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Hydroxy cycloalkyl fused pyridone carboxylic acid M_1 positive allosteric modulators

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ABSTRACT

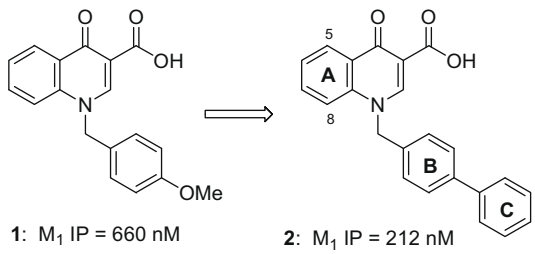
Incorporation of hydroxycycloalkyl fused pyridone carboxylic acids in lieu of quinolone carboxylic acids enhance free fraction without increased susceptibility to P-glycoprotein transport.

The central cholinergic nervous system serves essential functions and is activated by acetylcholine as the endogenous ligand, targeting nicotinic and metabotropic muscarinic receptors. The latter are class A G-protein coupled receptors (GPCR) of which there are five muscarinic subtypes, designated M_1–M_5.[1,2]

The progressive degeneration of cholinergic neurons in Alzheimer's disease (AD) is proposed as a leading cause of the resultant cognitive decline.[3] A therapeutic approach would be the direct activation of the M_1 receptor, which is highly expressed in the affected brain regions,[4] implying it may play a central role in memory and higher brain function.[5] Non-selective M_1 agonists exhibited improved cognitive performance in AD patients, but exhibited intolerable side effects attributed to activation of the highly conserved orthosteric acetylcholine binding site of other muscarinic sub-types.[6,7]

The activation of a less-highly conserved allosteric binding site in preference to the orthosteric domain, is one pathway to produce selectivity for M_1 over the other sub-types.[8,9] Quinolone carboxylic acid **1** has been described as a selective positive allosteric modulator of the M_1 receptor with excellent specificity for the M_1 subtype.[10,11] SAR evaluation of **1** led to the identification of biaryl replacements for the *para*-methoxybenzyl group such as biphenyl **2** (Fig. 1),[12] but higher plasma protein binding led to decreased free 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. The preceding paper described efforts to identify heterocyclic replacements for the phenyl A-ring.[13] This communication describes efforts to identify non-aromatic, cycloalkyl A-rings that would retain M_1 potency and show reduced protein binding leading to improved in vivo activity.

The synthesis of requisite A-ring modified biphenyls is shown in Scheme 1. Condensation of 3-aminocyclohexenone **3** with



1: M_1 IP = 660 nM
PB = 96-98%

2: M_1 IP = 212 nM
-Higher protein binding
-Reduced CNS exposure

Figure 1.

