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Pyridine containing M_1 positive allosteric modulators with reduced plasma protein binding

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ABSTRACT

Incorporation of pyridines and diazines into the biphenyl region of quinolone carboxylic acid derived M_1 positive allosteric modulators was investigated as a means of lowering plasma protein binding to enhance CNS exposure.

Cholinergic neurons serve critical functions in both the peripheral and central nervous systems (CNS). Acetylcholine is the key neurotransmitter in these systems, 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 to 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.

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 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 due to activation of other muscarinic subtypes via binding to the highly conserved orthosteric acetylcholine binding site.[6,7]

One avenue 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. recently reported the quinolone carboxylic acid **1** as a selective positive allosteric modulator of the M_1 receptor with exquisite selectivity for the M_1 subtype.[10] Recent efforts to improve the potency of **1a** led to the identification of biphenyl replacements for the *para*-methoxybenzyl group.[11] 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. It has been demonstrated in an unrelated series of compounds that insertion of a pyridine into a biphenyl motif can effectively reduce protein binding and enhance physical properties.[12] This Letter describes efforts to replace the proximal B-ring phenyl with a pyridine or other diazine and subsequent SAR observed on the distal C-ring phenyl (Fig. 1).

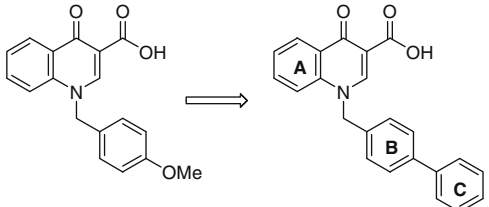

Figure 1.

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The chemistry employed to prepare target compounds **6–11** is shown in Scheme 1. The 8-fluoroquinolone ethyl ester **2** could be prepared by a Gould–Jacobs cyclization. Alkylation of the appropriate alkyl halide or mesylate **3a–f** was accomplished using potassium carbonate in DMF. Subsequent base mediated hydrolysis of **4a–f** followed by Suzuki coupling with the appropriate boronic acid afforded analogs **6–11**. This late stage cross-coupling allowed for greater diversity in a rapid analog fashion.

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.[13] The percent max represents the maximum potentiated EC_{20} response generated. Plasma protein binding was determined using the equilibrium dialysis method in the presence of rat and human serum.

Incorporation of the pyridine nitrogen at the 2-position of the B-ring (**6a**) enhanced functional activity relative to phenyl compound **1b** with an M_1 inflection point (IP) = 153 nM with an 80% maximal response, but had little effect on the rat or human plasma protein binding (Table 1). Location of the nitrogen at the 3-position (**7a**) lowered potency ~2-fold with 97% maximal response, but did not affect protein binding similar to the 2-pyridine **6a**. Pyrazine **8** did lower protein binding significantly, but with a notable loss (~4-fold) in M_1 potency. Much of this activity could be reclaimed in the form of pyridazine **9a**, which was similar to the phenyl analog **1b**, with a modest reduction in rat protein binding. Pyrimidines **10** and **11** lost significant M_1 activity. Due to the satisfying boost in M_1 potency seen with 2-pyridyl **6a**, variation of the distal phenyl C-ring was investigated in this series for further enhancements to M_1 functional activity and protein binding.

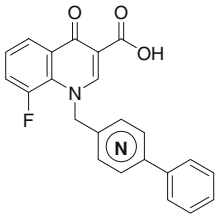
A library of boronic acids was employed to survey the SAR for analogs of **6a**. Overall the effects on potency were minimal when the C-ring was varied relative to phenyl comparator **6a**. Selected examples are shown in Table 2. For brevity, the % max responses are not shown, but were generally >90% unless otherwise indicated.

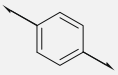
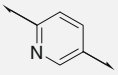
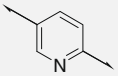
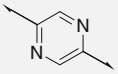
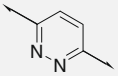
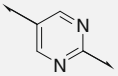
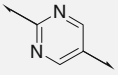
A variety of substituents such as fluorine (**6b**) or methoxy (**6c**) on the distal phenyl C-ring was found to modestly enhance M_1 activity with little effect on protein binding. Lipophilic heterocycles such as thiophene (**6d**) also reduced plasma free fractions as well. One exception was methyl sulfones **6e–g** that exhibited good M_1 activity (except the *para*-analog **6g**) and reduced protein binding, particularly for the *ortho* position. An amino group at the *meta* position (**6h**) was the most potent amongst the series with an M_1 IP = 21 nM and had an ~4% free fraction. Methylation of the group (**6i**) increased protein binding, but acetylation (**6j**) was similar to **6h**, albeit with a loss of M_1 activity.

