MedChemComm

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/medchemcomm

MedChemComm

ARTICLE

A Structure-Activity Relationship Study of the Positive Allosteric Modulator LY2033298 at the M_4 Muscarinic Acetylcholine Receptor

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/medchemcomm

Monika Szabo,[ab] Tracey Huynh,[a] Celine Valant,[b] J. Robert Lane,[b] Patrick M. Sexton,[b] Arthur Christopoulos*[b] and Ben Capuano*[a]

Positive allosteric modulators (PAMs) targeting the M_4 muscarinic acetylcholine receptor (mAChR) offer greater sub-type selectivity and unique potential as central nervous system agents through their novel mode of action to traditional orthosteric ligands. In an attempt to elucidate the molecular determinants of allostery mediated by the exemplar thienopyridine M_4 mAChR PAM, LY2033298, we report herein a systematic structure-activity relationship (SAR) study investigating different linkage points, halogen replacements to examine size and electronic effects, and different substitution combinations on the thienopyridine scaffold. We applied an operational model of allosterism to determine values of functional affinity (K_B), cooperativity ($\alpha\beta$) and intrinsic agonism (τ_B) for all compounds.

Introduction

The muscarinic acetylcholine receptors (mAChRs) are a class of G protein-coupled receptors (GPCRs) consisting of five subtypes (M_1-M_5) and are expressed in the central nervous system (CNS) and the periphery. The M_4 mAChR is of clinical interest as it has demonstrated an involvement in CNS disorders such as schizophrenia.[1] Specifically, the M_4 mAChR has been shown to alleviate the positive symptoms (hallucinations, delusions) and potentially benefit the cognitive deficits (memory, learning) associated with schizophrenia.[2,3] A significant problem associated with clinically prescribed antipsychotics designed to target a designated GPCR orthosteric site is their promiscuity and therefore lack of receptor selectivity. This phenomenon often results in an extensive side effect profile. Allosteric ligands offer a potential solution to this problem by targeting a topographically distinct site to the orthosteric site. While orthosteric sites tend to be conserved across receptor subtypes, allosteric sites can differ, making subtype selectivity a more eminent possibility.[4] Previous work on the M_4 mAChR has focused on positive allosteric modulators (PAMs), whereby they can exhibit several possible modes of action: potentiation of the binding of the endogenous ligand, acetylcholine (ACh); potentiation of the down-stream efficacy upon binding of the endogenous ligand; and/or direct activation by the allosteric ligand itself.[5] Earlier studies have deduced parameters for allosterism via implementing an operational model of allosterism. This model quantifies the magnitude and direction of the parameters of allosterism including modulator binding affinity, K_B, modulation of binding affinity of the orthosteric ligand, α, modulation of downstream efficacy upon binding of the orthosteric ligand, β, and intrinsic allosteric agonism, τ_B.[6]

There has been considerable research conducted in the field of M_4 PAMs, all of which focus on the thienopyridine scaffold.[7-9] Whilst significant progress has been made, none have matched the pharmacological profile of LY2033298 (Figure 1; **8a**). In attempts to move away from the 5-chloro-6-methoxy-4-methyl substitution pattern on **8a**, researchers at Vanderbilt University (VU) developed a number of analogues based on a 4,6-dimethyl substitution pattern on the thienopyridine motif, such as VU0152100 (Figure 1; **1a**) and VU10004 (**1b**). We have also published work on this scaffold investigating the pharmacological impact of incorporating modifications to the substitution pattern of the arylmethyl motif of VU0152100[10] and replacement of the cyclopropyl moiety of VU10004 with alternative primary cycloalkanamines and cyclic secondary amines.[11]

[a]*Medicinal Chemistry,* [b]*Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville 3052, Victoria, Australia.*
Email: Arthur.Christopoulos@monash.edu, Ben.Capuano@monash.edu
Electronic Supplementary Information (ESI) available: Experimental details for the synthesis and pharmacology for all compounds. See DOI: 10.1039/x0xx00000x

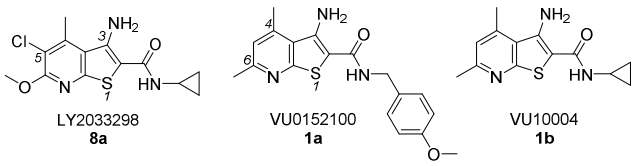


Figure 1 Muscarinic M_4 positive allosteric modulators LY2033298 (**8a**), VU0152100 (**1a**) and VU10004 (**1b**) with numbering systems.

