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Synthesis and Pharmacological Evaluation of Heterocyclic Carboxamides: Positive Allosteric Modulators of the M_1 Muscarinic Acetylcholine Receptor with Weak Agonist Activity and Diverse Modulatory Profiles

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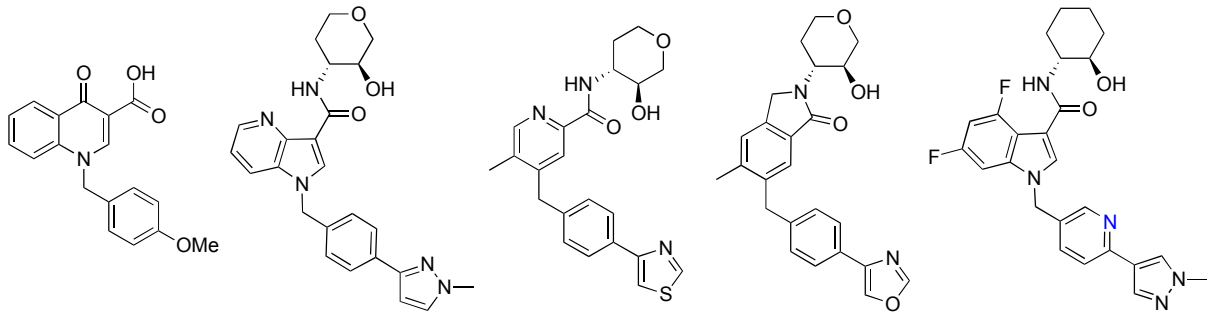
ABSTRACT: Targeting allosteric sites at M_1 muscarinic acetylcholine receptors is a promising strategy for the treatment of Alzheimer's disease. Positive allosteric modulators may not only potentiate binding and/or signaling of the endogenous agonist acetylcholine (ACh), but may also possess direct agonist activity (thus referred to as PAM–agonists). Recent studies suggest that PAM-agonists with robust intrinsic efficacy are more likely to produce adverse effects *in vivo*. Herein we present the synthesis and pharmacological evaluation of a series of pyrrole-3-carboxamides with a diverse range of allosteric profiles. We proposed structural modifications at top, core or pendant moieties of a prototypical molecule. Although, generally, there was a correlation between the degree of agonist activity and the modulatory potency of the PAMs, some derivatives displayed weak intrinsic efficacy yet maintaining strong allosteric modulation. We also identified molecules with the ability to potentiate mainly the affinity or both affinity and efficacy of ACh.

■ INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder manifested by the decline of cognitive function including memory, attention and language. The most recent World Alzheimer Report prevalence study estimated that 47 million people worldwide were suffering from AD in 2015 and further projected that without advances in rational drug therapy, the number of symptomatic cases might reach 131 million by 2050.[1] Dysfunction of cholinergic neurons and deficient acetylcholine (ACh) levels are believed to be the earliest events that trigger the pathology.[2–4] Based on these findings, targeting cholinergic receptors is a promising strategy to slow down the disease progression and ameliorate symptoms.[5] In particular, the M_1 muscarinic acetylcholine receptor (M_1 mAChR) subtype has been linked to the process of cognition, thus considered an attractive therapeutic target.[6,7] Consequently, current pharmacotherapy for AD includes acetylcholinesterase inhibitors (i.e. anticholinesterases), which are able to increase the levels of the endogenous orthosteric ligand ACh. Even though these drugs provide some symptomatic relief they are not well tolerated. The indiscriminate activation of all mAChR subtypes (M_1-M_5) leads to significant cholinergic toxicity, typically causing gastrointestinal and cardiovascular adverse effects, which are mainly mediated by activation of the peripheral M_2 and M_3 mAChRs.[8] For many years researchers had sought to develop M_1 mAChR selective agonists, and despite the numerous efforts in this area, little was achieved.[9] The recent high resolution crystal structures of the mAChRs[10–12] confirmed that this approach is unlikely to succeed due to the high homology of the orthosteric binding pocket across all five mAChR subtypes.

A new therapeutic opportunity has emerged from targeting the topographically distinct less-conserved allosteric site on M_1 mAChRs which proved to be a fruitful strategy to overcome the lack of receptor subtype selectivity of orthosteric ligands. BQCA (**1**, Figure 1) was the first selective positive allosteric modulator (PAM) of the M_1 mAChR to be described in the literature.[13] Subsequent to its disclosure a vast range of novel chemotypes was disclosed, and insights into the molecular basis of receptor-ligand interactions[12,14–17] as well as enriched structure-activity

relationship (SAR) studies[18–24] have been published. Allosteric molecules may also possess direct agonist activity in their own right, and are referred to as 'allosteric agonists' if they activate the receptor but do not modulate the orthosteric ligand, or 'PAM-agonists' (also 'ago-PAMs') if they possess both positive allosteric modulatory properties and agonism.[25] Although several M_1 PAM-agonists with aligned physicochemical properties and efficacy in rat cognitive function models were developed, recent studies reported evidence of cholinergic toxicity associated with highly selective M_1 PAMs.[22–24,26] The authors suggested that overactivation of the M_1 mAChR, as a result of the direct agonist activity of PAM-agonists, could underlie the AEs. It was therefore hypothesized that pure M_1 PAMs would have improved safety profiles.



BQCA (1)
PAM EC_{50} : 823 nM
Ago EC_{50} : NA
τ_B: 2.09

PF-06764427 (2)
PAM EC_{50} : 30 nM
Ago EC_{50} : 590 nM
τ_B: 13.2

PF-06767832 (3)
PAM EC_{50} : 60 nM
Ago EC_{50} : 3900 nM
τ_B: 10.5

PF-06827443 (4)
PAM EC_{50} : 47 nM
Ago EC_{50} > 5000 nM
τ_B: NA

VU6004256 (5)
PAM EC_{50} : 210 nM
Ago EC_{50} : 4800 nM
τ_B: NA

Figure 1. Examples of M_1 mAChR PAM-agonists from Merck **1**[13], Pfizer **2**,**3**,**4**[21,22,24] and Vanderbilt **5**[23] with the relevant pharmacological data. PAM EC_{50} concentrations were obtained using calcium mobilization assays in the presence of a fixed EC_{20} concentration of ACh (methodologies may differ slightly). Allosteric agonist EC_{50} concentrations (Ago EC_{50}) were obtained from concentration-response curves for the modulators in the absence of ACh; τ_B values represent a measure of the efficacy of the modulators derived from operational model-fitting, and were estimated in the present work from IP_1 accumulation assays. NA - not available.

Recently, Pfizer published the SAR associated with a series of potent azaindole M_1 mAChR PAM-agonists, with the lead compound being PF-06764427 (**2**).[21] Shortly after, the same group disclosed a pyridine series (lead compound PF-06767832 – **3**), also reporting *in vivo* adverse effects

for this class of compounds.[22] A subsequent study reported a series of γ- and δ-lactam PAMs (lead compound PF-06827443 – **4**), structurally related to pyridine derivative **3**, which, despite displaying less agonist activity, presented adverse effects.[24] An independent study by the Vanderbilt Center for Neuroscience Drug Discovery investigated the adverse effect liability of compound **2** together with their fluorinated indole derivative, VU6004256 (**5**).[23] They have reported that **5** acts as a M_1 mAChR PAM-agonist *in vitro*, and unlike compound **2** enhances recognition memory without severe behavioral convulsions and peripheral cholinergic effects in rodents. Nonetheless, it was not possible to draw a clear correlation between the subtle structural changes of the studied compounds and their pharmacological and safety profiles.[23] It has therefore remained unclear which structural features drive allosteric agonism relative to allosteric modulation or contribute to adverse effect liability.

Herein, we report the development of a pyrrole-3-carboxamide series closely related to compound **2** and detailed pharmacological characterization of selected PAMs in this series. We performed systematic modifications on three different regions (top, core and pendant, Figure 2) of a prototypical molecule **6a** in order to gain molecular diversity. Representative building blocks were selected to be incorporated in each of the regions of the molecule, which included revisiting some of the moieties used in previous studies by Pfizer[21,22,24] and Vanderbilt University.[23] We have obtained an enriched allosteric SAR profile by dissecting different pharmacological parameters (i.e. binding affinity; K_B, binding and functional cooperativity with ACh; α and $\alpha\beta$, respectively, and direct allosteric agonist efficacy; τ_B),[27,28] thus providing new insights into the structural basis of allosteric modulation.

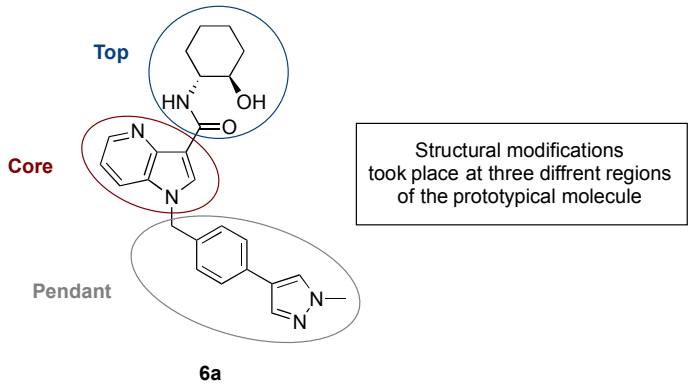


Figure 2. Chemical structure of the representative M_1 mAChR PAM-agonist **6a**, labeling top, core and pendant components of the molecule.

