Bioorganic & Medicinal Chemistry Letters 21 (2011) 2769–2772

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Fused heterocyclic M_1 positive allosteric modulators

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ARTICLE INFO

Article history:
Received 17 September 2010
Revised 5 October 2010
Accepted 6 October 2010
Available online 16 October 2010

Keywords:
Muscarinic
Alzheimer's
Allosteric modulator

ABSTRACT

Fused aromatics such as naphthalene were identified as highly potent and CNS penetrant M_1 positive allosteric modulators during an SAR study to replace the phenyl B-ring linkage.

Cholinergic neurons perform critical functions in both the peripheral and central nervous systems (CNS). Acetylcholine is the neurotransmitter in these processes, targeting nicotinic and metabotropic (muscarinic) receptors. Muscarinic receptors are class A family G-protein coupled receptors (GPCR) widely expressed in the CNS. There are 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.

One of the traits of Alzheimer's disease (AD) is the progressive and irreversible degeneration of cholinergic neurons in the basal forebrain leading to cognitive decline.[4] Consequently, direct activation of the M_1 receptor represents an approach to treat the symptoms of AD.[5] Along these lines, a number of non-selective M_1 agonists have shown potential to improve cognitive performance in AD patients, but further evaluation in the clinic was halted 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]

Quinolone carboxylic acid **1** is a selective positive allosteric modulator of the M_1 receptor.[8,9] Attempts to improve the potency of **1** led to the identification of biaryl replacements such as **2** for the *para*-methoxybenzyl group (Fig. 1).[10] 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.[11] This communication describes efforts to replace the phenyl B-ring with a less lipophilic linker in order to improve the potency, free fraction, and CNS exposure for this class of M_1 allosteric modulators.

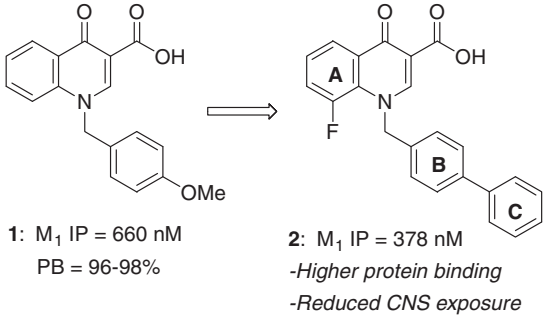

Figure 1. Lead potentiator **1**.

The chemistry employed to prepare the requisite test compounds is shown in Scheme 1. The quinolone ester **3** was prepared via a Gould–Jacobs cyclization.[12] Alkylation of **3** with the appropriate halide afforded **4a–c**. Subsequent ester hydrolysis afforded analog **7**.[13] Sonogashira coupling of **4a** with the appropriate halide or Suzuki cross coupling of the requisite boronic acid with bromide **4b** provided compounds **5** and **6a–p**, respectively, after ester hydrolysis.

Replacement of the central B-ring with an acetylenic moiety in the form of **5** led to ~10 fold loss of potency compared to the biphenyl derivative **2** (Fig. 2). However, the *trans*-olefin construct **6a** proved to modestly enhance M_1 activity relative to biaryl **2** and SAR analysis was subsequently investigated on this scaffold.

