Bioorganic & Medicinal Chemistry Letters 21 (2011) 2697–2701

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery and optimization of a novel, selective and brain penetrant M_1 positive allosteric modulator (PAM): The development of ML169, an MLPCN probe

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ARTICLE INFO

Article history:
Received 29 October 2010
Revised 29 November 2010
Accepted 2 December 2010
Available online 9 December 2010

Keywords:
Muscarinic
Acetylcholine
Positive allosteric modulator (PAM)
ML169
BQCA
Alzheimer's disease
MLPCN

ABSTRACT

This Letter describes a chemical lead optimization campaign directed at VU0108370, a weak M_1 PAM hit with a novel chemical scaffold from a functional HTS screen within the MLPCN. An iterative parallel synthesis approach rapidly established SAR for this series and afforded VU0405652 (ML169), a potent, selective and brain penetrant M_1 PAM with an in vitro profile comparable to the prototypical M_1 PAM, BQCA, but with an improved brain to plasma ratio.

The muscarinic acetylcholine receptors (mAChRs) are members of the family A G-protein-coupled receptors (GPCRs) and include five subtypes denoted M_1–M_5. All five of the mAChRs are known to play critical roles in multiple basic physiological processes and represent attractive therapeutic targets for a number of peripheral and CNS pathologies.[1–3] Within the mAChRs, a major challenge has been a lack of subtype selective ligands to study the specific contribution of discrete mAChRs in various disease states.[3,4] To address this limitation, we have focused on targeting allosteric sites on mAChRs as a means to develop subtype selective small molecules, both allosteric agonists and positive allosteric modulators (PAMs).[5–9] Moreover, the emerging phenomenon of ligand-biased signaling requires the development of diverse chemical scaffolds of M_1 ligands to successfully dissect of the roles of M_1 activation through multiple, discrete ligand-biased signaling pathways.[10,11]

As members of the Molecular Libraries Production Center Network (MLPCN),[12] we performed a real-time cell-based calcium-mobilization assay employing a rat M_1/CHO cell line (Z′ averaged 0.7) and screened a 63,656 member MLPCN compound library following a triple-add protocol to simultaneously identify M_1 antagonists, agonists (both orthosteric and allosteric) and positive allosteric modulators (PAMs). This screen proved to be a major success providing viable leads that were optimized into potent and highly selective M_1 ligands (Fig. 1): an M_1 antagonist (**1**, VU0255035, ML012),[13] an M_1 allosteric agonist (**2**, VU0357017, ML071),[14] and both an M_1 PAM (**3**, VU0366369, ML137)[15] and the first M_5 PAM (**4**, VU0238429, ML129)[16] derived from a pan-M_1,M_3,M_5-PAM (**5**, VU0119498).[17,18] However, the brain penetration ($brain_{AUC}/plasma_{AUC}$ = 0.1) and efficacy (60% ACh Max) of **3** were poor, as was the brain penetration of the prototypical M_1 PAM, BQCA (**6**, $brain_{AUC}/plasma_{AUC}$ = 0.1)[19–21]; therefore, M_1 PAM ligands with improved physicochemical properties for in vivo studies and novel scaffolds to address ligand-biased signaling are required. In this Letter, we describe the development of VU0405652

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doi:10.1016/j.bmcl.2010.12.015

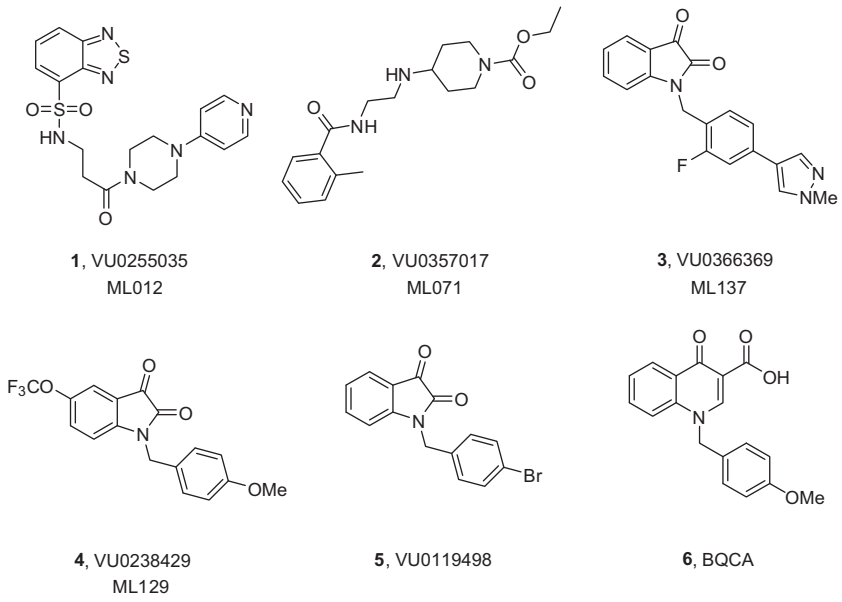


Figure 1. Structures of selective M_1 and M_5 MLPCN probes developed from hits from a triple-add functional M_1 HTS MLPCN screen (**1**–**5**) and BQCA (**6**).