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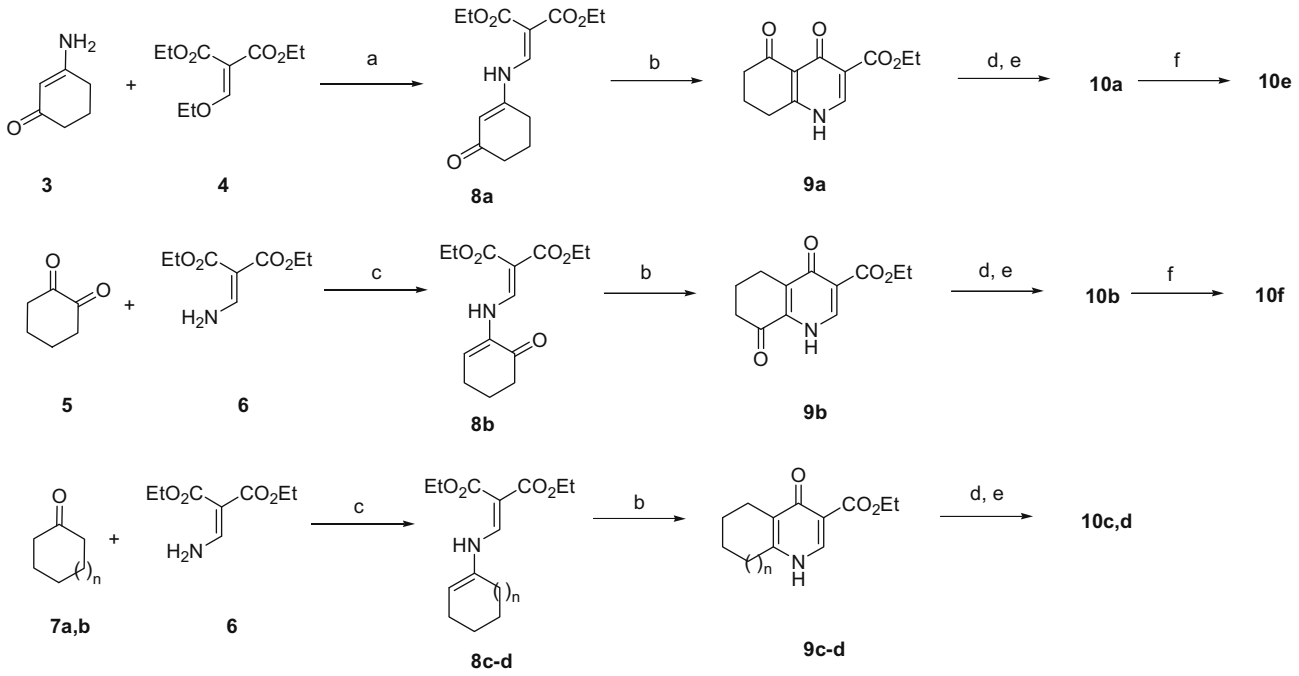
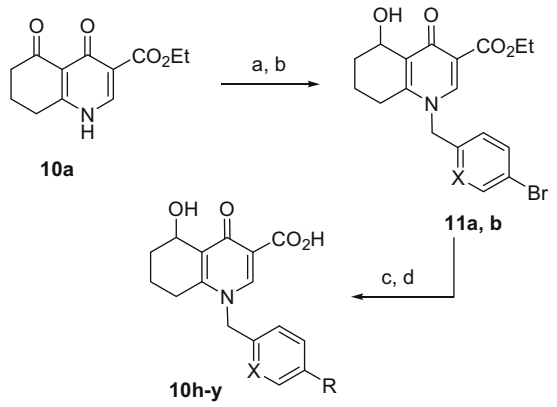


Scheme 1. Reagents and conditions: (a) 130 °C, neat; (b) diphenylether, 230 °C; (c) 4-toluene sulfonic acid, toluene, reflux; (d) 4-(bromomethyl)biphenyl, DMF, K_2CO_3; (e) 1 N NaOH, THF, EtOH; (f) $NaBH_4$, THF, MeOH.

ethoxymethylene malonate **4** produced **8a**. Similarly, condensation of di-ketone **5** or ketones **7a,b** with aminomethylene malonate **6** provided **8b** and **8c**. Cyclization of **8a–d** was carried out using a modified Gould–Jacobs cyclization[14] to afford heterocycles **9a–d**.[15] Alkylation of **9a–d** with 4-(bromomethyl)biphenyl followed by subsequent ethyl ester hydrolysis afforded carboxylic acids **10a–d**. The ketones present in **10a,b** were converted to alcohols **10e,f** via reduction with sodium borohydride.

The preparation of analogs bearing modified B/C-ring combinations is shown in Scheme 2. Alkylation of **10a** with appropriate halide followed by sodium borohydride reduction affords alcohols **11a** (X = N) or **11b** (X = CH). Subsequent Suzuki cross-coupling followed by ester hydrolysis affords acids **10h–y**.

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 represents the effect of compound and EC_{20} of acetylcholine relative to the maximal possible acetylcholine



Scheme 2. Reagents and conditions: (a) $ArCH_2X$, DMF, K_2CO_3; (b) $NaBH_4$, THF, MeOH; (c) $Pd(OAc)_2$, X-PHOS, K_2CO_3, CH_3CN, H_2O, 80 °C; (d) 1 N NaOH, THF, EtOH.

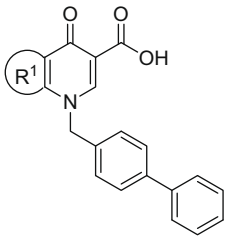
effect. Plasma protein binding was determined using the equilibrium dialysis method in the presence of rat and human serum.

