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Challenges in the development of an M_4 PAM preclinical candidate: The discovery, SAR, and *in vivo* characterization of a series of 3-aminoazetidine-derived amides

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ABSTRACT

This letter details the continued chemical optimization of a novel series of M_4 positive allosteric modulators (PAMs) based on a 5-amino-thieno[2,3-*c*]pyridazine core by incorporating a 3-amino azetidine amide moiety. The analogs described within this work represent the most potent M_4 PAMs reported for this series to date. The SAR to address potency, clearance, subtype selectivity, CNS exposure, and P-gp efflux are described. This work culminated in the discovery of VU6000918, which demonstrated robust efficacy in a rat amphetamine-induced hyperlocomotion reversal model at a minimum efficacious dose of 0.3 mg/kg.

Positive allosteric modulators (PAMs) of the muscarinic acetylcholine receptor (M_4) (**1**–**4**) have emerged as an exciting potential strategy for the treatment of numerous CNS disorders, including schizophrenia,[1–20] Huntington's disease,[21] and Alzheimer's disease.[22] Previous reports from our laboratory have described the discovery and characterization of VU0152100 (ML108, **1**), an *in vivo* tool compound which demonstrated efficacy in rodent models of anti-psychotic efficacy.[3,4] We subsequently reported related congener VU0467154 (**2**), based on a 5-amino-thieno[2,3-*c*]pyridazine core, which, despite its robust *in vivo* activity in multiple preclinical rodent models and a favorable pharmacokinetic (PK) profile, suffered from considerably lower potency at the human M_4 receptor as compared to rat.[11,19] In the course of our medicinal chemistry campaign to identify a compound with improved potency at the human M_4 receptor while maintaining suitable DMPK properties for a clinical candidate, we encountered steep SAR not only in potency at M_4, but in multiple DMPK properties as well.[13,14,19,20] Herein, we describe our efforts to replace the benzylic linker present in compounds **1**–**4** with substituted 3-amino azetidines (Fig. 1).

Observing that small cyclic amides afforded potent analogs in both Eli Lilly's and our M_4 PAM programs, we wished to examine the introduction of a cyclic linker between the 5-amino-thieno[2,3-*c*]pyridazine amide core and the appended aryl ring. Such a change may serve to decrease the planarity of the molecule, thus reducing its ability to form pi-stacking interactions and thereby improve solubility, restrict the conformations available for the aryl ring to adopt, and remove the benzylic methylene as a potential metabolic soft spot. Diamine linkers would provide a convenient synthetic handle by which to introduce substituents on the cyclic linker. Several potential linkers were examined, including monocyclic and bicyclic diamines; however, 3-amino substituted azetidines yielded the most potent analogs (Fig. 2).

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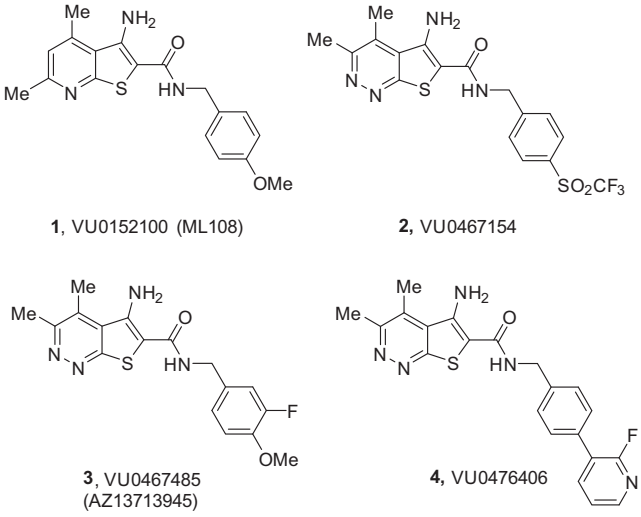
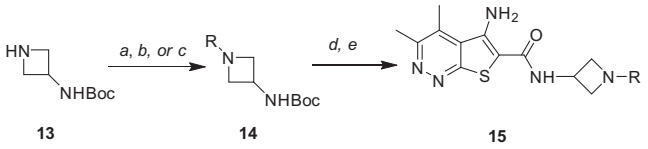


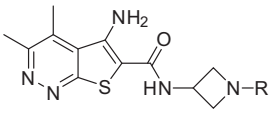
Fig. 1. Structures of representative M_4 PAMs **1**–**4**, highlighting the optimized rodent *in vivo* tool M_4 PAM, VU0467154 (**2**), the clinical candidate VU0467485/AZ13713945 (**3**) and the non-human primate *in vivo* tool VU0476406 (**4**).

