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

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ABSTRACT

This letter describes the further exploration of two series of M_1 allosteric 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.

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 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 and M_3 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 and M_3.[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

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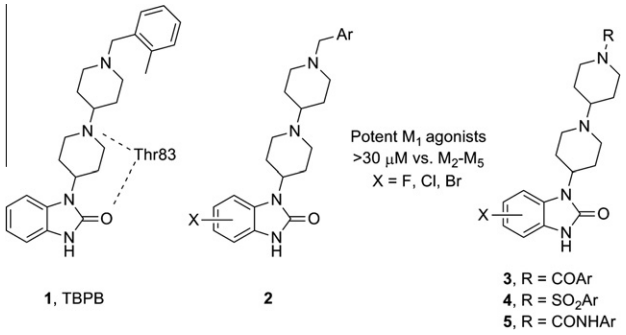


Figure 1. Structures of the M_1 allosteric agonist TBPB (**1**), highlighting the key H-bond interaction with Thr83 for allosteric binding at M_1, and analogs **2**–**5** of TBPB that retain selective M_1 agonism.

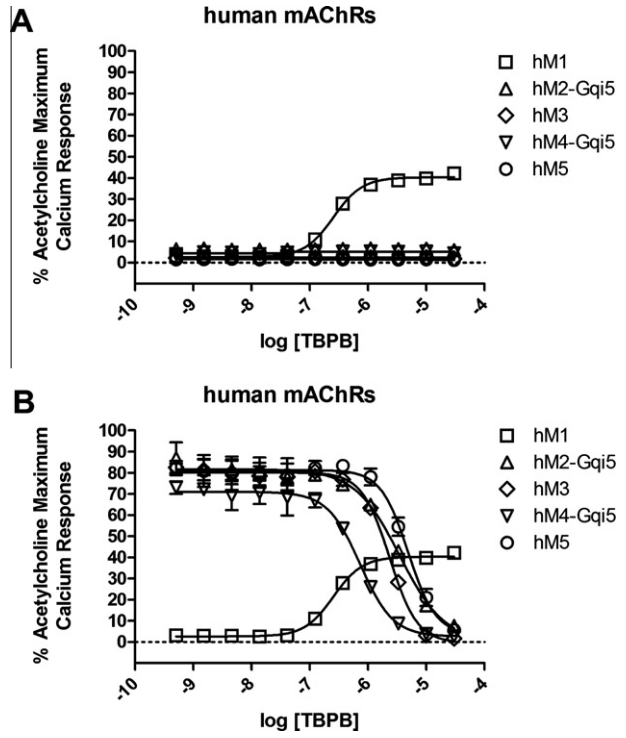


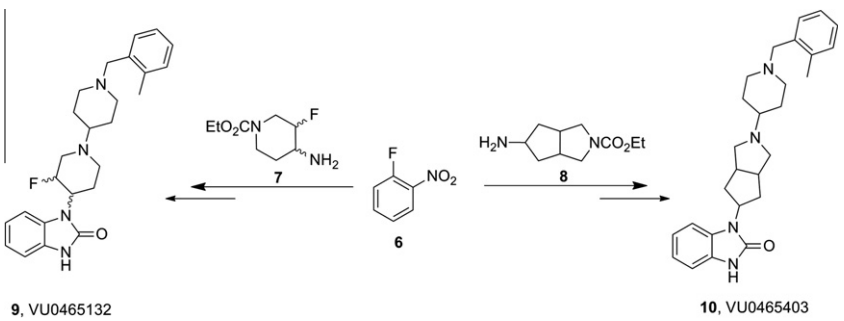
Figure 2. Pharmacological profile of TBPB. (A) rM_1–rM_5 concentration–response curves (CRCs) of TBPB screened in agonist mode. rM_1 EC_{50} = 79.6 nM (pEC_{50} = 7.10 ± 0.07), 69.3 ± 6.4% ACh Max, rM_2–rM_5 >30 μM. (B) rM_2–rM_5 CRCs of TBPB screened as antagonists. rM_2 IC_{50} = 1.29 μM (pIC_{50} = 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.

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 h for equilibrium to occur between atropine and [^{3}H]-NMS at room temperature for their dissociation kinetic studies whereas we allow 3 h at room temperature for this equilibrium to occur. Traditionally, when a 1 h equilibrium is utilized for M_1 dissociation studies, the studies are performed at 37 °C.[43] Incubation for only 1 h at room temperature



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