Bioorganic & Medicinal Chemistry Letters 20 (2010) 5617–5622

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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Heterobiaryl and heterobiaryl ether derived M_5 positive allosteric modulators

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ARTICLE INFO

Article history:
Received 20 July 2010
Revised 5 August 2010
Accepted 9 August 2010
Available online 12 August 2010

Keywords:
M5
mAChR
PAM
Allosteric

ABSTRACT

This Letter describes a chemical lead optimization campaign directed at VU0238429, the first M_5-preferring positive allosteric modulator (PAM), discovered through analog work around VU0119498, a pan G_q mAChR M_1, M_3, M_5 PAM. An iterative parallel synthesis approach was employed to incorporate basic heterocycles to improve physiochemical properties.

In the course of our program to develop allosteric ligands for GPCRs,[1–6] we recently described the identification of VU0119498 (**1**), a pan G_q muscarinic acetylcholine receptor (mAChR) M_1, M_3, M_5 positive allosteric modulator (PAM).[7] Application of an iterative parallel synthesis approach identified key structural elements within the VU0119498 scaffold that eliminated M_3 and M_5 activity affording VU0366369 (**2**), a highly selective M_1 PAM (M_1 EC_{50} = 0.83 μM, >30 μM vs M_2–M_5).[8] Further SAR discovered a critical 5-OCF_3 moiety on the isatin core that engendered selective M_5 activity that led to the development of the first M_5 PAM, VU0238429 (**3**).[9] Optimization of VU0238429, again employing iterative parallel synthesis, led to the development of VU0365114 (**4**) and VU0400265 (**5**) with excellent selectivity for the M_5 subtype (Fig. 1).[10] While **4** (M_5 EC_{50} = 2.7 μM, >30 μM vs M_1–M_4) and **5** (M_5 EC_{50} = 1.9 μM, >30 μM vs M_1–M_4) marked notable advances for the study of M_5 function with small molecule tools, both are lipophilic compounds (log *P*s >4.5) with limited solubility and overall poor physiochemical properties.[8–10] This Letter, describes efforts to identify alternatives for both the 5-OCF_3 moiety, and heterocyclic replacements for the phenyl rings that would retain M_5 potency and subtype selectivity while providing basic nitrogen atoms from which salts could be formed to improve solubility and physiochemical properties.

Our optimization strategy is outlined in Figure 2, and as SAR with allosteric ligands is often shallow, we employed an iterative parallel synthesis approach.[11] For the first round of parallel synthesis, we first held the biphenyl moiety constant and surveyed a diverse range of substituents (lipophilic, polar, basic, and acidic functionalities) in the 5-position as possible replacements for the 5-OCF_3 moiety. Libraries were prepared according to Scheme 1, wherein 5-methoxyisatin **6** is alkylated with 4-bromobenzyl bromide to provide **7** in 65% yield, followed by a microwave-assisted Suzuki coupling to install the biaryl motif affording **8** in 82% yield. A subsequent BBr_3-mediated demethylation delivered the key substrate **9** for library production in 83% yield. Depending on the electrophile, a number of alkylation/S_NAr/Mitsunobu conditions were employed to deliver the final 44 library members **10**.[12] The library was then triaged by a single point (10 μM) screen for their ability to potentiate an EC_{20} of ACh in M_5-CHO cells (Fig. 3). Surprisingly, not a single library member **10** was able to significantly potentiate the EC_{20} of ACh in M_5-CHO cells; however, three analogs **10ee**, **10gg**, and **10kk**, all containing a basic amine, did decrease the ACh EC_{20}, suggesting that they may be putative antagonists or NAMs. Thus, the 5-OCF_3 was essential for M_5 PAM activity and would be retained in future libraries.