To explore the structure activity relationships (SAR) of **8a**, we initially synthesised a focused series of compounds that encompassed alkyl chain extensions from several strategic points on the chemical structure of **8a** (Figure 2). The aim was to determine suitable tethering points and to observe if the incorporation of larger groups would be tolerated at these positions; with the future aim of structurally integrating either an orthosteric mAChR moiety to generate bitopic ligands[12] or generate dual acting ligands that incorporate a pharmacophore that is selective for another protein target.[13] This information may also be useful in determining the size of the allosteric binding pocket at the M_4 mAChR and therefore the scope for structural diversification. We also assessed the importance of the halogen at the 5-position in relation to both size and electronic effects by substituting with larger halogens (Br and I). In addition, we investigated an isosteric replacement of the C-Cl moiety for N to yield a thienopyrimidine bicyclic system in place of the thienopyridine. Although, the new scaffold is lacking a chlorine atom, it is predicted that the incorporation of an additional nitrogen atom will maintain a relatively electron-deficient six-membered ring of the bicyclic system comparable to that of LY2033298. We also explored different combinations of substituents on the thienopyridine ring of **8a** to ascertain the optimal substitution pattern essential for a PAM profile at the M_4 mAChR. To do this we looked at the following substitution patterns of the thienopyridine core, namely 6-methoxy-4-methyl, 5-chloro-4,6-dimethyl and 4,6-dimethyl. Although these substitutions have been previously reported,[9,14] the underlying operational parameters describing their allosteric effects have not been determined. All compounds were characterized in functional M_4 mAChR-mediated ERK1/2 phosphorylation (pERK1/2) assays and compounds that showed significant differences compared to **8a**, were further profiled in radioligand binding assays.

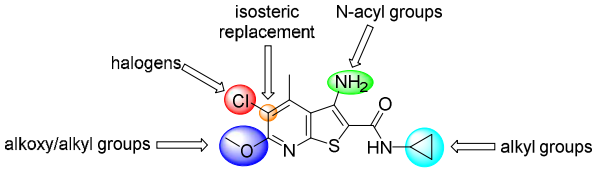
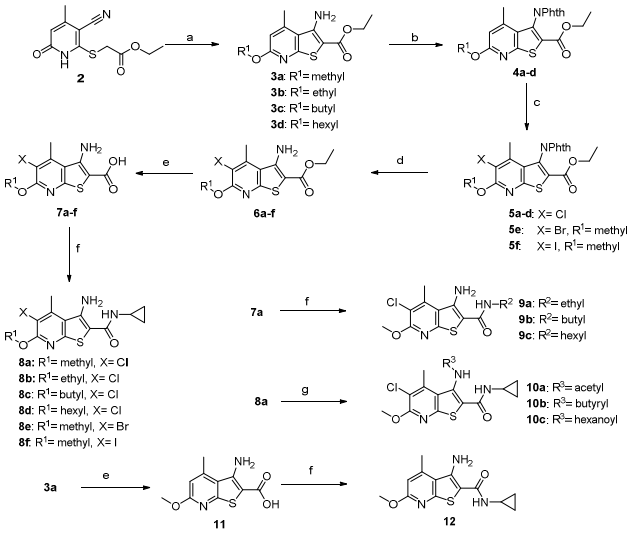


Figure 2 General overview of structural modifications to LY2033298 (**8a**) investigated in this study.

