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Discovery of Naphthyl-Fused 5-Membered Lactams as a New Class of M_1 Positive Allosteric Modulators

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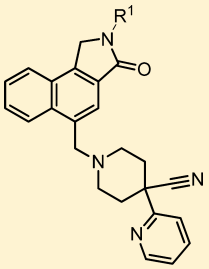
Supporting Information

ABSTRACT: Selective activation of the M_1 muscarinic receptor via positive allosteric modulation represents an original approach to treat the cognitive decline in patients with Alzheimer's disease. A series of naphthyl-fused 5-membered lactams were identified as a new class of M_1 positive allosteric modulators and were found to possess good potency and in vivo efficacy.



KEYWORDS: *M_1, muscarinic, positive allosteric modulators, Alzheimer's disease, acetylcholine*

Alzheimer's disease (AD) is the most common neurodegenerative disease representing one of the largest unmet medical needs in human heath today. One of the hallmarks of AD is the progressive degeneration of cholinergic neurons in the basal forebrain leading to cognitive decline.[1] Acetylcholine, the key neurotransmitter in cholinergic neurons, targets both nicotinic and muscarinic receptors. Muscarinic receptors are G-protein coupled receptors (GPCRs) widely expressed in the central nervous system (CNS). There are five subtypes in the muscarinic family, designed M_1–M_5,[2,3] of which M_1 receptor is the most abundantly expressed in the hippocampus, cortex,[4] and striatum, suggesting a prominent role in memory and cognition.

As a result, significant interest has been placed on developing selective M_1 agonists in order to minimize adverse gastrointestinal events associated with activation of the other muscarinic subtypes.[5] One approach to achieve selectivity is to target allosteric sites on M_1 that are less conserved than the orthosteric site.[6−8] To this end, we have previously reported several highly promising selective allosteric positive modulators of M_1, derived from the original HTS lead, quinolone carboxylic acid **1** (BQCA).[9−14] Efforts to improve the potency and brain penetration led to structural modification in the form of a quinolizidinone ring system, such as **2** (PQCA).[15−17] More recent efforts focused on identification of replacements for the carboxylic acid to enhance CNS exposure and avoid clearance of the parent via the glucuronidation pathway.[18] Accordingly, quinolizidinone carboxamide **3** was identified and provided significant improvement with respect to potency while maintaining excellent selectivity over other muscarinic receptors.[19] However, compound **3** was found to be a substrate for the multidrug resistant (MDR) efflux transporter P-glycoprotein (P-gp), which serves as the major efflux transporter of xenobiotics at the blood−brain barrier.[20] Amides were identified that dialed out the P-gp efflux and led to M_1 PAMs with good brain penetration and free drug concentration.[19] However, it was hoped that the 1*S*,2*S*-2-hydroxy cyclohexyl group present in **3**, which provided excellent potency, could be utilized in a scaffold distinct from the quinolizidinone amide. Herein we report the discovery of a novel class of M_1 allosteric modulators derived from a naphthyl lactam core (**4**) that maintain the high potency without being substrates for P-gp.

The preparation of the analogues of naphthyl lactam **4** is shown in Scheme 1. Starting from 1-hydroxy-2-naphthoic acid methyl ester **5**, triflate (**6**) formation is followed by Stille cross-coupling to provide 1-methyl derivative **7**. Selective bromination at the C-4 position with bromine provided compound **8**. A second bromination at the C-1 methyl group with NBS provided dibromo intermediate **9**. Condensation with amines afforded lactam **10**. The bromo group at C-4 was then transformed to an aldehyde via the standard two-step protocol: vinylation via Suzuki followed by ozonolysis. To complete the synthesis, reductive amination with previously reported piperidine **12**[17] led to final product **4**.

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. A number of analogues with

