Published in final edited form as:
Bioorg Med Chem Lett. 2013 January 1; 23(1): 223–227. doi:10.1016/j.bmcl.2012.10.132.

Further exploration of M_1 allosteric agonists: Subtle structural changes abolish M_1 allosteric agonism and result in *pan*-mAChR orthosteric antagonism

Douglas J. Sheffler[a,b,c,^], **Christian Sevel**[a,^], **Uyen Le**[a,b,c], **Kimberly M. Lovell**[a,b,c], **James C. Tarr**[b,c], **Sheridan J. S. Carrington**[a,b], **Hyekyung P. Cho**[b,c], **Gregory J. Digby**[a], **Colleen M. Niswender**[a,b,c], **P. Jeffrey Conn**[a,b,c], **Corey R. Hopkins**[a,b,c,d], **Michael R. Wood**[a,b,c,d], and **Craig W. Lindsley**[a,b,c,d,*]

[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

[d]Department of Chemistry, Vanderbilt University, Nashville, TN 37232, USA

Abstract

This letter describes the further exploration of two series of M_1allosteric agonists, TBPB and VU0357017, previously reported from our lab. Within the TPBP scaffold, either electronic or steric perturbations to the central piperidine ring led to a loss of selective M_1 allosteric agonism and afforded *pan*-mAChR antagonism, which was demonstrated to be mediated via the orthosteric site. Additional SAR around a related M_1 allosteric agonist family (VU0357017) identified similar, subtle 'molecular switches' that modulated modes of pharmacology from allosteric agonism to *pan*-mAChR orthosteric antagonism. Therefore, all of these ligands are best classified as bi-topic ligands that possess high affinity binding at an allosteric site to engender selective M_1 activation, but all bind, at higher concentrations, to the orthosteric ACh site, leading to non-selective orthosteric site binding and mAChR antagonism.

Keywords

TBPB; M1; Allosteric agonist; Muscarinic receptor

Schizophrenia is a complex psychiatric disorder characterized by a combination of positive and negative symptoms along with significant cognitive dysfunction.[1,2] Current antipsychotic therapies can address the positive symptoms, but the negative and cognitive symptoms remain poorly managed, if at all, and are key predictors of functional disability.[3–6] A large number of anatomical, molecular, genetic, preclinical behavioral and

[*]To whom correspondence should be addressed: craig.lindsley@vanderbilt.edu.
[^]These authors contributed equally to this work

human clinical studies have provided strong evidence that agents able to enhance cholinergic transmission or activate muscarinic acetylcholine receptors (mAChRs, M_1-M_5), notably M_1, have exciting therapeutic potential for the treatment of the positive, negative and cognitive symptoms of schizophrenia as well as cognitive dysfunction in other CNS disorders.[7–16] However, previous compounds developed to selectively activate M_1 receptors have failed in clinical development due to a lack of true specificity for this receptor subtype.[7–18] Often, many of the compounds bind to the orthosteric ACh binding site, which can result in adverse side effects as a result of M_2-M_5 activation.[7–18] Recently, multiple industrial and academic laboratories, including ours, have targeted less conserved allosteric sites on the M_1 receptor in an attempt to develop highly selective M_1 activators (both M_1 allosteric agonist and M_1 positive allosteric modulators, PAMs) and avoid activation of M_2-M_5.[17–39]

For example, we have previously reported on TBPB **1**, a potent, CNS penetrant and highly selective M_1 allosteric agonist that displays robust efficacy in multiple preclinical antipsychotic and cognition models, as well as significant impact on Aβ production.[19] Mutagenesis and modeling efforts identified a key H-bond interaction between the central piperidine nitrogen of TBPB and Thr83 of M_1, likely contributing to TBPB's affinity for this M_1 allosteric binding site.[40] In multiple Letters, we have also described SAR around TBPB **1**, and found that halide substitutions were well tolerated on the benzimidazole core **2**, as well as amide **3**, sulfonamide **4** and urea **5** replacements for the benzyl amine of the distal basic piperidine nitrogen (Fig. 1); however, SAR was 'shallow'.[21] Moreover, these subtle structural modifications could engender D_2 antagonism, along with M_1 allosteric agonism, affording molecules with an attractive pharmacological profile for the treatment of schizophrenia.[21] In this Letter, we describe a more detailed pharmacological profile of **1**, along with the as yet unexplored SAR of the central piperidine ring of **1**, and the discovery of subtle 'molecular switches'[41] that modulate modes of pharmacology from allosteric agonism to *pan*-mAChR orthosteric antagonism.