In parallel with the library work, we prepared a number of singleton analogs (Fig. 4) following the synthetic routes outlined in Scheme 1. In the related M_1 PAM series, we found that strategic introduction of fluorine atoms maintained M_1 selectivity while increasing potency.[8,13] In the present case, as with **11** and **12**, this strategy did not translate well into the M_5 PAM series, leading to a loss in both potency and efficacy. In our M_4 PAM series, a piperonyl moiety was equipotent to a 4-OMe group (as in **3**); this modification was also productive here, providing **13**, a highly efficacious M_5 PAM

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0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.08.042

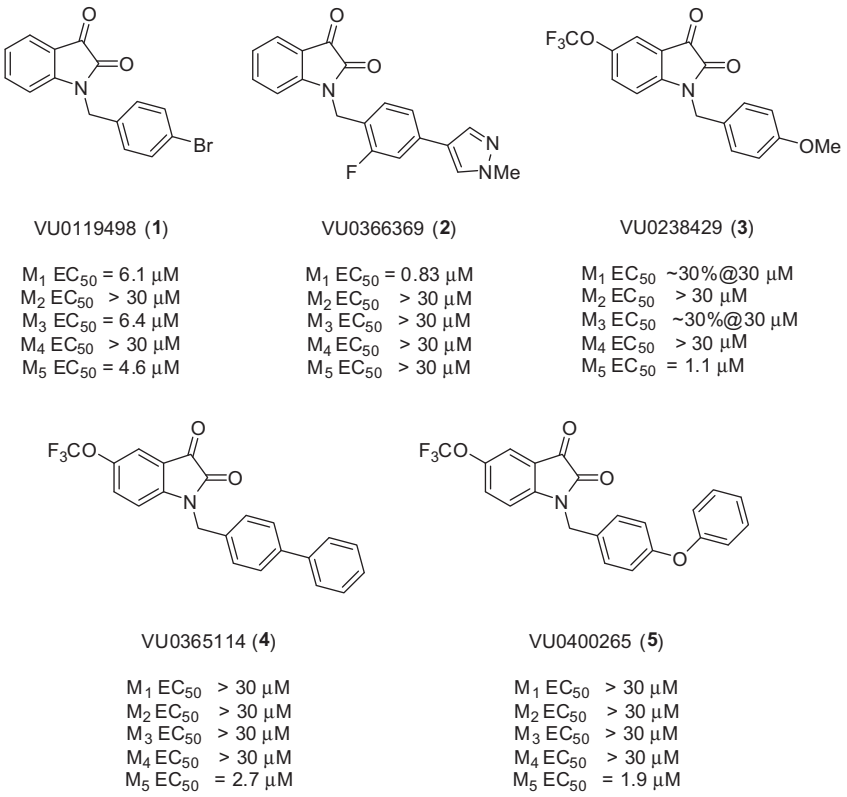


Figure 1. HTS lead VU0119498, a pan G_q mAChR M_1, M_3, M_5 PAM which was optimized to provide both an M_1 selective PAM, VU0366369, and an M_5-preferring PAM, VU0238429. Further optimization of VU0238429 led to the development of two highly selective M_5 PAMs, VU0365114 and VU0400265. Data represent means from at least three independent determinations with similar results using mobilization of intracellular calcium in M_1–M_5 CHO cells (M_2 and M_4 cells co-transfected with G_{qi5}).

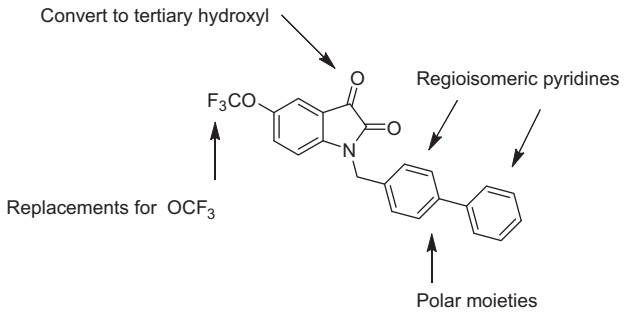


Figure 2. Optimization strategy for VU0365114 (**4**), a highly selective M_5 PAM.