Our optimization efforts were focused towards the development of M_1 mAChR PAMs with distinct pharmacological properties, in particular PAMs with weak agonist activity. The full pharmacological characterization of six selected PAMs in our series and three literature compounds, in binding and functional assays, indicated that there was no correlation between the binding and functional cooperativity with ACh, suggesting that these PAMs could modulate only the affinity or both affinity and efficacy of ACh. However, there was a significant correlation between the intrinsic efficacy of PAMs and their functional cooperativity with ACh, consistent with a two-state model of action.[28,29]

■ RESULTS AND DISCUSSION

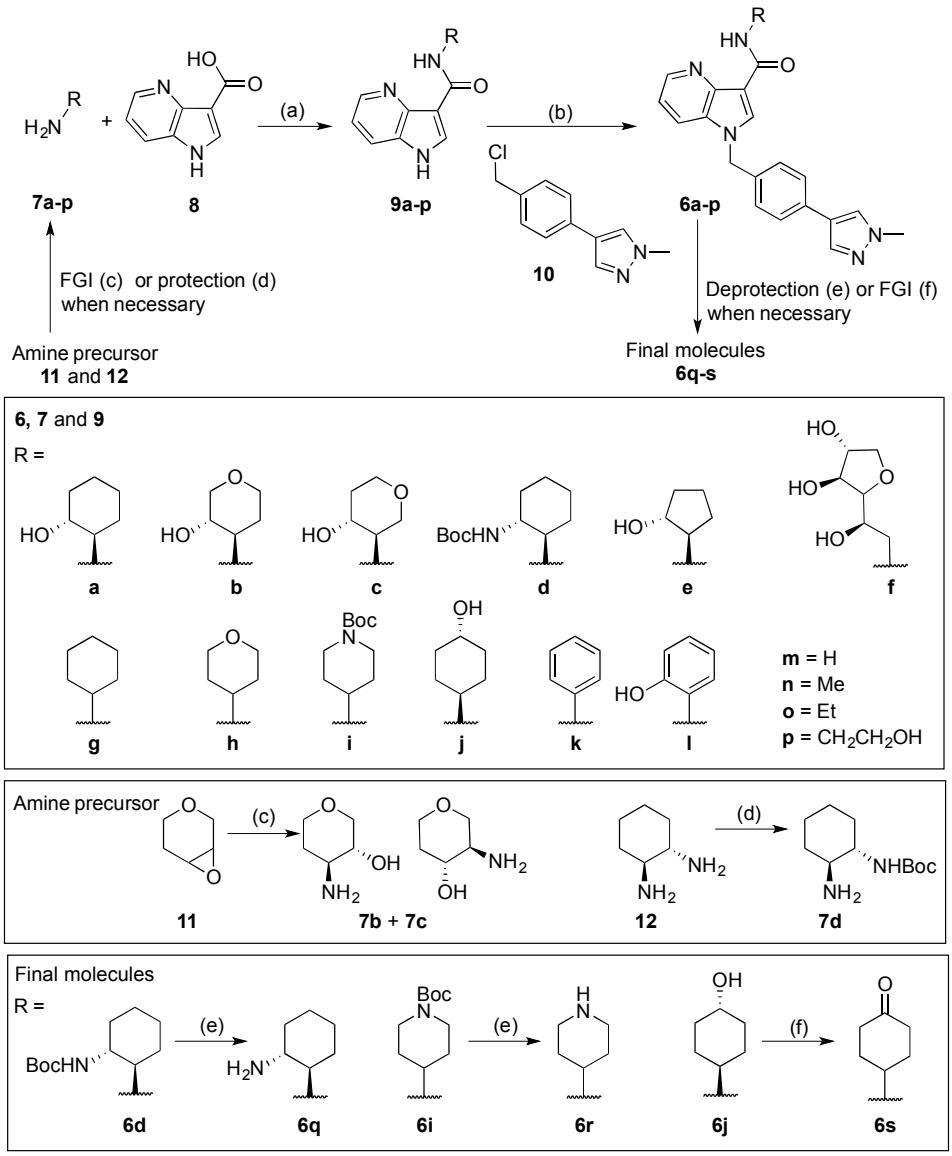
Chemistry. We first synthesized our prototypical compound **6a**, which consists of a hydroxycyclohexyl top, 1*H*-pyrrolo[3,2-*b*]pyridine core and a 4-(1-methyl-1*H*-pyrazol-4-yl)benzyl pendant. The 2-hydroxycyclohexylcarbamoyl motif was first employed in Merck analogues[30] and has been present in many M_1 PAMs ever since. It appears to be well tolerated and maintain cooperativity in a range of different scaffolds. The 1*H*-pyrrolo-[3,2-*b*]pyridine core (also referred to as 4-azaindole) was maintained in order to preserve the intramolecular hydrogen bond (IMHB) between the fused pyridine nitrogen and the carboxamide hydrogen in some of our analogues. This IMBH was observed in both ^{1}H NMR and computational torsional energy experiments of **2** and it was considered key to pre-organize the ligand into a putative bioactive conformation thus diminishing interaction-energy requirements to a favorable binding.[21] Finally, the 4-(1-methyl-1*H*-pyrazol-4-yl)benzyl moiety was chosen as it has been used in a range of M_1 mAChR allosteric ligands (similar to the moiety in the PAM-agonist **5**), and has shown to contribute to affinity towards the receptor.[18–20] While studying structural modifications in certain regions of the molecule the remaining two other key moieties were kept unaltered allowing a direct comparison with **6a**.

We used a practical two-step synthetic route (Schemes 1-4), thereby taking advantage of two key disconnection points of prototypical compound **6a**, namely the C-N bonds of the carboxamide group and the *N*-alkylated nitrogen of the azaindole core, to obtain a variety of compounds. A collection of 1*H*-indole-3-carboxylic acids and related building blocks were commercially available, thus allowing the installation of a variety of amines on the top part of the molecule via amide coupling. *N*-Alkylation was achieved by nucleophilic substitution by the secondary amine of the core structure with the corresponding benzyl halide pendant. The modifications to the top region included *N*-substituted homo- or heterocyclic alcohols and their amine bioisosteres as well as acyclic and aromatic amides (Scheme 1-2). The core modifications were largely based on bicyclic scaffolds related to indole, halogenated bicyclic analogues and the truncated pyrrole, where the peripheral fused aryl ring was absent (Scheme 3). Finally, for the pendant modifications, we

deleted or replaced the *N*-methylpyrazole moiety at the 4-position of the benzyl group by bioisosteric motifs (oxazole, thiazole) or more simple substituents (–OMe, –COOMe, –COOH or –$CONH_2$) (Scheme 4). These modifications allowed us to explore the structural determinants of the different allosteric features (affinity, cooperativity with ACh and intrinsic efficacy of PAMs).

The synthesis of *N*-substituted carboxamides from a variety of primary amines **7a-p** is shown in Scheme 1. The reaction of *trans*-2-aminocyclohexanol (**7a**) and 4-azaindole-3-carboxylic acid (**8**) using HCTU (2-(6-chloro-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate) as the coupling agent afforded the amide **9a** in 46% yield. Attempts with different coupling reagents, including HATU (2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate) and EDC•HCl (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride), produced significantly lower yields of the desired product (29% and 15% yield, respectively). Amides **9b-p** were obtained from their parent amines **7b-p** by applying the same methodology used for the synthesis of **9a**, giving yields ranging from 21 to 45%. Some of the amines were prepared from suitable starting materials or Boc-protected prior to incorporation (Scheme 1, bottom). The reaction of 3,7-dioxabicyclo[4.1.0]heptane (**11**) and concentrated ammonium hydroxide aqueous solution resulted in both positional isomers *trans*-aminotetrahydro-2*H*-pyranol **7b** and **7c** in a 1:1 ratio, which were separable by column chromatography yielding 30% of each isomer. Boc-protected amine **7d** was prepared using an excess of *trans*-1,2-diaminocyclohexane **12** to avoid the doubly protected product. The desired amine **7d** was obtained in 15% yield after column chromatography.

Scheme 1. Synthetic route to obtain a diverse range of top motifs[a,b]



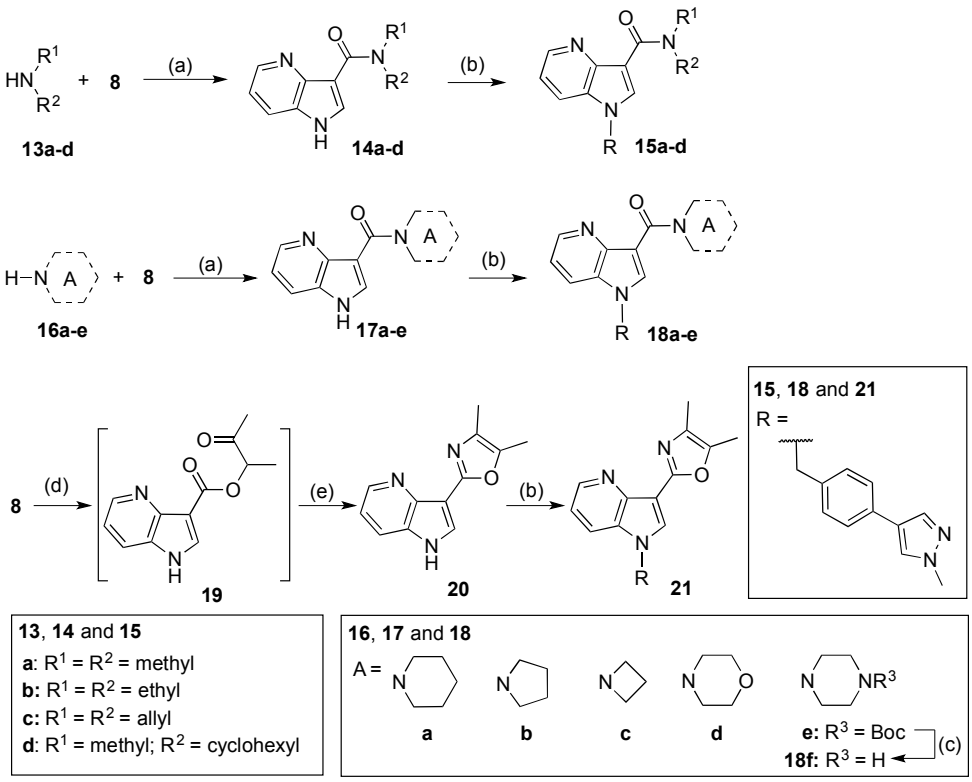
[a]Reagents and conditions: (a) HCTU, DIPEA, DMF; 21–46% yield, (b) **10**, K_2CO_3, DMF; 58–94% yield (c) $NH_4OH_{(aq)}$, MeOH; 38% and 33% yield for **7b** and **7c** respectively, (d) Boc_2O, DCM/dioxane, 0 ˚C to rt; 15% yield, (e) TFA, DCM, 0 ˚C to rt; 67% and 86% yield for **6q** and **6r** respectively (f) CrO_3, H_2SO_4, H_2O, acetone, 0 ˚C to rt; 13% yield. [b]All reactions were conducted overnight at rt, unless otherwise stated.

