and 8-F (**8**) variants of pyrazole **6b** were prepared and evaluated (Table 3). Pyrazole **6b** had limited brain exposure in rat as measured by the CSF to unbound plasma ratio of 0.05, which may be in part due to the susceptibility of this compound as a rat P-gp substrate. CSF is viewed as a surrogate for free brain levels compared to total drug level in the brain homogenate. The addition of the 8-fluorine in **7** led to a modest reduction in M_1 activity and free fraction, but did improve permeability and brain exposure despite still having some degree of rat P-gp susceptibility. The single fluorine containing analog **8** was similarly potent to **6b**, but with markedly higher protein binding. Thus, the 5-fluorine enhanced free fraction while the 8-fluorine has a role in improving the permeability.

Compound **7** was evaluated for the ability to fold potentiate a dose response of acetylcholine with a fixed concentration of potentiator. As can be seen from Figure 2, in the presence of 1 μM of potentiator, a left-shift of 46-fold was observed in the acetylcholine dose response showing **7** is a potent positive allosteric modulators of the human M_1 receptor. It should be noted that some degree of agonism is observed at 10 μM and higher concentrations.

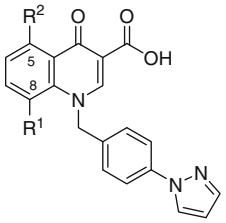
We have previously shown that incorporation of a pyridine or pyridazine ring in place of the B-ring phenyl in the biaryl series maintains good M_1 receptor activity and augments the free frac-

Table 2
Passive permeability and P-gp effects for selected compounds

Compd	R^1	Papp[a]	MDR1[b]	MDR1a[b]
6b		17	2.0	5.1
6c		30	4.4	16.7
6f		5	4.6	9.8
6g		9	1.0	3.9
6i		24	0.8	5.7
6o		6	15.9	17
6p		23	1.7	11.8
6r		7	7.8	8.7

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

Table 3
Evaluation of substituted A-rings



Compd	R^1	R^2	M_1 IP (nM)	Rat PB	Human PB	Papp[a]	MDR1[b]	MDR1a[b]	B/P[c]	CSF/U_{plasma}[d]
6b	H	F	94	89.8	88.4	17	2.0	5.1	0.04	0.05
7	F	F	171	94.9	95.7	29	1.4	4.1	0.03	0.1
8	F	H	96	98.3	98	—	—	—	—	—

[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.

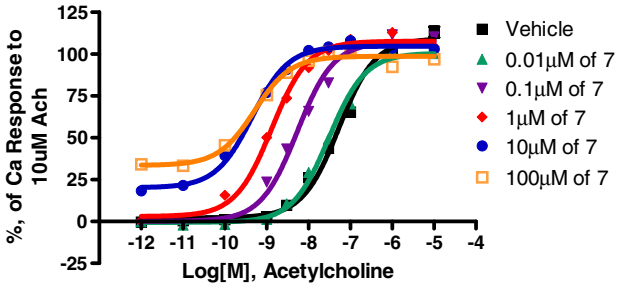
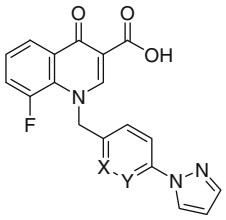


Figure 2. Fold potentiation plot for compound **7**.

Table 4
Evaluation of B-ring pyridine or pyridazine incorporation



Compd	X	Y	M_1 Pot IP[a] (nM)
6b	CH	CH	96
9	N	CH	1400
10	CH	N	864
11	N	N	24,000

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

tion.[12] Accordingly, this strategy was investigated utilizing a C-ring pyrazole as shown in Table 4. Interestingly, incorporation of a 2-pyridine (**9**), 3-pyridine (**10**), and pyridazine (**11**) B-rings led to dramatic decreases, 9–240-fold, in functional activity relative to **6b** showing the previously reported biaryl SAR did not translate to this N-heterocyclic series.

Due to the high potency, good free fraction, and relative degree of brain penetration, pyrazole **7** was chosen for further evaluation for performance in a mouse contextual fear conditioning assay, which serves as a model of episodic memory. In this model, mice were treated with scopolamine before introduction to a novel environment to block association with a novel environment. Mice treated with 10 mpk of **7** (dosed ip) exhibited a significant reversal compared to mice treated with scopolamine alone (see Supplementary data). The corresponding plasma levels were ~9 μM, a significant improvement over compound **1**, where ~33 μM plasma concentration was required for efficacy. Additional in vivo evaluation of pyrazole **7** will be the subject of a pending manuscript.

In summary, the synthesis and SAR of N-heterocyclic containing quinolone carboxylic acid M_1 positive allosteric modulators has been detailed in an effort to identify potent compounds with reduced plasma protein binding. A number of N-linked heterocycles were found to be acceptable replacements for the phenyl C-ring, but many of them presented lower free fractions or were substrates for P-gp. A pyrazole in the form of compound **7** retained good potency, showed improved free fraction, and was not a human P-gp substrate. Limited brain exposure was observed as **7** was a rat P-gp substrate. In spite of this, pyrazole **7** showed efficacy in a mouse model of cognition at lower plasma levels relative to previously reported quinolone carboxylic acids.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.01.013.

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