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Development of a Highly Selective, Orally Bioavailable and CNS Penetrant M_1 Agonist Derived from the MLPCN Probe ML071

Evan P. Lebois[a], **Gregory J. Digby**[a], **Douglas J. Sheffler**[a,b], **Bruce J. Melancon**[a,b,c], **James C. Tarr**[a,b,c], **Hyekyung P. Cho**[a,b,c], **Nicoel R. Miller**[d], **Ryan Morrison**[a,b,c], **Thomas M. Bridges**[a,b], **Zixiu Xiang**[a], **J. Scott Daniels**[a,b,c], **Michael R. Wood**[a,b,c], **P. Jeffrey Conn**[a,b,c], and **Craig W. Lindsley**[a,b,c,*]

[a]Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

[b]Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University Medical Center, Nashville, TN 37232, USA

[c]Vanderbilt Specialized Chemistry Center for Probe Development (MLPCN), Nashville, TN 37232, USA

Abstract

Herein we report the discovery and SAR of a novel series of M_1 agonists based on the MLPCN probe, ML071. From this, VU0364572 emerged as a potent, orally bioavailable and CNS penetrant M_1 agonist with high selectivity, clean ancillary pharmacology and enantiospecific activity.

Keywords

Muscarinic acetylcholine receptor 1; mAChR1 (M_1); ML071; Allosteric agonist

The muscarinic acetylcholine receptors, mAChRs or M_1–M_5, are members of the class A G protein-coupled receptors (GPCRs) that mediate a broad range of actions of the neurotransmitter acetylcholine (ACh) in the central nervous system and other tissues.[1–3] Previous attempts to develop compounds that are highly selective for M_1 have failed because of the high conservation of the orthosteric ACh binding site and difficulty in developing truly specific compounds, not only for M_1 (versus M_2–M_5) but also other biogenic amine receptors (BARs).[4–6] Despite these major shortcomings, multiple orthosteric M_1 'preferring' agonists have provided proof of concept in Phase II and III clinical trials for both Alzheimer's disease (AD) and schizophrenia, generating great enthusiasm for selective M_1 activation.[7–14]

Current efforts are focused on the selective activation of M_1 by targeting less conserved allosteric sites, and this approach is proving highly successful for multiple GPCRs.[4,5,15,16] Allosteric and/or bi-topic M_1 partial agonists first appeared, such as **1**–**4**, with improved mAChR selectivity in many instances, but a general lack of selectivity versus BARs (D_2,

*To whom correspondence should be addressed: craig.lindsley@vanderbilt.edu.
[d]Current Affiliation: NIH Chemical Genomics Center, National Institute of Health, Bethesda, MD USA

$5HT_{2C}$, $5HT_{2B}$, opiate, etc.) and poor DMPK properties precluded their utility as tools to probe selective M_1 activation *in vivo* (Fig. 1).[6,17–20] Recently, numerous additional M_1 full and partial agonists have been reported with improved properties and efficacy in cognition models.[21–23] Positive allosteric modulators (PAMs) from three structural classes, **5**–**7**, have also been reported, with exquisite selectivity for M_1 versus M_2–M_5 and BARs, allowing limited *in vivo* proof of concept studies to be conducted.[24–26,28,29,31,32] For all of these PAMs, both DMPK properties and CNS penetration are barely adequate for *in vivo* studies; [24–26,28,29,31,32] however, two scaffolds variants of the BQCA PAM scaffold have overcome the DMPK issues and afforded PAMs with good properties and CNS exposure.[27,30]