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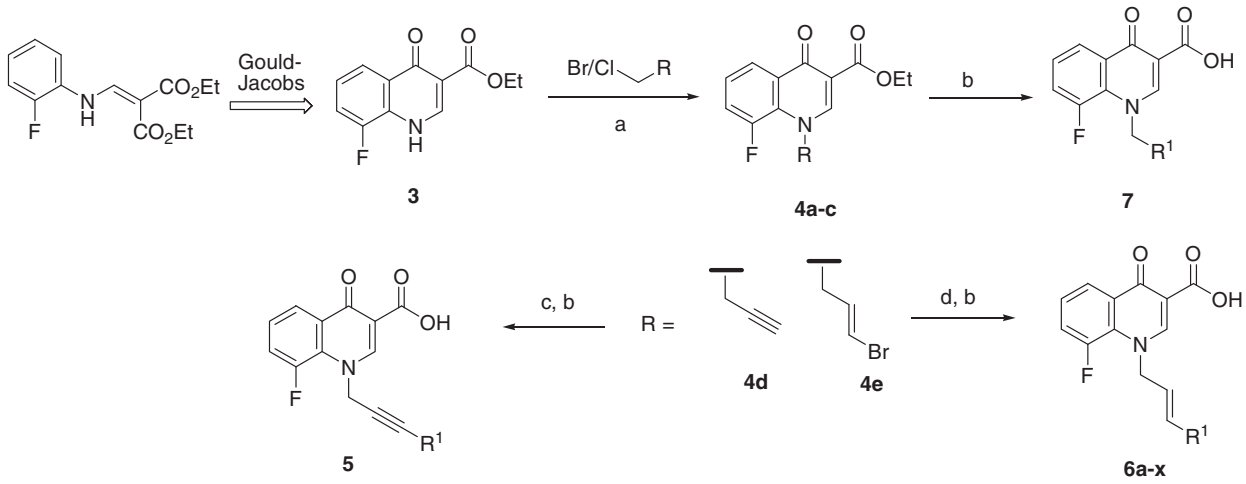
doi:10.1016/j.bmcl.2010.10.028



Scheme 1. Reagents and conditions: (a) K_2CO_3, KI, DMF, rt–50 °C; (b) LiOH, dioxane; (c) $Pd(PPh_3)_4$, R^1I/Br, CuI, TEA, THF or DMSO, 60 °C; (d) $Pd(t\text{-}Bu_3P)_2$, $R^1B(OH)_2$, THF, 1 N Cs_2CO_3, 100 °C.

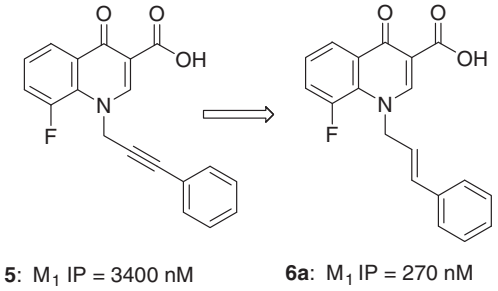
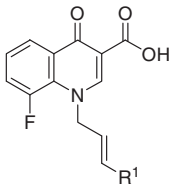


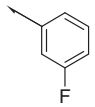
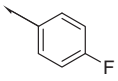
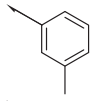
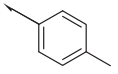
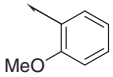
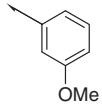
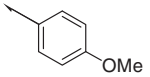
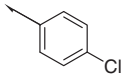
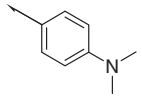
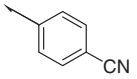
Figure 2. Alkyne to alkene analogs.

The SAR data for select analogs of phenyl **6a** 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 $FLIPR_{384}$ fluorometic imaging plate reader. Plasma protein binding was determined using the equilibrium dialysis method in the presence of rat and human serum.

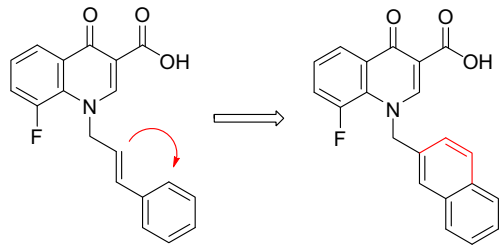
As can be seen in Table 1, a fluorine (**6b**) was the only tolerated *ortho*-substituent as **6e** (R^1 = Me) and **6h** (R^1 = OMe) were not active. There was a trend among **6b–m** indicating *para*-subsitution was preferred, with fluorine (**6d**) representing ~2-fold improvement over **6a**. Little improvement was seen by varying the elec-

Table 1
M_1 potentiation for compounds **6b–s**



Compds	R^1	M_1 pot IP (nM)[a]	Compds	R^1	M_1 pot IP (nM)[a]	Compds	R^1	M_1 pot IP (nM)[a]
6b		240	**6c**		370	**6d**		120
6e		>10,000	**6f**		250	**6g**		910
6h		>10,000	**6i**		1400	**6j**		380
6k		320	**6l**		860	**6m**		1000
6n		5200	**6o**		>10,000	**6p**		610

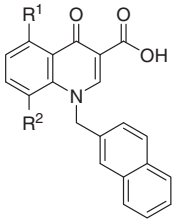
[a] Values represent the numerical average of at least two experiments. Interassay variability was ±30% (IP, nM), unless otherwise noted.