The second step was the *N*-alkylation to attach the 4-(1-methyl-1*H*-pyrazol-4-yl)benzyl moiety to intermediates **9a-p**. The benzyl chloride **10** was prepared in-house (see Scheme 4). We used a convergent synthesis, preparing this moiety prior to the nucleophilic substitution in order to avoid one additional step for the synthesis of each individual analogue. Alternatively, intermediates **9a-p**

would have had to be *N*-alkylated with bromobenzyl bromide followed by a Suzuki reaction to introduce the pyrazole moiety. The *N*-alkylations were performed in DMF using potassium carbonate as base obtaining compounds **6a-p** in good yields from 58-94% (except for compound **6l**). The synthesis of **6l** afforded two byproducts due to *O*-alkylation of the phenolic group. The more complex mixture forced us to adopt a different purification strategy, which included the use of preparative HPLC, resulting in a final yield of 18%. Boc-protection was essential for the synthesis of intermediates **6d** and **6i** to prevent alkylation of the free amino groups. These derivatives were subsequently subjected to Boc-deprotection (Scheme 1, bottom) under acidic conditions to give the final compounds **6q** and **6r** in good yields. Ketone analogue **6s** was obtained in 13% yield by oxidation of the hydroxyl group of derivative **6j** using Jones reagent. The reaction was stopped as soon as byproducts were detected by TLC, even though total consumption of the starting material was not observed at this point. Although alternative oxidizing agents could be considered, ample material was obtained for pharmacological evaluation therefore optimization of this reaction was not attempted.

One of the most promising derivatives of our initial series was the primary amide **6m**. Therefore, we designed structurally related *N,N*-disubstituted amides (Scheme 2). Both acyclic **13a-d** and cyclic **16a-e** amines were installed using the same approach presented before. Intermediates **14a-d** (open carbon chains) were obtained in 17-25% yield and **17a-e** (cyclic analogues) in 30-51% yield. Final compounds **15a-d** and **18a-e** were obtained upon *N*-alkylation of the corresponding amide intermediates (**14**, **17**) with the benzyl chloride pendant **10**. The unprotected piperazine derivative **18f** was afforded after Boc-deprotection of **18e** under acid conditions (Scheme 2). As a bioisostere of **6m**, compound **21** was synthesized by functionalizing the carboxylic acid group in **8** to the corresponding ester in **19** and then undergoing a cyclocondensation to obtain the oxazole top motif in **20**. The next step consisted of the introduction of the pendant group to afford **21**. We failed to obtain the mentioned oxazole compound by reacting carboxamide **6m** and 2-chloro-3-butanone following literature methodologies.[31,32]

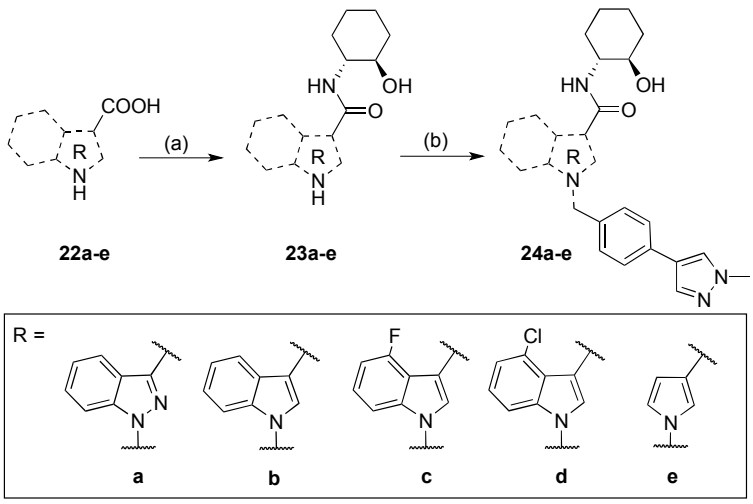
Scheme 2. Synthesis of *N,N*-disubstituted amides and oxazole top motifs[a,b]



[a]Reagents and conditions: (a) HCTU, DIPEA, DMF; 17-25% for **14a-d** and 30-51% yield for **17a-e** (b) **10**, K_2CO_3, DMF; 46-55% for **15a-d**, 58-98% for **18a-e** and 19% yield for **21** (c) TFA, DCM, 0˚C to rt; 56% yield (d) 2-hydroxy-3-butanone, EDC•HCl, DMAP, DMF; **19** was not isolated (e) NH_4OAc, AcOH, 120 ˚C; 2% yield. [b]All reactions were conducted overnight at rt, unless otherwise stated.

A smaller series of analogues with modifications on the core region of **6a** was achieved by attaching the key moieties (**7a** and **10**) to commercially available heterocyclic carboxylic acids (**22a-e**) (Scheme 3). Amide intermediates **23a-e** were obtained from their corresponding parent carboxylic acids **22a-e** by applying the same amide coupling conditions used in the synthesis of **9a**, in yields ranging from 34-66%. The benzyl pendant **10** was installed as described previously. The electronic withdrawing character of the core moiety had the opposite effect on the yields of the *N*-alkylation since the nucleophile strength depends on the availability of the lone pair of electrons on the aryl-1*H*-pyrrole nitrogen. As a result, the lowest yields were observed for **24c** and **24d** (54% and 47%, respectively), while **24b** was synthesized in 82% yield.

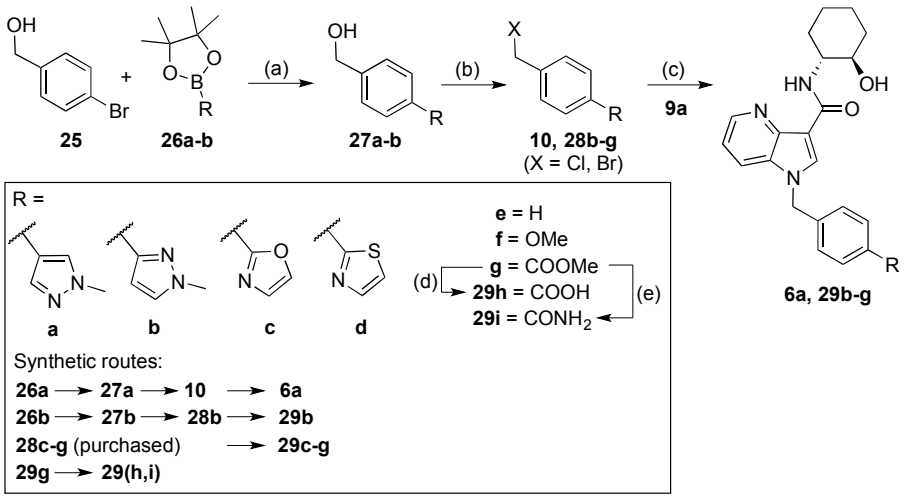
Scheme 3. Synthetic route to obtain a diverse range of core motifs[a,b]



[a]Reagents and conditions: (a) HCTU, *trans*-2-aminocyclohexanol **7a**, DIPEA, DMF; 34-66% yield (b) **10**, K_2CO_3, DMF; 47-82% yield. [b]All reactions were conducted overnight at rt.

The final set of compounds shown in Scheme 4 possess different benzyl pendants. Benzyl alcohols **27a** and **27b** were prepared in-house through Suzuki coupling between 4-bromobenzyl alcohol **25** and the corresponding 1-methylpyrazole boronic acid pinacol esters. Next, the alcohol was activated using thionyl chloride to give the corresponding substituted benzyl chlorides **10** (from **27a**) and **28b** (from **27b**). Attempts to produce the benzyl bromide analog of **10** were unsuccessful. All classic methodologies of bromination (using hydrogen bromide, phosphorus tribromide and NBS) failed to yield the expected product or resulted in a complex mixture of products. Benzyl halides **28c-g** were purchased from commercial suppliers rather than prepared in-house. Compounds **29b-g** were obtained after *N*-alkylation of carboxamide **9a** with the respective benzyl halide **28b-g**. Final derivatives **29h** and **29i** were afforded by hydrolysis or aminolysis of the methyl ester **29g**, respectively.

Scheme 4. Synthetic route to obtain compounds with a range of pendant motifs[a,b]



[a]Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, THF/1 M Na_2CO_3, reflux; 70-72% yield (b) $SOCl_2$, DCM, 0 ^oC to rt; 95% yield (c) **9a**, K_2CO_3, DMF; 26-83% yield (d) $EtOH/NaOH_{(aq)}$, reflux; 45% yield (e) $MeOH/NH_4OH_{(aq)}$, reflux, 3 d; 32% yield. [b]All reactions were conducted overnight at rt, unless otherwise stated.

Pharmacology. We previously established a rational analysis methodology to describe allosterism and provided enriched SAR of BQCA analogues[18] as well as exploring different M_1 - PAM chemotypes, 4-phenylpyrphenylpyridinones[19] and fused arylpyrimidinones.[20] This strategy allowed us to correlate chemical modifications to the changes in parameters that describe allosteric modulation and agonism at the M_1 mAChR. Here, we adopted a similar approach to evaluate the allosteric features of our selected heterocyclic carboxamide derivatives.