Based on the need for additional M_1 tools, we recently performed a functional M_1 HTS of the 65,000 compound MLPCN library using a high-expressing M_1 rat CHO cell line.[33,34] From this effort, we identified M_1 agonists, antagonists and PAMs, which were quickly optimized to afford the M_1 selective antagonist ML012 (VU0255035),[33] M_1 PAMs ML137 (VU0366369)[31] and ML169 (VU0405562)[32] as well as the M_1 agonist ML071 (VU0357017).[34] ML071 (**8**) proved to be a highly selective M_1 partial agonist (EC_{50} = 200 nM, 81% ACh Max, >30 μM versus M_2–M_5) with clean ancillary pharmacology (no activity greater than 50% in a 10 μM radioligand binding panel of 68 GPCRs, ion channels and transporters, including the BARs) and favorable DMPK properties and CNS penetration (Fig. 2).[31] ML071 demonstrated that selective M_1 activation potentiated NMDA receptor currents, provided a significant increase in soluble APP (sAPPα) in cell culture, and dose-dependently reversed scopolamine-induced disruption of contextual fear conditioning responses.[34] However, ML071 also displayed functional D_2 antagonism (IC_{50} = 4.5 μM), which we hoped to eliminate through chemical optimization. As previously detailed, SAR for ML071 was shallow, with few modifications tolerated.[34] In this Letter, we describe the introduction of cyclic constraints to impart improved potency, efficacy and DMPK properties (Fig. 2).

A number of cyclic constraints ((*R*)- and (*S*)-3-aminopyrroldines (**9**–**12**), piperazine (**13**), [2.2.1] (**14**) and [3.3.0] (**15**) congeners) were synthesized (Fig. 3A) and evaluated, affording less than 30% M_1 activation at 10 μM (Fig. 3B). However, the (*R*)-aminopiperidine constraint **17** (VU0364572) afforded full activation of M_1, while the (*S*)-enantiomer **16** was inactive, providing the first reported example of enantioselective M_1 activation. VU0364572 proved to be highly selective for M_1 (Fig. 3C) with an EC_{50} of 110 nM and ~95% ACh Max.

This finding led us to synthesize libraries of analogs around both **16** and **17** wherein we varied the amide moiety; interestingly, all (*S*)-enantiomers **16** were inactive (M_1 EC_{50}s >10 μM). Analogs **17** were synthesized according to Scheme 1. As shown in Table 1, SAR was shallow, as with ML071,[34] with only 12 of 36 analogs possessing M_1 potencies below 5 μM; moreover, **17** proved to be the best compound within this series in terms of M_1 potency, ACh Max and mAChR selectivity. Efforts now focused on full characterization of VU0364572, **17**.

We then evaluated VU0364572 (**17**) in a radioligand binding panel of 68 GPCRs, ion channels and transporters (including all the BARs),[35] and no significant activities were reported (no inhibition >30% @10 μM). Importantly, **17** showed minimal activity at hERG (22%@10 μM), despite possessing a classical hERG pharmacophore. Furthermore, based on the previously observed weak functional D_2 antagonism of ML071 (D_2 IC_{50} = 4.5 μM), we evaluated **17**, and were delighted to find no functional activity at the D_2 receptor (Fig. 4). Based on this, VU0364572 is a highly selective M_1 agonist with exceptionally clean

ancillary pharmacology, and a valuable small molecule tool to dissect the role of direct and selective M_1 activation.[1–20]

Further in vitro characterization followed, evaluating the ability of **17** to shift APP processing towards the non-amyloidogenic pathway. Employing our standard model[20,22,28,29] in TREx293-hM_1 cells, carbachol (CCh) (10 μM) affords a significant increase in soluble APP (sAPPα), whereas an identical concentration of **17** provides a more robust increase (~3-fold) in sAPPα. Based on this data, selective activation of M_1, via **17**, may have a disease modifying role in AD.

Activation of NMDA receptor currents by M_1 is postulated to play a critical role in the cholinergic regulation of cognitive function and circuitry that underlie the efficacy of mAChR agonists in schizophrenia (the NMDA receptor hypofunction hypothesis of schizophrenia)[36] and AD. As shown in Figure 5, VU0364572 (**17**), was found to potentiate NMDA receptor currents in hippocampal CA1 pyramidal cells, further validating selective M_1 activation as a means to promote synaptic plasticity.