6a: M_1 IP = 270 nM **7**: M_1 IP = 80 nM

Figure 3. Connection to naphthalene **7**.

Table 2
M_1 potentiation, rat and human protein binding for compounds **7**–**9**



Compds	R^1	R^2	M_1 pot IP (nM)[a]	Rat PB	Human PB
7	H	F	80	99.2	99.6
8	F	F	266	100	100
9a	F	H	69	94.2	96.7

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

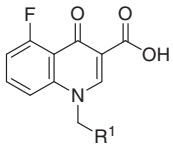
tronic and steric nature of the *para*-substituents (**6g,j,k–m**). Pyridines **6n–p** showed a marked drop-off in M_1 potency, with the 4-pyridyl **6p** being the best (M_1 IP = 610 nM) among the group. Overall, the SAR for substitution on the phenyl ring was generally flat.

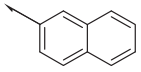
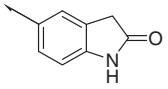
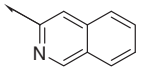
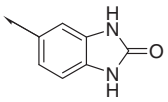
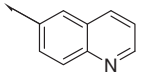
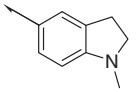
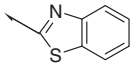
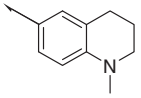
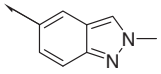
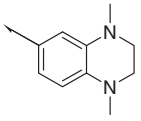
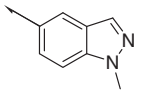
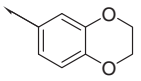
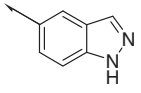
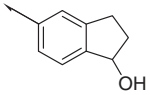
Since compounds **1** and **2** possessed a benzylic group off the N-1 position in the form of the B-ring in Figure 1, it was decided to fuse the olefin group present in **6a** onto the phenyl to form a naphthyl ring as shown in Figure 3. Gratifyingly, naphthalene **7** was found to be ~3-fold more potent (M_1 IP = 80 nM) than **6a**.[14]

Although naphthalene **7** in combination with the fluorine at 8-position of the quinolone was highly potent, the human and rat plasma protein binding was greater than 99% (Table 2). Addition of a fluorine at the 5-position (**8**) led to a decrease in potency and full protein binding. However, the single 5-fluoro quinoline A-ring analog **9a**, was highly potent (M_1 IP = 69 nM) with reasonable free fractions in rat (5.8%) and human (3.3%) plasma protein. To follow up on this result, a number of fused heterocycles were prepared in lieu of the naphthyl within the context of the 5-fluoroquinoline motif. Select examples are shown in Table 3.

Incorporation of a nitrogen atom (**9b,c**) or a benzothiazole (**9d**) led to a marked drop-off in M_1 functional activity. Interestingly, indazoles **9e–g** exhibited good activity, with the N-1 methyl isomer **9f** being equipotent (M_1 IP = 72 nM) to naphthyl **9a**. Additionally, all three possessed higher free fractions relative to naphthyl **9a**. The related lactam **9h** also possessed a similar profile in terms of potency and free fraction compared to the indazoles, but urea variant **9i** showed a considerable loss of activity. Interestingly, the *N*-methyl-dihydroindole **9j** was not active, while modest potency was obtained in the six-membered variant **9k**. While the corresponding fused piperazine was weakly potent, the analogous

Table 3
M_1 potentiation, rat and human protein binding for compounds **9a**–**n**



