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Parallel synthesis of *N*-biaryl quinolone carboxylic acids as selective M_1 positive allosteric modulators

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ABSTRACT

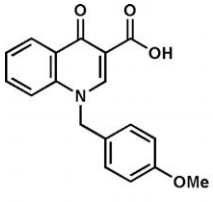
An iterative analog library synthesis approach was employed in the exploration of a quinolone carboxylic acid series of selective M_1 positive allosteric modulators, and strategies for improving potency and plasma free fraction were identified.

One of the pathological hallmarks of Alzheimer's disease (AD) is the progressive degeneration of the cholinergic neural system.[1] The cholinergic neurons release acetylcholine and are critical for higher brain function and memory, so their deterioration leads to irreversible loss of cognitive faculties. Cholinesterase inhibitors such as donepezil, galantamine, and rivastigmine were developed based on this hypothesis and were the first successful symptomatic treatments for AD.[2] The efficacy of cholinesterase inhibitors is modest, of brief duration, and limited by tolerability, but indicates that enhancing cholinergic function is an effective strategy to combat AD.

The muscarinic receptors are metabotropic, class A, G-protein coupled receptors (GPCRs) activated by acetylcholine.[3] The M_1 receptor is one of the five muscarinic receptor subtypes (M_1 through M_5) that have been identified to date, and its high expression in the hippocampus, striatum, and cortex suggest a role in learning and memory.[4] Direct activation of M_1 as an approach to treat the symptoms of AD has been explored in the clinical setting with a number of nonselective muscarinic agonists.[5] While cognitive improvement was observed, the studies were limited by cholinergic side effects hypothesized to result from nonselective action on other muscarinic receptor subtypes.

High orthosteric site conservation across muscarinic receptor subtypes has made agonist selectivity for the M_1 receptor difficult to achieve. As a strategy for attaining subtype selectivity, we explored the possibility of identifying positive allosteric modulators (potentiators).[6,7] Allosteric binding sites are under less evolutionary pressure than the orthosteric site, so potentiators of M_1 may display good subtype selectivity. A high-throughput screen for M_1 potentiators identified compound **1** (Fig. 1), recently reported by Ma et al., as a selective positive allosteric modulator of M_1.[8] This N-benzylated quinolone carboxylic acid potentiates the response of M_1 to acetylcholine with no effect on other muscarinic receptor subtypes, and was further characterized as a potential lead compound. This Letter describes efforts to optimize **1** through modifications to the quinolone core and the *N*1-benzyl substituent employing an iterative analog library synthesis approach.

Compound potencies were determined in the presence of an EC_{20} of acetylcholine in human M_1-expressing CHO cells using calcium mobilization readout on a $FLIPR_{384}$ fluorometric imaging plate reader.[7,9] Plasma protein binding was determined using the



1

M_1 Pot IP = 820 nM
PB (% bound; h,r) = 99.1, 95.7%
Rat PK: CL = 6.3 mL/min/kg
$t_{1/2}$ = 3.4 h
F = 97%
Dog PK: CL = 6.8 mL/min/kg
$t_{1/2}$ = 0.9 h
F = 76%

Figure 1. In vitro profile of quinolone carboxylic acid **1**.

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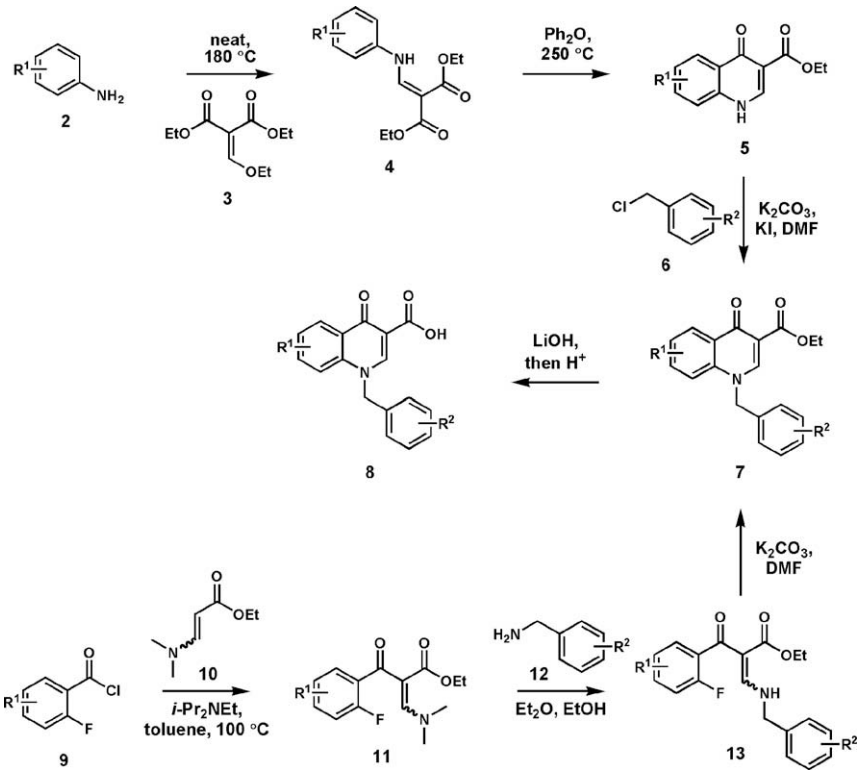