The *in vitro* data was tremendously exciting, prompting the evaluation of **17** in our tier 1 DMPK battery. **17** displayed low plasma protein binding for both human (f_u = 5.8%) and rat f_u = 14.9%) and had a clean CYP profile (3A4, 2C9, 1A2 and 2D6; IC_{50} >25 μM). Intrinsic clearance experiments (rat CL_{INT} = 23.4 mL/min/kg and human CL_{INT} = 11.2 mL/min/kg) suggested **17** would be a low to moderate clearance compound, and rat PK confirmed a good *in vitro/in vivo* correlation. A standard rat IV(1 mg/kg)/PO (10 mg/kg) study found **17** to possess a CL of 14.7 mL/min/kg, with a V_{ss} of 0.98 L/kg and a $t_{1/2}$ of 46 min. Importantly, **17** was orally bioavailable with a %F of 37 (AUC_{IV} = 1.1 μg*hr/mL, AUC_{PO} = 4.2 μg*hr/mL). In parallel, we performed an oral plasma:brain level (PBL) study with **17** in male Sprague-Dawley rats (Table 2). At a dose of 10 mg/kg with a 90 minute endpoint, **17** achieved an average $Brain_{AUC}$/$Plasma_{AUC}$ of 1.35, providing excellent CNS exposure.

As it became time to profile **17** on human M_1–M_5, our research team had moved away from the high-expressing HTS lines and developed both rat and human M_1–M_5 with expression more closely resembling native expression levels. In these new cell lines, both **17** and ML071 experienced an ~10-fold right shift in potency (EC_{50}s of 1.3 μM and 2.3 μM, respectively) with slightly lower ACh Max, yet still remained highly selective (M_2–M_5 EC_{50}s >30 μM). As M_1 expression levels vary amongst neuronal tissues and brain regions, compounds such as **17** may behave as full agonists in one population and as weak, partial agonists in another. The ramifications of this, and further in depth *in vitro* and *in vivo* pharmacological studies are in progress and will be reported shortly.[37]

In summary, the chemical lead optimization of the MLPCN M_1 agonist probe ML071 (VU0357017), led to the discovery of VU0364572 (**17**), an M_1 agonist with high selectivity (versus M_2–M_5 as well as the BARs), exceptional PK and brain exposure, robust effects on APP processing and potentiation of NMDA receptor currents and enantiospecific M_1 activation. Further in depth *in vitro* and *in vivo* pharmacological studies with VU0364572 (**17**) are in progress and will be reported shortly.

