



Spirocyclic replacements for the isatin in the highly selective, muscarinic M₁ PAM ML137: The continued optimization of an MLPCN probe molecule

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ARTICLE INFO

Article history:

Received 20 November 2012

Revised 19 December 2012

Accepted 2 January 2013

Available online 11 January 2013

Keywords:

Muscarinic acetylcholine receptor 1

M₁

Spirocyclic

Positive allosteric modulator (PAM)

ML137

VU0413162

ABSTRACT

This Letter describes the further optimization of an MLPCN probe molecule (ML137) through the introduction of 5- and 6-membered spirocycles in place of the isatin ketone. Interestingly divergent structure–activity relationships, when compared to earlier M₁ PAMs, are presented. These novel spirocycles possess improved efficacy relative to ML137, while also maintaining high selectivity for the human and rat muscarinic M₁ receptor subtype.

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The tremendous potential for truly selective muscarinic acetylcholine (ACh) ligands in a variety of disease states is well appreciated; in reality, however, this potential has been difficult to achieve due to a historical reliance on targeting the highly conserved orthosteric ACh binding site shared by the five muscarinic ACh receptor subtypes (mAChRs, M₁ through M₅).¹ As such, many of our current beliefs pertaining to the potential utility of muscarinic ligands in central nervous system (CNS) disorders has come from the phenotypes of M₁₋₅ knock out (KO) mice,² CNS receptor localization and the promising clinical outcomes observed with the muscarinic agonist xanomeline in both Alzheimer's disease and schizophrenia patients.^{3,4} Unfortunately, xanomeline's limited muscarinic subtype selectivity has given rise to unacceptable levels of adverse events consistent with peripheral activation of the M₃ receptor. Although switching focus to allosteric agonists is a credible tactic for circumventing the selectivity issues associated with engaging the orthosteric site, a fair number of allosteric agonists have recently been shown to bind in a bitopic sense to the mAChRs, bringing the orthosteric site issues back into unwelcome consider-

ation.^{5,6} Another tactic for achieving high mAChR subtype selectivity has been the development of positive allosteric modulators (PAMs), compounds with selectivity profiles that benefit from binding solely at sites distinct from the ACh binding site where amino acid homology between the muscarinic receptors has the potential to be reduced. This approach has been validated through the development of numerous, highly selective PAMs for M₁,⁷ M₄,⁸ and M₅.⁹

Recently, we described our initial success at replacing the isatin portion of ML137 and were able to arrive at a variety of novel structures partially summarized by compounds **1** and **2** in Figure 1.¹⁰ Reduction or modification of the ketone carbonyl in ML137 served as the starting point for these benzo-fused bicyclic replacements. Alternatively, it might be envisioned that this carbonyl could be partially hydrated when water is available (in vitro/in vivo) or even preferentially present as the hydrate when bound to the allosteric site on the hM₁ receptor. This idea formed the rationale for the preparation of the spirocyclic ketal **3** (Fig. 1) as a discrete and stable entity. When tested against the human M₁ (hM₁) receptor for functional activity, **3** provided a hM₁ PAM EC₅₀ of 2.0 μM with an ACh_{max} response of 62%, showing reasonable potency and efficacy, respectively. Although not as potent

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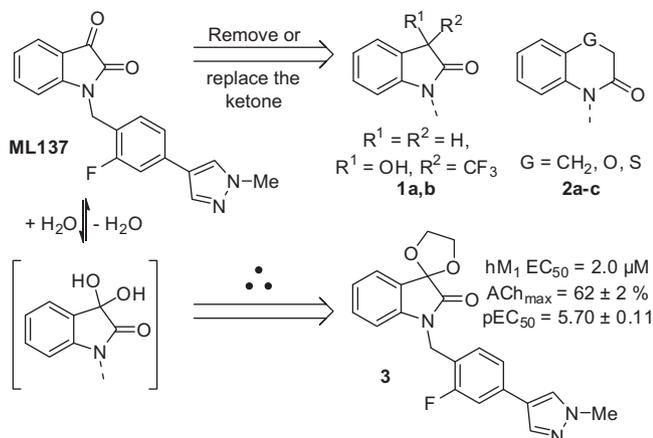