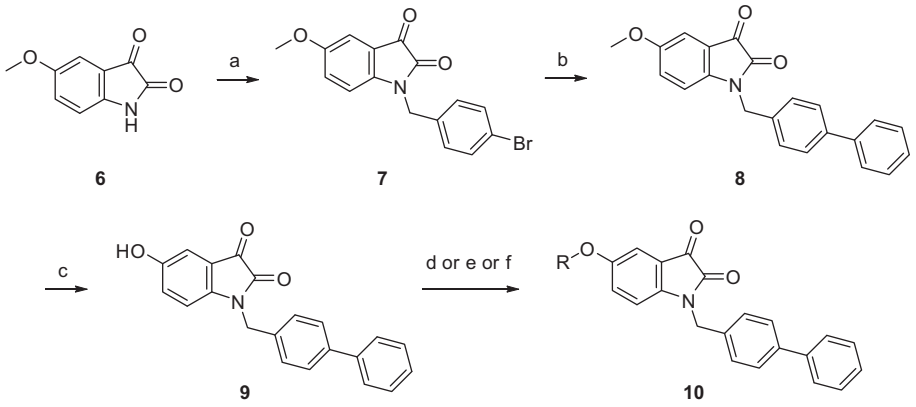
of comparable potency to **4**. Finally, we surveyed installation of the biphenyl and phenyl ether moieties in the 3-position versus the 4-position, **14** and **15**, respectively, but all M_5 PAM activity was lost (M_5 EC_{50} >30 μM). In addition, replacement of the isatin ketone carbonyl with either a tertiary alcohol or a spirocyclopropane resulted in analogs devoid of M_5 PAM activity. These data influenced the design of subsequent heterocyclic-containing libraries.

Based on the preceding data, the next library was designed wherein the 5-OCF_3 was held constant, the benzylic ring was retained as phenyl and the 4-position of the benzylic phenyl ring was substituted with various heterocycles (Scheme 2). 5-Trifluoromethoxyisatin **16** was alkylated with 4-bromobenzyl bromide to provide **17** in 99% isolated yield. Suzuki couplings with heterocyclic boronic acids delivered analogs **18** in low yields ranging between 9% and 18%. From this effort (Fig. 5), three analogs displayed M_5 PAM activity comparable to **4** (M_5 EC_{50}s 1.6–2.8 μM), but with low efficacy (M_5 ACh Max <60%).

We then replaced the benzylic phenyl ring with either a 2- or 3-pyridyl ring and explored aryl and heteroaryl rings in the 4-position (Scheme 3). Thus, 5-trifluoromethoxyisatin **16** was alkylated with either 5-bromo-2-(bromomethyl)pyridine or 5-(bromomethyl)2-chloropyridine to provide **19** and **20**, respectively, in 98% isolated yields. Suzuki couplings with heterocyclic boronic acids delivered libraries of analogs **21** and **22**.

As shown in Table 1, this effort produced several active analogs with a balance of M_5 potency and efficacy. Notably, the 3-pyridyl analogs **22** and the chloro intermediate in this series **20** proved to be uniformly active, affording M_5 PAMs with modest potency (M_5 EC_{50}s 2.4–4.4 μM and 50–80% M_5 ACh Max). Interestingly, heterobiaryl analogs **21b** and **21c** were devoid of M_5 PAM activity, while the phenyl congener **21a** was of comparable potency to **4**, but with diminished efficacy (47% M_5 ACh Max). Gratifyingly, HCl salts could be generated for analogs **21** and **22** with improved solubility across vehicles as compared to **4**. Moreover log *P* was reduced by an order of magnitude ($\log P = 4.6$ for **4** whereas $\log P = 3.6$ for **22a** and $\log P = 3.0$ for **22b**).

Finally, we prepared a small library based on biaryl ether **5**, replacing the distal phenyl ring with various heterocycles. Chemistry to access these analogs proved arduous under Ullmann coupling conditions. Therefore, our synthetic route (Scheme 4) employed methyl 4-hydroxybenzoate **23** for S_NAr reactions with heteroaryl chlorides to produce analogs **24**. Reduction with DIBAL delivered benzylic alcohols **25** which were then converted to the corresponding bromides **26** and employed in alkylation chemistry with **16** to deliver analogs **27**.



Scheme 1. Reagents and conditions: (a) 4-bromobenzyl bromide, K_2CO_3, KI, DMF, rt, 48 h (65%); (b) $PhB(OH)_2$, 5 mol % $Pd(PPh_3)_4$, 1.0 M aq Cs_2CO_3, THF, mw, 120 °C, 20 min (82%); (c) BBr_3, DCM, 0 °C to rt, 2 h (83%); (d) R-X, Cs_2CO_3, KI, DMF, mw, 120 °C, 30 min (avg. 20% for 31 analogs **10**); (e) ROH, PS-PPh_3, DIAD, THF, rt (avg. 4% for 10 analogs **10**); (f) Het-X, Cs_2CO_3, DMF, 160 °C, 20 min (avg. 10%, three analogs **10**).