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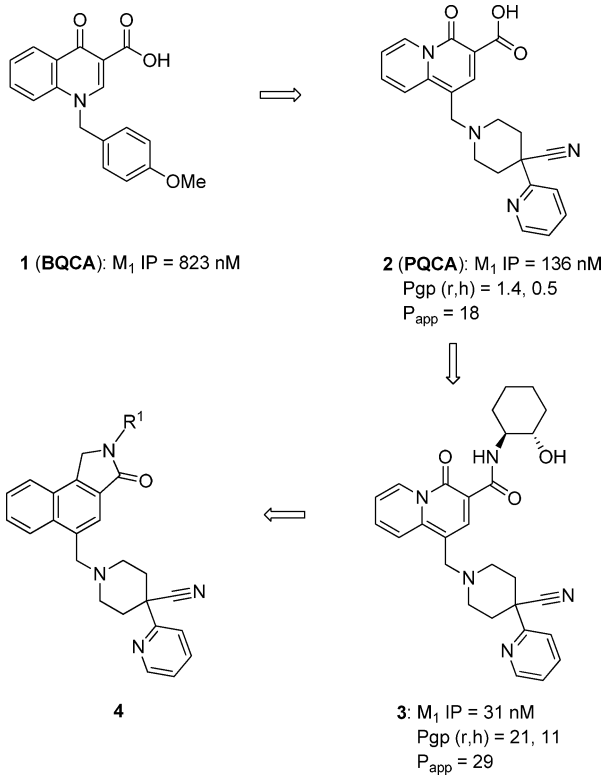
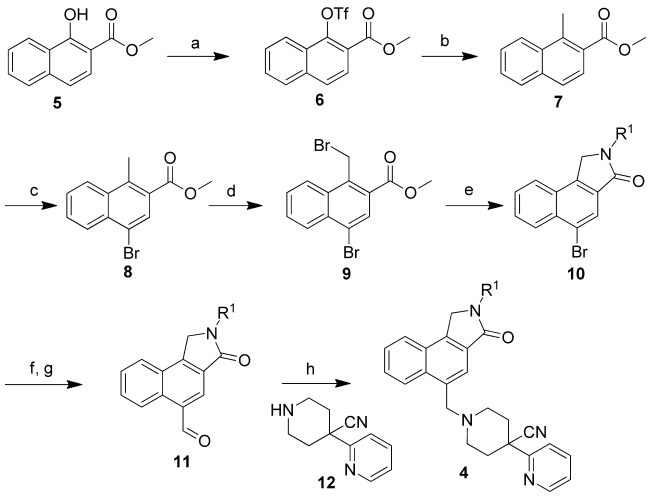


Figure 1. Evolution of M_1 PAM.

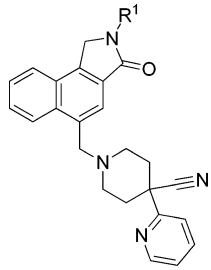
Scheme 1[a]



[a]Reagents and conditions: (a) Tf_2O, Py. −5 °C; (b) $SnMe_4$, LiCl, $Pd(PPh_3)_2Cl_2$, DMF, 110 °C; (c) Br_2, HOAc, 90 °C; (d) NBS, $(BzO)_2$, CCl_4, 90 °C; (e) R_1NH_2, THF; (f) vinylBF_3K, $Pd_2(dba)_3$, PCy_3, K_3PO_4, dioxane, 140 °C; (g) O_3, CH_2Cl_2, −78 °C, then resin-bound PPh_3; (h) $NaBH(OAc)_3$, CH_2Cl_2.

varied substituents on the lactam nitrogen were synthesized as described in Scheme 1. Representative examples are shown in Table 1. Not surprisingly, compound **4d** bearing the aforementioned 1*S*,2*S*-2-hydroxy cyclohexyl group off the lactam is the most potent compound among various R1 groups explored and displayed an M_1 IP value of 15 nM. This specific stereochemistry was critical for potency as the associated 1*R*,2*R*-trans isomer **4e** and cis isomer **4f** were both significantly less active. *ortho*-Fluoro phenyl lactam **4a** gave only moderate

Table 1. SAR on N-Substitution of Lactams



Compds	R^1	M_1 IP (nM)[a]	Compds	R^1	M_1 IP (nM)
4a		290	**4d**		15
4b		1500	**4e**		540
4c		280	**4f**		150

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

potency with an M_1 IP value of 290 nM, approximately 5-fold less potent than **4d**. Similar potency was observed with 4-tetrahydropyran lactam **4b** (M_1 IP = 280 nM). However, the corresponding 3-pyran (**4c**) was ∼5-fold weaker. It is worth noting that this SAR pattern at the R^1 position was consistent with previously reported quinolizidinone amides.[19] Moreover, it is important to recognize that this new naphthyl fused lactam still provides potent M_1 PAMs such that cyclization of the amide in **3** into a lactam is tolerated and that the quinolizidinone can indeed be replaced by the naphthalene ring system. This shows that the carbonyl moiety present in previous quinolone and quinolizidinone M_1 PAMs is not required for activity. As a novel class of M_1 PAM, selected lactams were profiled in functional assays at other muscarinic subtypes and showed no activity at M_2, M_3, or M_4 receptors, indicating that lactams maintain selectivity for M_1.

