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Isatin replacements applied to the highly selective, muscarinic M₁ PAM ML137: Continued optimization of an MLPCN probe molecule

Bruce J. Melancon^{a,c,d}, Michael S. Poslusney^{a,c,d}, Patrick R. Gentry^{a,d}, James C. Tarr^{a,c,d}, Douglas J. Sheffler^{a,c}, Margrith E. Mattmann^{a,c}, Thomas M. Bridges^{a,c,d}, Thomas J. Utley^{a,c,d}, J. Scott Daniels^{a,c,d}, Colleen M. Niswender^{a,c,d}, P. Jeffrey Conn^{a,c,d}, Craig W. Lindsley^{a,b,c,d}, and Michael R. Wood^{a,b,c,d,*}

^aDepartment of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

^bDepartment of Chemistry, Vanderbilt University, Nashville, TN 37232, USA

^cVanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University Medical Center, Nashville, TN 37232, USA

^dVanderbilt Specialized Chemistry Center for Probe Development (MLPCN), Nashville, TN 37232, USA

Abstract

This Letter describes the continued optimization of an MLPCN probe molecule (ML137) with a focused effort on the replacement/modification of the isatin moiety present in this highly selective M₁ PAM. A diverse range of structures were validated as viable replacements for the isatin, many of which engendered sizeable improvements in their ability to enhance the potency and efficacy of acetylcholine when compared to ML137. Muscarinic receptor subtype selectivity for the M₁ receptor was also maintained.

Keywords

Muscarinic acetylcholine receptor 1; M₁; Allosteric; Positive allosteric modulator (PAM); ML137; VU0448350

Acetylcholine (ACh) is a critical neurotransmitter influencing a broad range of physiological processes both inside and outside of the central nervous system by binding to both nicotinic (nAChR) and muscarinic acetylcholine receptors (mAChR). The challenges associated with developing highly selective mAChR ligands interacting at the orthosteric binding site of ACh are most significantly impacted by the high level of receptor homology displayed across the five mAChR subtypes (M₁ through M₅).¹ Despite this seemingly insurmountable roadblock towards the production of truly selective muscarinic agonists, a great deal of information has been learned from M₁₋₅ KO mice² and the testing of less-than-selective muscarinic agonists in preclinical and clinical experiments. The clinical promise for dual, or

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*To whom correspondence should be addressed: michael.r.wood@vanderbilt.edu.

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potentially individual, M₁/M₄ mAChR activators in treating aspects of both Alzheimer's disease and schizophrenia has been demonstrated with the orthosteric agonist xanomeline;^{3,4} however, this clinical candidate's limited selectivity (particularly against M₃) ultimately caused its withdrawal from further clinical trials irrespective of the encouraging positive outcomes. A new and exciting chapter in the search for selective/specific mAChR activators commenced with the identification of novel positive allosteric modulators (PAMs) and allosteric agonists through the use of functional high throughput screening (HTS) assays.⁵ These next-generation ligands have the novel benefit of interacting at sites removed from the orthosteric binding site, where the potential for selective interactions with just one of the five mAChR is greatly enhanced. This revitalized interest in selectively activating the mAChRs has been very successful, providing a number of M₁ PAMs (BQCA,⁶ ML137,⁷ and ML169⁸), M₄ PAMs (ML108⁹, ML173,¹⁰ ML253,¹¹ and ML293¹²) and M₅ PAMs (ML129¹³, and ML172¹⁴). Here we describe the continued structural exploration around the M₁ PAM, MLPCN probe molecule ML137.

Figure 1 shows the development of two M₅ PAMs and one M₁ PAM from a common HTS lead, VU0119498, all of which share the isatin core in common. Although VU0119498 displayed PAM activity at the M₁, M₃, and M₅ mAChRs,¹³ specific structural modifications resulted in highly preferring (ML129) or completely selective (ML172) M₅ PAMs and a highly selective M₁ PAM (ML137). The ability to dial in muscarinic receptor subtype selectivity was facilitated by the wide variety of commercially available substituted isatins and the efficiency of analog preparation, clearly demonstrating that this scaffold can be very advantageous. However, it was also appreciated that the isatin motif has an established history of appearing in a wide range of medicinal chemistry targets across the full spectrum of potential clinical indications.¹⁵ Additionally, HTS hits containing the isatin core are so frequent that the isatin scaffold has earned a position of notoriety in some measures of HTS promiscuity.¹⁶ As such, it seemed reasonable to explore replacements, or modifications, to the isatin core in the context of ML129, ML137, and ML172.

Our first efforts focused on a large and extensive library which thoroughly and rapidly explored small modifications to the isatin core employing commercially available benzyl, biphenyl and biaryl ethers similar to those found in ML129 and ML172. As is often the case with steep allosteric SAR,⁵ this effort proved unfruitful (data and structures not shown). We then hypothesized that a more focused effort directed solely at replacing the isatin moiety, while retaining the exact biaryl from ML137, could be more productive. These analogs and the requisite phenylpyrazoles were prepared according to the general synthesis route appearing in Scheme 1.