Acknowledgments

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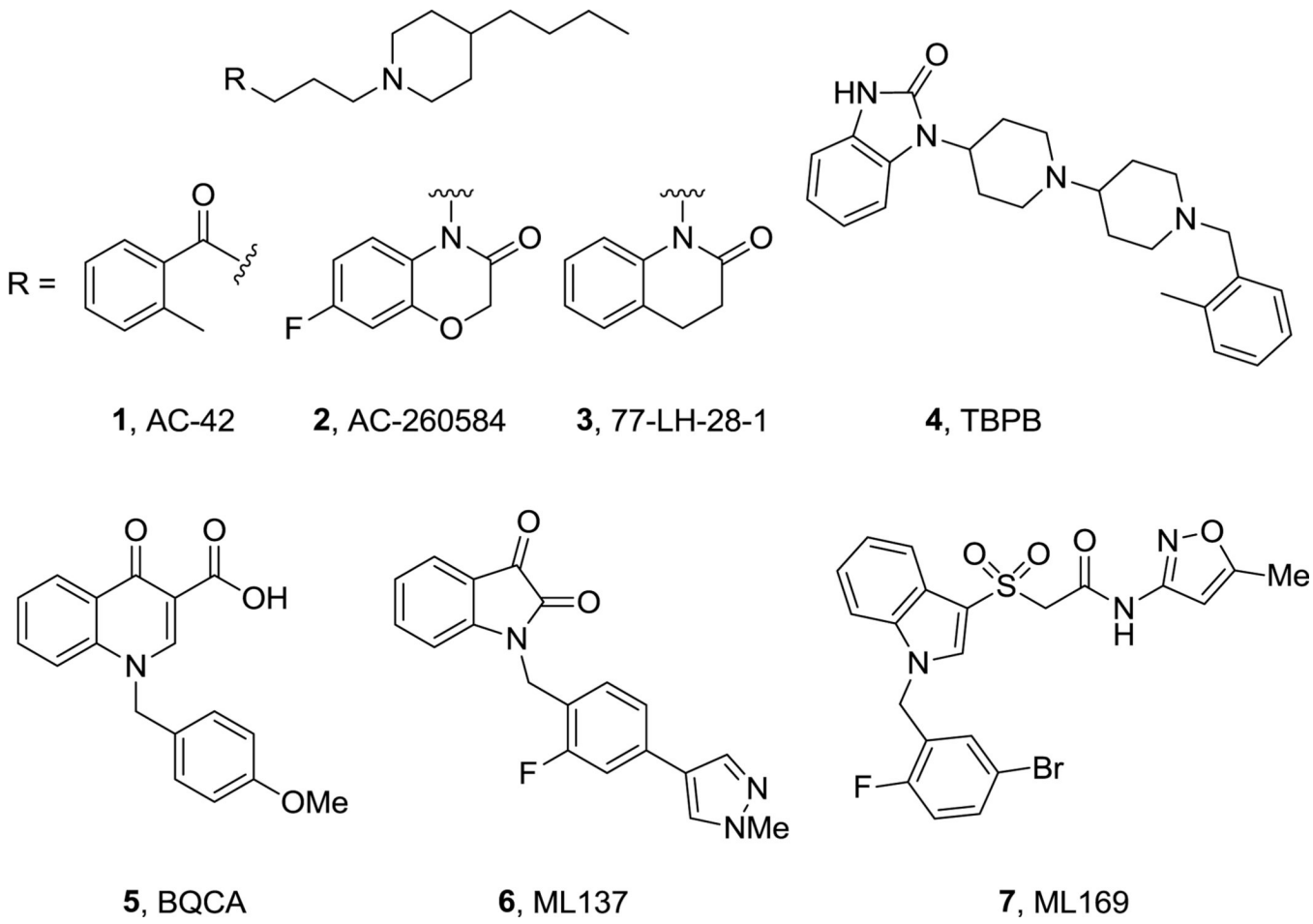


Figure 1.
Prototypical 'M_1-preferring' allosteric (bi-topic) agonists **1**–**4**, and M_1 PAM series **5**–**7**.