Our previous characterization of TBPB revealed that this compound activates M_1 receptors.[19] Here, we confirm that TBPB is a selective M_1 partial agonist and induces responses in CHO cells expressing rM_1 receptors, but not rM_2-M_5Rs (Fig. 2A). Interestingly, TBPB also inhibits ACh-induced responses in cells expressing M_2-M_5 receptors, suggesting additional activity at an orthosteric site (Fig. 2B). Based on this finding, TBPB can be described as a bi-topic ligand that binds M_1 at both an allosteric site and the orthosteric site, the former of which confers functional M_1 agonism and the latter of which confers orthosteric antagonism. These findings are similar to previously characterized M1 agonists including AC-42,[32,33] 77-LH-28-1,[36] VU0364572,[28,30] and VU0357017[26,30], which have been characterized as bi-topic ligands.[42,43]

As the central piperidine nitrogen of TBPB is thought to be critical (H-bond with Thr83) for allosteric binding,[40] we sought to disrupt this key H-bond both electronically and conformationally by installation of a β-fluorine atom (to attenuate basicity and H-bond acceptor ability) and replacement of the piperidine with a [3.3.0] system, respectively.[21] Our expectation was that these structural modifications would abolish binding at the allosteric site, and that these analogs would bind solely at the orthosteric site and function as *pan*-mAChR antagonists. The synthesis was straightforward (Scheme 1), following the synthetic route previously developed.[22]

When screened as an agonist, neither **9** nor **10** elicited M_1 activation, suggesting that binding at the allosteric site had been abolished by disrupting the key H-bond interaction with Thr83.[40] Interestingly, both **9** and **10** proved to be weak, micromolar *pan*-mAChR antagonists when screened in the presence of an EC_{80} of ACh against M_1-M_5. The [3.3.0]

analog **10** was a weak M_1 antagonist (pIC_{50} = 5.20±0.04, IC_{50} = 6.4 μM, −1.07±1.3% ACh Max) and IC_{50}s >10 μM against M_2-M_5). The β-fluoro analog **9** (Fig. 3A) was slightly more potent at M_1 (Rat M_1 IC_{50} = 4.9 μM (pIC_{50} = 5.31±0.17), 13.6±1.5% ACh Max, M_2, M_3 M_4, and M_5 IC_{50} > 10 μM (pIC_{50}s <5) (Human M_1-M_5 data not shown, but similar)). Radioligand binding experiments with [^{3}H]-NMS (Fig. 3B) and dissociation kinetic experiments (Fig. 3C) are consistent with **9** acting as an ACh orthosteric site antagonist. Thus, a single fluorine atom serves as 'molecular switch' to modulate the pK_a of the central piperidine nitrogen atom (from ~11 to 9.4),[44] and likely prohibits its ability to accept a key H-bond from Thr83 in the M_1 allosteric site, an interaction that may confer M_1 functional agonist activity.[40]

Interestingly, our dissociation kinetics experiments with TBPB indicate that this compound also does not alter the off-rate of [^{3}H]-NMS. These data are inconsistent with those of Jacobson et al., 2010, where TBPB was shown to slow the off-rate of [^{3}H]-NMS.[40] The most likely explanation for the discrepancy between our results and those of Jacobson et al., 2010 is a difference in our assay protocols. Jacobson et al. allow 1 hour for equilibrium to occur between atropine and [^{3}H]-NMS at room temperature for their dissociation kinetic studies whereas we allow 3 hours at room temperature for this equilibrium to occur. Traditionally, when a 1 hour equilibrium is utilized for M_1 dissociation studies, the studies are performed at 37°C.[43] Incubation for only 1 hour at room temperature may not allow enough time for a proper equilibrium to be established between atropine and [^{3}H]-NMS and thus Jacobson et al. may have performed their TBPB dissociation studies under non-equilibrium conditions.

