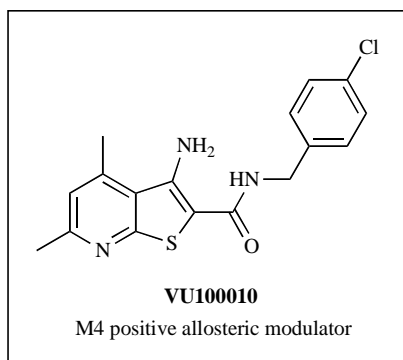


Molecule of the Month

Discovery of mAChR subtype selective M4 positive allosteric modulators. There are two types of cholinergic receptors through which the neurotransmitter acetylcholine (ACh) acts: nicotinic acetylcholine receptors (nAChRs), ligand gated ion channels, and muscarinic acetylcholine receptors (mAChRs), class A G protein-coupled receptors. To date, five mAChR subtypes have been identified (M1-M5) and are thought to mediate the majority of the actions of ACh in the peripheral and central nervous systems. Of these, M1 and M4 are the most heavily expressed in the CNS and represent attractive therapeutic targets for cognition, Alzheimer's disease, schizophrenia and movement disorders such as dystonia and Parkinson's disease. In contrast, the adverse effects of cholinergic agents are due to activation of peripheral M2 and M3 mAChRs [1-3]. Phase III clinical trials with Xanomeline an M1/M4 preferring orthosteric agonist, demonstrated efficacy as both a cognition enhancing agent, are more interestingly, as an antipsychotic agent [4]. In follow-up studies in rats, xanomeline displayed an antipsychotic phenotype comparable to clozapine [5]. However, was the antipsychotic phenotype mediated by activation of M1, M4 or a combination of M1/M4? Data from mAChR knock-out mice suggest that a selective M1 agonist would be beneficial for cognition whereas an M4 agonist would provide antipsychotic effects for the treatment of schizophrenia [6]. This proposal is further supported by recent studies demonstrating that M4 receptors modulate the dynamics of cholinergic and dopaminergic neurotransmission and that loss of M4 function results in a state of dopamine hyperfunction - a hallmark of schizophrenia [7]. These data, coupled with new information that schizophrenic



patients possess altered hippocampal M4, but not M1, receptor expression argue well for the need for selective activators of M4 as a novel treatment for schizophrenia [8]. Due to the high sequence homology and conservation of the orthosteric ACh binding site among the mAChRs, development of chemical agents that are selective for a single subtype have been largely unsuccessful - until now [1-7].

In a recent paper from the Conn laboratory at Vanderbilt (Shirey et.al. *Nat. Chem. Bio.* **2008**, *4* (1), 42-50), a novel, mAChR subtype-selective M4 positive allosteric modulator, **VU100010**, was disclosed [9]. Employing a cheminformatics approach, coupled with medicinal chemistry, Conn identified a new series of ligands that interact with an allosteric site on the M4 receptor (does not displace [³H]-NMS) which not only activates the receptor, but also confers complete selectivity versus M1, M2, M3 and M5 - a 'Holy Grail' for molecular pharmacology as this tool will enable researchers to probe the pharmacological role of selective M4 activation and evaluate the potential of M4 as a novel therapeutic target

for multiple CNS disorders. VU100010 possesses an EC₅₀ for potentiation of ~400 nM and potentiates the ACh response curve 47-fold to the left. Mechanistic studies indicate that VU100010 exerts its allosteric activation of M4 by increasing the affinity of ACh and coupling to G proteins. In electrophysiology studies, VU100010 was shown to modulate hippocampal synaptic transmission. Specifically, the M4 positive allosteric modulator increased carbachol-induced depression of transmission at excitatory, but not inhibitory synapses in the hippocampus. Importantly, this effect was absent when inactive analogues of VU100010 were employed or in M4 knockout mice [9].

No *in vivo* data was disclosed for VU100010, but the *in vitro* profile of this novel and highly selective M4 positive allosteric modulator represents a major advance in the muscarinic field and will allow many important studies to be performed to dissect the contribution of M4 to the efficacy of xanomeline and ultimately, to the potential of selective M4 activation as a therapeutic mechanism for CNS disorders.

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