Four analogs **27a**–**27d** displayed M_5 PAM activity (Fig. 6). The phenyl moiety of **5** could be replaced with 2-pyridyl (**27a**, M_5 EC_{50} = 3.2 μM, 46% ACh Max), 6-fluoro-2-pyridyl (**27b**, M_5 EC_{50} = 1.5 μM, 62% ACh Max), a 6-methoxy-2-pyridyl (**27c**, M_5 EC_{50} = 2.2 μM, 51% ACh Max), or a 2-thiazolyl (**27d**, M_5 EC_{50} = 2.8 μM, 58% ACh Max). Again, HCl salts could be generated for analogs **27** with improved solubility across vehicles as compared to **5**. Moreover log *P* was reduced by an order of magnitude.

From this effort, two analogs emerged **22a** (VU0415478), a heterobiaryl derivative, and **27b** (VU0414747), a heterobiaryl ether congener, worthy of further evaluation. In acetylcholine fold-shift assays at a standard 30 μM concentration, **22a** elicited a robust 14-fold leftward shift and **27b** displayed a sixfold leftward shift of the ACh concentration response curves (Fig. 7). Compound **22a** was found to possess ~20% allosteric agonism. Notably, the fold-shifts were equivalent to those of the phenyl analogs **4** (10-fold shift) and **5** (fivefold shift). We then evaluated selectivity of these heterocyclic analogs versus the other mAChRs. Using a 30 μM, fold-shift selectivity assay protocol,[2–7] neither **22a** nor **27b** showed any significant effect on M_1–M_4; thus, **22a** and **27b** are highly selective M_5 PAMs. Moreover, the diminished lipophilicity translated directly into a cleaner ancillary pharmacology profile. For example,

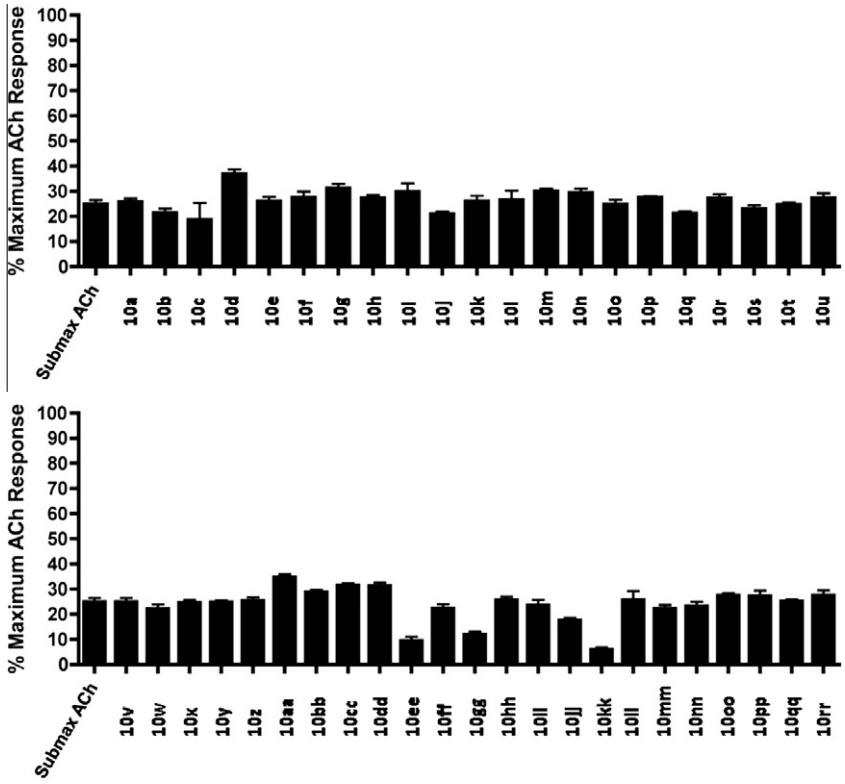


Figure 3. ACh EC_{20} triage screen of 44 analogs **10a**–**10rr** at 10 μM in M_5-CHO cells by intracellular calcium mobilization assay. Data represent means from at least three independent determinations with similar results.