Analogs were readily prepared following functionalization of commercially available 3-(Boc-amino)-azetidine via nucleophilic substitution or Buchwald-Hartwig[23,24] cross-coupling reactions, followed by Boc deprotection and amide coupling to the thieno[2,3-*c*]pyridazine core (Scheme 1). Our initial library examined the effect of tertiary carbamates, sulfonamides, and amides (Table 1). Basic tertiary azetidine amines were poorly tolerated and led to a sharp decrease in human M_4 (hM_4) potency (data not shown). Carbamates proved to be the most potent compounds in this class, with analog **6b** displaying an EC_{50} of 23 nM. However, upon further profiling, **6b** was found to have weak activity at human M_2 (hM_2, EC_{50} = 2.65 μM) and a short elimination half-life *in vivo* in rat ($t_{1/2}$ <30 min) due to facile hydrolysis of the carbamate, which proved to be the case in general for the carbamate series and thus precluded their advancement. Azetidine sulfonamides (**6e**), ureas (**6d**), and amides (**6f-h**) were also tolerated, albeit with lower potency as compared to the carbamates. Compound **6h** was selected for further assessment, which gratifyingly found an improved profile compared to the carbamate series with reduced activity at hM_2 (EC_{50} >10 μM) and low *in vivo* clearance (rat CL_p = 3.1 mL/min/kg). Unfortunately, **6h** was found to have low



Scheme 1. Synthesis of M_4 PAM analogs **6**, **16**, **17**. Reagents and conditions: (a) R-X, DCM, DIPEA, rt. (b) R-Het-X, Cs_2CO_3, DMF, heat (c) Ar-X, $Pd_2(dba)_3$, *rac*-BINAP, Cs_2CO_3, toluene, 100 °C (d) TFA, DCM, rt, 3 h (e) 5-amino-3,4-dimethylthieno[2,3-*c*]pyridazine-6-carboxylic acid, HATU, DIPEA, DMF, 2 h.

Table 1
Structures and activities for M_4 PAM analogs **6**.



Cmpd	R	hM_4 EC_{50} (nM)[a] [% ACh Max ± SEM]	hM_4 pEC_{50} (±SEM)
6a	CO_2Bn	30 [81 ± 8]	7.62 ± 0.19
6b	CO_2Ph	23 [96 ± 3]	7.65 ± 0.03
6c	CO_2(3-Me)Ph	67 [85 ± 9]	7.23 ± 0.17
6d	C(O)NHPh	217 [89 ± 6]	6.66 ± 0.03
6e	SO_2Ph	268 [70 ± 8]	6.58 ± 0.07
6f	C(O)Ph	773 [85 ± 6]	6.22 ± 0.25
6g	C(O)2-pyridyl	564 [91 ± 5]	6.25 ± 0.03
6h	C(O)4-pyridyl	179 [85 ± 7]	6.75 ± 0.03

[a] Calcium mobilization assays with $hM_{4/Gqi5}$-CHO cells performed in the presence of an EC_{20} fixed concentration of acetylcholine; values represent means from three (n = 3) independent experiments performed in triplicate.

CNS exposure (rat brain:plasma K_p = 0.03, $K_{p,uu}$ = 0.37 at 0.25 h post-IV cassette dose) likely due to P-gp efflux (MDCK-MDR1 ER = 96).Table 2

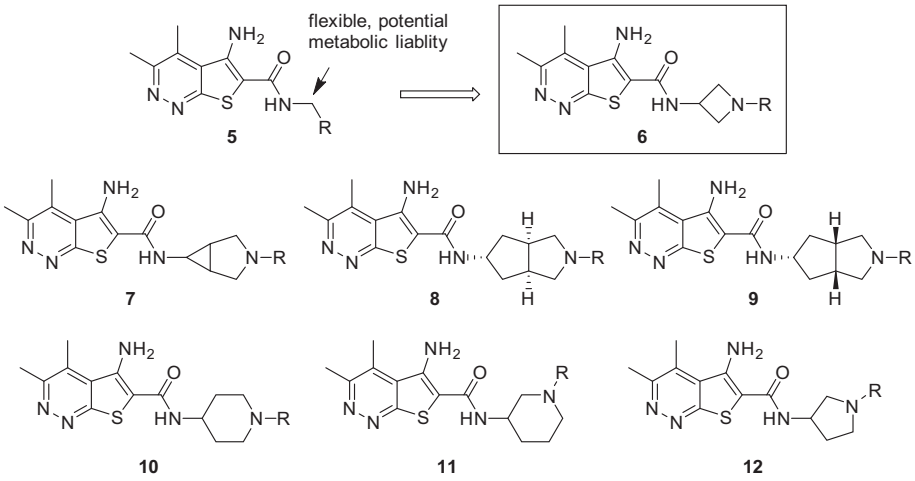


Fig. 2. Cyclic diamines examined as alternative amide linkers to thieno[2,3-*c*]pyridazine core.