Having identified the naphthyl lactam as a potent new structural class, it was important to verify if it showed an advantage over amide **3** with respect to reduced P-gp efflux. Plasma protein binding was also determined using the equilibrium dialysis method in the presence of rat and human serum. The most potent lactam **4d** did indeed display significantly reduced efflux and was a borderline substrate for human and rat P-gp with efflux ratios (ERs) of 3.2 and 4.3, respectively (Table 2). This is a significant improvement over the analogous quinolizidinone amide **3**, with efflux ratios (ERs) of 11 and 21, respectively (Figure 1). Compound **4d** also displayed excellent passive-permeability and good unbound fraction in plasma (5% in rat and 8% in human).

In order to further identify non-P-gp substrates, an SAR campaign was initiated on the amine motif. When the 2-pyridyl at R^3 was replaced with other pyridines (**4g** and **4h**) or less basic diazines (**4i**−**k**), the M_1 potency was maintained but the P-gp ERs increased. Next, a methyl group was placed at key positions on the pyridine ring. The *ortho*-methyl analogue **4l** was found not to be a human P-gp substrate, while the rat P-gp ER was also improved. The *para*-methyl compound **4m** also showed similar advance on human P-gp but not on rat.

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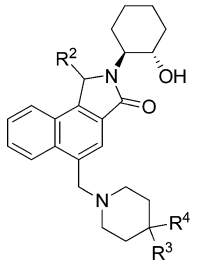
Table 2. M_1 FLIPR, Protein Binding, and Pgp Data for Selected Compounds



Compds	R^2	R^3	R^4	M1 IP (nM)[a]	Rat PB	Human PB	Papp[b]	MDR1(human)[c]	MDR1a(rat)[c]
4d	H		CN	15	95	92	34	3.2	4.3
4g	H		CN	22	ND	ND	30	26	49
4h	H		CN	22	ND	ND	32	nd	nd
4i	H		CN	17	93	91	29	6	20
4j	H		CN	31	85.8	91.4	31	8	11
4k	H		CN	27	85.4	85.6	27	nd	nd
4l	H		CN	27	99	99	28	1.5	3.1
4m	H		CN	16	99.3	99.4	22	1.3	4.5
4n	H		CN	83	99.4	98.9	23	1.7	3.4
4o	H		F	58	98.5	97	34	2.1	2.7
4p	Me		CN	19	98.6	94	29	1.7	4.1

[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%.

Although both methyl analogues showed similar potency and reduced P-gp efflux compared to **4d**, they were considerably more lipophilic and showed very high plasma protein binding at ~99%. When the 2-pyridine was replaced with a phenyl group, compound **4n** showed reduced P-gp efflux as expected, but at the expense of a ~5-fold loss of potency. The phenyl

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Table 3. Brain and Plasma Distribution in Rats for Selected Compounds

compds	R^2	R^3	R^4	Plasma Conc. (nM)[a]	CSF(nM)[a]	CSF/U_{plasma}[b]	Solubility (uM, pH=2/7)	LogD (HPLC)
4d	H		CN	5175	134	0.51	195/11	2.9
4p	Me		CN	1122	4.7	0.3	198/19	3.4
4m	H		CN	2342	8.6	0.52	179/3	3.3

[a]Sprague–Dawley rats, concentration at 2 h postdose. Oral dose 10 mg/kg in 0.5% methocel. Interanimal variability was less than 20% for all values. [b]Determined using rat plasma protein binding from Table 2.

replacement also resulted in higher plasma protein binding for **4n**. A similar SAR trend was observed when exchanging CN group with F: compound **4o** showed a loss of ~4-fold of M_1 potency but did have reduced P-gp efflux. In addition, the methyl group could be introduced onto benzylic position of lactam ring (R^2). In this case, racemic product **4p** maintained potency and showed improved human P-gp efflux. Compound **4p** also retained a reasonable unbound free fraction despite the addition of the methyl group but was more lipophilic with a logP of 3.3.

On the basis of the potency, P-gp profile, and free fraction properties, compounds **4d**, **4m**, and **4p** were selected as candidates to determine the CNS exposure in rats (Table 3). Plasma and cerebrospinal fluid (CSF) levels were measured after 2 h following a 10 mg/kg oral dose. Compound **4d** gave significant plasma (5.2 uM) and CSF (134 nM) levels with a CSF/Uplasma ratio of 0.51. Although compound **4p** provided a similar CSF/Uplasma ratio (0.52) compared to **4d**, the absolute CSF level (8.6 nM) was significantly lower. The other methyl analogue **4m** gave a moderately reduced CSF/Uplasma ratio (0.3) as well as low absolute CSF level (4.7 nM). The high CSF level of **4d** was believed to be driven by higher unbound drug concentration than the corresponding methyl analogues **4m** and **4p**. Consistent with their increased protein binding, methyl analogues **4m** and **4p** have higher measured logP values than **4d**. All three compounds showed excellent solubility at pH 2 (existed as salt form) but poor solubility at pH 7 (neutral form).