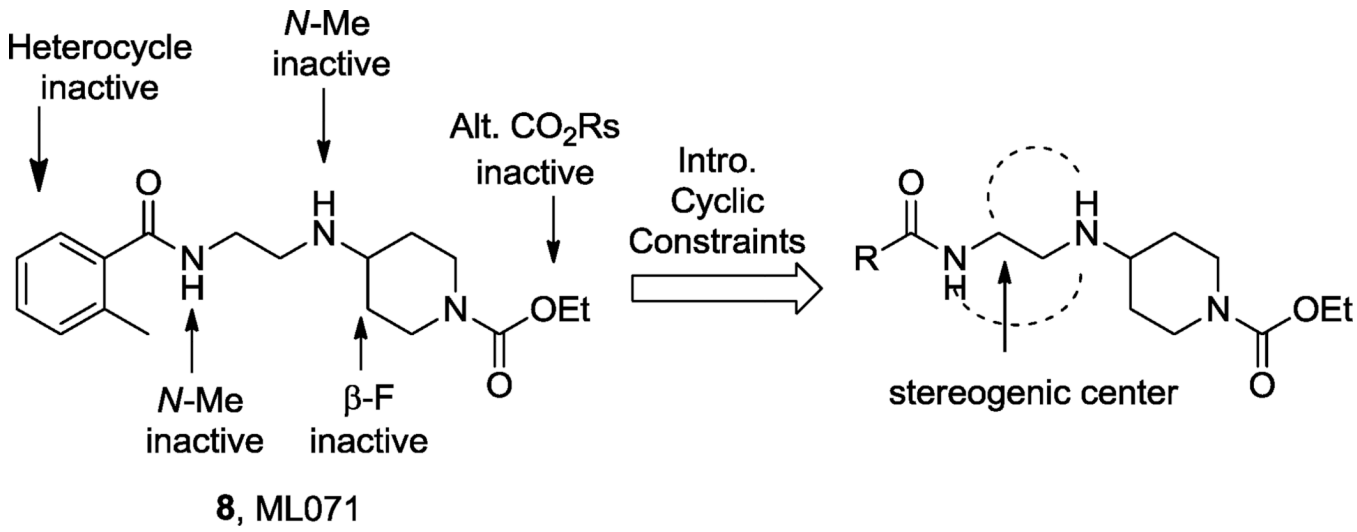


Figure 2.
Prior SAR for ML071 and proposal to introduce cyclic constraints.