While 'molecular switches' that modulate modes of pharmacology and subtype selectivity are common in Family C GPCRs (eg., mGlu receptors),[41] we have rarely encountered them in Family A GPCRs. Within the mAChRs, we had previously only encountered 'molecular switches' that modulate mAChR subtype selectivity.[27,41] We recently reported on a chemically distinct series of M_1 allosteric agonists, represented by **11** and **12** (Fig. 4), where mutagenesis work indicated a novel allosteric binding site in the third extracellular loop of M_1.[26] Serendipitously, when preparing additional analogs around **12**, a chemical vendor sent the incorrect starting material, leading to the synthesis of the alternative regioisomer of **12**, compound **13.13** proved to be a potent antagonist of M_1 (IC_{50} = 273 nM, pIC_{50} = 6.32±0.11), as well as M_2-M_5, and detailed molecular pharmacology showed that **13** was an orthosteric mAChR antagonist. Note, the modulation from M_1 allosteric agonist to *pan*-mAChR orthosteric antagonist was due to major structural modifications (ie., 3° to 2° amine and 2° to 3° amide).

These data, coupled with the results from TBPB and **9**, led us to scrutinize additional analogs of **12**, in an effort to discover more subtle 'molecular switches' that can modulate modes of pharmacology within the VU0364572 bi-topic ligand scaffold. One of our initial efforts led to the development of VU0419757 (**14**) which contains a 3-bromophenyl amide (Fig. 5) and proved to be a weak partial M_1 allosteric agonist due to the addition of a 3-bromophenyl amine (EC_{50} = 2.0 μM, pEC_{50} = 5.69±0.08, 37.4±8.7% ACh Max). Modification with an additional bromine atom to the 5-position afforded VU0430613 (**15**), an equipotent M_1 antagonist (IC_{50} = 3.4 μM, pIC_{50} = 5.47±0.03). Furthermore, when the aryl amides in **12** were replaced with heterocyclic amides, *pan*-mAChR antagonists resulted. Even when fluorine atoms were employed to attenuate the basicity of the heterocycles, M_1 allosteric agonism was lost. For example, a 6-trifluomethoxy nicotinamide derivative, VU0362058 (**16**), proved to be another pan-mAChR antagonist (Fig. 6), favoring inhibition of M_2 (IC_{50} = 1.1 μM, pIC_{50} = 5.97) over M_1 (IC_{50} = 3.2 μM, pIC_{50} = 5.50±0.06) and M_3-M_5 (IC_{50}s >10 μM).

In summary, we have explored additional SAR around two M_1 allosteric agonists TBPB and VU0364572 (**12**). Within the TBPB scaffold, either electronic or steric perturbations to the benzimidazolone piperidine ring led to a loss of selective M_1 allosteric agonism and afforded *pan*-mAChR orthosteric antagonists. Based on mutagenesis and modeling studies with TBPB, the electronic and steric perturbations disrupted a key H-bond with Thr83 in the allosteric site on the M_1 receptor. Similar subtle 'molecular switches' were found within the VU0364572 series, converting potent M_1 agonists into *pan*-mAChR antagonists. These findings, coupled with our recent studies showing brain-region-dependent and receptor reserve-dependent pharmacology of functionally selective M_1 bi-topic agonists[42] show the challenges associated with development of selective M_1 agonists. Therefore, we propose that positive allosteric modulation of M_1 receptors, as opposed to allosteric agonism, may be the preferred mechanism by which to develop selective M_1 activators as therapeutic agents.

Acknowledgments

This work was supported by grants from the NIH and NIMH (1RO1MH082867). Vanderbilt is a Specialized Chemistry Center within the Molecular Libraries Probe Centers Network (U54 MH84659).

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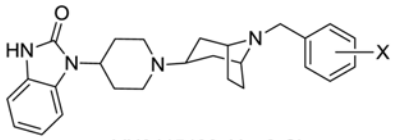
21. In addition to the [3.3.0] system, we also synthesized and evaluated tropane replacements for the piperidine ring, and these also lost M_1 agonism and afforded M_1 antagonism (IC_{50}s 950 nM to 1.6 μM).