Our initial IP_1 accumulation assays provided a robust method to assess the modulation of ACh response by 41 novel putative M_1 mAChR PAMs at 1 μM and 10 μM. The ability of the novel PAMs to modulate the ACh concentration-response curves was determined by calculating the difference in pEC_{50} (ΔpEC_{50}) as a surrogate for the cooperativity ($\alpha\beta$), and the increase in baseline (Δbaseline) was used as a surrogate for the intrinsic efficacy (τ_B) of the test allosteric ligand. Figure 3 shows the magnitude of the increase in potency of the ACh concentration-response curve and the elevation in the baseline in the presence of 10 μM of the test compound compared to ACh alone (ΔpEC_{50} vs. Δbaseline). In this manner, we could systematically track the chemical modifications

yielding distinct pharmacological properties, such as weak intrinsic efficacy (low Δbaseline) while preserving cooperativity (large ΔpEC_{50}).

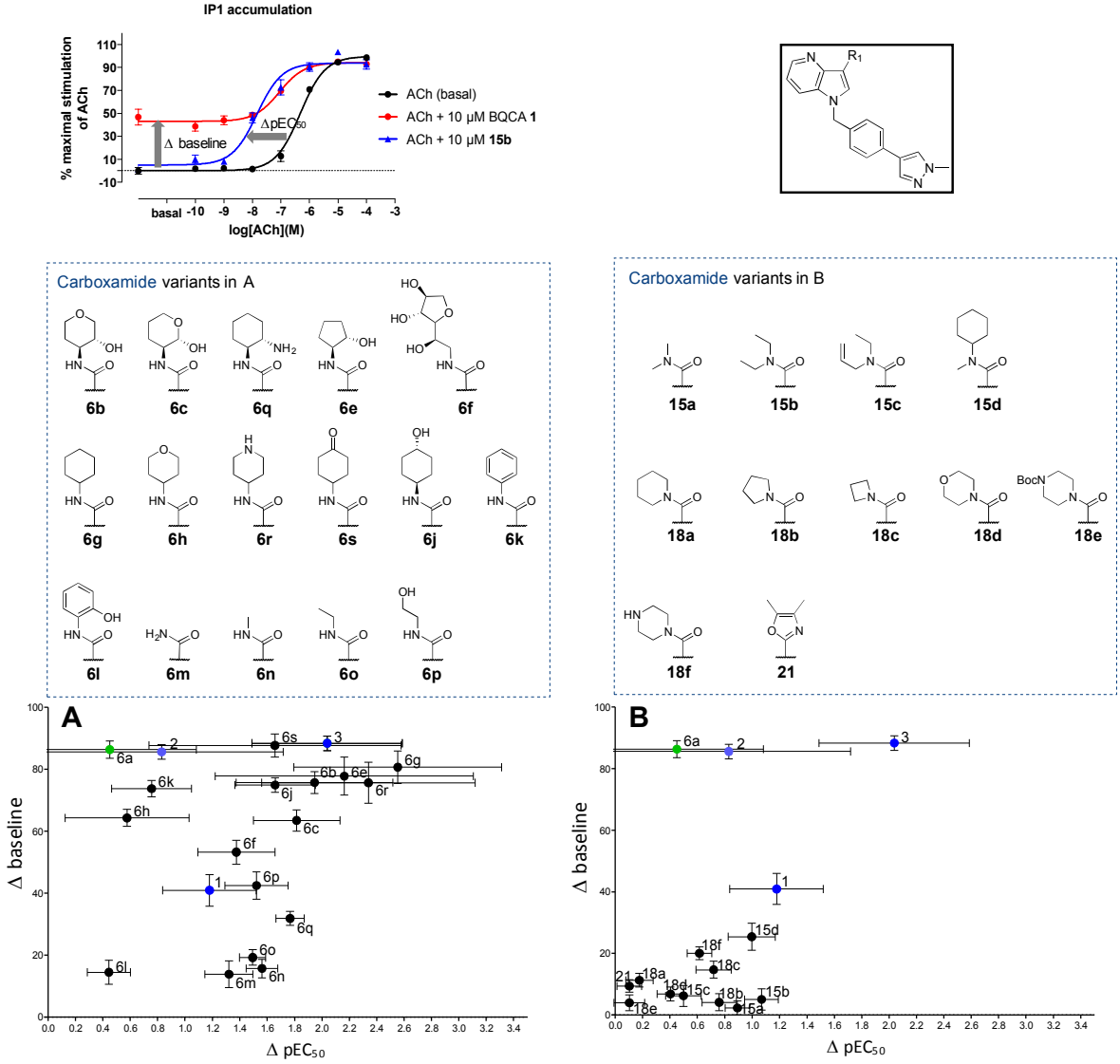


Figure 3. Plot of ΔpEC_{50} *vs* Δbaseline for carboxamide variants. Parameters were estimated from ACh concentration-response curves in the presence or absence of 10 μM of each compound in IP_1 accumulation assays in FlpIn CHO cells stably expressing the hM_1 mAChR. Plotted values represent the means ± SEM of at least three experiments performed in duplicate. ΔpEC_{50} = pEC_{50} of (ACh + 10 μM compound) – pEC_{50} of ACh (basal). Δbaseline = maximal stimulation of (ACh basal + 10 μM compound) – maximal stimulation of ACh (basal). ΔpEC_{50} was used as a surrogate of functional cooperativity for 10 μM of the modulator and Δbaseline as a surrogate of its direct agonism at the same concentration. In all graphs **1**, **2**, **3** (in blue) and **6a** (green) were included as reference compounds. Graphs: **A**. Plot of *N*-substituted carboxamides **6b-s** – top variants. **B**. Plot of *N,N*-disubstituted carboxamides **15a-d**, **18a-f** and **21** – top variants. The estimated ΔpEC_{50} and Δbaseline values are listed in Supplementary Table 1.

Figure 3A shows the effect of the modifications to the top motif. Compounds **6a** (2-hydroxyclyclohexyl), **6b** (2-hydroxytetrahydopyran-3-yl), **6c** (2-hydroxytetrahydopyran-4-yl), **6e** (2-hydroxycyclopentyl), **6f** (1-deoxy-3,6-anhydrogalactitol), **6g** (cyclohexyl), **6h** (tetrahydropyranyl), **6j** (4-hydroxyclohexyl), **6k** (phenyl), **6r** (piperidinyl) and **6s** (4-oxocyclohexyl) displayed varying degrees of cooperativity with ACh but stronger intrinsic efficacy than BQCA (**1**) when tested at 10 μM, suggesting that the presence of cyclic substituents at the top of the molecule correlates with strong intrinsic efficacy of the allosteric modulator. Compound **6l** (2-hydroxyphenyl), was an exception to this general phenomenon, displaying low intrinsic efficacy and only weakly potentiating the ACh-mediated IP_1 response. The ^1H NMR spectrum of the compound showed deshielding of the phenolic group, with the –OH signal moving downfield (10.02 ppm), supporting the presence of an additional IMHB. This may impair the activity of **6l** by increasing the energy required to adopt its bioactive conformation upon the receptor-ligand interaction. Deletion of –OH group as in **6k** effectively rescued the intrinsic efficacy of the molecule (Δbaseline **6l** ~14%; **6k** ~73%). Interestingly, whereas compound **6q** (2-aminocyclohexyl top motif) displayed a stronger potentiation of the ACh-mediated IP_1 response, its intrinsic efficacy was significantly lower than that observed for compound **6a** (2-hydroxycyclohexyl), suggesting that positively charged groups in the top part of the molecule may have an important influence on intrinsic efficacy. The primary carboxamide **6m** and acyclic *N*-substituted carboxamide **6n** and **6o** also reduced the intrinsic efficacy of the compounds relative to **1** while maintaining a similar potentiation of the ACh response. However, by the simple addition of a hydroxyl group to the carbon chain, as in **6p**, the intrinsic efficacy of the molecule was returned to that in the range observed with BQCA (**1**).

The presence of H-bond donors (–OH, –NH) and acceptors (O / N) commonly has a substantial impact on the receptor-ligand interactions, possibly creating (or deleting) interactions within amino acids in the binding site.[33] In our series, the deletion of the hydroxyl group in compound **6a** (ΔpEC_{50}: 0.44 ± 0.63) resulted in a significant increase in the pEC_{50} (compound **6g** – ΔpEC_{50}: 2.55

± 0.75). This top moiety has never been reported in previous SAR of indoles or azaindoles M_1 mAChR PAMs.[21,23] However, the presence or absence of hydroxyl groups or –O– or HN– heteroatoms on the cyclohexyl top motif did not have a significant impact on the agonist activity of the derivatives (Figure 3A). This represents an opportunity to modify physicochemical properties without losing activity. Polyhydroxylated compound **6f**, for example, is unlikely to cross the BBB (predicted cLogP = 0.177[34] – while preferred range for brain penetration is cLogP 2-4).[35] Its activity as M_1 mAChR PAM-agonist could therefore be repurposed to treat peripheral disorders in which the M_1 mAChR is involved (e.g. exocrine glands and gut smooth muscle conditions).

Figure 3B shows that addition of an *N,N*-disubstituted amide to the top part of the molecule, as seen in analogues **15a-d** and **18a-f**, decreased the intrinsic efficacy of the compounds when compared to BQCA **1**. Compounds **15a** (*N,N*-dimethyl), **15b** (*N,N*-diethyl), **15d** (*N*-methyl-*N*-cyclohexyl) and **18b** (pyrrolidine) potentiated the ACh response to a similar extent as seen with BQCA **1** (ΔpEC_{50} for **1:** 1.17 ± 0.34; **15a**: 0.89 ± 0.09; **15b**: 1.07 ± 0.12; **15d**: 1.00 ± 0.17; **18b**: 0.76 ± 0.12), while having lower intrinsic efficacy. Compound **15d** also had lower intrinsic efficacy than its mono-substituted counterparts, **6a** and **6g**. However, the piperidinyl and *N*-Boc-piperazinyl top motifs (e.g. in **18a**, **18e**) resulted in a complete loss of ACh potentiation. Interestingly, the piperazine top motif, as in compound **18f**, increased the intrinsic efficacy relative to the bioisostere **18d** (morpholine), while the bioisostere **18a** (piperidine) remained essentially inactive. The more rigid incorporation of a dimethyloxazole (compound **21**) was not well tolerated and completely obliterated the intrinsic efficacy and cooperativity with ACh. Overall, the *N,N*-disubstituted compounds (**15** and **18**) proved that the removal of the capacity to form an IMHB by replacing the amide hydrogen with an alkyl chain, is not significantly detrimental to the modulation of ACh response and that this modification may contribute to the diminished intrinsic efficacy of these PAMs. However, compounds **6m-o** which have the IMHB intact, also showed reduced intrinsic efficacy compared to **1**, and retained strong cooperativity with ACh.