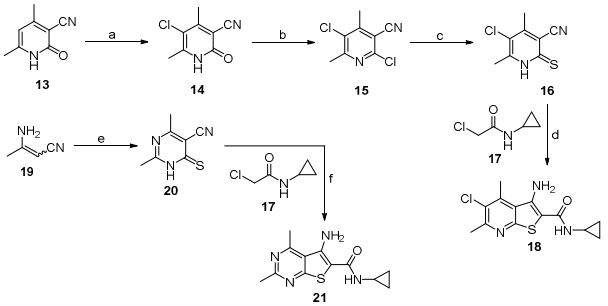
Synthesis

The synthesis of derivatives with alkyl chains extending from the methoxy group at the 6-position (O-alkyl derivatives) are represented in Scheme 1. We commenced the sequence with the monocyclic pyridinone (**2**); the chemistry of which has been previously described.[15] In one step, we combined **2** with the required primary alkyl iodide under mild basic conditions to effect O-alkylation then subsequently added a stronger base (1 M aqueous KOH) to convert the monocyclic intermediates to bicyclic analogues (**3a-d**).[10] The primary aromatic amine of all analogues was protected as the phthalimide (**4a-d**) in good yield using phthalic anhydride prior to halogenating the 5-position (**5a-f**) with the appropriate *N*-halosuccinimide. This strategy was implemented to circumvent unwanted *N*-halogenation. Subsequent phthalimide de-protection under standard conditions of hydrazine monohydrate afforded **6a-f** in good to excellent yield (46-89%). Base-promoted ester hydrolysis (following acidic work-up) furnished the carboxylic acids **7a-f** in similar yields. A BOP-mediated amide coupling reaction was then employed between cyclopropanamine and carboxylic acids (**7a-f**) to generate the final O-alkyl analogues (**8a-f**). The N-alkyl analogues (**9a-c**) were furnished by simply coupling the intermediate carboxylic acid **7a** with the required acyclic alkanamines. Synthesis of the 'amine modified' analogues was carried out by direct acylation of LY2033298 (**8a**) with the required acid chlorides to furnish the target carboxamide derivatives (**10a-c**) in moderate yield (45-48%).



Scheme 1 Synthesis of O-alkyl, N-alkyl, N-acyl and des-chloro derivatives of LY2033298. Reagents and conditions: (a) (i) Alkyl-I, K_2CO_3, DMF, 4 h, RT; (ii) 1 M KOH, 15 min, 58-94%; (b) Phthalic anhydride, AcOH, reflux, 20 h, 48-86%; (c) *N*-halosuccinimide, conc. HCl, EtOH, reflux, 1.5 h, 80-99%; (d) $(NH_2)_2.H_2O$, EtOH, reflux, 3 h, 46-

89%;(e) 2 M NaOH, EtOH, reflux, 1.5 h, 49-96%; (f) Alkanamine, BOP reagent, DIPEA, DMF, RT., 1-12 h, 15-72%; (g) Acid chloride, Et_3N, DCM, N_2, reflux, 2 days, 45-48%.



Scheme 2 Synthesis of LY2033298/VU hybrid and thienopyrimidine analogues. Reagents and conditions: (a) SO_2Cl_2, 1,2- DCE, reflux 6 h, 42%; (b) $POCl_3$, MW, 100 °C, 1h, 88%; (c) Thiourea, EtOH, relux, 24 h, 29%; (d) NaOMe, MeOH, relux, overnight, 30%; (e) Acetyl chloride, NH_4SCN, dioxane, 23%; (f) Na metal, EtOH, reflux 4 h,75%.

Synthesis of des-chloro-LY2033298 (**12**) was achieved in good yield by firstly converting the bicyclic ethyl ester (**3a**) to the free carboxylic acid (**11**) via base hydrolysis and subsequent acidic work-up, followed by a BOP-mediated coupling reaction with cyclopropanamine to install the cyclopropyl moiety.

We envisaged the synthesis of the LY2033298/VU hybrid analogue (**18**) commencing from the pyridinone precursor (**13**) as illustrated in Scheme 2. The first chlorine atom was successfully installed at the 5-position of the substituted pyridinone core using sulfuryl chloride to give intermediate **14**. Subsequent chlorination to furnish the dichloro substituted pyridine (**15**) was effected using phosphoryl chloride. Treatment of **15** with thiourea in ethanol at reflux afforded the versatile pyridinethione (**16**) in moderate yield. Reaction of **16** with pre-synthesised 2-chloro-*N*-cyclopropylacetamide (**17**) under basic conditions produced the target thienopyridine (**18**) with the 5-chloro-4,6-dimethyl substitution pattern. Synthesis of the thienopyrimidine ana-logue (**21**) was successfully achieved by firstly reacting 3-aminocrotononitrile (**19**), acetyl chloride and ammonium thiocyanate to generate the substituted pyrimidine core (**20**), followed by treatment with chloroacetamide **17** and sodium metal in ethanol at reflux to furnish the product in very good yield (75%).