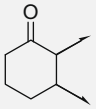
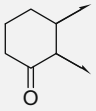
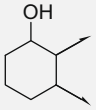
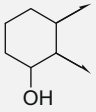
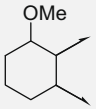
Data for modified A-ring compounds is shown in Table 1. Cyclohexanone **10a** lost ~fourfold in terms of M_1 activity relative to quinolone **2**, but exhibited a markedly reduced plasma protein binding profile. The corresponding 8-isomer **10b** was substantially less active (M_1 IP = 4.8 μM), while the cyclohexyl **10c** and cyclopentyl **10d** were comparable with minimal improvement in free fraction compared to **2**. Reduction of **10a** to secondary alcohol **10e** provided the most potent compound amongst the series (M_1 IP = 440 nM) with high maximal acetylcholine activity, representing only a two fold potency decrease with respect to **2**. The 8-hydroxy isomer **10f** and the methyl ether of **10e** were substantially less potent.[17] Based upon the good potency and higher free fraction (~5%) of **10e** relative to **2**, additional SAR evaluation of the B and C rings with the 5-hydroxycyclohexane in place was investigated with selected compounds as shown in Table 2.

Previously, it was observed in the quinolone series that incorporation of a 2-pyridyl (**10h,i**) in place of the phenyl B-ring reduced protein binding and was neutral in terms of potency.[18] Compound **10h** did possess enhanced free fraction, but both analogs lost M_1 activity implying a poor SAR translation from the quinolone series. In addition, a range of substitutions at the three positions of the phenyl C-ring were evaluated (**10j–o**), and the SAR was flat, with the *meta* isomers proving to be the most well tolerated.

Lipophilic heterocycles such as thiophene **10p** and furan **10q** showed no potency advantages. In earlier quinolone SAR, N-linked heterocycles exhibited good properties over their C-linked counterparts,[19] but pyrazole **10r** and imidazole **10s** had reduced M_1 activity. Substituted pyridines (**10t–w**) also were not particularly advantageous, with only methoxypyridine **10u** showing improved M_1 potency. Lastly, substituted pyrazoles were investigated with isobutyl **10y** exhibiting the best potency of all B/C-ring analogs examined with a similar free fraction relative to biphenyl **10e**. Overall, the SAR for B/C-ring combinations was flat[20] and did not translate well from the quinolone SAR to this hydroxycyclohexane class of compounds.

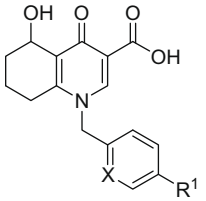
Table 1
M_1 FLIPR and protein binding data for select compounds



Compd	R^1	M_1 Pot IP[a] (nM)	% Max	Rat PB	Human PB
2		212	92	98.7	99.5
10a		800	94	86.7	91.4
10b		4890	70	—	—
10c		751	91	98.5	98.8
10d		810	99	97.9	98.5
10e		440	99	93.6	94.4
10f		8190	59	—	—
10g		1100	90	—	—

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

Table 2
M_1 FLIPR and protein binding data for select compounds



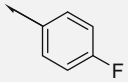
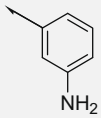
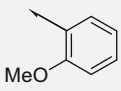
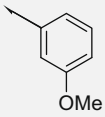
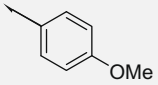
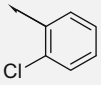
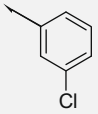
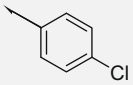
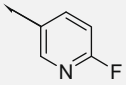
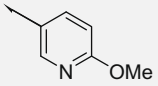
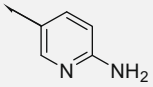
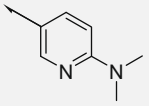
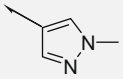
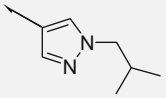
Compd	R^1	M_1 Pot IP[a] (nM)	Rat PB	Human PB
10h (X = N)		1100	78.6	75.4
10i (X = N)		420	—	—
10j		3100	—	—

Table 2 (*continued*)

Compd	R^1	M_1 Pot IP[a] (nM)	Rat PB	Human PB
10k		410	—	—
10l		1386	—	—
10m		1400	—	—
10n		340	—	—
10o		340	—	—
10p		2640	—	—
10q		750	—	—
10r		1369	62.3	56.1
10s		2500	21.2	40.9
10t		14790	—	—
10u		320	—	—
10v		2425	—	—
10w		1095	—	—
10x		908	59.4	82.1
10y		200	84.4	94.9

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

Selected compounds were examined for CNS exposure as shown in Table 3. Since P-glycoprotein (P-gp) is a major efflux transporter of xenobiotics at the blood–brain barrier (BBB), P-gp efflux in human (MDR1) and rat (MDR1a) P-gp, as well as passive permeability, were evaluated to triage potential candidates. Biphenyl **10e** and phenylpyrazole **10x** exhibited good permeability (Papp >15), but N-linked phenylpyrazole **10r** was low removing it from further consideration.