(ML169),[22] a highly selective M_1 PAM MLPCN probe, with a novel chemical scaffold and improved brain penetration.

Perusal of the HTS data, which also yielded the non-selective hit **5**, identified a second weak M_1 PAM hit **7**, VU0108370, with an EC_{50} of ~13 μM. Confirmation of **7** from fresh powder and counter-screening against M_2–M_5 increased our enthusiasm for this highly M_1 mAChR selective hit (Fig. 2); however, the CRC did not plateau, suggesting the M_1 EC_{50} was actually >13 μM. Despite the weak potency, the confirmation of a novel M_1 PAM scaffold with high M_1 selectivity initiated a lead optimization campaign to improve M_1 potency while maintaining the high M_2–M_5 selectivity.

Our initial optimization strategy is outlined in Figure 3, and as SAR with allosteric ligands is often shallow, we employed an iterative parallel synthesis approach, along with targeted syntheses for structures encompassing more speculative modifications. Attempted modifications of the Eastern oxazole-amide, although not extensive, met with no success, returning only compounds with undetectable activity. In a straightforward attempt to reduce molecular weight the benzyl group attached to the indole nitrogen was omitted, but met with a similar lack of success (EC_{50} >10 μM) as did the sulfide and sulfoxide congeners.

Thus, we planned to hold the northern portion of **7** constant, and survey diversity on the southern benzyl moiety employing a

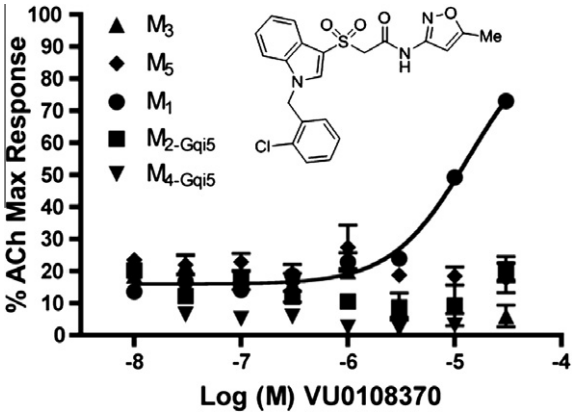


Figure 2. Concentration response curves (CRCs) for M_1–M_5 for HTS hit VU0108370. M_1 EC_{50} ~13 μM (does not plateau) and M_2–M_5 EC_{50} >30 μM.

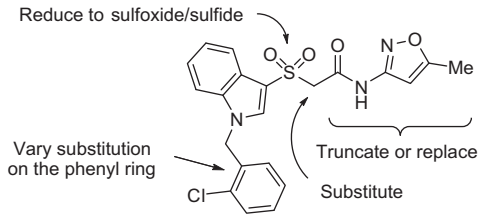
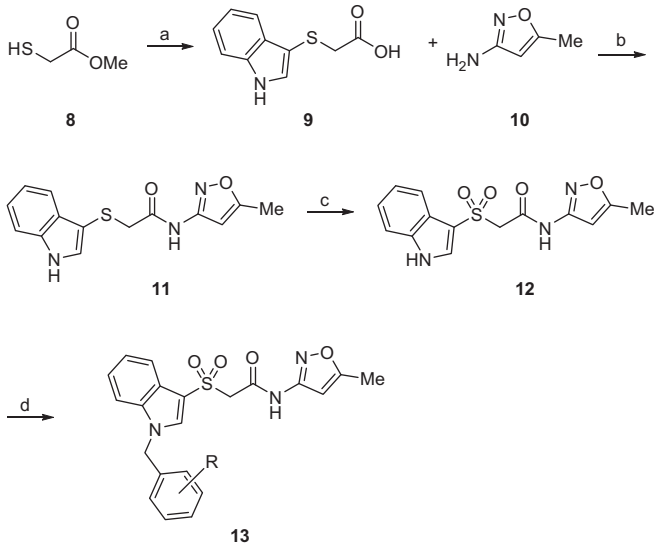


Figure 3. Initial optimization strategy for VU0108370, **7**.



Scheme 1. Reagents and conditions: (a) (i) indole, I_2, KI, MeOH, H_2O; (ii) 2 M LiOH, THF (38%); (b) PyClu, DCE, 110 °C, 20 min, mw (71%); (c) Oxone, MeOH, H_2O (88%); NaH, DMF, BnX (50–90%).

library synthesis approach. As shown in Scheme 1, the key library scaffold **12** was readily prepared in three steps from methyl thioglycolate **8**. A PyClu-mediated microwave-assisted coupling between **9** and **10** provided **11** in 71% yield, which was then oxidized to the corresponding sulfone **12** with Oxone in 88% yield.