Results and discussion

To characterize the biological activity of the synthesized compounds incorporating O-alkyl (**8b-d**) and N-alkyl chains (**9a-c**) on the LY2033298 scaffold, halogen replacements (**8e-f**) and modification to the aromatic amine functionality (**10a-c**), we tested all analogues in a functional pERK1/2 assay using intact FlpIn-CHO cells stably transfected with the human M_4 mAChR. Compounds were initially tested in time-course assays (data not shown) to determine the optimal incubation time for maximum ERK1/2 phosphorylation for each compound. As the response to all compounds peaked at a similar time to ACh, interaction studies were performed using varying concentrations of ACh (10 μM to 10 nM) at a stimulation time of 6 minutes. The results of these interaction studies are summarised in Table 1. By applying an operational model of allosterism, we derived values of functional affinity (pK_B), intrinsic efficacy (τ_B) and overall cooperativity ($\alpha\beta$) of the allosteric ligand's effect on ACh.

All ligands displayed no notable enhancement in functional affinity when compared across their respective groups, i.e. O-alkyl, N-alkyl or halogen derivatives. For the O-alkyl analogues, there was a significant loss in intrinsic efficacy when proceeding from methyl (**8a**) and ethyl (**8b**) to the butyl (**8c**) and hexyl (**8d**) derivatives. The intrinsic efficacy was maintained between **8a** and **8b** confirming that the addition of the extra carbon at-om has little effect and that anything larger is detrimental to the compound's agonism. The cooperativity of the O-alkyl derivatives with ACh was also maintained, however again the larger butyl (**8c**) and hexyl (**8d**) substituents resulted in a substantial loss in the cooperativity (96- and 120-fold, respectively) when compared with **8a**. Replacement of the chlorine at the 5-position of **8a** with bromine (**8e**) or iodine (**8f**), afforded no significant gain or loss in intrinsic efficacy and cooperativity compared with the parent compound. As such, the thienopyridine scaffold possessing larger halogen atoms with reduced electronegativity is well tolerated by the M_4 mAChR. For the series of N-alkyl analogues synthesized, only the N-hexyl derivative (**9c**) showed a significant drop in intrinsic agonism (22-fold) and cooperativity (185-fold) when compared to the N-cyclopropyl compound (**8a**). There were no significant differences between the N-butyl compound **9b** compared to **8a**, confirming that a butyl linker is tolerated but the additional two carbon atoms significantly affects the agonistic and PAM properties of the ligand. The N-ethyl compound **9a** maintained similar allosteric properties to **8a**, as the ethyl group occupies a similar chemical and physical space to that of the cyclopropyl group.

The general trend observed between the O-alkyl and N-alkyl analogues was as we sequentially increased the alkyl chain length, we observed a decrease in both the intrinsic efficacy and the cooperativity of the compounds with ACh. This is illustrated in Figure 3 with N-alkyl analogues **9a-c** exhibiting a smaller decrease in activity as a function of chain length. As such the allosteric pocket on the M_4 mAChR appears to have a greater degree of space to accommodate larger groups on this side (**9a-c**), which is in agreement with the VU compounds that incorporate larger alkyl and substituted benzyl groups at this position.[9] However, recent evidence on the binding mechanism of allosteric ligands to the M_2 mAChR showed that upon receptor activation and binding of an allosteric ligand, the allosteric site encloses the modulator, creating a tight fitting binding pocket.[16] Therefore major structural changes to PAMs may affect how they can fit in the allosteric site, and