In Figure 4A, the effects of modifications to the core part of the molecule and the benzyl pendant were explored. Compound **6a** and other bicyclic scaffolds, **24a-d**, showed essentially a PAM-agonist profile, and were all more potent allosteric agonists than **1**.[21] Creating a new scaffold by simplification of the azaindole core, as shown in compound **24e**, caused a dramatic drop in intrinsic efficacy, while still maintaining cooperativity with ACh (7-fold potentiation of ACh activity). Similar to the *N,N*-disubstituted carboxamide series, the absence of a IMHB did not result in a critical decrease in the modulatory effect.

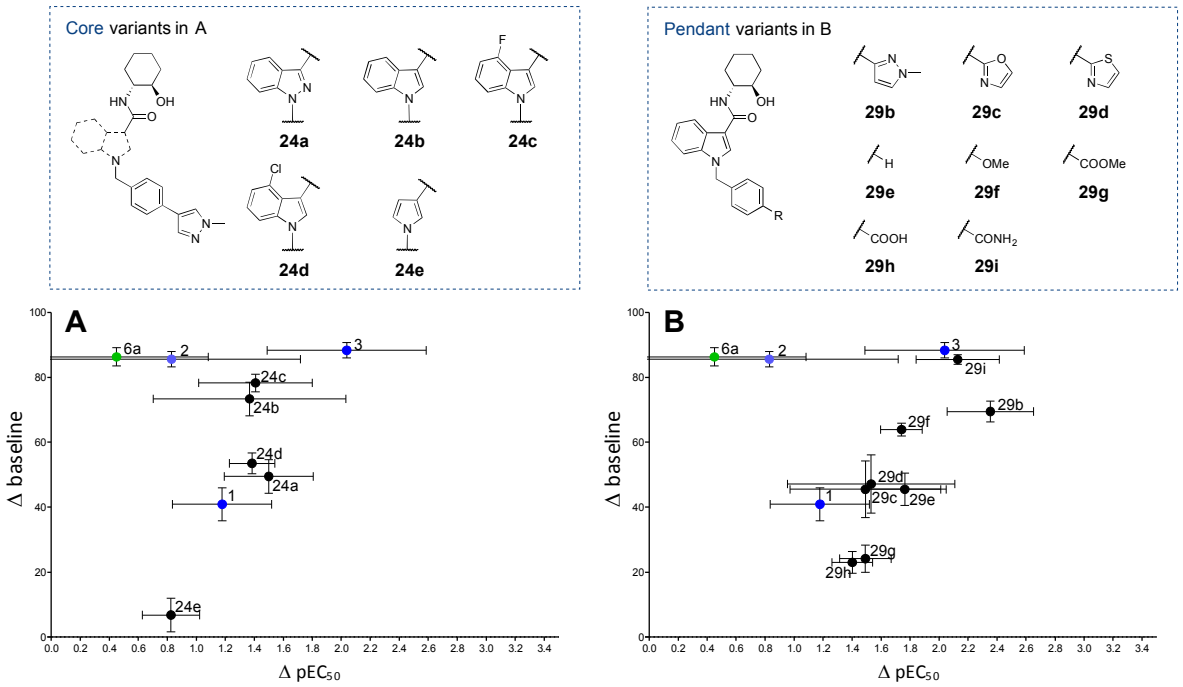


Figure 4. Plot of ΔpEC_{50} *vs* Δbaseline for core and pendant variants. Parameters were estimated from ACh concentration-response curves in the presence or absence of 10 μM of each compound in IP_1 accumulation assays in FlpIn CHO cells stably expressing the hM_1 mAChR. Plotted values represent the means ± SEM of three experiments performed in duplicate. Plotted values represent the means ± SEM of at least three experiments performed in duplicate. ΔpEC_{50} = pEC_{50} of (ACh + 10 μM compound) – pEC_{50} of ACh (basal). Δbaseline = maximal stimulation of (ACh basal + 10 μM compound) – maximal stimulation of ACh (basal). ΔpEC_{50} was used as a surrogate of functional cooperativity for 10 μM of the modulator and Δbaseline as a surrogate of its direct agonism at the same concentration. In all graphs **1**, **2**, **3** (in blue) and **6a** (green) were included as reference compounds. Graphs: **A**. Plot of compounds **24a-e** – core variants. **B**. Plot of compounds **29b-i** – pendant variants. The estimated ΔpEC_{50} and Δbaseline values are listed in Supplementary Table 1.

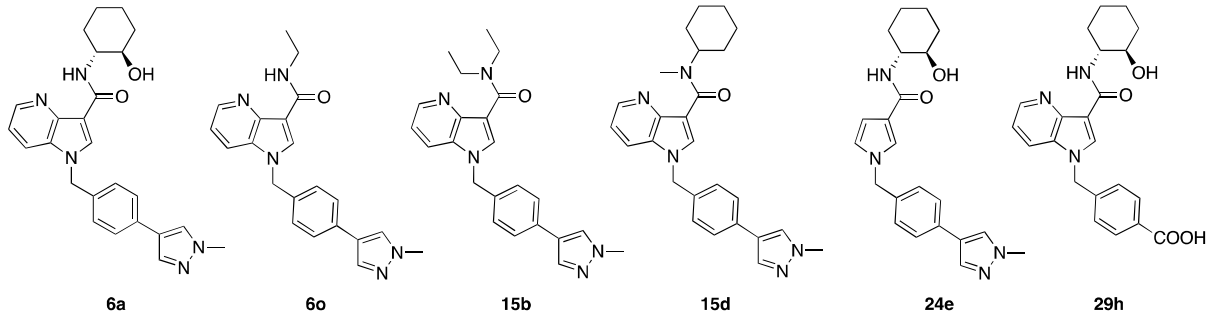
Figure 4B shows the effects of alterations to the pendant moiety on the intrinsic efficacy and cooperativity of the novel PAMs. The positional isomers 4- and 3-*N*-methylpyrazole motif, as in compounds **6a** and **29b**, had significantly different cooperativity values ($\Delta pEC_{50:}$ 0.45 ± 0.63 and 2.35 ± 0.30, respectively), consistent with previously published data by Rook and colleagues.[24] By replacing the methylpyrazole motif in **6a** with an –OMe group (**29f**), the cooperativity with ACh was decreased while the strong intrinsic efficacy was maintained, in agreement with previously published results by Davoren and colleagues.[22] Changing the pendant group to an oxazole (compound **29c**), thiazole (**29d**) or hydrogen (**29e**) had minimal overall effects on the cooperativity or intrinsic efficacy of these compounds when compared to **1**. However, changing the pendant group to a $–CONH_2$ group (**29i**) afforded a significant increase in intrinsic efficacy and improved cooperativity with ACh, consistent with our previous findings on other scaffolds.[18–20] In contrast, its bioisosteres **29h** (-COOH) and **29g** (-COOMe) had similar cooperativity but lower intrinsic efficacy compared to **1**.

Overall, in search of compounds with improved cooperativity with ACh and reduced intrinsic efficacy compared to the prototypical allosteric modulator BQCA **1**, compounds **6o**, **15b**, **15d** (top part modified), **24e** (core modified) and **29h** (pendant modified); as well as **6a** and the literature compounds **2** and **3** (for comparison); were selected for further pharmacological characterisation.

To determine the affinity and binding cooperativity of the allosteric modulators with the radiolabeled antagonist, [^{3}H]NMS, at the M_1 mAChR, whole cell equilibrium binding studies were performed. Increasing concentrations of the endogenous agonist, ACh, competed for [^{3}H]NMS binding to the M_1 mAChR (pK_i = 5.41 ± 0.08). Compounds **1**, **2**, **3** and **6a** also almost completely inhibited [^{3}H]NMS binding (i.e. via high negative cooperativity), and their binding affinity (pK_B) values could therefore be estimated from these experiments (Table 1 and Supporting Information Figure 1A). All the other PAMs tested had no or weak effects in their own right. To determine the binding cooperativities (Logα) between ACh and PAMs, increasing concentrations of the

modulators were titrated against an EC_{20} concentration of ACh (3 μM) (Supporting Information, Figure 1B), and the data were analyzed using an allosteric ternary complex model (Eq. 1, Experimental section).[36] The binding affinities for **6o**, **15b**, **15d**, **24e** and **29h** were also obtained from these analyses (Table 1).