VU0415488, X = 2-Cl
VU0415455, X = H

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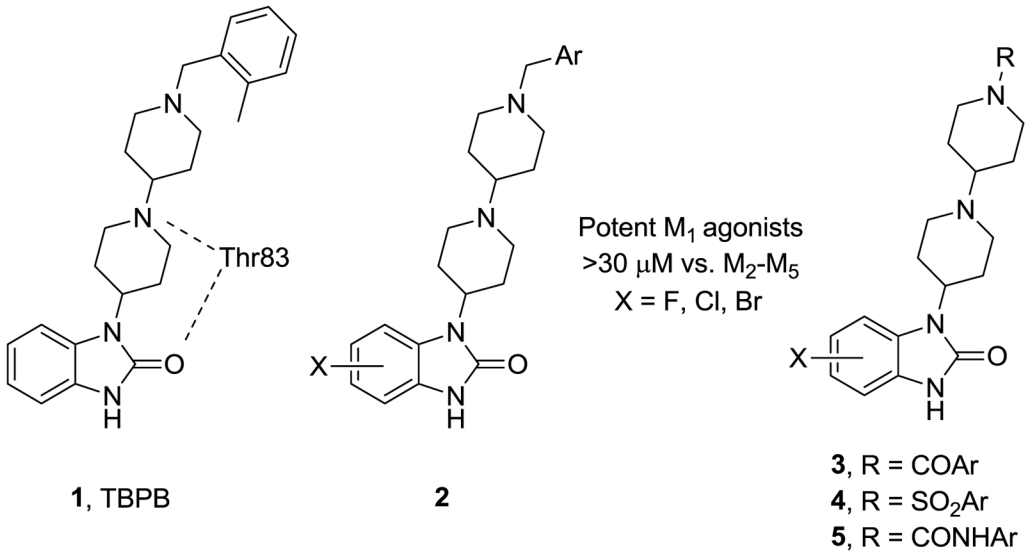


Figure 1.

Structures of the M_1 allosteric agonist TBPB (**1**), highlighting the key H-bond interaction with Thr83 for allosteric binding at M_1, and analogs **2**–**5** of TBPB that retain selective M_1 agonism.

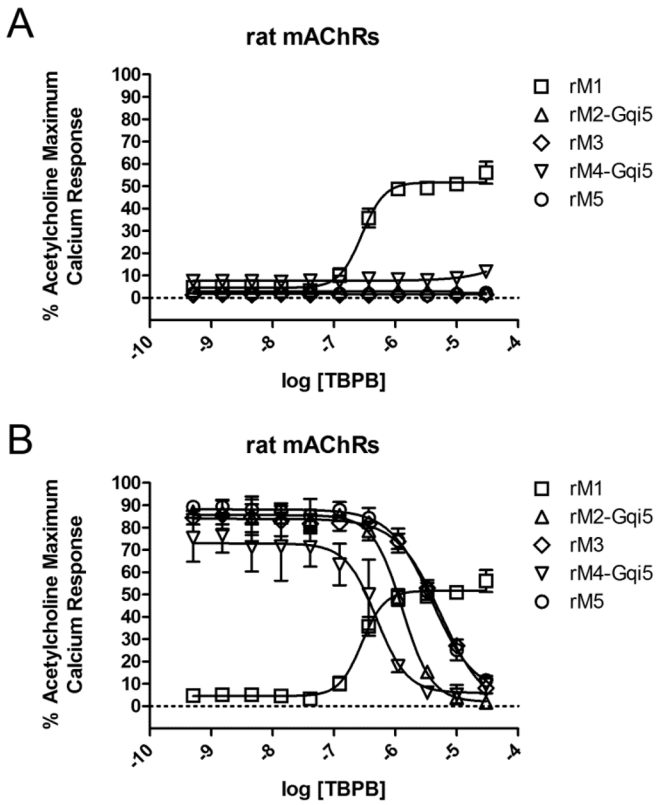


Figure 2.

Pharmacological profile of TBPB. A) rM_1-rM_5 concentration-response curves (CRCs) of TBPB screened in agonist mode. rM_1 EC_{50} = 79.6 nM (pEC_{50} = 7.10±0.07), 69.3±6.4% ACh Max, rM_2-rM_5 >30 μM B) rM_2-rM_5 CRCs of TBPB screened as antagonists. rM_2 IC_{50} = 1.29 μM (pIC_{50} = 5.89), rM_3 IC_{50} = 5.48 μM (pIC_{50} = 5.26), rM_4 IC_{50} = 493 nM (pIC_{50} = 6.31), rM_5 IC_{50} = 3.97 μM (pIC_{50} = 5.40), and all reduce an ACh EC_{80} to baseline. Human CRCs not shown. Screened as agonists, hM_1 EC_{50} = 255 nM (pEC_{50} = 6.59), 40% ACh Max, M_2-M_5 >30 μM. Screened as antagonists, hM_2 IC_{50} = 3.5 μM (pIC_{50} = 5.46), hM_3 IC_{50} = 2.3 μM (pIC_{50} = 5.63), hM_4 IC_{50} = 734 nM (pIC_{50} = 6.13), hM_5 IC_{50} = 4.9 μM (pIC_{50} = 5.31), and all reduce an ACh EC_{80} to baseline.