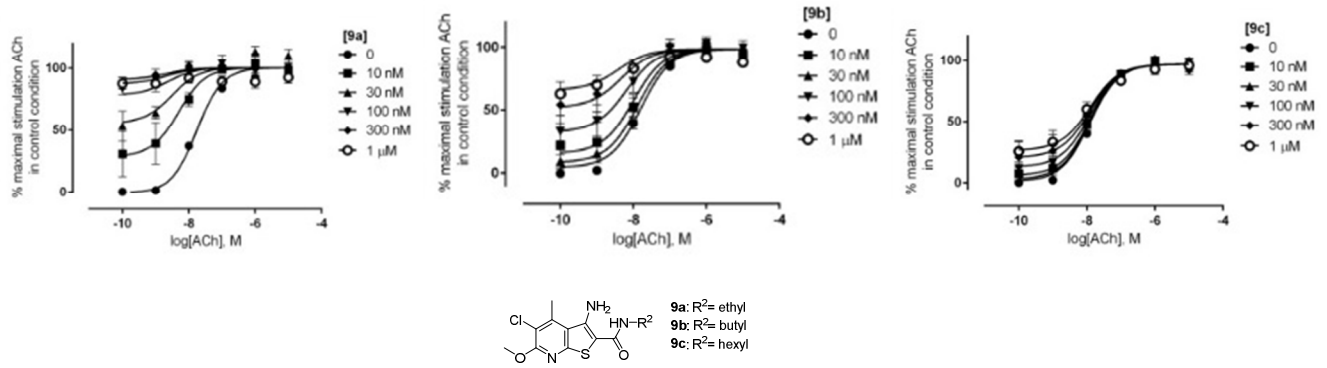


Figure 3 Effect of increasing alkyl chain length on the ability of *N*-alkyl analogues **9a-c** to allosterically modulate the activity of ACh in M_4 mAChR-mediated pERK1/2 assays. Data points represent the mean of three experiments performed in duplicate.

Table 1 Functional ERK1/2 phosphorylation and binding data for LY2033298 (**8a**), *O*-alkyl analogues (**8b-d**), halogen replacement analogues (**8e-f**) and *N*-alkyl analogues (**9a-c**) at the M_4 mAChR

					Functional ERK1/2 phosphorylation data[a,b]			Binding data[a,c]	
C#	**R^1**	**R^2**	**R^3**	**X**	**pK_B(K_B, μM)**	**log τ_B (τ_B)**	**log $\alpha\beta$ ($\alpha\beta$)**	**pK_i (K_i, μM)**	***log α (α)**
8a	Methyl		H	Cl	6.41 (0.4)*	0.93 ± 0.14 (8.5)	2.38 ± 0.23 (240)	6.41 ± 0.11 (0.4)	3.24 ± 0.14 (1738)
8b	Ethyl		H	Cl	6.10 ± 0.37 (0.8)	0.97 ± 0.32 (9.3)	1.79 ± 0.44 (61.7)	6.71 ± 0.16 (0.2)	1.98 ± 0.12 (95.5)
8c	Butyl		H	Cl	6.81 ± 0.40 (0.2)	-0.51 ± 0.20 (0.3)	0.40 ± 0.16 (2.5)	5.57 ± 0.40 (2.7)	1.35 ± 0.16 (22.4)
8d	Hexyl		H	Cl	7.42 ± 0.32 (0.04)	-0.75 ± 0.18 (0.2)	0.30 ± 0.11 (2.0)	nt	nt
8e	Methyl		H	Br	6.52 ± 0.44 (0.3)	1.25 ± 0.40 (17.8)	1.91 ± 0.52 (81.3)	6.28 ± 0.07 (0.52)	2.54 ± 0.11 (347)
8f	Methyl		H	I	6.66 ± 0.33 (0.2)	0.37 ± 0.18 (2.3)	0.97 ± 0.44 (9.3)	5.78 ± 0.09 (1.7)	2.43 ± 0.12 (269)
9a	Methyl	Ethyl	H	Cl	6.73 ± 0.39 (0.2)	0.94 ± 0.33 (8.7)	1.84 ± 0.53 (69.2)	5.37 ± 0.32 (4.3)	2.75 ± 0.36 (562)
9b	Methyl	Butyl	H	Cl	6.29 ± 0.46 (0.5)	0.44 ± 0.30 (2.8)	1.19 ± 0.51 (15.5)	5.50 ± 0.14 (3.2)	2.09 ± 0.18 (123)
9c	Methyl	Hexyl	H	Cl	6.70 ± 0.32 (0.2)	-0.35 ± 0.12 (0.4)	0.13 ± 0.15 (1.3)	nt	nt
10a	Methyl		$COCH_3$	Cl	n/a	n/a	n/a	nt	nt
10b	Methyl		COC_3H_7	Cl	n/a	n/a	n/a	nt	nt
10c	Methyl		COC_5H_{11}	Cl	n/a	n/a	n/a	nt	nt