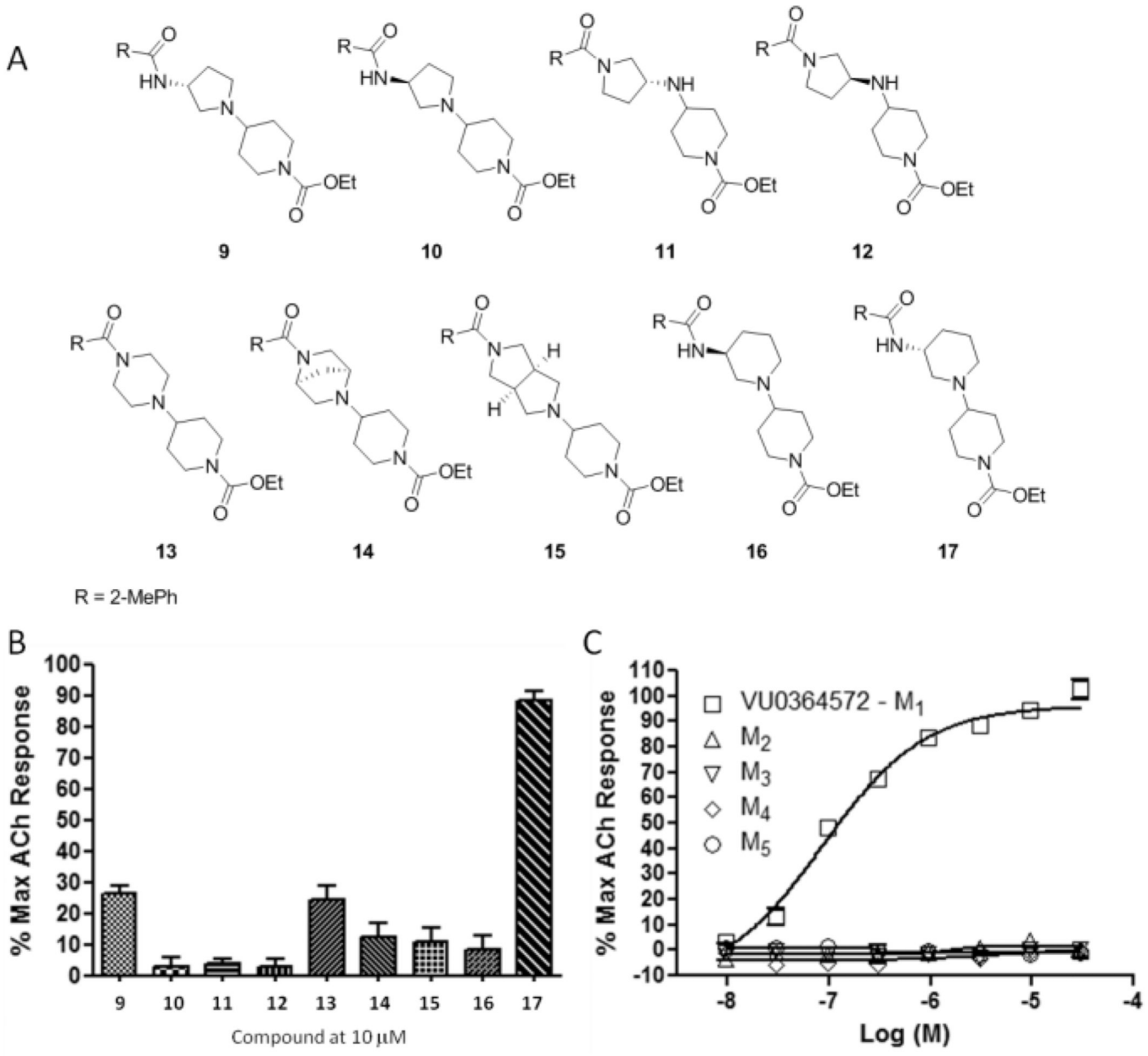


Figure 3.

A) Structures of cyclic constrained analogs of **8**. B) 10 μM M_1 single point screen of cyclic constraint variants of ML071. C) Full CRCs at M_1–M_5 for VU0364572 (**17**).

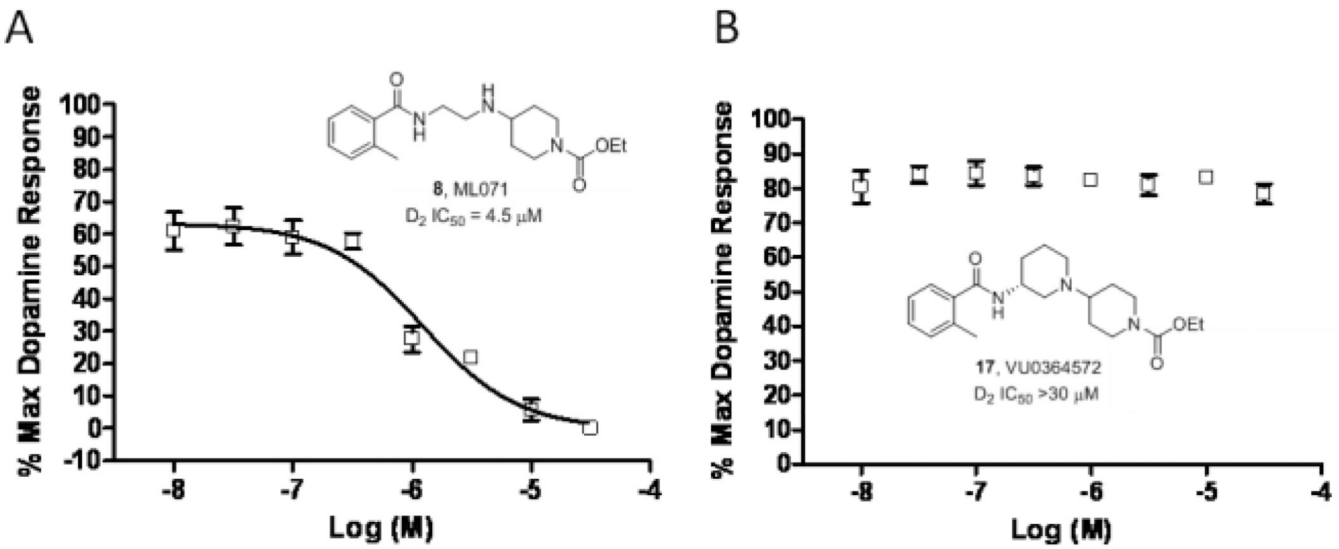


Figure 4.