Table 1. Binding and functional allosteric parameters for selected carboxamides and literature compounds at the M_1 mAChR.



	radioligand binding ([^{3}H]NMS)			IP$_1$ accumulation	
	pK_B	**Logα'**[c]	**Logα (α)**[d]	**Log$\alpha\beta$ ($\alpha\beta$)**[e]	**Logτ_B (τ_B)**[f]
1	5.48 ± 0.09[a]	-0.91 ± 0.12	1.58 ± 0.09 (38.0)	1.44 ± 0.10 (27.5)	0.32 ± 0.05 (2.09)
2	6.41 ± 0.05*[a]	-1.28 ± 0.05	1.52 ± 0.08 (33.1)	1.94 ± 0.10 (87.1)*	1.12 ± 0.03 (13.2)*
3	6.20 ± 0.04*[a]	-1.32 ± 0.12	1.71 ± 0.08 (51.3)	1.97 ± 0.08 (93.3)*	1.02 ± 0.03 (10.5)*
6a	5.92 ± 0.09*[a]	-1.35 ± 0.28	1.66 ± 0.07 (45.7)	2.66 ± 0.07 (457)*†	1.36 ± 0.03 (22.9)*
6o	4.61 ± 0.02*[b]	ND	1.55 ± 0.09 (35.5)	2.19 ± 0.05 (155)*†	0.56 ± 0.04 (3.63)
15b	4.43± 0.09*[b]	-0.11 ± 0.04	1.28 ± 0.06 (19.0)	1.34 ± 0.08 (21.9)	0
15d	4.91 ± 0.09*[b]	ND	1.23 ± 0.07 (17.0)*	1.73 ± 0.07 (53.7)†	-0.24 ± 0.11 (0.57)*
24e	4.19 ± 0.06*[b]	-0.05 ± 0.01	1.81 ± 0.08 (64.6)	2.06 ± 0.11 (115)*	0.52 ± 0.09 (3.31)
29h	4.63 ± 0.20*[b]	-0.26 ± 0.15	1.76 ± 0.12 (57.5)	1.97 ± 0.06 (93.3)*	0.46 ± 0.04 (2.88)

[a]Values were estimated from one way competition with [^{3}H]NMS. [b]Values were estimated from competition with [^{3}H]NMS in the presence of an EC_{20} concentration of ACh. For compound **1**, **2**, **3** and **6a**, the Logα' with [^{3}H]NMS was fixed to the value obtained in (*a*), for all other ligands was fixed to 0. [c]Binding cooperativity with [^{3}H]NMS. [d]Binding cooperativity with ACh. [e]Functional cooperativity with ACh. For compound **1**, **2**, **3** and **6a**, pK_B was fixed to the value obtained in *a*, for all other PAMs was fixed to the value obtained in *b*. [f] Intrinsic efficacy of the modulator. *Significant difference ($p < 0.05$) was determined using one-way ANOVA with Dunnett's post test compared to **1** as the reference PAM. †Significant difference ($p < 0.05$) between Logα and Log$\alpha\beta$ values for each compound was determined using unpaired t-test. Data represent the mean ± SEM of at least four individual experiments in duplicate.

As shown in Table 1, the binding affinities were improved for **2**, **3** and our prototypical PAM, **6a** (2-hydroxycyclohexyl top motif) compared to BQCA **1**. However, modifications to the core, top and pendant motif of **6a** reduced affinities of PAMs at the M_1 mAChR. Davoren and coworkers[21] have proposed that the π–π interactions between the azaindole core and Trp400 and Tyr179 residues are important to the overall potency of this class of compounds. Similarly, interaction between the heterocycle at the 4-position of the benzyl pendant is supposed to be key for eletrostatic and π interactions within a tyrosine and aromatic pocket. However, no information about the relevance of the hydroxycyclohexyl top moiety interactions within the allosteric pocket is available in the literature. Interestingly, all PAMs tested in our work, except for **15d**, displayed similar binding cooperativities with ACh, as seen by BQCA **1**, although having lower affinities.

To investigate the effects of selected PAMs on receptor function, concentration-response curves for ACh-stimulated IP_1 accumulation were generated in the absence or presence of increasing concentrations of the test compounds (Supporting Information Figure 2). An operational model of allosterism and agonism was applied to estimate their intrinsic efficacy (τ_B) and functional cooperativity with ACh ($\alpha\beta$). The binding affinities were fixed to the values obtained in the binding studies, shown in Table 1. Consistent with previously published data,[22,23] we observed approximately 5-fold increase in intrinsic efficacy of **2** and **3** compared to **1** (Table 1). However, in contrast to previous studies,[23,24] where **3** displayed lower agonism than **2** (Ago EC_{50} = 590 nM for **2** and 3900 nM for **3**, Figure 1), we obtained similar τ_B values for these compounds. In our series, the two *N,N*-disubstituted carboxamides (**15b** and **15d**) displayed the weakest intrinsic efficacy, whereas the agonist activity of only **6a** was increased compared to **1**.

Interestingly, unlike the binding cooperativities, the functional cooperativities of all the PAMs tested, except for **15b** and **15d**, were significantly increased compared to **1** (Table 1). The Log$\alpha\beta$ values for compounds **6a**, **6o** and **15d** were significantly higher than the Logα values, whereas for all other PAMs tested there were no significant differences between Logα and Log$\alpha\beta$ values,

suggesting that the modulation of ACh signaling by these PAMs is mainly derived from their binding cooperativity. Overall, there was no significant correlation between the Logα and Log$\alpha\beta$ values (Figure 5A), indicating that the differences are not solely due to the different experimental conditions in binding and IP_1 accumulation assays. Compounds **6a**, **6o** and **15d** may therefore modulate both the affinity and efficacy of ACh whereas compounds **1**, **2**, **3**, **15b**, **24e** and **29h** may act as only affinity modulators.

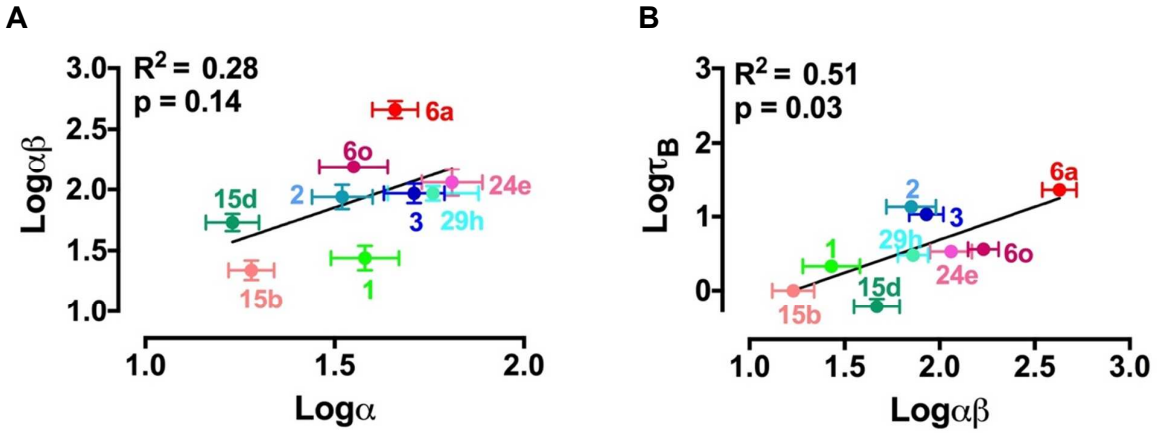


Figure 5. Correlation analysis of the binding and functional parameters for the selected PAMs at the M_1 mAChR. **A**. No significant correlation between binding cooperativity (α) estimates from whole cell radioligand binding assays and functional cooperativity ($\alpha\beta$) estimates from IP_1 accumulation assays. **B**. Significant correlation between functional cooperativity and intrinsic efficacy (τ_B) values estimated from IP_1 accumulation assays. The solid line is the line of best fit.

As it was suggested by Alt and co-workers,[26] M_1 PAMs with weak agonist activity and appreciable potentiation of ACh response might have better *in vivo* safety profiles by fine-tuning native receptor signaling. Our previous studies demonstrate that generally the magnitude of ACh potentiation by an allosteric modulator ($\alpha\beta$) is correlated to an increase in the intrinsic efficacy (τ_B) of the PAM. However, particular structural modifications can tune specifically one of the allosteric features over the other.[18–20] The results of the present study also show a direct correlation between Logτ_B and Log$\alpha\beta$ (Figure 5B), consistent with a two-state model of action, where the functional cooperativity of a PAM tracks with the degree of orthosteric agonist efficacy and stimulus-response

coupling.[28,29] Although such a mechanism can result in higher degrees of potentiation for stronger agonists or for more amplified (e.g. downstream) signaling pathways, it does not predict biased modulation. Intriguingly a recent study by Davoren *et al.*[24] suggested that even M_1 PAMs with weak agonist activity could exhibit cholinergic side effects (lead compound **4**, Figure 1). However, it remains unclear whether this was a chemotype-specific effect since the fused lactam ring in **4** is solely a covalent formalization of the IMHB present in **3**.

Notably, a fluorinated indole derivative **5**, which potentially lacks the IMHB, typically present on structural related compounds (as seen in **2**), was reported to display stronger modulatory effects than direct agonist activity. Although the extirpation of the IMHB in our series also resulted in compounds with lower τ_B values, further studies are required to link structural features of M_1 PAMs to their *in vivo* profile.

■ CONCLUSIONS

In this study, we reported the synthesis and optimization of the PAM activity of azaindole, indole and pyrrole derivatives inspired by prototypical M_1 PAM-agonist, PF-06764427 (**2**),[21] and provided a detailed *in vitro* pharmacological evaluation of selected compounds within our series and three reference M_1 PAMs from literature, BQCA (**1**), PF-06764427 (**2**) and PF-06767832 (**3**). Although some of the indole and azaindole scaffolds were evaluated in recent studies by Pfizer[21] and Vanderbilt University,[23] we were able to obtain a series of compounds bearing a wider range of structural modifications. Typically, the 2-hydroxyclyclohexyl (as in **6a**) and 2-hydroxytetrahydopyran-3-yl motifs (as in **6b**) are present in the structure of several M_1 mAChR PAM-agonists; here we have demonstrated that many other cyclic motifs, such as the cyclohexyl as in **6g**, the bulky polyhydroxylated furanoside as in **6f** and the aromatic phenyl group as in **6k**, are tolerated on the top part of the molecule without suffering significant changes to the allosteric effects. This represents an exceptional opportunity to optimize physicochemical properties, and to link structural features to pharmacological parameters, and ultimately to *in vivo* response profiles of the M_1 PAMs.

We showed that unprecedented modifications, such as the substitution of the amide hydrogen by short alkyl chains (as in **15b**, **15d**) and the deletion of the core fused aryl ring (as in **24e**), yielded promising PAMs with weak agonist activities. Interestingly, the removal of the cyclic top part (**6m-p**, Figure 3A) and the elimination of the IMHB (in **15a**, **15b**, **15d**, **18b** and **18c**, Figure 3B) were not too detrimental to the modulatory effect of the M_1 PAMs. Similarly, the deletion of the heteroaromatic nitrogen in the fused pyridine ring, by either replacing it with a carbon (**24a-d**) or removing the aromatic ring (compound **24e**) did not affect the modulatory response (Figure 4A). Modifications to the benzyl pendant in the original structure **6a** (Figure 4B) provided compounds with diverse pharmacological behaviors. The *N,N*-disubstituted series standout with compounds presenting a modulatory effect similar to the reference PAM **1** but much lower inherent agonism.