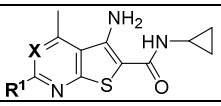
[a]Data represent the mean ± SEM of three-four separate experiments performed in duplicate. [b]Values are obtained from pERK1/2 assays via interaction with varying concentrations of Ach. [c]Values are obtained via radioligand ([^{3}H]NMS) binding assays through interaction with varying concentrations of ACh.*Log α' is calculated by fixing log α to -100. n/a= Compound not active. nt= Not tested in binding assays as cooperativity ($\alpha\beta$) in pERK1/2 assays was very low or compound was not active.*= The pK_B is fixed to the pK_i obtained from binding experiments.

may result in a loss of their ability to exert effects upon the ligand in the orthosteric site.

Due to many analogues showing very similar functional affinity, allosteric modulation and/or intrinsic efficacy to **8a,**

we performed radioligand binding assays to gain greater insight into any subtle differences between the compounds, because the pERK1/2 experiments reflect functional responses that are determined by both ligand binding to the receptor (i.e., affinity) and activation of the receptor (i.e., efficacy), and thus cannot differentiate the individual contributions of allosteric modulator effects on orthosteric ligand affinity (α) versus efficacy (β) on the overall estimated functional cooperativity ($\alpha\beta$). An advantage of performing binding assays is that they provide direct measures of agonist affinity only, and thus any allosteric modulation observed in these assays can be used to derive a direct estimate of the α parameter. Therefore this information can be used in conjunction with the functional assay analysis to deduce the individual contributions of both α and β parameters in driving the PAM effect on ACh. All compounds in Table 1 were tested, excluding **8d** and **9c** as both were very weak PAMs in the pERK1/2 signalling pathway. Compared to **8a**, there was a significant loss in affinity modulation for O-alkyl analogues **8b** and **8c** (18- and 77-fold, respectively). The halogen substituted analogues **8e** and **8f** also had a small but significant loss in affinity modulation (5- and 6-fold, respectively) to that of **8a**. The N-alkyl analogues showed that **9a** was able to maintain a similar affinity modulation to **8a**, whereas extending to the butyl analogue (**9b**) resulted in a 14-fold loss in receptor affinity. This suggests that increasing the size of O-alkyl substituent at the 6-position on the thienopyridine affects modulation of ACh binding affinity. In comparison, substitution of alkyl groups at the amide nitrogen bearing the cyclopropyl group is tolerated, but diminishes with greater than two carbon length alkyl chains compared to **8a**. Table 1 highlights that changes to the thienopyridine scaffold of **8a** can be tolerated, however larger structural changes such as hexyl chains on both the O-alkyl and N-alkyl analogues are detrimental to the allosteric modulatory properties of the ligand.

Table 2 LY2033298 core variants (**12**, **18** and **21**) in functional ERK1/2 phosphorylation assays at the M_4 mAChR



Compd	R^1	X	pK_B (K_B, μM)	log τ_B (τ_B)	log $\alpha\beta$ ($\alpha\beta$)
12	OMe	CH	5.55 ± 0.38 (2.8)	0.66 ± 0.30 (4.6)	1.46 ± 0.39 (28.8)
18	Me	C-Cl	6.45 ± 0.28 (0.4)	1.11 ± 0.26 (12.9)	1.89 ± 0.36 (77.6)
21	Me	N	4.90 ± 0.26 (12.5)	0.49 ± 0.16 (3.1)	1.15 ± 0.27 (14.1)
VU10004[11]	Me	CH	5.37 ± 0.14 (4.3)	0.77 ± 0.11 (5.9)	1.72 ± 0.17 (52.5)

Data represent the mean ± SEM of three separate experiments performed in duplicate. Values are obtained via interaction with varying concentrations of ACh.