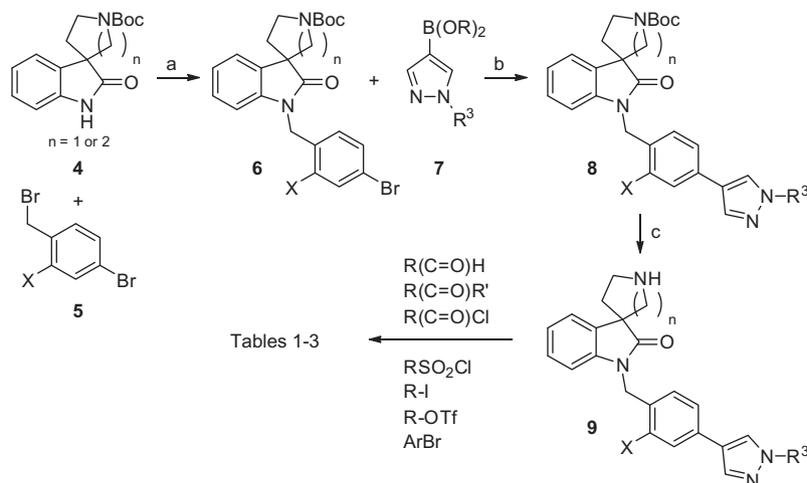
Figure 1. Prior replacements for the isatin in ML137 and a rationale for the preparation of the spirocyclic ketal **3**.

(EC_{50}) as its parent ML137 ($hM_1 EC_{50} = 0.60 \mu M$, $ACh_{max} = 45 \pm 3\%$, $pEC_{50} = 6.22 \pm 0.02$),¹¹ compound **3** produced improved efficacy ($\%ACh_{max}$) at the highest concentration tested of $30 \mu M$. This novel spirocycle was the impetus for exploring a wider range of diverse

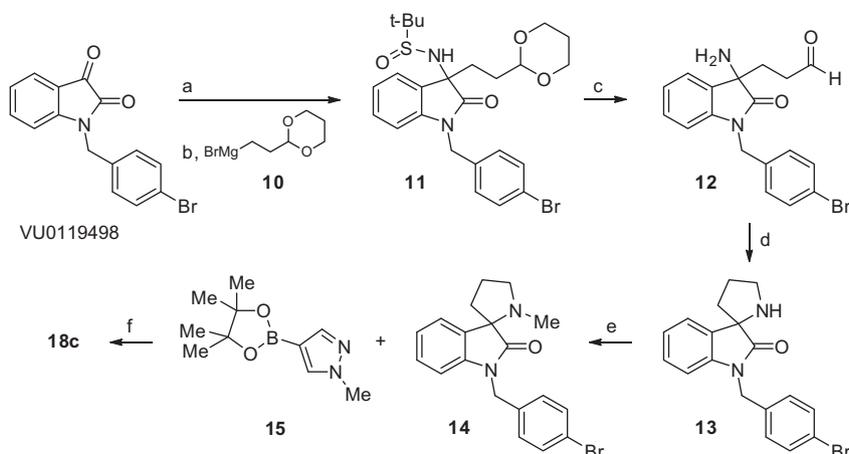
spirocycles at this location. Some of these spirocycles were available from commercial sources and were alkylated with the appropriate benzyl chloride as described previously.¹⁰ However, the majority of subsequent analogs were prepared according to Schemes 1–3.

Scheme 1 shows the alkylation of commercially available Boc-protected spirocyclic amines **4** with benzylic bromides **5** employing potassium carbonate in acetonitrile to provide aryl bromides **6**. Compound **6** can then undergo Suzuki couplings with a variety of boronic acids/esters, such as **7**, to form the *N*-Boc protected biaryl compounds **8**. The *N*-Boc protecting group can then be removed with a strong acid (TFA or anhydrous HCl) to give the secondary amine **9**. Amine **9** was an invaluable intermediate poised to engage in a wide variety of well-precedented reactions with a near limitless number of electrophiles.

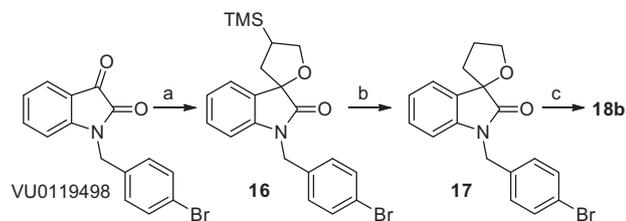
Scheme 2 depicts the construction of an isomeric spiropyrrolidine analog, which was not commercially available, starting from the known, non-selective $M_{1,3,5}$ PAM VU0119498.^{9a} Condensation of this isatin with *tert*-butyl sulfonamide promoted by titanium (IV) tetraisopropoxide in DCM was followed, in a separate step, by the addition of Grignard **10** to yield **11**. In this manner, both the nitrogen and the carbon chain required to form the spiropyrrolidine were efficiently introduced. Removal of both protecting groups from **11** with HCl in dioxane gave the aminoaldehyde **12**



Scheme 1. Reagent and conditions: (a) K_2CO_3 , MeCN; (b) $PdCl_2(dppf)$ -DCM, Cs_2CO_3 , THF/ H_2O , $160^\circ C$, 10 min; (c) TFA or HCl, $0^\circ C$ to rt.