Scheme 1. General synthesis of quinolone carboxylic acids.

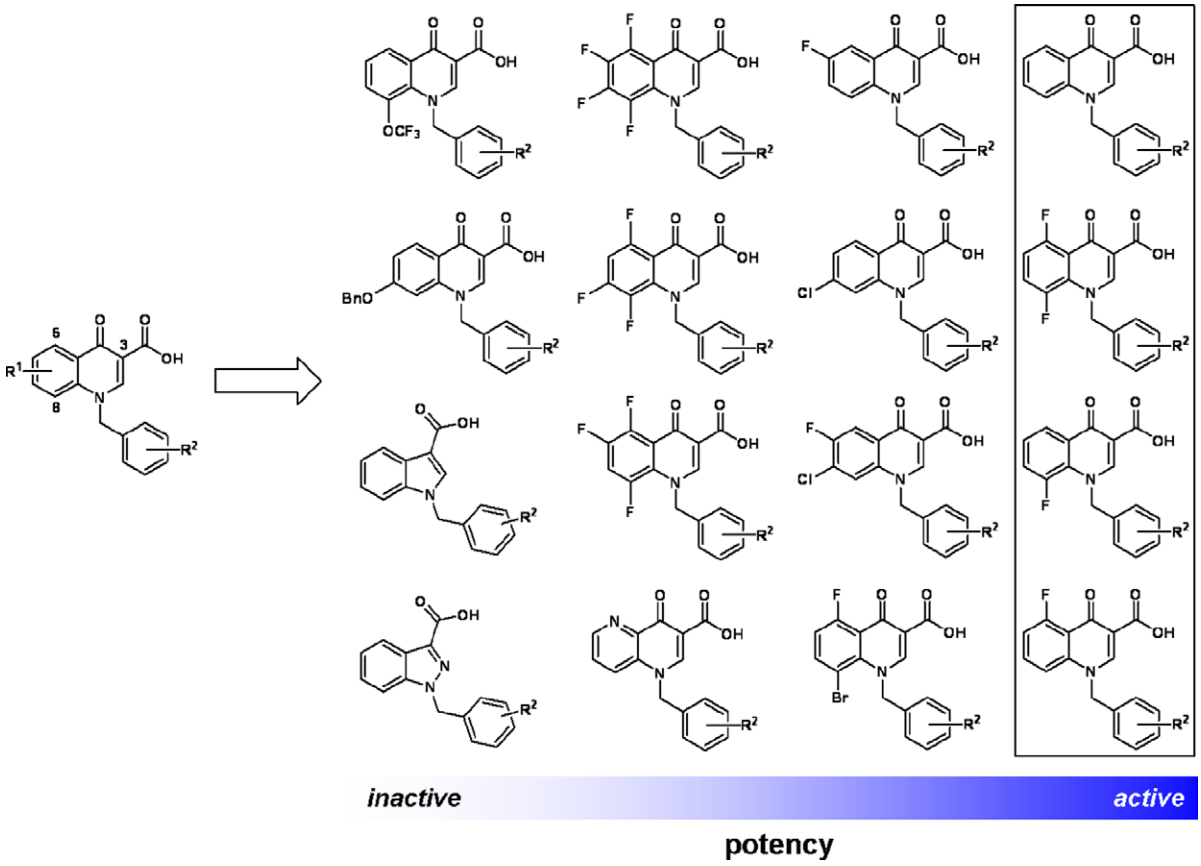
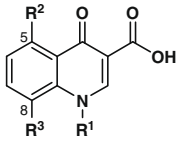


Figure 2. Representative scaffolds explored.