There are examples of ligands in the literature whereby substitution at the 6-position of the thienopyridine scaffold with pyridinylmethyl or morpholine derivatives show activity, however groups longer and more lipophilic tend to decrease the efficacy of the compounds.[8] While it has been previously reported that the combination of the VU scaffold (4,6-dimethyl thienopyridine) and substitution at the primary aromatic amine is mostly tolerated but results in a loss of activity[9], we found that the scaffold of **8a** (5-chloro-4-methyl-6-methoxy thienopyridine) in combination with acylation of the primary aromatic amine (**10a-c**) abolished activity completely. The next part of our study was to elucidate if structural changes to the pyridine ring of the thienopyridine scaffold of **8a** had any major influences on its allosteric properties and to pinpoint what substitutions are crucial for its activity. The results of compounds **12**, **18**, **21** and VU10004 are summarised in Table 2. The pyrimidine derivative (**21**), which was used to mimic the electronegativity and ring deactivating effects of the chlorine at that position whilst also reducing the chemical and physical space, had no significant advantages as compared to the chlorine at that same position. There was no statistical significance for compounds **12**, **18**, **21** and VU10004 when compared to **8a** and compared to each other in relation to the functional affinity, intrinsic agonism and cooperativity of the compounds. As such these results highlight that the thienopyridine is able to tolerate small structural changes to its pyridine ring, however it is not clear what substitutions are essential for its activity and allosteric properties. Combined with the results from Table 1, the pharmacology of thienopyridine scaffold appears to be more affected when substituted with longer and more lipophilic groups. Changes to the scaffold itself, neither enhance nor diminish the pharmacological profile. The results do however reveal that a simple change from a butyl to a hexyl chain can significantly alter the pharmacological profile. It is also possible that the O- and N-alkyl chains may be too flexible and therefore more rigid functional groups should be employed at these relative positions of **8a**.

Conclusions

We performed a comprehensive SAR study of the PAM **8a** by (i) substituting progressively longer O- or N-alkyl chains from the scaffold through the ether linkage (**8b-d**), (ii) halogen replacement (**8e-f**), (iii) amide derivatives via acylation of the primary aromatic amine (**9a-c**) and (iv) different substitution combinations around the pyridine ring (**12**, **18** and **21**). The allosteric properties of the ligands progressively decreased when substituting alkyl chains

greater than two carbon atoms from either side of **8a** (Table 1 and Figure 2). Additionally, halogen replacements were tolerated, but significantly decreased the ability of the compounds to positively modulate agonist affinity as compared to **8a**, confirming that compound **8a** largely modulates the affinity of ACh in the orthosteric pocket of the M_4 mAChR rather than modulating the efficacy. Acylation of the primary aromatic amine (**10a-c**) completely abolished activity. Due to the small molecule nature of PAMs at the M_4 mAChR and the recent advances into the understanding of mechanisms of how they bind to muscarinic receptors[16], the possibility of tethering larger functional groups for a dual acting or a potential bitopic mode of action based on the scaffold of **8a** is likely to prove challenging. Furthermore, whilst we can identify subtle differences between PAMs by utilizing the operational model of allosterism, it is difficult to determine exactly what functional groups on the thienopyridine scaffold of **8a** are important for its PAM mode of action (Table 2). Nonetheless, these results show the benefits of profiling ligands using operational models of allosterism to gain a more comprehensive appraisal into the contribution of each of the parameters for a PAM. Additionally the results represent useful SAR towards the understanding and development of the thienopyridine scaffold of PAMs targeting the M_4 mAChR.

Acknowledgements

This research was supported by the project grant APP1049564 and program grant APP1055134 of the National Health and Medical Research Council (NHMRC). A.C. is a principal research fellow (NHMRC). J.R.L. is a R.D. Wright Biomedical Career Development Fellow (APP1052304, NHMRC) and a Larkin's Fellow (Monash University, Australia). M.S. and T.H. acknowledge an Australian Postgraduate award and M.S. acknowledges a Monash Post Graduate Publication Award.