On the basis of the robust CSF levels of compound **4d**, further studies were performed to investigate the properties of this new class of lactam-derived M_1 modulators. Fold potentiation with a fixed concentration of modulator **4d** was evaluated on the M_1 dose response with acetylcholine as the agonist. As shown in Figure 2, with increasing concentration of compound **4d**, a left shift was observed up to ~40-fold at 1.8 μM in the acetylcholine dose–response curve, indicating that **4d** is a potent positive allosteric modulator of human M_1 receptor. It worth noting that PAM **4d** displayed dose-dependent partial agonism as indicated by an upward shift of acetylcholine dose–response curves at the two highest concentrations tested.

To evaluate the in vivo efficacy, PAM **4d** was tested in a mouse contextual fear conditioning (CFC) assay, which serves as a model of episodic memory (Figure 3). In this study, mice were treated with scopolamine, a nonselective muscarinic antagonist, prior to exposure to a novel environment to impair a new association. Mice dosed by intrapertitoneal injection with **4d** exhibited a significant reversal of scopolamine-induced

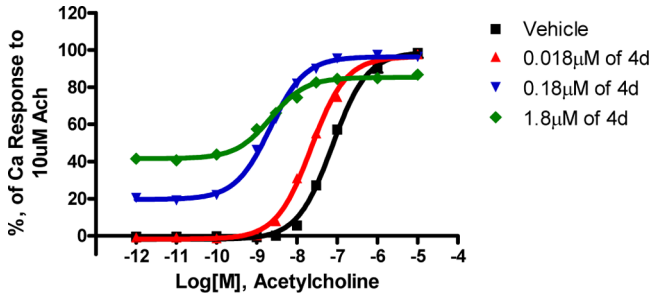

Figure 2. Fold potentiation of **4d**. Mean values from four replicate wells are plotted; data are representative of 12 independent experiments.

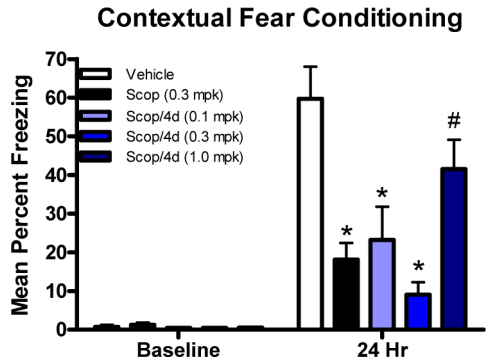

Figure 3. Evaluation of lactam **4d** in the mouse CFC model. Data are representative of four experiments. *, Different from vehicle; #, different from scopolamine + vehicle ($P < 0.05$, Dunnet test).

deficit at 1 mpk relative to mice treated with scopolamine alone. The corresponding plasma level at 1 mpk was 1.5 μM. By way of comparison, the previous lead, carboxylic acid **2** showed significant reversal at ~1 μM plasma levels. The result demonstrated robust proof-of-action for the new series despite the fact that lactam **4d** is a rodent P-gp substrate. Pharmacokinetics of lead compound **4d** was also evaluated in rat and dog (Table 4). Low clearance was observed in these two species, further highlighting the potential to provide potent M_1 PAMs with potential for good human pharmacokinetic properties.

Table 4. Pharmacokinetics of 4d in Rat and Dog

	dose (mg/kg)	route	Cl (min/ml/kg)	V_{dss} (L/kg)	$T_{1/2}$ (h)
rat	2	iv	4.1	2.8	12
dog	0.125	iv	2.1	1.2	6.7

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In summary, naphthyl-fused 5-membered lactams have emerged as a new class of M_1 positive allosteric modulators. This naphthyl fused lactam is novel not only because it shows that cyclization of the amide in **3** into a lactam is tolerated but that the quinolizidinone can be replaced by the naphthalene meaning that the carbonyl moiety present in previous quinolone and quinolizidinone M_1 PAMs is not required for activity. The *trans*-1*S*,2*S*-2-hydroxy cyclohexyl group was found to be the most potent group off the amide position, and significant attenuation of P-gp efflux could be garnered. Compound **4d** demonstrated high CSF drug levels and good efficacy in a mouse contextual fear model of episodic memory despite being a rodent P-gp substrate. Further SAR study of these lactams with respect to improving solubility at neutral pH and reducing P-gp efflux is expected to provide optimized M_1 PAMs and will be reported in due course.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Representative assay and experimental procedures and data for test compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

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