Compds	R^1	M_1 pot IP (nM)	Rat PB	Human PB	Compds	R^1	M_1 pot IP (nM)	Rat PB	Human PB
9a		69	94.2	96.7	**9h**		81	58.5	72.5
9b		897	nd	nd	**9i**		617	nd	nd
9c		459	nd	nd	**9j**		>10 k	nd	nd
9d		1797	nd	nd	**9k**		624	nd	nd
9e		142	80.2	76.5	**9l**		3800	nd	nd
9f		72	86.7	88.3	**9m**		84	90.8	80.4
9g		104	83.9	65.9	**9n**		97	56.9	67.6

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

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

Compds	Papp[a]	MDR1[b]	MDR1a[b]	Plasma concn. (nM)[c]	Brain concn. (nM)[c]	CSF concn. (nM)[c]	B/P	CSF/U_{plamsa}[d]
9a	23	0.9	1.7	676	178	18	0.26	0.60
9f	30	1.8	5.3	13,239	538	155	0.04	0.09
9h	1.6	0.9	2.1	—	—	—	—	—
9m	36	1.8	5.6	3704	577	119	0.15	0.16
9n	8.5	4.9	12.8	—	—	—	—	—

[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 Table 3.

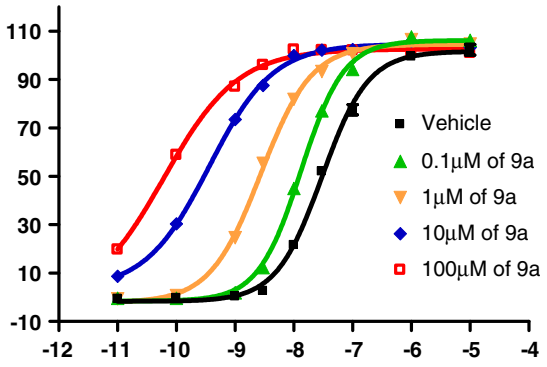


Figure 4. Fold potentiation of **9a**.

dihydro-benzodioxine **9m** gave excellent M_1 activity (M_1 IP = 84 nM) and free fraction (10–20%). Lastly, hydroxy-indane **9n** possessed an intriguing balance of potency and free fraction to warrant further characterization.

In order to further evaluate compounds for their CNS exposure potential, select analogs were evaluated for passive permeability and for their potential as substrates for the CNS efflux transporter P-gp (Table 4). With the exception of lactam **9h** and hydroxy-indane **9n**, all compounds showed good passive permeability (Papp >15) warranting further consideration. The other three compounds (**9a**, **9f**, and **9m**) were not P-gp substrates (efflux ratios <2.5) and were subsequently evaluated for CNS exposure in rat utilizing oral dosing at 10 mpk. Naphthalene **9a**, exhibited the highest CSF:U_{plasma} ratio (0.6) as well as total brain to plasma among the three. *N*-methyl indazole **9f**, gave a very low CSF:U_{plasma} ratio (<0.1) despite the high plasma levels and good free fraction. Dihydro-benzodioxine **9m** afforded a modestly higher ratio (0.16). These results could also reflect the fact that **9a** is not a substrate for rat P-gp, while **9f** and **9m** are.

Naphthalene **9a** was evaluated for the ability to potentiate a dose response of acetylcholine with a fixed concentration of potentiator. As can be seen from Figure 4, in the presence of 1 μM of potentiator, a left-shift of ~100-fold was observed in the acetylcholine dose response showing it is a potent positive allosteric modulator of the human M_1 receptor. It should be noted that a very minor amount of agonism is observed in the presence of **9a** at 100 μM concentration.

In summary, while looking for less lipophilic linkers than the B-ring phenyl found in quinolone **2**, fused heterocycles such as naphthalene were identified as highly potent M_1 positive allosteric modulators. Moreover, naphthalene analog **9a** also showed improved brain penetration and effectively potentiated the M_1 receptor in the presence of acetylcholine. Continued SAR incorporating these fused heterocycles into non-quinolone carboxylic acid scaffolds is ongoing.

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13. Analogs **9a–n** were prepared as described in Scheme 1, substituting ethyl 5-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate for **3**.
14. The *c* Log *P*'s of **2** and **9a** were 2.9 and 2.3, respectively.