Compound **24e** bears a pyrrole core and represents a new scaffold to M_1 PAMs, thus offering a promising lead for future structure-activity studies of this class of compounds.

Our detailed analysis of the allosteric features of selected M_1 PAMs revealed diverse allosteric profiles (Table 1). Overall, when compared to the reference molecule **1**, all the selected compounds but our prototypical PAM **6a** have lower affinities (pK_B) at the M_1 mAChR, however, except for **15d**, they all display similar binding cooperativities with ACh (α). Interestingly, all modifications, except *N,N*-disubstituted carboxamides, which display the weakest intrinsic efficacies (**15b** and **15d**), result in higher functional cooperativities with ACh ($\alpha\beta$), when compared to **1**. The significant difference between Logα and Log$\alpha\beta$ values for **6a**, **6o** and **15d** indicates that the potentiation of ACh signaling by these PAMs is derived from both modulation of ACh affinity and efficacy, whereas all the other PAMs are mainly affinity modulators. Within our series, only **6a** has higher intrinsic efficacy compared to **1**, and generally, the degree of ACh potentiation correlates with the intrinsic efficacy of PAMs, consistent with a two-state model of allosterism. Taken all together, we suggest compound **15d** as a promising PAM for future studies as it has appreciable affinity at the M_1 mAChR, functional cooperativity in the same range of the reference PAM **1** yet possessing significantly lower intrinsic efficacy (Table 1). Our detailed pharmacological evaluations provide novel insights into the SAR at the M_1 mAChR, which may aid in predicting *in vivo* efficacy and AEs liability of M_1 PAMs. However, further studies on more physiologically relevant systems, such as mouse primary culture neurons, pharmacokinetic and toxicology studies are needed prior to testing this compound in animal models.

■ EXPERIMENTAL SECTION

Chemistry. Chemicals and solvents were purchased from standard suppliers and used without further purification. Davisil silica gel (40−63 μm), for flash column chromatography (FCC), was supplied by Grace Davison Discovery Sciences (Victoria, Australia), and deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. (distributed by Novachem PTY. Ltd., Victoria, Australia). PF-06764427 and PF-06767832 were purchased from Sigma-Aldrich. Reactions were monitored by thin layer chromatography on commercially available precoated aluminium-backed plates (Merck Kieselgel 60 F_{254}). Visualization was by examination under UV light (254 and 366 nm). Appropriate staining was carried out using a solution of ninhydrin (in ethanol) to visualize primary and secondary amines. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance Nanobay III 400 MHz Ultrashield Plus spectrometer at 400.13 and 100.62 MHz, respectively. Chemical shifts (δ) were recorded in parts per million (ppm) with reference to the chemical shift of the deuterated solvent. Coupling constants (*J*) were recorded in Hz, and the significant multiplicities described by singlet (s), doublet (d), triplet (t), quadruplet (q), broad (br), multiplet (m), doublet of doublets (dd), and doublet of triplets (dt). Two-dimensional (2D) experiments including HSQC and HMBC where used to assign the compounds only when necessary. LC-MS were run to verify reaction outcome and purity using the following system: Agilent 6120 Series Single Quad coupled to an Agilent 1260 Series HPLC; Buffers: A, 0.1% formic acid in H_2O; B, 0.1% formic acid in MeCN; The gradient was: 0−1 min 95% buffer A and 5% buffer B, from 1 to 2.5 min up to 0% buffer A and 100% buffer B, held at this composition until 3.8 min, 3.8−4 min 95% buffer A and 5% buffer B, held until 5 min at this composition; the flow rate was 0.5 mL/min and total run time was 5 min at a Poroshell 120 EC-C18 50 × 3.0 mm 2.7 μm column. Retention times (t_R) are quoted, in minutes, for all final coumpounds. Mass spectra were acquired in positive and negative ion mode with a scan range of 100−1000 *m/z*. UV detection was carried out at 214 and 254 nm. HRMS analyses were carried out on an Agilent 6224 TOF LC/MS spectrometer coupled to an Agilent 1290 Infinity (Agilent, Palo Alto, CA). Preparative HPLC was

performed using an Agilent 1260 infinity coupled with a binary preparative pump and Agilent 1260 FC-PS fraction collector, using Agilent OpenLAB CDS software (Rev C.01.04), and an Agilent 7 μm XDB-C8 21.2 × 250 mm column. The following buffers were used: buffer A, 0.1% TFA in H_2O; buffer B, 0.1% TFA in MeCN, with sample being run at a gradient of 5% buffer B to 100% buffer B over 10 min, at a flow rate of 20 mL/min. All screening compounds were of >95% purity.

General Procedure A. Amide Coupling. A mixture of the carboxylic acid (1.0 equiv) and HCTU (1.2 equiv) in DMF (1 mL) was stirred for 5 min until a homogenous suspension was observed. A solution of the respective amine (1.2 equiv) and DIPEA (2 equiv) in DMF (1 mL) was added dropwise and the reaction was stirred overnight at rt DMF was co-evaporated with water and ethanol under reduced pressure at 50 ˚C. The residue was diluted with 1 M Na_2CO_3 solution (80 mL) and extracted with EtOAc (3 × 80 mL). The combined organic layers were washed with brine (80 mL), filtered over anhydrous Na_2SO_4 and dried under reduced pressure. Unless stated otherwise stated, the crude products were purified by flash column chromatography.

General Procedure B. *N*-Alkylation. A mixture of the carboxamide (1 equiv), the respective benzyl halide (1 equiv) and K_2CO_3 (2 equiv) in DMF (1 mL/0.2 mmol of starting material) was stirred at rt overnight. DMF was co-evaporated with water and ethanol under reduced pressure at 50 ˚C. Unless stated otherwise, crude products were purified by flash column chromatography.

***N*-((1*S*,2*S*)-2-Hydroxycyclohexyl)-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6a).** Compound **9a** (50 mg, 0.19 mmol) and **10** (40 mg, 0.19 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **6a** as a white solid (73 mg, 89%). ^{1}H NMR (DMSO-d_6) δ 8.77 (d, *J* = 7.7 Hz, 1H), 8.49 (dd, *J* = 4.7/1.2 Hz, 1H), 8.39 (s, 1H), 8.09 (dd, *J* = 8.3/1.2 Hz, 1H), 8.08 (s, 1H), 7.81 (s, 1H), 7.52 (d, *J* = 8.2 Hz, 2H), 7.30 (dd, *J* = 8.3/4.7 Hz, 1H), 7.27 (d, *J* = 8.2 Hz, 2H), 5.50 (s, 2H), 4.79 (d, *J* = 5.4 Hz, 1H), 3.84 (s, 3H), 3.77–3.69 (m, 1H), 3.46–3.38 (m, 1H), 2.08–2.00 (m, 1H), 1.92–1.85 (m, 1H), 1.68–1.60 (m, 2H), 1.37–1.20 (m, 4H). ^{13}C NMR (DMSO-d_6) δ

165.2, 143.5, 143.3, 135.9, 135.4, 134.1, 132.4, 129.8, 127.8, 127.6, 125.4, 122.4, 118.8, 117.2, 109.5, 72.8, 54.4, 50.0, 37.5, 33.7, 31.2, 24.0, 23.6. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{25}H_{28}N_5O_2$ calcd: 430.2243; found: 430.2247. LC-MS t_R = 2.99.

***N*-((3*R*,4*S*)-3-Hydroxytetrahydro-2*H*-pyran-4-yl)-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6b).** Compound **9b** (25 mg, 0.10 mmol) and **10** (20 mg, 0.10 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **6b** as a white solid (26 mg, 62%). ^{1}H NMR (DMSO-d_6) δ 8.83 (d, *J* = 7.4 Hz, 1H), 8.50 (dd, *J* = 4.7/1.2 Hz, 1H), 8.42 (s, 1H), 8.08 (dd, *J* = 8.3/1.2 Hz, 1H), 8.06 (s, 1H), 7.80 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.24 (dd, *J* = 8.3/4.7 Hz, 1H), 5.50 (s, 2H), 5.18 (d, *J* = 5.4 Hz, 1H), 3.94–3.87 (m, 1H), 3.87–3.78 (m, 5H), 3.52–3.45 (m, 1H), 3.45–3.40 (m, 1H), 3.16–3.10 (m, 1H), 2.12–2.05 (m, 1H), 1.58–1.46 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 163.0, 144.0, 143.1, 136.5, 136.3, 134.8, 132.7, 129.7, 128.5, 128.2, 125.7, 121.8, 119.8, 117.7, 110.3, 71.6, 69.3, 66.1, 52.2, 50.0, 39.1, 32.3. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{24}H_{26}N_5O_3$ calcd: 432.2036; found: 432.2046. LC-MS t_R = 2.87.

N-((3*S*,4*S*)-4-Hydroxytetrahydro-2*H*-pyran-3-yl)-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo [3,2-*b*]pyridine-3-carboxamide (6c). Compound **7c** (25 mg, 0.10 mmol) and **10** (20 mg, 0.10 mmol) was reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford the **6c** as a white solid (25 mg, 60%). ^{1}H NMR (DMSO-d_6) δ 8.85 (d, *J* = 7.7 Hz, 1H), 8.50 (dd, *J* = 4.4 Hz, 1H), 8.41 (s, 1H), 8.07 (m, 2H), 7.80 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.30 (m, 3H), 5.50 (s, 2H), 5.12 (d, *J* = 5.4 Hz, 1H), 4.01–3.95 (m, 1H), 3.83 (s, 3H) , 3.83–3.78 (m, 1H), 3.73–3.66 (m, 1H), 3.53–3.45 (m, 1H), 3.37–3.34 (m, 1H), 2.27–2.17 (m, 1H), 1.97–1.90 (m, 1H), 1.57–1.49 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 163.3, 144.1, 143.1, 136.5, 136.4, 134.8, 132.7, 129.7, 128.5, 128.3, 125.7, 121.9, 119.9, 117.8, 110.3, 68.3, 68.0, 65.0, 51.8, 50.0, 39.1, 33.4. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{24}H_{26}N_5O_3$ calcd: 432.2036; found: 432.2048. LC-MS t_R = 2.88.