The two remaining compounds (**10e**, and **10x**) were evaluated for brain exposure in rat, utilizing oral dosing (10 mpk) and sampling at a 2 h time point. Biphenyl **10e** gave a modest CSF/U_{plasma} ratio of 0.22 and a suitably high plasma concentration. Phenylpyr-

Table 3
Permeability, P-gp, and bioanalysis of plasma, brain, and CSF levels for selected compounds

Compd	Papp[a]	MDR1[b]	MDR1a[b]	Plasma concn[c] (nM)	Brain concn[c] (nM)	CSF concn[c] (nM)	B/P	CSF/U_{plasma}[d]
10e	20	1.5	1.9	6145	146	87	0.03	0.22
10r	2.8	3.5	8.1	—	—	—	—	—
10x	19	2.2	7.3	26677	368	110	0.01	0.10

[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 1 and 2.

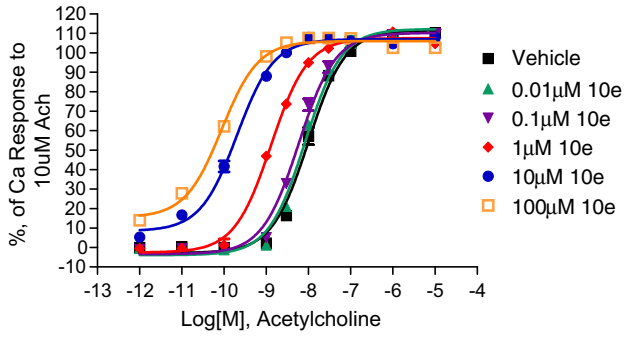


Figure 2.

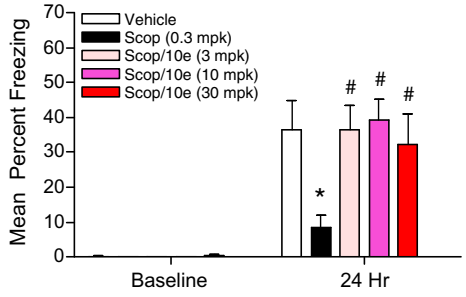


Figure 3.

azole **10x** exhibited robust plasma levels, but a lower CSF/U_{plasma} ratio of 0.10, which could be attributed to the result of **10x** being a substrate for rat P-gp.

Compound **10e** was evaluated for the ability to fold potentiate a dose response of acetylcholine (Fig. 2). In the presence of 1 μM or greater concentration of potentiator **10e**, a leftward-shift was observed in the acetylcholine dose response curve showing it is a potent positive allosteric modulator of the human M_1 receptor. No effects were seen at concentrations that were below the inflection point (440 nM) of **10e**.

Based on the observed M_1 potency and reasonable CSF/U_{plasma} ratio, compound **10e** was evaluated in a mouse contextual fear conditioning assay, which serves as a model of episodic memory (Fig. 3). In this experiment, mice were treated with scopolamine before introduction to a novel environment to block this new association. Mice dosed ip with all three doses of **10e** exhibited a full reversal compared to mice treated with scopolamine alone. The corresponding plasma levels at 3 mpk were 6 μM,[21] a notable improvement over compound **1**, where ~33 μM plasma was required for in vivo efficacy.

In summary, a series of substituted cycloalkyl fused pyridone carboxylic acids in lieu of quinolone carboxylic acids were prepared and evaluated. Optimal A-rings were the cyclohexane and 5-hydroxy cyclohexane **10e**. The SAR for B/C-ring combinations of **10e** was flat relative to previous data with the quinolones. Potentiator **10e** showed adequate CNS exposure and performed very well in a mouse model of episodic memory. Additional structural types employing the hydroxycyclohexane A-ring motif are undergoing evaluation.

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