A) CRC for ML071 for D_2 antagonism, affording an IC_{50} of 4.5 μM. B) CRC for VU0364572 for D_2 antagonism, affording an IC_{50} > 30 μM.

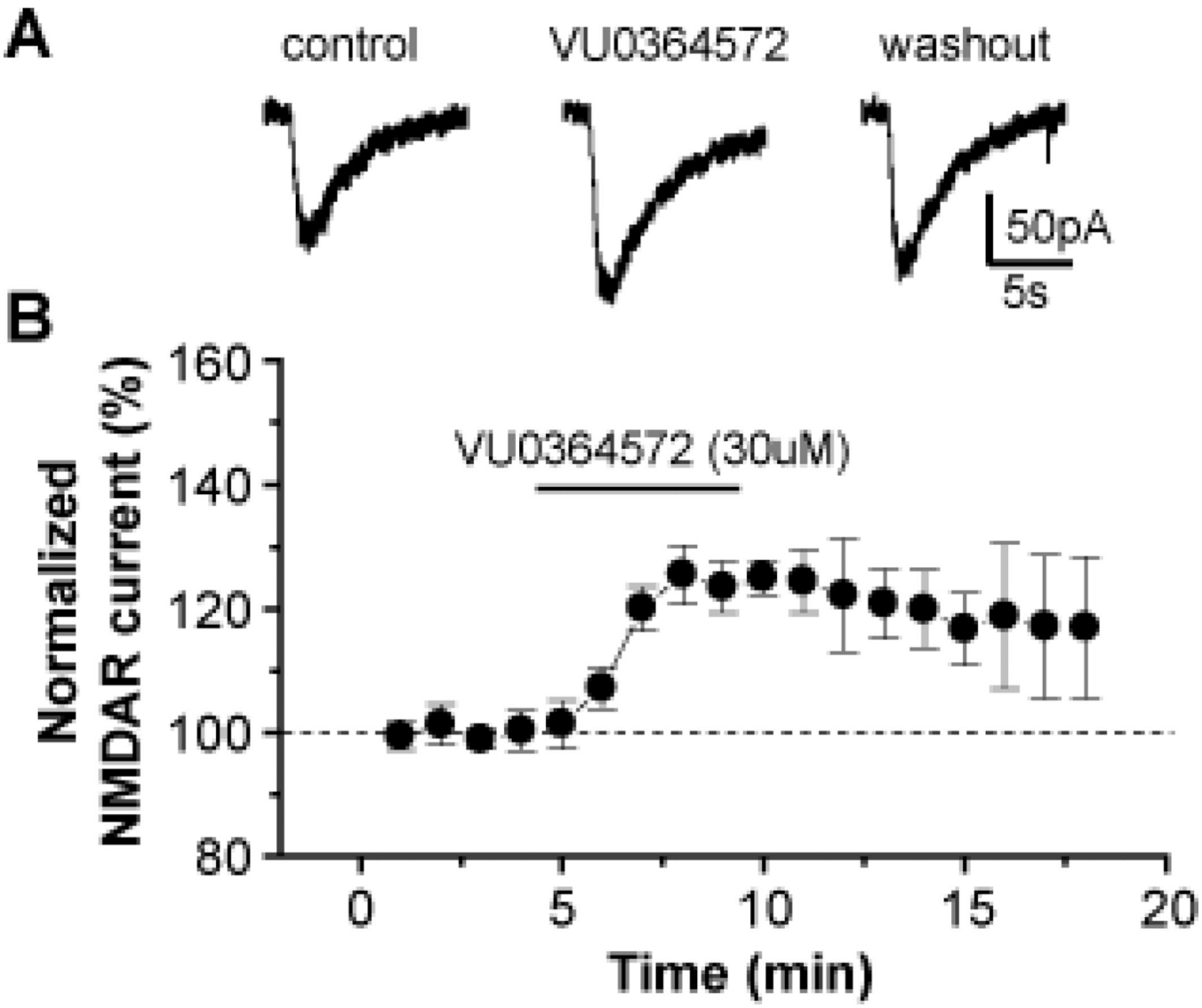
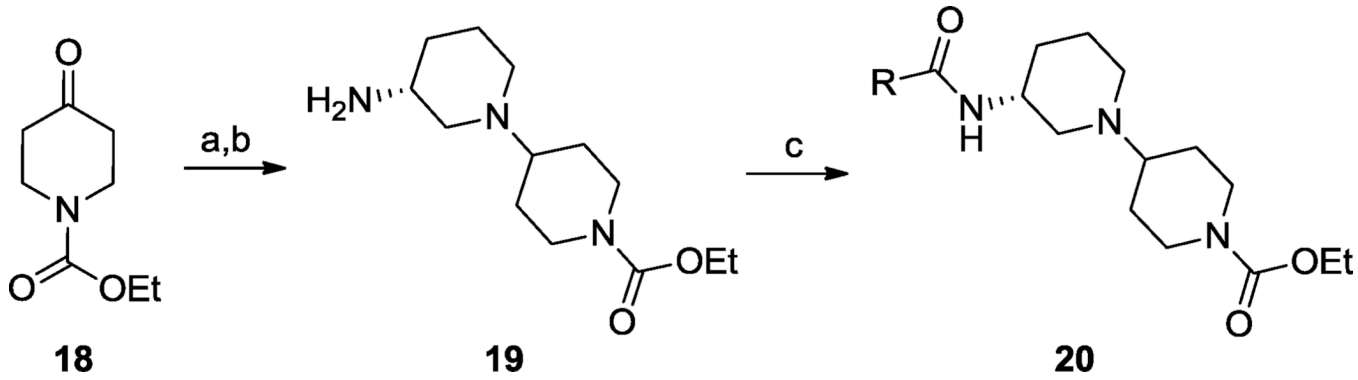