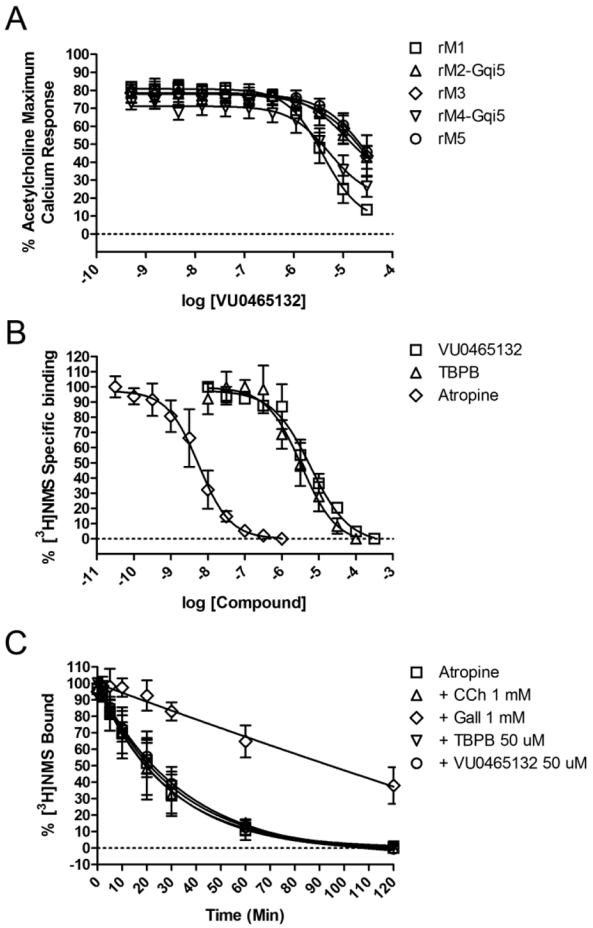


Figure 3.

Pharmacological profile of β-F-TBPB **9** (VU0465132). A) Rat M_1-M_5 CRCs screened as antagonists. Rat M_1 IC_{50} = 4.9 μM (pIC_{50} = 5.31±0.17), 13.6±1.5% ACh Max, M_2, M_3 and M_5 IC_{50} > 10 μM (pIC_{50}s <5), M_4 IC_{50} = 5.2 μM (pIC_{50} = 5.29±0.09), 26.6±3.4% ACh Max (Human M_1-M_5 data not shown, but comparable). B) [^{3}H]-NMS competition binding studies. At higher concentrations than the control atropine (Ki = 1.4 nM, pKi = 8.86±0.24), both **9** (Ki = 1.35 μM, pKi = 5.87±0.18) and TBPB (Ki = 0.60 μM, pKi = 6.22±0.14) fully displace [^{3}H]-NMS, consistent with interactions at the orthosteric ACh site. C) Dissociation kinetics with TBPB, **9**, atropine, carbachol (CCh) and Gallamine (Gall). In our assays, only the allosteric modulator Gall altered the rate of [^{3}H]-NMS dissociation, providing further evidence that **9** acts as an orthosteric antagonist. K_{off} rates were as follows ± SEM: Atropine (0.0379±0.0125 min^{-1}), CCh(0.0393±0.0125 min^{-1}), Gall (0.0012±0.0006 min^{-1}), TBPB (0.0335±0.0095 min^{-1}), VU0465132 (0.0308±0.0103 min^{-1}).