Readily available 4-bromobenzyl alcohols **1** undergo Suzuki reactions with pyrazole boronic acids/esters **2** employing standard conditions and provided the biaryl benzyl alcohols **3**. Ghosez' reagent (1-chloro-*N,N*,2-trimethylprop-1-en-1-amine) in DCM smoothly converted the benzylic alcohol into the corresponding benzyl chloride **4**. With ample quantities of **4** in place, isatin or a variety of isatin replacements could be alkylated, employing a range of bases, to give analogs **5** and the majority of structures appearing in Figure 2 and Table 1. Alternative routes towards the preparation of these initial analogs can be seen in Schemes 2 and 3.

Scheme 2 depicts the alkylation of isatin with a 4-bromobenzyl bromide **6** using a carbonate base in acetonitrile to give *N*-benzylated isatins **7**. Next, a trifluoromethyl group was introduced at the ketone carbon employing the conditions of Shreeve, et al.,¹⁷ to yield the aryl bromides **8**. A Suzuki reaction analogous to the first step in Scheme 1 could then provide analogs **9**, as well as a diverse range of alternative biaryls, simply by changing the boronate in the final step.

Scheme 3 shows the route used in the preparation of sultam **13** (See Figure 2 for structure), which was necessary due to a lack of reactivity and selectivity (N versus C benzylation) associated with the desired parent sultam. Starting from commercially available benzylic amine **11** and sulfonyl chloride **10**, the linear sulfonamide **12** was prepared in 93% yield. The nitrogen-aryl bond was then formed using Cu(I) catalysis in degassed toluene to give the desired sultam **13**, in 59% yield.

Figure 2 shows the functional activity for two active analogs along with a host of unsuccessful isatin replacements (inactive at human M₁ (hM₁) up to a concentration of 30 μ M). Although not as potent (EC₅₀) as their parent ML137 (hM₁ EC₅₀ = 0.60 μ M, ACh_{max} = 45 $\pm$ 3 %, pEC₅₀ = 6.22 $\pm$ 0.02),¹⁸ both **13** and **14** produced comparable efficacy (% ACh_{max}) at the highest concentration tested of 30 μ M and, more importantly, no longer contained the isatin core. Indolinone **14** appeared to be more promising than sultam **13** from activity and synthesis standpoints and so was explored further. While reduction of the isatin ketone was gratifyingly tolerated, attachment of the biaryl at the resultant methylene (as in **15**) or further reduction of the amide carbonyl to the indoline **16** was not tolerated. The overall planarity of the indolinone core appeared to be important for PAM activity since scission of the 5-membered lactam to give the *N*-acetyl amide **17** provided an inactive compound. However, annulation of a second aryl ring, as in **18**, proved detrimental to activity as well, despite maintaining a planar system. The remaining analogs in Figure 2 (**19–22**) attempted to mimic various heteroatom interactions potentially occurring with ML137 or **14**, but all retained no hM₁ PAM activity.

While full reduction of the isatin ketone to arrive at **14** appeared to be the most tolerated modification so far, the decrease in potency suggested that the introduction of various substituents to this methylene might restore some of the lost activity. Numerous small modifications were made to **14** based on the chemistries appearing in Schemes 1 and 2, as well as through straightforward derivatization of ML137 to provide the compounds appearing in Table 1. The introduction of a methyl group onto the indolinone methylene diminished hM₁ PAM activity for **23a**. However, the introduction of an amino group at the same location, increased the potency of **23b** 2-fold over **14**. Unfortunately, this primary amino group instilled an undesirable level of chemical instability, presumed to be air oxidation or self-condensation based on the brightly colored byproducts observed. Attempts to block this degradation with methyl groups provided only weakly active PAMs (**23c** and **23d**). Replacing the amino group with a hydroxyl group provided the stable compound **23e**, now with improved efficacy relative to **23c**. Fluorination of the methyl group in **23e** was beneficial, providing racemic **23g** which displayed a measurable EC₅₀ of 4.8 μ M. Although not routinely performed during these early stages of structure-activity relationship (SAR) development, the enantiomers of **23g** were resolved employing HPLC chiral column chromatography to demonstrate that all the hM₁ PAM activity resided in a single enantiomer of unknown absolute configuration (compare **23h** and **23i**).