Table 1
M_1 FLIPR data for select compounds



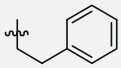
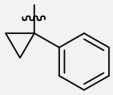
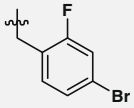
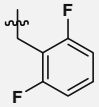
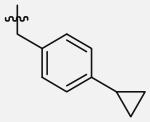
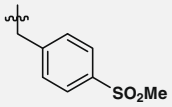
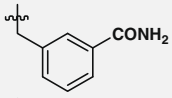
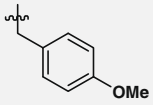
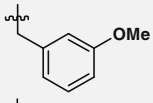
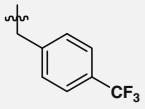
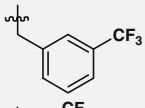
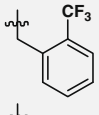
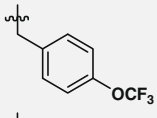
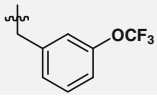
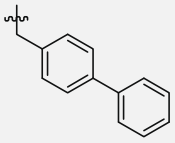
#	R^1	M_1 Pot IP (nM)[a]			
		a R^2 = H R^3 = H	**b** R^2 = H R^3 = F	**c** R^2 = F R^3 = H	**d** R^2 = F R^3 = F
14		1700	4000	ND	3000
15		>100,000	24,000	ND	ND
16		13,000	22,000	ND	ND
17		ND	1200	1100	860
18		ND	1000	1300	610
19		ND	1200	830	ND
20		18,000	13,000	ND	16,000
21		4300	3400	ND	ND
22		820	1100	2000	ND
23		2800	2700	2100	4200
24		ND	980	1500	760
25		7200	2700	ND	1600
26		4300	6900	ND	2300
27		1800	2100	2800	1300
28		4100	3600	5900	3200

Table 1 (*continued*)

#	R^1	M_1 Pot IP (nM)[a]			
		a R^2 = H R^3 = H	**b** R^2 = H R^3 = F	**c** R^2 = F R^3 = H	**d** R^2 = F R^3 = F
29		210	380	84	270

ND = not determined.
[a] Values represent the numerical average of at least two experiments. Interassay variability was ± 30% (IP, nM), unless otherwise noted.

equilibrium dialysis method in the presence of human and rat serum. Compound **1** had an M_1 inflection point (IP) of 820 nM (Fig. 1) and a plasma free fraction that varied between human and rat (0.9% and 4.3%, respectively). The pharmacokinetic profile of **1**, characterized by low clearance and excellent oral bioavailability across preclinical species, was very encouraging and gave us confidence to further pursue this structural series. Our efforts sought to improve on the M_1 potency of **1** while maintaining an acceptable physicochemical profile.

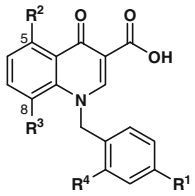
We viewed the structure of lead compound **1** as a context suitable for iterative parallel synthesis, so a general synthesis of quinolone analogs was employed as outlined in Scheme 1. Anilines **2** were heated with diethyl ethoxymethylenemalonate (**3**) in the absence of solvent to provide enamines **4**. These intermediates were dissolved in diphenyl ether and further heated to elicit cyclization to the quinolone nucleus (**5**). Under basic conditions, **5** was regioselectively alkylated with activated halides **6** to furnish N-substituted quinolones **7**, and subsequent basic hydrolysis of the ethyl ester yielded the carboxylic acid targets **8**. To increase the scope of our efforts, we also used a route wherein primary amines **12** could serve as a source of structural diversity. Here, *o*-fluorobenzoyl chlorides were used to acylate ethyl 3-(dimethylamino)acrylate (**10**) to produce the *N*,*N*-dimethyl enamines **11**, which underwent smooth substitution with amines **12**. The resultant enamines **13** could be cyclized to the quinolones **7** under basic conditions.

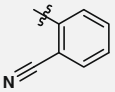
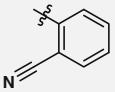
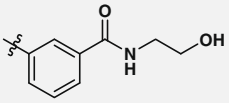
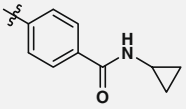
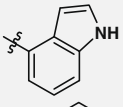
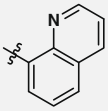
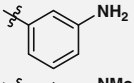
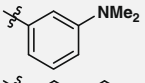
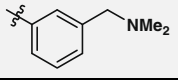
Optimization of the substitution pattern on the quinolone core required beginning the synthesis with diversely substituted anilines **2** or benzoyl chlorides **9**. Each scaffold was substituted with a number of common benzyl groups at the *N*1-position to enable a rough stack-ranking assessment across scaffolds. After canvassing a variety of fused bicyclic scaffolds, in most cases derived using the chemistry described above, we concluded that fluorine or hydrogen atom substitution at the 5- and/or 8-position of a quinolone nucleus was preferred for M_1 potency (Fig. 2).