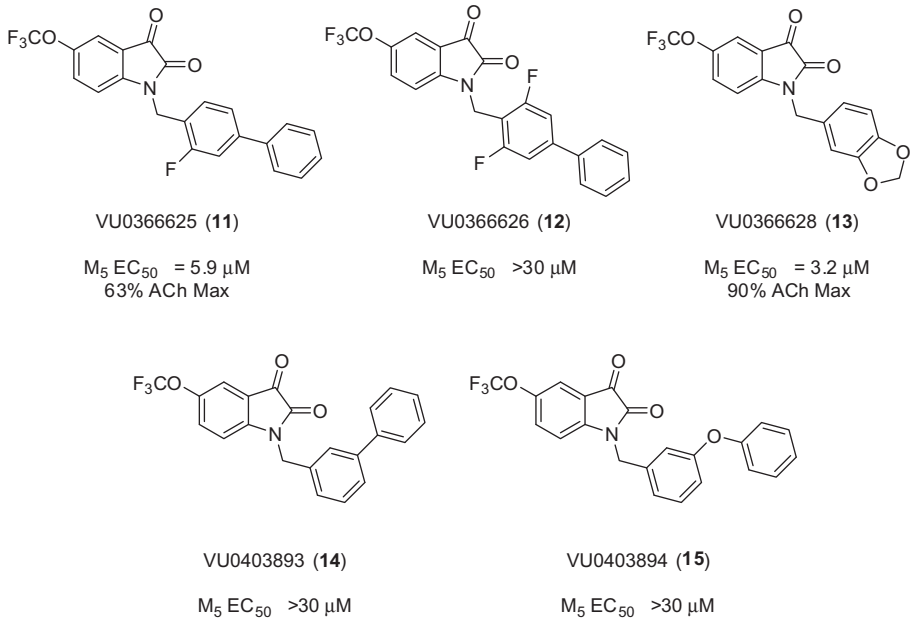
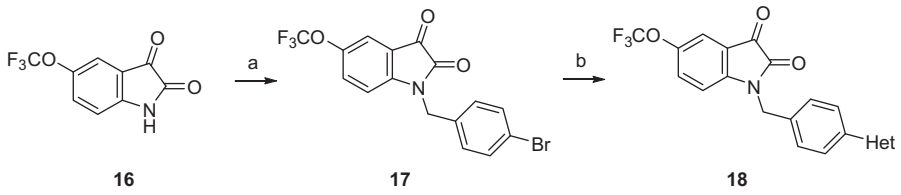


Figure 4. Singleton analogs designed to test key design elements to influence heterocyclic library design.



Scheme 2. Reagents and conditions: (a) 4-bromobenzyl bromide, K_2CO_3, KI, DMF, rt, 48 h (99%); (b) Het-B(OH)$_2$, 5 mol % Pd(PPh$_3$)$_4$, 1.0 M aq Cs_2CO_3, THF, mw, 120 °C, 20 min (9–18%).

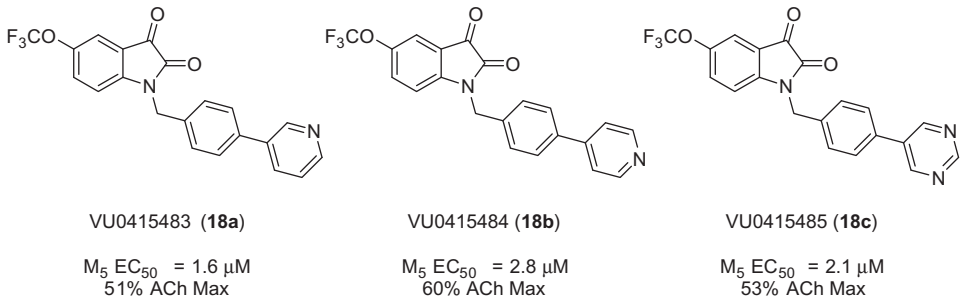
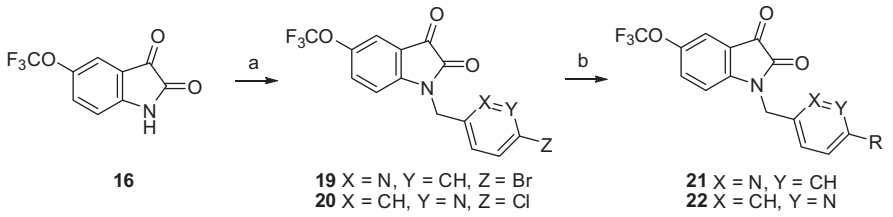
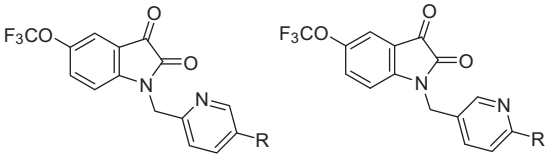


Figure 5. M_5 PAM analogs **18a**–**18c** with heterocycles in the 4-position of the biaryl motif.