Pyridines were also examined in lieu of the distal phenyl C-ring to see if additional gains in free fraction could be obtained. The 3-pyridine (**6l**) looked the most promising in maintaining M_1 activity similar to that of **6a**, and did provide an enhanced free fraction. A range of substituents (**6m–q**) were found to vary these properties with **6o** showing a good balance of potency and free fraction for further study. Other nitrogen containing heterocycles were also examined. Amongst them, *N*-methyl pyrazole **6r**, exhibited M_1 potency similar to the phenyl group and appeared to show a large species dependent drop in rat protein binding. The highly active potentiator, indole **6s**, was extensively bound in both human and rat plasma.

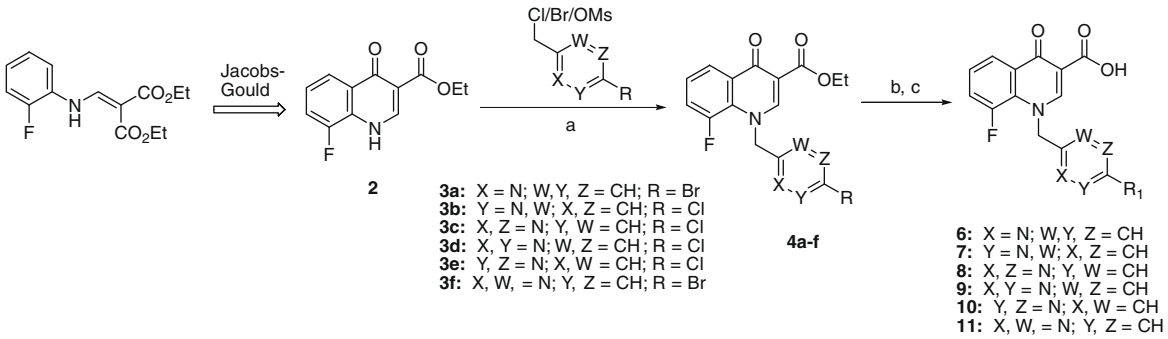
Select compounds from Table 2 were evaluated for their ability to potentiate a dose–response of acetylcholine with a fixed concentration of potentiator.[10] As can be seen from Table 3, in the presence of 30 μM of potentiator, a left-shift between 29- and 81-fold was observed in the acetylcholine dose–response showing they are potent positive allosteric modulators of the human M_1 receptor.

Table 1
M_1 FLIPR and protein binding data for select compounds



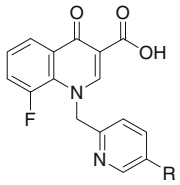
Compds	R^1	M_1 Pot IP[a] (nM)	% Max	Rat PB	Human PB
1b		378	84	98.0	98.4
6a		153	80	97.3	98.4
7a		604	97	98.0	98.2
8		1300	85	95.1	97.1
9a		390	97	96.1	98.4
10		4000	88	nd	nd
11		2324	90	nd	nd

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



Scheme 1. Reagents and conditions: (a) K_2CO_3, KI, DMF, rt–50 °C; (b) LiOH, dioxane; (c) $Pd(t\text{-}Bu_3P)_2$, $R_1B(OH)_2$, DMSO, 1 *N* Cs_2CO_3, *u*wave-160 °C, 10 min.

Table 2
M_1 FLIPR and protein binding data for select compounds



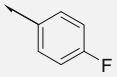
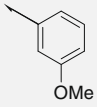
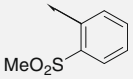
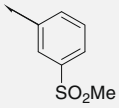
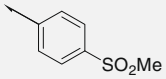
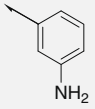
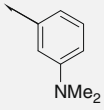
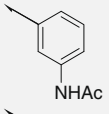
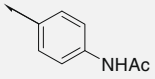
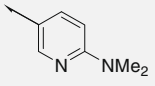
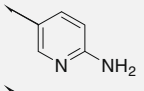
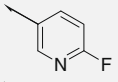
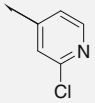
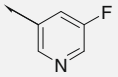
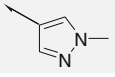
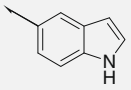
Compds	R^1	M_1 Pot IP[a] (nM)	Rat PB	Human PB
6b		67	98.7	98.1
6c		89	98.4	97.8
6d		150	99.4	98.1
6e		97	91.7	85.8
6f		97	96.8	95.5
6g		260	nd	nd
6h		21	96.8	95.5
6i		110	99.3	98.7
6j		131	95.8	95.9
6k		78	92.3	97.0
6l		370	82.5	95.1
6m		82	98.1	99.0
6n		200	83.9	90.9
6o		100	86.3	97.6
6p		169	97.5	96.5
6q		680	90.9	92.9
6r		160	89.0	98.4