Scheme 2. Reagents and conditions: (a) *tert*-butyl sulfonamide, $Ti(OiPr)_4$, $0^\circ C$ to rt, DCM, 84% yield; (b) THF, $-78^\circ C$ to rt, 72% yield; (c) HCl in dioxane (4 M), $0^\circ C$ to rt; (d) $NaBH(OAc)_3$, DCE, rt, 58% yield, 2 steps; (e) $(CH_2O)_n$, CO_2H , H_2O , $100^\circ C$, 2 h, 70% yield; (f) $PdCl_2(dppf)$ -DCM, Cs_2CO_3 , THF/ H_2O , $160^\circ C$, 10 min, 40% yield.



Scheme 3. Reagents and conditions: (a) allyltrimethylsilane, SnCl_4 , DCM, 0 °C, 5 h, 60% yield; (b) TBAF, THF, 60 °C, 18 h, 28% yield; (c) **15**, $\text{PdCl}_2(\text{dppf})$ -DCM, Cs_2CO_3 , THF/ H_2O , 160 °C, 10 min, 75% yield.

(alternatively in a protonated/cyclized version, not shown) which readily underwent reductive cyclization to form the spiropyrrolidine **13**. The secondary amine of **13** was reductively methylated with formaldehyde in an aqueous mixture of formic acid at elevated temperatures to give the tertiary amine **14**. Suzuki coupling with **15** provided the fully elaborated spiropyrrolidine **18c** (see Table 1 for structure). The analogous spirotetrahydrofuran was prepared starting from the same isatin (Scheme 3). Tin (IV) chloride promoted the formal dipolar cycloaddition of allyltrimethylsilane to the ketone in VU0119498 giving the spirocycle **16** in a single step and in good yield (60%).¹² Heating **16** with TBAF in THF yielded the unadorned spirotetrahydrofuran **17**, which was efficiently converted to the desired biaryl **18b** under our standard Suzuki coupling conditions.

Table 1 presents a survey of spirocycles and their functional PAM activity at hM_1 . Compounds **18a** and **18f** showed an interesting divergence in the SAR between these spirocycles and our earlier series of non-spiro isatin replacement analogs in that deletion of the amide carbonyl (**18a**) or deletion of the phenyl ring (**18f**) did not abolish PAM activity, as was previously observed.¹⁰ Although an EC_{50} could not be determined for these two compounds, a measurable positive allosteric modulation of ACh's activity was observed ($\text{ACh}_{\text{max}} > 20\%$) and presumably indicates beneficial interactions between the receptor and portions of the new spirocycle ring.¹³ Relative to **3**, the tetrahydrofuran **18b** and *N*-methyl pyrrolidine **18c** lost potency but retained a measurable level of hM_1 PAM efficacy. This loss in potency is most likely not related to the absence of a fluorine on the phenyl ring (vide infra, compare **19d** with **20i** from Tables 2 and 3, respectively). The isomeric *N*-methyl spiropyrrolidine **18d** displayed measurable potency with a hM_1 EC_{50} of 3.2 μM . However, the desmethyl analog **18e** lacked any PAM activity at concentrations up to 30 μM . While the *N*-methyl spiropiperidine **18g** was about twofold less potent than the lead spirocycle **3**, for this structure, removal of the *N*-methyl group did not abolish all the PAM activity for **18h**.