Scheme 3. Reagents and conditions: (a) 5-bromo-2-(bromomethyl) pyridine or 5-(bromomethyl)2-chloropyridine, K_2CO_3, KI, DMF, rt, 48 h (98%); (b) (Ph)Het-B(OH)$_2$, 5 mol % Pd(PPh$_3$)$_4$, 1.0 M aq Cs_2CO_3, THF, mw, 120 °C, 20 min (5-11%).

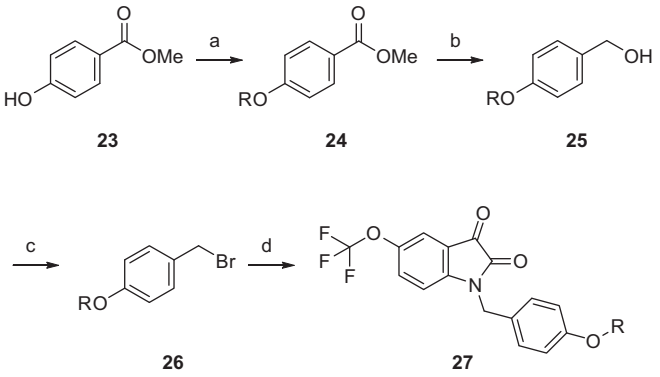
Table 1
Structures and activities of analogs **21** and **22**



21 **22**

Compd	R	M_5 Ec_{50} (μM)[a]	M_5 %ACh Max[a]
20	Cl	2.4	80
21a	Ph	3.1	47
21b	3-Pyridyl	>30	ND
21c	4-Pyridyl	>30	ND
22a	Ph	3.8	76
22b	3-Pyridyl	4.0	53
22c	4-Pyridyl	4.4	50

[a] Average of at least three independent determinations.



Scheme 4. Reagents and conditions: (a) Cs_2CO_3, DMF, 130 °C, 5–12 h (57–91%); (b) 1 M DIBAL, toluene, 0 °C to rt, 2 h (86–94%); (c) PBr_3, CH_2Cl_2, 0 °C (19–53%); (d) **16**, K_2CO_3, KI, DMF, mw, 120 °C, 30 min (5–32%).

5 showed significant activity (>50% inhibition at 10 μM) for 32 targets in a panel[14] of 68 GPCRs, ion channels, and transporters which limited its utility as a tool compound to study selective M_5 activation. In the same assay panel, **27b** was found to possess modest activities for 11 of the 68 targets, and only significant activity at NET and H_1.

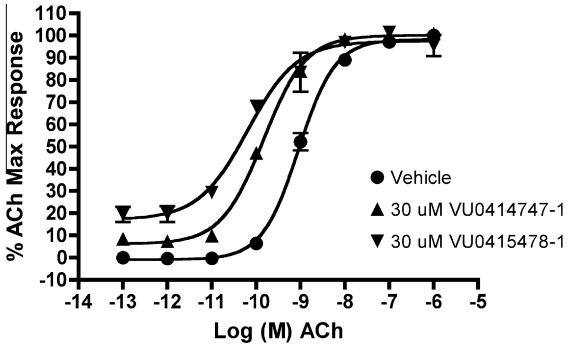
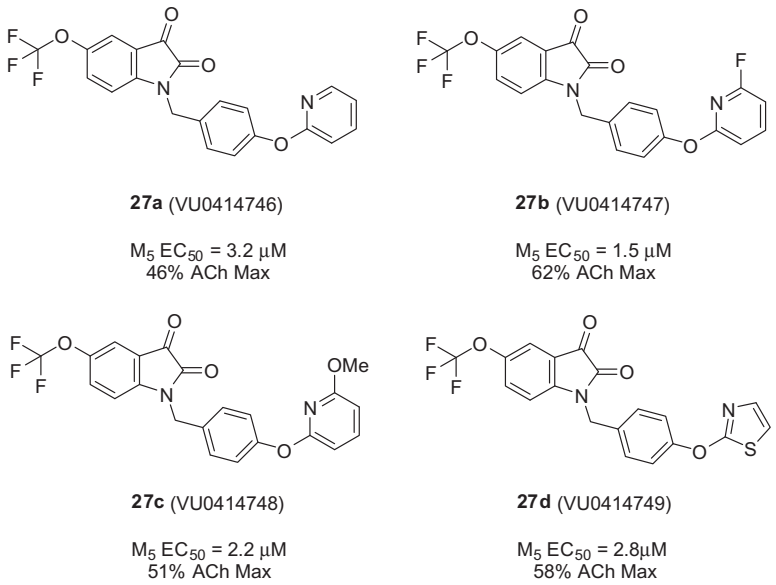