We then turned to the optimization of the *N*1-substituent, and chose to operate on these four 'preferred' scaffolds. A broad selection of monomers was employed in the diversity-yielding step, and the products were screened in the FLIPR assay. While it was difficult to make predictive generalizations on the basis of the data (Table 1), some trends did appear. Benzyl substitution at the 1-position was strongly preferred over alkyl (**14** vs **15**), and branching at the benzyl position was poorly tolerated (**14** vs **16**). Halogenated and lipophilic substituents were better tolerated than polar groups (**17**–**19** vs **20**–**21**). A *para* substituent on the benzyl group was preferred over *meta*- or *ortho*-substitution (**22** vs **23**, **24** vs **25**–**26**, **27** vs **28**). These data did little to differentiate the four quinolone scaffolds, and relatively few compounds matched the potency of the HTS lead **1** (=**22a**). However, the four biphenyl compounds **29a–d** that combined lipophilicity with placement at the *para* position represented a potency breakthrough.

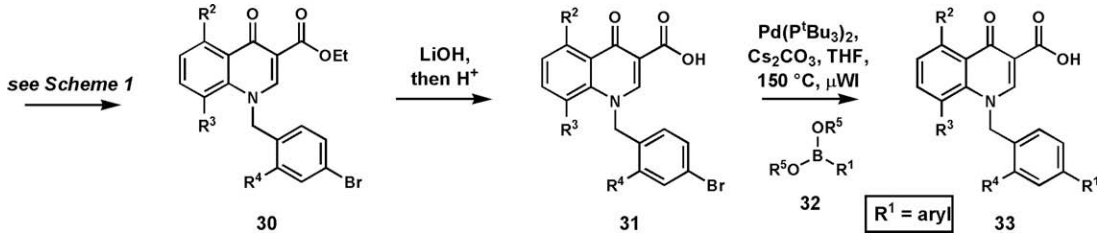
Unsurprisingly, **29a–d** displayed a high degree of plasma protein binding, most likely owing to the lipophilicity of the biphenyl substituent (Table 2). During the course of our efforts, we found that high protein binding correlated with decreased CNS exposure in vivo, so we next focused on addressing this limitation. To this end, we designed biaryl compound libraries to explore variation of the distal aryl group with *para* disubstitution on the proximal phenyl ring (Scheme 2). Employing the N1-benzylation route described in Scheme 1, *p*-bromobenzyl quinolone carboxylic esters **30** were prepared, and basic hydrolysis yielded the corresponding carboxylic acids **31**. These aryl bromides were combined with substituted aryl pinacolboronate esters or boronic acids **32** under Suzuki–Miyaura cross-coupling reaction conditions to produce the target biaryl compounds **33**.

Table 2
M_1 FLIPR and plasma protein binding data for select compounds



#	R^1	R^2	R^3	R^4	M_1 Pot IP[a] (nM)	Human PB[b]	Rat PB[b]	*c* Log *P*[c]
29a		H	H	H	210	99.5	98.8	2.78
29b		H	F	H	380	98.0	97.8	2.99
29c		F	H	H	84	99.2	98.7	2.99
29d		F	F	H	270	99.6	99.5	3.20
34		H	H	F	50	98.8	96.3	2.99
35		H	H	Cl	120	>99.9	>99.9	3.45
36		H	F	H	540	99.4	99.4	2.87
37		F	H	H	400	99.1	98.5	2.87
38		H	F	H	99	97.6	98.3	1.66
39		H	F	H	110	98.1	99.6	2.69
40		H	F	H	76	99.8	99.8	3.28
41		F	H	H	190	99.0	98.5	3.18
42		H	F	H	210	96.4	97.8	2.24
43		H	F	H	94	99.0	98.8	3.15
44		H	F	H	220	95.2	95.2	1.50

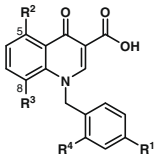
[a] Values represent the numerical average of at least two experiments. Interassay variability was ±30% (IP, nM), unless otherwise noted.
[b] Percent bound.
[c] *c* Log *P* values were calculated using the Cerius[2] product marketed by Accelrys Software.

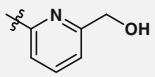
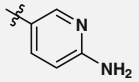
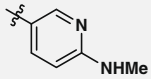
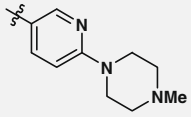
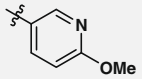
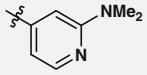
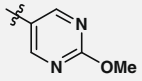
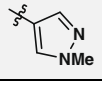


Scheme 2. General synthesis of biaryl quinolone carboxylic acids.