Figure 5.

VU0364572 potentiates NMDA receptor currents in hippocampal CA1 pyramidal cells. A) Representative whole cell traces of NMDA-evoked currents and B) time course of normalized amplitude of NMDAR currents before, during and after application of 30 μM VU0364572 (**17**). Notably, even after washout, the potentiation persists for 10 min. Error bars represent mean ± SEM for five independent determinations.

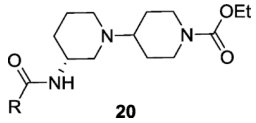


Scheme 1.

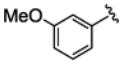
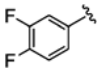
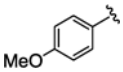
Reagents: (a) (*R*)-3-amino-3-*N*-Boc-piperidine, $NaBH(OAc)_3$, DCE, 95%; (b) HCl, dioxane, rt, 96%; (c) RCOCl, DIEA, DCM, rt, 65–95%.

Table 1

Structures and activities of M_1 agonist analogs **20a**–**k**.



20

Cmpd	R	M_1 EC_{50} (μM)[a]	ACh Max (%)[a]	M_2–M_5 EC_{50}s (μM)[a]
8		0.2	81	>30
17		0.11	96	>30
20a		0.06	77	>10
20b		0.13	91	>10
20c		0.14	92	>10
20d		0.25	91	>10
20e **20f**		0.31	84	>10
20g		0.89	41	>10
20h		0.69	44	>10
20i		1.3	61	>10
20j		0.96	63	>10
20k		2.6	57	>10
		5.0	35	>10

[a] Average of at least three determinations in our rat HTS high-expressing CHO cell line

Table 2

10 mg/kg Oral Plasma:Brain Level Study with **17**.

Dose (mg/Kg)	Animal	Plasma (μM)	Brain (μM)	Brain:Plasma
10	1	1.45	2.23	1.54
10	2	1.62	1.89	1.17