Figure 7. Human M_5 ACh fold-shift assay at a standard 30 μM concentration for heterocyclic analogs **22a** (VU0415478), a heterobiaryl derivative, and **27b** (VU0414747), a heterobiaryl ether congener.

In summary, an iterative parallel synthesis approach rapidly identified key heterocyclic replacements for phenyl moieties in two related biaryl and biaryl ether M_5 PAM scaffolds. SAR was steep, but key analogs **22a** and **27b** maintained M_5 PAM activity, robust leftward shifts of the ACh CRC and mAChR subtype selectivity. In addition, HCl salts could be formed and log *P* was reduced by an order of magnitude. Further in vitro pharmacology and electrophysiology studies with **22a** and **27b** are in progress and will be reported in due course.

Acknowledgments

The authors thank NIMH (1RO1 MH082867) and the MLPCN (1U54 MH084659) for support of our program in the development of subtype selective allosteric ligands of mAChRs. Vanderbilt is a Specialized Chemistry Center within the MLPCN.



27a (VU0414746)
M_5 EC_{50} = 3.2 μM
46% ACh Max

27b (VU0414747)
M_5 EC_{50} = 1.5 μM
62% ACh Max

27c (VU0414748)
M_5 EC_{50} = 2.2 μM
51% ACh Max

27d (VU0414749)
M_5 EC_{50} = 2.8μM
58% ACh Max

Figure 6. M_5 PAM heterobiaryl ether analogs **27a**–**27d**.

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11. Kennedy, J. P.; Williams, L.; Bridges, T. M.; Daniels, R. N.; Weaver, D.; Lindsley, C. W. *J. Comb. Chem.* **2008**, *10*, 345.
12. Library members **10**, R=: **a**, trifluopropyl; **b**, carboxymethyl; **c**, methyl; **d**, cyclobutylmethyl; **e**, *n*-propyl; **f**, *n*-butyl; **g**, cyclohexylmethyl; **h**, benzyl; **i**, 4-OMeBn; **j**, 3-OMeBn; **k**, 2-FBn; **l**, 4-FBn; **m**, 2,6-diFBn; **n**, 2-ClBn; **o**, 2-CF_3Bn; **p**, 4-CF_3Bn; **q**, 2,4-diFBn; **r**, 4-OCF_3Bn; **s**, 2-MeBn; **t**, 4-ClBn; **u**, 3-ClBn; **v**, 3-CF_3Bn; **w**, 2-CF_3Bn; **x**, 3-OCF_3Bn; **y**, 2,5-diFBn; **z**, *i*-Bu; **aa**, Et; **bb**, 3-FBn; **cc**, 3-CF_3-4-OMeBn; **dd**, morpholinoethyl; **ee**, 3-methyl-1-methylpiperidine; **ff**, 2-OMeBn; **gg**, *N*,*N*-dimethylethyl; **hh**, 4-pyridylmethyl; **ii**, 3-pyridylmethyl; **jj**, 2-pyridylmethyl; **kk**, pyrrolidinyl ethyl; **ll**, 2-F phenethyl; **mm**, 2-CF_3 phenethyl; **nn**, 2-Cl phenethyl; **oo**, 2-Me phenethyl; **pp**, trifluoroethyl; **qq**, 2-pyridyl; **rr**, 2-pyrimidyl.
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14. For information on the ancillary pharmacology panel see the Lead Profiling Screen at www.ricerca.com.