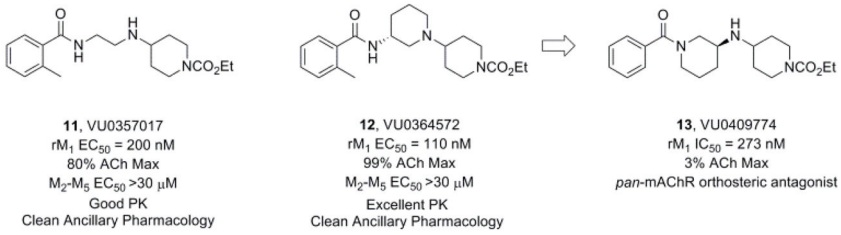
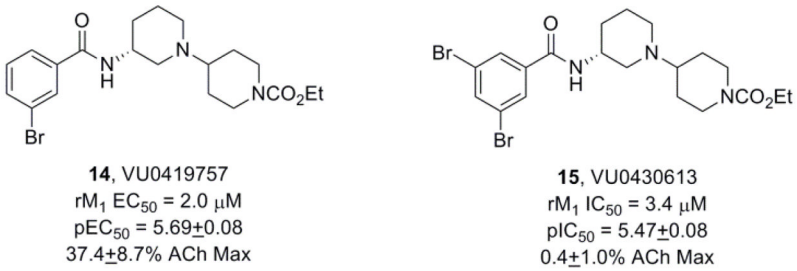


Figure 4.

Structures and activities of M_1 allosteric agonists **11** and **12**, and a mAChR orthosteric antagonist **13** (regioisomer of **12**).



14, VU0419757
rM_1 EC_{50} = 2.0 μM
pEC_{50} = 5.69±0.08
37.4±8.7% ACh Max

15, VU0430613
rM_1 IC_{50} = 3.4 μM
pIC_{50} = 5.47±0.08
0.4±1.0% ACh Max

Figure 5.
Structures and activities of M_1 allosteric agonist **14** and a 3,5-dibromo congener **15**, an M_1 antagonist.

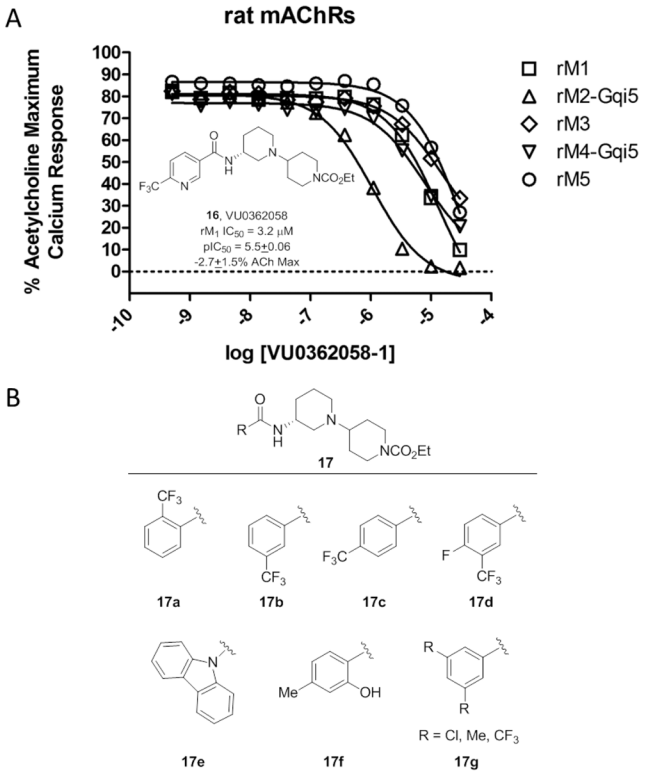
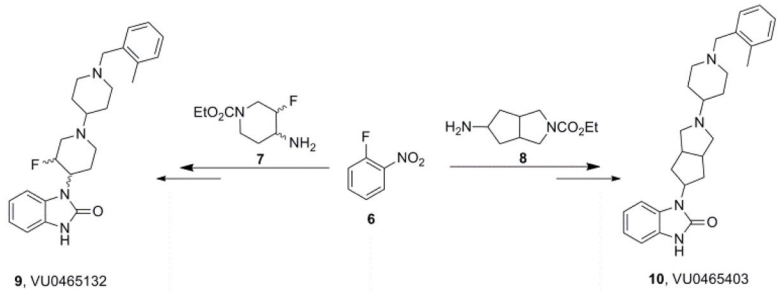


Figure 6.

A) Structure of VU0362058 (**16**) and full M_1-M_5 CRCs, highlighting the *pan*-mAChR antagonism via a subtle modification to the M_1 allosteric agonist **12**. B) Representative additional amide analogs of **12** (**17**) where the nature of substitutions on the aromatic ring conferred M_1 antagonism as opposed to M_1 agonism. While some SAR trends are evident, other switches in the mode of M_1 pharmacology are random and confound chemical optimization efforts.



Scheme 1.
Synthesis of TBPB analogs **9** (VU0465132) and **10** (VU0546403).