Because compound **18d** showed measurable potency, with the potential for a twofold potency increase upon chiral resolution, and was readily available for rapid analog preparation, this spiropyrrolidine was chosen for the additional modifications shown in Table 2. HPLC chiral column chromatography resolution of **18d** provided the individual enantiomers (>95% ee) **19a** and **19b** which surprisingly shared the identical potency and only slightly different efficacy, favoring enantiomer **19a**. A series of *N*-cycloalkyl groups were explored (**19c–f**), showing the cyclopentyl ring to be the preferred size, which exhibited an equivalent potency to the *N*-methyl version (**18d**). The increase in alkyl group size from R = Me to R = *c*-pentyl may help to explain why there was now an enantioselective preference for one enantiomer (**19g**) over the other (**19h**), both with unknown absolute configurations. Given the range of activities seen with the various cycloalkyl groups, it was surprising to see such a large decline in activity upon going to the isopropyl analog **19i**. The *N*-ethyl analog **19j** shared the same potency as the *N*-methyl analog, but as fluorines were introduced onto the ethyl terminus, potency decreased (**19k–m**).

Table 1

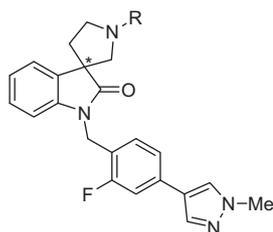
Structures and activities of analogs **3**, **18a–h** showing spirocyclic alternatives to the isatin core

Compd	X	hM_1 EC_{50}^a (μM)	$\text{ACh}_{\text{max}}^{a,b}$ (%)	pEC_{50}^a
3	F	2.0	62 ± 2	5.70 ± 0.11
18a	F	>10	43 ± 2	
18b	H	>10	43 ± 3	
18c	H	>10	53 ± 4	
18d	F	3.2	65 ± 3	5.49 ± 0.10
18e	F	–		
18f	F	>10	28 ± 3	
18g	F	3.8	49 ± 4	5.42 ± 0.06
18h	F	>10	29 ± 2	

^a Values represent the mean ± standard error of the means of at least three independent determinations performed in triplicate. '–' Indicates an inactive compound showing no PAM activity up to the highest concentration tested (30 μM).

^b When hM_1 EC_{50} is reported as >10, this value represents the percent maximal ACh response when the test compound was present at its highest concentration (30 μM).

Whether this trend in potency resulted from decreasingly basic pyrrolidine nitrogens, an increase in steric size, or a combination of both factors was not rigorously explored. Carbonate, acyl and sulfonyl groups were also attached to this nitrogen (**19n–r**) but resulted only in compounds with less functional activity than the parent analog **18d**. Interestingly, the *N*-phenyl pyrrolidine **19s** was prepared and found to be inactive at the hM_1 receptor, whereas the 2-pyrimidine analog **19t** produced low micro-molar activity. Table 2 serves to illustrate the type of shallow SAR frequently observed with allosteric ligands, where even minor changes often result in few gains, and more frequently, significant losses in functional activity.

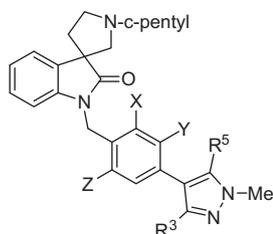
Table 2
Structures and activities of spiro pyrrolidine analogs **19a–t**

Compd	* ^a		hM ₁ EC ₅₀ ^b (μM)	ACh _{max} ^{b,c} (%)	pEC ₅₀ ^b
19a	A	Me	2.9	67 ± 3	5.54 ± 0.06
19b	B	Me	2.9	50 ± 3	5.54 ± 0.07
19c	±	<i>c</i> -Butyl	>10	48 ± 3	
19d	±	<i>c</i> -Pentyl	3.1	57 ± 3	5.51 ± 0.02
19e	±	<i>c</i> -Hexyl	5.2	60 ± 2	5.29 ± 0.06
19f	±	<i>c</i> -Heptyl	7.5	50 ± 3	5.13 ± 0.05
19g	A	<i>c</i> -Pentyl	1.6	69 ± 1	5.80 ± 0.03
19h	B	<i>c</i> -Pentyl	>10	33 ± 3	
19i	±	<i>i</i> -Pr	>10	30 ± 2	
19j	±	Et	3.2	59 ± 2	5.49 ± 0.03
19k	±	CH ₂ CH ₂ F	4.1	60 ± 3	5.39 ± 0.02
19l	±	CH ₂ CHF ₂	>10	52 ± 3	
19m	±	CH ₂ CF ₃	—		
19n	±	Boc	—		
19o	±	Ac	>10	41 ± 2	
19p	±	(C=O) <i>c</i> -butyl	6.3	45 ± 3	5.20 ± 0.07
19q	±	(C=O) <i>c</i> -hexyl	5.0	50 ± 4	5.30 ± 0.05
19r	±	SO ₂ Me	>10	41 ± 1	
19s	±	Ph	—		
19t	±	2-Pyrimidyl	2.5	64 ± 1	5.61 ± 0.07

^a Absolute stereochemistry is unknown, but each resolved enantiomer displayed an ee of >95% as determined by analytical chiral HPLC.