Table 2 (*continued*)

Compds	R^1	M_1 Pot IP[a] (nM)	Rat PB	Human PB
6s		57	99.4	99.8

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

Table 3
Fold potentiation data for select compounds

Compds	Fold shift
6a	37
6b	29
6g	33
6h	45
6o	81
6r	37

Having identified a number of interesting C-rings from Table 2, combinations of these were prepared and evaluated with the 3-pyridyl (**7**) and pyridazine (**9**) B-rings to assess whether further improvements could be gained. Results for select compounds are shown in Table 4. Overall, the SAR for the 3-pyridyl compounds (**7b**–**e**) translated very well from the 2-pyridine series, while the pyridazine analogs **9b**–**e** were all less potent across the board. For example, *meta*-methylsulfone **7e** maintained good M_1 activity, while the pyridazine version **9e** gave an M_1 IP = 1.5 μM. This finding was unfortunate as the pyridazines showed very good free fractions as exemplified by **9c**, which had protein binding in the 58–68% range. Amongst the 3-pyridines (**7b**–**i**), *meta*-amino analog **7f** possessed the most interesting potency and protein binding profile.

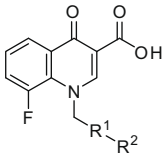
With a number of potent compounds available with good free fractions, evaluation of brain exposure was required. P-glycoprotein (P-gp) is an ATP-driven efflux transporter at the blood–brain barrier (BBB), responsible for the efflux of a number of xenobiotic substances from the brain. Accordingly, P-gp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

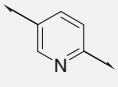
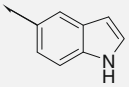
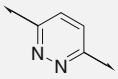
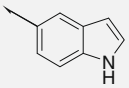
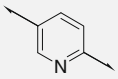
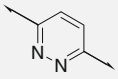
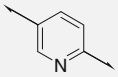
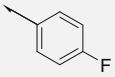
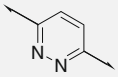
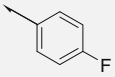
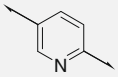
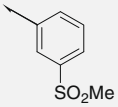
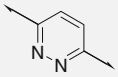
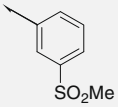
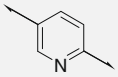
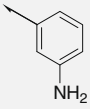
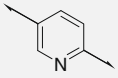
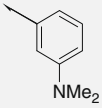
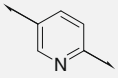
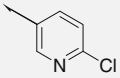
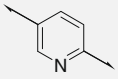
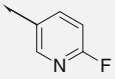
As can be seen in Table 5, with the exception of pyrazole **6r** (MDR1a = 6.3), the majority of compounds exhibited efflux ratios less than 3, indicating that they were not substrates for human or rat P-gp. However, four compounds possessed sub-adequate passive permeability (P_{app} <15), another important component necessary to achieve permeation across the BBB. These include all three methylsulfones **6e**–**g** as well as aminopyridine **6n**.

The four remaining compounds (**6l**, **6o**, **6r**, and **7e**) were evaluated for brain exposure in rat, utilizing oral dosing (10 mpk) and sampling at a 2 h time point. With the exception of **7e**, which also had the lowest plasma exposure, all compounds gave very low total brain levels (5% or less). Bi-pyridyl **6l** and pyrazole **6r**, also have quite poor CSF levels relative to the free plasma concentrations (<10%). While **6l** did have the highest protein binding of the four compounds, which may explain the low brain exposure observed, it is less clear why **6r** gave such poor results despite the very high plasma levels. Only fluoro-pyridine **6o** gave a suitable CSF/U_{plamsa} ratio of 0.32, and also exhibited a very high plasma concentration.[14] It appears that more than P-gp efflux potential and free fraction are involved in the CNS disposition of these quinolone carboxylic acids.[15]