A series of substituted biphenyl compounds was first made in an attempt to improve plasma free fraction (Table 2). Halogen substitution on the phenyl ring proximal to the quinolone core improved M_1 potency (**29a** vs **34**–**35**) but only marginally improved free fraction in the case of **34**. Inclusion of polar groups (**36**–**39**) and fused bicyclic heterocycles (**40**–**41**) was generally ineffective,

Table 3
M_1 FLIPR and plasma protein binding data for select compounds



#	R^1	R^2	R^3	R^4	M_1 Pot IP[a] (nM)	Human PB[b]	Rat PB[b]	*c* Log *P*[c]
45		H	F	H	250	94.2	97.8	1.88
46		F	H	H	61	94.5	94.9	1.84
47		H	F	H	130	95.3	95.4	1.63
48		H	F	H	48	98.7	99.3	2.19
49		H	F	H	51	98.2	99.1	0.94
50		H	F	H	63	99.3	99.5	2.36
51		H	F	H	100	97.6	98.0	1.84
52		H	F	H	45	98.5	99.2	2.54
53		H	F	H	830	87.3	96.5	1.21
54		H	H	F	510	95.4	96.3	1.72
55		H	H	F	56	94.1	89.8	1.43
56		F	H	F	41	95.6	98.0	1.64
57		H	H	F	37	98.0	98.6	1.64

[a] Values represent the numerical average of at least two experiments. Interassay variability was ±30% (IP, nM), unless otherwise noted.
[b] Percent bound.
[c] *c* Log *P* values were calculated using the Cerius[2] product marketed by Accelrys Software.

but aniline **42** represented an incremental improvement. While its *N,N*-dimethyl analog **43** was highly protein bound, the homologous basic tertiary amine **44** had a free fraction of nearly 5% in both human and rat.

In addition to providing improved M_1 potency with greater consistency, libraries incorporating heterocyclic biaryls were even more fruitful in the search for compounds with decreased protein binding (Table 3). A number of pyridines (**45**–**52**) were examined, and among this group were two (**46**–**47**) with ~5% free fraction. Pyrimidines **53** and **54** also lowered protein binding significantly, but with a considerable loss of potency (~10-fold with respect to pyridine **46**). Pyrazole **55** represents another example combining excellent potency with appreciable free fraction. Inclusion of a fluorine atom at the quinolone 5-position (**56**) or an *N*-methyl group on the pyrazole (**57**) maintained potency, but had a detrimental effect on the free fraction. Comparison of calculated Log *P* values for the biphenyls as a group versus the heterocyclic biaryls largely demonstrates the expected inverse relationship between *c* Log *P* and plasma free fraction. However, within the group of heterocyclic biaryls, **45** and **46** showed a higher free fraction than might have been predicted on the basis of the *c* Log *P*, providing some validation of our choice to pursue this series of compounds with parallel synthesis.

There is evidence to suggest that drug concentration in cerebrospinal fluid (CSF) is a reasonable predictor for unbound drug concentration in the brain.[10] A number of compounds in this series were therefore orally dosed to rats at 10 mg/kg, and CSF was used as a surrogate for in vivo assessment of CNS exposure. Compounds **44** and **46** are discussed as representative of the biphenyl and heterocyclic biaryl subseries, respectively. At a time point of 1 h, compound **44** showed a concentration of only 11 nM in the CSF; **46** a concentration of 24 nM. In a P-glycoprotein efflux assay, **44** and **46** both showed efflux ratios greater than 3, indicating that they are substrates for P-gp. Thus, the lower than expected exposures observed here may result from the action of these or other efflux transporters at the blood–brain barrier.

Selective M_1 positive allosteric modulators were discovered based on N-benzylated quinolone carboxylic acid lead **1**. Efforts driven by parallel synthesis identified that fluorine atom substitution at the 5- and/or 8-positions of the quinolone ring system were preferred in terms of M_1 potency. Analog libraries also led to the discovery that biaryl substituents at *N*1 further enhanced potency, providing a 20-fold improvement over lead compound **1**. Incorporation of a basic tertiary amine or certain heterocycles distal to the quinolone core were found to be effective strategies for decreasing plasma protein binding,[11] but these compounds nevertheless showed poor brain exposure, possibly as a result of the action of CNS transporters. Further studies are underway to identify selective M_1 allosteric modulators with improved CNS exposure.

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