Discovery of M5-Preferring Muscarinic Receptor Antagonists: Evaluation of N-phenyl-N-(1-phenylethyl) Carbamic Acid Esters of Various Azacyclics
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Purpose
Drugs of abuse produce their rewarding effects by releasing dopamine from ventral tegmental area neurons. M5 muscarinic acetylcholine receptors (mAChR) are localized on ventral tegmental area dopaminergic neurons. Consistent with M5 mAChR modulation of dopamine release, behavioral studies using M5 knockout mice show a reduced rate of cocaine self-administration, reduced reward and withdrawal responses following morphine and cocaine administration. Thus, M5 mAChRs may be a potential pharmacotherapeutic target for the treatment of drug abuse. Based on the scaffold of 1,2,5,6-tetrahydropyridine-3-carboxylic acid, we recently discovered a series of M5-preferring antagonists by modifying the substituents on the ester phenyl ring of the phenethyl group (Zheng et al. 2013). In the current study, a series of azacyclic alcohol carbamates, which contain a substituted phenethyl group, were synthesized and investigated for their binding affinity and selectivity at M5 mAChRs.

Methods
Analogs were synthesized using a three-step parallel synthesis method, starting with a microwave assisted N-alkylation of aniline, followed by forming N,N-disubstituted chloroformamide and microwave assisted coupling with various azacyclic alcohols. M1 and M5 receptor affinities were determined by measuring inhibition of [3H]N-methylscopolamine binding to Chinese hamster ovary cell membranes expressing either human M1 or M5 mAChRs.

Results
A dozen analogs were synthesized. Preliminary results show that analogs containing a quinuclidiny1 core, i.e. GZ-10130A and GZ-10128A, are potent at both M5 (Ki = 4 and 30 nM, respectively) and M1 (Ki = 8 and 30 nM, respectively) receptors; however, both these analogs are not selective for M5 receptors. In addition, a tropine containing analog, GZ-10128B, exhibited modest affinity (Ki = 400 nM), but demonstrated preferential interaction (3.5-fold selectivity) with M5 receptors.

Conclusion
This preliminary SAR study provides fundamental structural information to continue to build the pharmacophore for potent selective interaction of ligands with the M5 mAChRs.
This work was supported by NIH DA030667 and UL1TR000117