The SAR of the substitution pattern on the quinolone A-ring was also examined utilizing compound **6r** as the scaffold (Table 6). Prior SAR has revealed that only the 5- and 8-positions on the quinolinone tolerate substitution, ideally with a small replacement

Table 4
M_1 FLIPR and protein binding data for select compounds



Compds	R^1	R^2	M_1 Pot IP[a] (nM)	Rat PB	Human PB
7b			65	99.6	100
9b			8662	nd	nd
7c			951	86.4	90.5
9c			778	58.6	68.0
7d			190	98.6	99.1
9d			466	nd	nd
7e			28	94.7	93.5
9e			1543	nd	nd
7f			21	94.7	93.5
7g			130	98.9	97.8
7h			169	97.5	95.5
7i			202	86.3	97.6

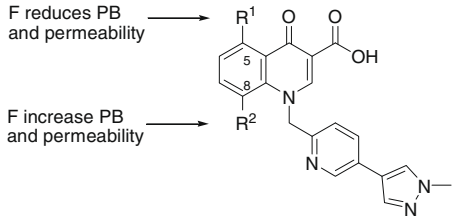
[a] Values represent the numerical average of at least two experiments. Interassay variability was ±30% (IP, nM), unless otherwise noted.

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

Compds	Papp[a]	MDR1[b]	MDR1a[b]	Plasma concn[c] (nM)	Brain concn[c] (nM)	CSF concn[c] (nM)	B/P	CSF/U_{plamsa}[d]
6e	9	1.2	1.8	nd	nd	nd	nd	nd
6f	6	1.1	1.5	nd	nd	nd	nd	nd
6g	7	0.9	1.0	nd	nd	nd	nd	nd
6l	30	0.7	2.5	2794	154	25	0.05	0.05
6n	11	1.1	1.5	nd	nd	nd	nd	nd
6o	28	0.5	1.0	12,837	311	97	0.05	0.32
6r	28	1.6	6.3	33,274	430	40	0.03	0.08
7e	19	2.1	4.5	382	154	0	0.4	—

[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 4.

Table 6
SAR for fluorine substitution on the A-ring



Compds	R^1	R^2	M_1 IP[a]	Rat PB	Human PB	Papp[b]	MDR1[c]	MDR1a[c]
6r	H	F	118	88.9	98.4	28	1.6	6.3
6t	F	H	176	90.6	78.6	6.6	2.6	6.8
6u	F	F	114	92	77.0	11	1.4	8.1

[a] Values represent the numerical average of at least two experiments. Interassay variability was ±30% (IP, nM), unless otherwise noted.
[b] Passive permeability (10^{-6} cm/s).
[c] MDR1 Directional Transport Ratio (B to A)/(A to B). Values represent the average of three experiments and interassay variability was ±20%.

such as fluorine.[16] While moving the fluorine to the 5-position (**6t**) or the 5,8-di-fluoro variant (**6u**) has little effect on M_1 activity, substantial effects are noted on permeability and protein binding. Interestingly, the 5-fluoro group decreased protein binding, but has the undesired effect of eroding permeability. The 8-fluoro **6r** had much higher protein binding (in human), but good permeability, while the combination **6u** has attenuated properties that appear largely modulated by the 5-fluorine. As a result the 8-fluoro is a preferred A-ring scaffold, at least for this particular pyridine containing class of quinolone carboxylic acid allosteric modulators.

In summary, the synthesis and SAR of pyridine containing quinolone carboxylic acid M_1 positive allosteric modulators has been detailed in an effort to identify potent compounds with reduced plasma protein binding. It was initially found that pyridine and pyridazines were acceptable replacements for the phenyl B-ring, but further analogs showed the pyridines presented to be the most flexible with respect to SAR via modification of the C-ring. The bi-pyridyls generally retained the most potency and gave analogs with the best free fraction. However, good permeability and not being substrates for P-gp did not necessarily translate into higher CNS exposure, indicating other factors may be in play, such as the potential for the involvement of other CNS transporters. Studies are underway to investigate these possibilities.

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13. Selected compounds were examined in functional assays at other muscarinic receptors and showed no activity at M_2, M_3, or M_4 receptors.
14. As a typical example of other pharmacokinetic properties, acid **6o** gave low clearance (Cl = 6.0 ml/min/kg), V_{dss} = 0.7, and a half-life of 5.1 h in dog.
15. A number of isosteres have been prepared in an effort to alter transport affinity, but the carboxylic acid group is required for M_1 functional activity.
16. Shipe, W.D. et al., in preparation.