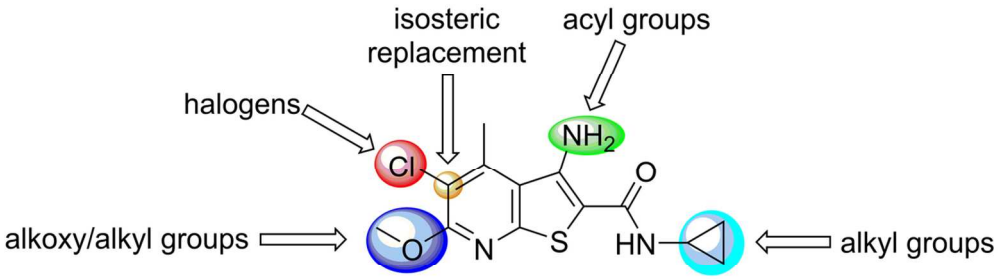
Notes and references

1. Langmead, C. J., Watson, J., Reavill, C., *Pharmacol. Ther.*, 2008, **117**, 232-243.
2. Money, T. T., Scarr, E., Udawela, M., Gibbons, A. S., Jeon, W. J., Seo, M. S., Dean, B., *CNS Neurol. Disord.- Drug Targets*, 2010, **9**, 241-256.
3. Raedler, T. J., *Psychiat. Times*, 2008, **25**, 14-15+16.
4. May, L. T., Leach, K., Sexton, P. M., Christopoulos, A., *Annu. Rev. Pharmacol. Toxicol.*, 2007, **47**, 1-51.
5. Gregory, K. J., Sexton, P. M., Christopoulos, A., *Curr. Neuropharmacol.*, 2007, **5**, 157-167.
6. Keov, P., Sexton, P. M., Christopoulos, A., *Neuropharmacology*, 2011, **60**, 24-35.
7. Brady, A. E., Jones, C. K., Bridges, T. M., Kennedy, J. P., Thompson, A. D., Heiman, J. U., Breininger, M. L., Gentry, P. R., Yin, H., Jadhav, S. B., Shirey, J. K., Conn, P. J., Lindsley, C. W., *J. Pharmacol. Exp. Ther.*, 2008, **327**, 941-953.
8. Kennedy, J. P., Bridges, T. M., Gentry, P. R., Brogan, J. T., Kane, A. S., Jones, C. K., Brady, A. E., Shirey, J. K., Conn, P. J., Lindsley, C. W., *ChemMedChem*, 2009, **4**, 1600-1607.
9. Shirey, J. K., Xiang, Z., Orton, D., Brady, A. E., Johnson, K. A., Williams, R., Ayala, J. E., Rodriguez, A. L., Wess, J., Weaver, D., Niswender, C. M., Conn, P. J., *Nat. Chem. Biol.*, 2008, **4**, 42-50.
10. Huynh, T., Valant, C., Crosby, I. T., Sexton, P. M., Christopoulos, A., Capuano, B., *J. Med. Chem.*, 2013, **56**, 8196-8200.
11. Huynh, T., Valant, C., Crosby, I. T., Sexton, P. M., Christopoulos, A., Capuano, B., *ACS Chem. Neurosci.*, 2015, **6**, 838-844.
12. Lane, J. R., Sexton, P. M., Christopoulos, A., *Trends Pharmacol. Sci.*, 2013, **34**, 59-66.
13. Mohr, K., Tränkle, C., Kostenis, E., Barocelli, E., De Amici, M., Holzgrabe, U., *Brit. J. Pharmacol.*, 2010, **159**, 997-1008.
14. Le, U., Melancon, B. J., Bridges, T. M., Vinson, P. N., Utley, T. J., Lamsal, A., Rodriguez, A. L., Venable, D., Sheffler, D. J., Jones, C. K., Blobaum, A. L., Wood, M. R., Daniels, J. S., Conn, P. J., Niswender, C. M., Lindsley, C. W., Hopkins, C. R., *Bioorg. Med. Chem. Lett.*, 2013, **23**, 346-350.
15. Szabo, M., Klein Herenbrink, C., Christopoulos, A., Lane, J. R., Capuano, B., *J. Med. Chem.*, 2014, **57**, 4924-4939.
16. Kruse, A. C., Ring, A. M., Manglik, A., Hu, J., Hu, K., Eitel, K., Hubner, H., Pardon, E., Valant, C., Sexton, P. M., Christopoulos, A., Felder, C. C., Gmeiner, P., Steyaert, J., Weis, W. I., Garcia, K. C., Wess, J., Kobilka, B. K., *Nature*, 2013, **504**, 101-106.

MedChemComm

ARTICLE

TOC graphic



M_4 muscarinic positive allosteric modulator, **LY2033298**

58x28mm (600 x 600 DPI)