With **23g** showing promise as another viable replacement for the isatin core, we explored alternatives to the *N*-methyl pyrazole. Although a wide range of heterocycles could be efficiently introduced at the terminal location of the biaryl utilizing the chemistry in Scheme 2, none was found to be superior to the pyrazole (data and structures not shown for about 30 diverse alternative aryl groups). Only the most minor of modifications was tolerated in that the *N*-ethyl pyrazole **23j** was found to be of comparable potency, but with reduced efficacy. Alkyl groups larger than ethyl (**23k** and **23l**), or the non-alkylated pyrazole **23m** resulted in a complete loss of activity. Additional modifications to **23g** appeared detrimental towards hM₁ PAM activity (**23n–q**) with the exception of deleting the fluorine on the central phenyl ring providing **23r**, a compound of similar potency when compared to **23g**.

Unfortunately, none of the analogs appearing in Table 1 dramatically surpassed the potency of **14**. However, the relative simplicity of **14** inspired us to explore more benzo-fused bicycles which did not contain a 5-membered ring as the point of attachment to the biaryl southern region. In direct analogy to **14**, expansion of the 5-membered lactam to a 6- or 7-membered version caused a drop in activity (Table 2, **24a** and **24b**). Although **24a** retained a measurable hM₁ EC₅₀ of 5.6 μ M, its unsaturated quinolinone analog **24c** possessed a hM₁ EC₅₀ > 10 μ M, but with slightly improved efficacy. Deletion of the phenyl group from **24c** to arrive at pyridone **24d** resulted in a loss of all PAM activity, indicating the importance of this benzo-fused motif. Placing an oxygen adjacent to the aryl ring (**24e**) resulted in a compound of similar potency to **24a**, while the carbamate and urea analogs **24f** and **24g**, respectively, maintained reasonable hM₁ PAM efficacy but with EC₅₀s greater than 10 μ M. The thio version of **24e**, compound **24h**, appears to have improved potency relative to **24e**; however, these two potency values are not statistically different as seen by comparing their experimentally determined pEC₅₀ values. Oxidized versions of **24h** and its carbonyl-reduced analog **24k** all showed moderately reduced activity (compounds **24i** and **24j**) or no activity at all (compound **24k**). This complete lack of activity for **24k** was not a surprise given the results obtained for **16**, and served to reiterate the importance of this carbonyl. This survey of expanded-ring analogs in Table 2 barely scratches the surface of possible structures and a more focused optimization of any slightly active structure could prove highly productive.

The utility of selective M₁ PAMs as a therapeutic agent has not been explored to the extent where it is definitively known whether a high efficacy (high fold shift of an ACh concentration-response) PAM or a low efficacy PAM is preferable under the various disease states for which they might provide clinical efficacy. However, ML137, with an ability to shift the ACh concentration-response by just 2.7, rests firmly in the latter category. Therefore, it was desirable to characterize tool compounds spanning a wide range of fold-shift values. Although none of the hM₁ PAM potencies presented here rivaled that of the lead compound ML137, improvements with respect to efficacy were being realized and likely contributed to the improved ACh fold-shift values appearing in Table 3. Reduction of the isatin carbonyl (ML137 to **14**) caused a 4-fold decrease in hM₁ PAM EC₅₀, but at the same time resulted in a 10-fold improvement in ACh fold-shift. As a group, the novel compounds appearing in Table 3 all shared a reduced potency relative to ML137, while at the same time, displayed improved fold-shifts ranging from 6.5 to 27.2. A graphical representation of four hM₁ PAMs, and the vehicle control for ACh, as part of a fold-shift experiment is shown in Figure 3. Compound **14** (VU0448350-1), with the highest fold-shift, shifted the ACh concentration-response curve (CRC) furthest to the left, indicating its superior ability to enhance the activity of ACh among this set of compounds. By way of comparison, ML137 (VU0366369-1), which displays an order of magnitude lower fold-shift value relative to **14**, produces the first concentration-response curve just to the left (open triangles) of the vehicle control (ACh alone, open squares).

Seemingly minor modifications to allosteric ligands can result in large changes to both potency and receptor subtype selectivity (off-target activity in general) and this observation has been embodied within the concept of the 'molecular switch'.^{5, 19} With this idea in mind, we chose to profile the highest efficacy compound (**14**) across all five of the human muscarinic receptor subtypes; encouragingly, we found little-to-no erosion of muscarinic subtype selectivity (VU0448350-1, Figure 4). Although it would appear from the graph that **14** displays very weak hM₄ PAM activity, examination of the raw calcium traces from the functional assay indicated this was most likely an artifact resulting from an unexplained baseline increase at the two highest concentrations tested (data not shown). Fundamentally, the identification of a selective M₁/M₄ PAM would be a significant achievement in light of the positive clinical outcomes observed with xanomeline;^{3,4} unfortunately, compound **14** does not represent a step along that path.

In summary, a number of bicyclic benzo-fused isatin replacements have been identified which retain reasonable hM₁ PAM activity and may represent novel starting points for continued optimization. Compound **14**, which showed the largest improvement in efficacy compared to ML137, was also demonstrated to be very selective for the hM₁ receptor over the other muscarinic receptor subtypes. The continued optimization of ML137 along alternative lines will be reported shortly.