^b Values represent the mean ± standard error of the means of at least three independent determinations performed in triplicate. '—' Indicates an inactive compound showing no PAM activity up to the highest concentration tested (30 μM).

^c When hM₁ EC₅₀ is reported as >10, this value represents the percent maximal ACh response when the test compound was present at its highest concentration (30 μM).

Table 3
Structure and activities of analogs **20a–i**

Compd	X	Y	Z	R ³	R ⁵	hM ₁ EC ₅₀ ^a (μM)	ACh _{max} ^{a,b} (%)	pEC ₅₀ ^a
20a	F	H	H	Me	H	3.0	41 ± 4	5.52 ± 0.02
20b	F	H	H	CF ₃	H	—		
20c	F	H	H	H	Me	—		
20d	F	H	H	Me	—	—		
20e	H	F	H	H	H	2.0	28 ± 3	5.70 ± 0.09
20f	F	H	F	H	H	2.6	66 ± 2	5.58 ± 0.07
20g	F	F	H	H	H	>10	43 ± 2	
20h	H	F	F	H	H	4.1	51 ± 3	5.39 ± 0.05
20i	H	H	H	H	H	1.8	44 ± 4	5.74 ± 0.05

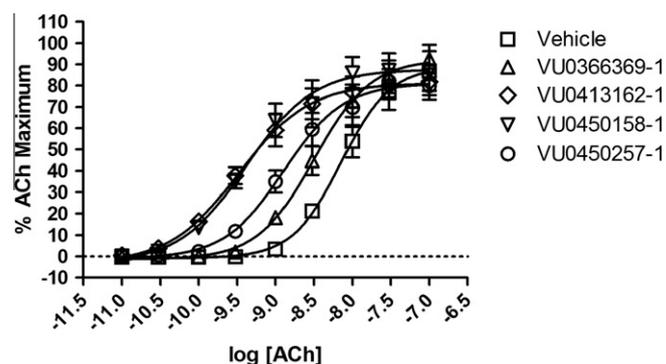
^a Values represent the mean ± standard error of the means of at least three independent determinations performed in triplicate. '—' Indicates an inactive compound showing no PAM activity up to the highest concentration tested (30 μM).

^b When hM₁ EC₅₀ is reported as >10, this value represents the percent maximal ACh response when the test compound was present at its highest concentration (30 μM).

Table 4
ACh fold-shifts for selected hM₁ PAMs

Compd	hM ₁ ACh fold-shift ^a
ML137	2.7 ± 0.2
3	27 ± 3
19a	21 ± 2
19b	7.2 ± 0.2
19g	28 ± 2
19j	8.3 ± 0.8
19k	13.5 ± 0.7
19l	5.9 ± 0.5
19t	17.4 ± 0.9

^a Leftward shift of an ACh CRC in the presence of 30 μM compound relative to the ACh CRC control. Values represent the mean ± standard error of the means of at least three independent determinations.

**Figure 2.** Fold-shift CRCs for the hM₁ PAMs: ML137 (VU0366369-1), **3** (VU0413162-1), **19a** (VU0450158-1), **19b** (VU0450257-1).

Having explored the spirocycle region of these new M₁ PAMs, we decided to re-examine the biaryl region in the context of the *N*-cyclopentyl spiro pyrrolidine **19d**. The results appearing in Table 3 indicated that the 3-position of the pyrazole could be substituted with a methyl group, leaving PAM activity virtually unaffected (**20a**). However, if a larger and more electron withdrawing trifluoromethyl group was introduced at the same location, all PAM activity was absent. Methyl substitution at the 5-position eliminated PAM activity (**20c**) and simultaneous methylations at both the 3- and 5-positions provided an inactive compound (**20d**). Although not shown in Table 3, compounds not methylated on the *N*-1 position of the pyrazole were devoid of PAM activity. Fluorination patterns on the central ring provided relatively flat SAR. The slightly preferred location for a single fluorine atom was that found in **20e**. Of the difluorides tested, compound **20f** was equipotent with the best monofluoride, while the other difluorides brought significantly reduced activity. Unexpectedly, compound **20i** demonstrated that an absence of fluorination on the central phenyl was potentially superior with respect to potency. This fluorine SAR is very different from the SAR elucidated during the development of ML137,^{7b} and exemplifies the potential pitfalls associated with trying to transfer established SAR from one series into even a closely related PAM series.