Acknowledgments

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Figure 1.
HTS lead VU0119498 parlayed into selective *M₁* and *M₅* PAMs.

Figure 2.
Rapid foray into isatin replacements while holding the benzyl pyrazole constant.

Figure 3.
Fold-shift CRCs for the hM₁ PAMs: ML137 (VU0366369-1), **14** (VU0448350-1), **23g** (VU0422337-1), and **23r** (VU0449055-1).

Figure 4.
Muscarinic selectivity for **14** (VU0448350-1).

Scheme 1.

Reagents and conditions: (a) $\text{PdCl}_2(\text{dppf}) \cdot \text{DCM}$, Cs_2CO_3 , THF/ H_2O , 160 °C, 10 min; (b) Ghosez' reagent (1-chloro-N,N,2-trimethylprop-1-en-1-amine), DCM, rt, 15 h; (c) base (i.e. K_2CO_3 , NaH, etc.), aprotic solvent (i.e. DCM, DMF, MeCN), 0 °C to rt.

Scheme 2.

Reagents and conditions: (a) K_2CO_3 , MeCN, rt, overnight; (b) TMS- CF_3 , CsF, THF, rt, overnight; (c) **2**, $PdCl_2(dppf) \cdot DCM$, Cs_2CO_3 , THF/ H_2O , 160 °C, 10 min.

Scheme 3.

Reagents and conditions: (a) NEt_3 , DCM, 30 min., 93% yield; (b) $\text{CuI}(\text{cat.})$, K_3PO_4 , DMEDA (N, N'-dimethylethylene-diamine), toluene, degassed with argon (10 minutes), sealed, 110 °C for 3 h, 59% yield.

Table 1

Structure and activities of analogs **23a–r**.

Compd	^a	R ¹	R ²	X	R ³	hM ₁ EC ₅₀ (μM) ^b	ACh _{max} (% ^b) ^c	pEC ₅₀ ^b
23a	±	Me	H	F	Me	>10	53 ± 2	
23b	±	NH ₂	H	F	Me	1.3	50 ± 3	5.90 ± 0.04
23c	±	NH ₂	Me	F	Me	>10	28 ± 2	
23d	±	NMe ₂	Me	H	Me	>10	48 ± 2	
23e	±	OH	Me	F	Me	>10	49 ± 3	
23f	±	OH	CH ₂ F	F	Me	>10	59 ± 2	
23g	±	OH	CF ₃	F	Me	4.8	51 ± 3	5.32 ± 0.07
23h	A	OH	CF ₃	F	Me	3.3	57 ± 1	5.48 ± 0.07
23i	B	OH	CF ₃	F	Me	-		
23j	±	OH	CF ₃	F	Et	3.3	37 ± 2	5.48 ± 0.07
23k	±	OH	CF ₃	F	<i>n</i> -Pr	-		
23l	±	OH	CF ₃	F	<i>i</i> -Pr	-		
23m	±	OH	CF ₃	F	H	-		
23n	±	OMe	CF ₃	F	Me	-

Compd	^a	R ¹	R ²	X	R ³	hM ₁ EC ₅₀ (μM) ^b	ACh _{max} (%) ^{b,c}	pEC ₅₀ ^b
23o	±	F	CF ₃	F	Me	>10	25 ± 2	
23p	±	F	F	F	Me	-		
23q	±	OH	CF ₃	Cl	Me	>10	29 ± 2	
23r	±	OH	CF ₃	H	Me	4.0	66 ± 2	5.40 ± 0.05

^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 2Structures and activities of analogs **24a–k** attached to the preferred biaryl.

Compd	hM ₁ EC ₅₀ (μM) ^a	ACh _{max} (%) ^{a,b}	pEC ₅₀ ^a
24a	5.6	44 ± 3	5.25 ± 0.07
24b	-		
24c	>10	51 ± 3	
24d	-		
24e	5.2	52 ± 3	5.29 ± 0.09
24f	>10	62 ± 2

	Compd	hM ₁ EC ₅₀ (μM) ^a	ACh _{max} (%) ^{a,b}	pEC ₅₀ ^a
24g		>10	53 ± 2	
24h		3.2	30 ± 2	5.49 ± 0.12
24i		>10	46 ± 3	
24j		>10	36 ± 2	
24k		-		

^aValues 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).

^bWhen 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 3ACh fold-shifts for selected hM₁ PAMs.

Compd	hM ₁ ACh fold-shift ^a
ML137	2.7 ± 0.2
14	27.2 ± 0.6
23g	7.4 ± 0.6
23h	12 ± 2
23e	6.5 ± 0.3
23f	8.0 ± 0.3
23r	15.2 ± 0.5

^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.