Even after a considerable amount of effort was invested to explore a great deal of structural diversity, no improvements in potency were forthcoming relative to the lead compound ML137. However, as shown in Table 4, encouraging improvements in efficacy were realized in the arena of hM₁ ACh fold-shift values. Our initial foray into spirocycles, ketal **3**, demonstrated an order of magnitude increase in its ability to shift the ACh concentration-response relative to ML137. Perhaps the most interesting pair of compounds in Table 4 was the pair of enantiomers **19a** and **19b**.

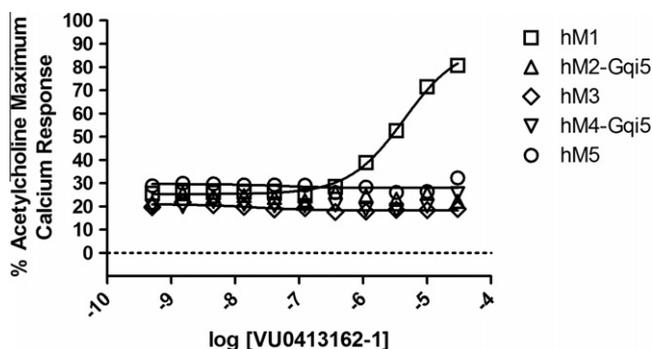


Figure 3. Muscarinic selectivity for **3** (VU0413162-1).

Although both compounds share the same potency of 2.9 μM (hM_1 EC_{50}), the enantiomer with a slightly better efficacy (**19a**: $\text{ACh}_{\text{max}} = 67\%$ versus **19b**: $\text{ACh}_{\text{max}} = 50\%$) is roughly 3-times better at enhancing the potency of ACh at the hM_1 receptor. This can be seen graphically in Figure 2 by comparing **19a** (open inverted triangles) and **19b** (open circles). Returning to Table 4, in the context of the *N*-ethyl spiropyrrolidines **19j** and **19k**, the potency trend relative to the number of fluorines present was not strictly recapitulated in their abilities to produce an ACh fold-shift. For example, although **19j** was slightly more potent than **19k**, with both sharing the same ACh_{max} , **19k** elicited a higher fold-shift of the full ACh concentration-response curve (CRC). For the final compound in Table 4, **19t**, it was encouraging to see that such a structurally diverse analog could also show such robust efficacy.

To round out the characterization of these novel spirocyclic M_1 PAMs, we profiled **3** and **18d** across all five human and all five rat muscarinic acetylcholine receptors. Up to the highest concentrations tested (30 μM) both compounds were completely selective for the M_1 receptors. A representative example of these results is shown in Figure 3 for compound **3** (VU0413162-1) against the human receptors.

In summary, a number of isatin replacements containing various spirocycles have been explored and these novel structures have been confirmed as M_1 PAMs. Although flat SAR was observed with respect to potency, significant improvements in efficacy were achieved for a range of structures. Very high receptor subtype selectivity for **3** and **18d** was observed across the spectrum of muscarinic subtypes (M_2 through M_5). Further optimization within any of these scaffolds holds great promise for the development of highly active and selective M_1 PAMs and will be reported in due course.

Acknowledgments

The authors thank the NIH (U54MH084659), NIMH (1R01MH082867) and William K. Warren, Jr., who funded the

William K. Warren, Jr. Chair in Medicine (to CWL), for support of our M_1 program. Vanderbilt University is a Specialized Chemistry Center within the MLPCN, and all ML# probes are freely available upon request.¹⁴

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- Although the M_1 PAM EC_{50} has been previously reported as 0.83 μM with a $\% \text{ACh}_{\text{max}} = 65\%$ (Ref. 7b), the inherent variability associated with functional assays supports that the most recent $\text{EC}_{50} = 0.60 \mu\text{M}$ for ML137 is equally valid and can serve as a baseline comparator between the two papers.
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- It is worth noting that intermediates **13**, **14**, **16** and **17** (Schemes 2 and 3), which bear a close resemblance to the $\text{M}_{1,3,5}$ PAM VU0119498, were found to be inactive at hM_1 .
- For information on the MLPCN and information on how to request any of the ML# probe compounds, such as ML137, see: <http://mli.nih.gov/mli/mlpcn/>.