***tert*-Butyl(2-(1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamido)cyclohexyl)carbamate (6d).** Compound **9d** (89 mg, 0.25 mmol) and **10** (51 mg, 0.25 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **6d** as a colourless oil (97 mg, 74%). ^{1}H NMR ($CDCl_3$) δ 8.94 (d, *J* = 8.2 Hz, 1H), 8.46 (dd, *J* = 4.7/1.2 Hz, 1H), 8.03 (s, 1H), 7.68 (s, 1H), 7.57 (dd, *J* = 8.3/1.2 Hz, 1H), 7.54 (s, 1H), 7.37 (d, *J* = 8.2 Hz, 2H), 7.10–7.06 (m, 3H), 5.35 (d, *J* = 7.0, 1H), 5.25 (s, 2H), 3.99–3.90 (m, 1H), 3.88 (s, 3H), 3.50–3.40 (m, 1H), 2.23–2.16 (m, 1H), 2.11–2.04 (m, 1H), 1.78–1.67 (m, 2H), 1.57–1.30 (m, 4H), 1.21 (s, 9H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 529.2.

***N*-((1*S*,2*S*)-2-Hydroxycyclopentyl)-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6e).** Compound **9e** (61 mg, 0.25 mmol) and **10** (51 mg, 0.25 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **6e** as a white solid (84 mg, 80%). ^{1}H NMR (CD_3OD) δ 8.43 (dd, *J* = 4.6/0.8 Hz, 1H), 8.13 (s, 1H), 7.81 (dd, *J* = 8.3/0.8 Hz, 1H), 7.80 (s, 1H), 7.71 (s, 1H), 7.42 (d, *J* = 8.2 Hz, 2H), 7.17 (dd, *J* = 8.3/4.6 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 2H), 5.33 (s, 2H), 4.22–4.11 (m, 2H), 3.84 (s, 3H), 2.28–2.17 (m, 1H), 2.06–2.96 (m, 1H), 1.88–1.74 (m, 2H), 1.69–1.57 (m, 2H). ^{13}C NMR (CD_3OD) δ 165.0, 143.6, 143.2, 135.9, 135.2, 134.0, 132.4, 129.8, 127.8, 127.6, 125.4, 122.3, 118.8, 117.2, 109.2, 77.6, 58.0, 50.0, 37.6, 31.7, 29.7, 20.4. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{24}H_{26}N_5O_2$ calcd: 416.2087; found: 416.2087. LC-MS t_R = 3.00.

***N*-((2*R*)-2-((3*R*,4*S*)-3,4-Dihydroxytetrahydrofuran-2-yl)-2-hydroxyethyl)-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6f).** Compound **9f** (36 mg, 0.11 mmol) and **10** (24 mg, 0.11 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **6f** as a white oil (32 mg, 58%). ^{1}H NMR (CD_3OD) δ 8.40 (d, *J* = 4.2 Hz, 1H), 8.11 (s, 1H), 7.79 (s, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.70 (s, 1H), 7.37 (d, *J* = 8.2 Hz, 2H), 7.10 (m, 3H), 5.29 (s, 2H), 4.14 (br s, 1H), 4.07 (br s, 1H), 4.00–3.94 (m, 2H), 3.85–3.81 (m, 4H), 3.79–3.75 (m, 1H), 3.74–3.72 (m, 1H), 3.62–3.55 (m, 1H). ^{13}C NMR (CD_3OD) δ 165.3, 143.6, 143.2, 136.0, 135.3, 134.0, 132.4, 129.7, 127.8, 127.7, 125.4,

122.3, 118.7, 117.2, 109.4, 86.6, 78.8, 77.4, 73.3. 70.1, 50.0, 42.0, 37.6. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{25}H_{28}N_5O_5$ calcd: 478.2090; found: 478.2085. LC-MS t_R = 2.77.

***N*-Cyclohexyl-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6g).** Compound **9g** (61 mg, 0.25 mmol) and **10** (51 mg, 0.25 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **6g** as a white solid (68 mg, 66%). ^{1}H NMR ($CDCl_3$) δ 8.85 (d, *J* = 4.9 Hz, 1H), 8.48 (dd, *J* = 4.2/1.2 Hz, 1H), 8.08 (s, 1H), 7.71 (s, 1H), 7.59–7.53 (m, 2H), 7.39–7.35 (m, 2H), 7.13–7.07 (m, 3H), 5.29 (s, 2H), 4.08 (br s, 1H), 3.91 (s, 3H), 2.07–1.95 (m, 2H), 1.82–1.73 (m, 2H), 1.65–1.23 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 162.9, 143.7, 143.6, 136.7, 135.2, 133.3, 132.2, 129.6, 127.6, 127.0, 126.3, 122.4, 118.0, 117.1, 111.4, 50.8, 47.4, 47.3, 39.1, 33.3, 33.2, 25.8, 24.8. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{25}H_{28}N_5O$ calcd: 414.2294; found: 414.2289. LC-MS t_R = 3.00.

1-(4-(1-Methyl-1*H*-pyrazol-4-yl)benzyl)-*N*-(tetrahydro-2*H*-pyran-4-yl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6h). Compound **9h** (25 mg, 0.10 mmol) and **10** (20 mg, 0.10 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **6h** as a white solid (26 mg, 63%). ^{1}H NMR ($CDCl_3$) δ 8.96 (d, *J* = 7.8 Hz, 1H), 8.48 (dd, *J* = 4.7/1.2 Hz, 1H), 8.08 (s, 1H), 7.71 (s, 1H), 7.60 (dd, *J* = 8.3/1.2 Hz, 1H), 7.57 (s, 1H), 7.39 (d, *J* = 8.2 Hz, 2H), 7.20–7.10 (m, 3H), 5.30 (s, 2H), 4.33–4.25 (m, 1H), 4.03–3.97 (m, 2H), 3.91 (s, 3H), 3.61–3.54 (m, 2H), 2.07–2.00 (m, 2H), 1.78–1.67 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 163.2, 143.8, 143.6, 136.7, 135.2, 133.2, 132.9, 129.7, 127.7, 127.0, 126.0, 122.4, 118.2, 117.2, 111.0, 66.8, 66.7, 50.9, 44.8, 44.7, 39.1, 33.3. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{24}H_{26}N_5O_2$ calcd: 416.2087; found: 416.2095. LC-MS t_R = 2.95.

***tert*-Butyl 4-(1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamido)piperidine-1-carboxylate (6i).** Compound **9i** (34 mg, 0.10 mmol) and **10** (20 mg, 0.10 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **6i** as a light yellow oil (30 mg, 60%). ^{1}H NMR ($CDCl_3$)

δ 8.96 (d, *J* = 7.7 Hz, 1H), 8.46 (dd, *J* = 4.6/1.2 Hz, 1H), 8.07 (s, 1H), 7.70 (s, 1H), 7.60 (dd, *J* = 8.3/1.2 Hz, 1H), 7.56 (s, 1H), 7.38 (d, *J* = 8.2 Hz, 2H), 7.14–7.06 (m, 3H), 5.29 (s, 2H), 4.28–4.20 (m, 1H), 4.05–3.95 (m, 2H), 3.90 (s, 3H), 3.12–3.01 (m, 2H), 2.06–1.98 (m, 2H), 1.65–1.55 (m, 2H), 1.46 (s, 9H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 515.2.

***N*-((1*R*,4*R*)-4-Hydroxycyclohexyl)-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6j).** Compound **9j** (40 mg, 0.15 mmol) and **10** (31 mg, 0.15 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 20:1) to afford **6j** as a white solid (62 mg, 94%). ^{1}H NMR ($CDCl_3$) δ 8.80 (d, *J* = 7.9 Hz, 1H), 8.44 (dd, *J* = 4.7/1.1 Hz, 1H), 8.08 (s, 1H), 7.69 (s, 1H), 7.58 (dd, *J* = 8.4/1.1 Hz, 1H), 7.55 (s, 1H), 7.38 (d, *J* = 8.2 Hz, 2H), 7.10 (m, 3H), 5.27 (s, 2H), 4.08–3.97 (m, 1H), 3.88 (s, 3H), 3.74–3.64 (m, 1H), 2.50–2.32 (m, 1H), 2.19–2.09 (m, 2H), 2.07–1.98 (m, 2H), 1.52–1.40 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 163.3, 143.7, 143.6, 136.7, 135.4, 133.3, 132.8, 129.7, 127.7, 127.1, 126.0, 122.4, 118.2, 117.2, 111.3, 69.7, 50.8, 47.0, 46.9, 39.0, 34.0, 30.9, 30.8. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{25}H_{28}N_5O_2$ calcd: 430.2243; found: 430.2247. LC-MS t_R = 2.93.

1-(4-(1-Methyl-1*H*-pyrazol-3-yl)benzyl)-*N*-phenyl-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6k). Compound **9k** (15 mg, 0.06 mmol) and **10** (15 mg, 0.06 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **6k** as a white solid (16 mg, 64%). ^{1}H NMR (DMSO-d_6) δ 10.96 (s, 1H), 8.61–8.58 (m, 2H), 8.16 (dd, *J* = 8.3/1.2 Hz, 1H), 8.08 (s, 1H), 7.81 (s, 1H), 7.77 (d, *J* = 8.2 Hz, 2H), 7.53 (d, *J* = 8.2 Hz, 2H), 7.41–7.32 (m, 5H), 7.12–7.07 (m, 1H), 5.55 (s, 2H), 3.83 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 161.6, 144.2, 142.8, 139.4, 137.2, 136.5, 134.7, 132.8, 129.9, 129.2, 128.6, 128.3, 125.7, 123.7, 121.8, 120.4, 119.6, 118.1, 109.9, 50.2, 39.1. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{25}H_{22}N_5O$ calcd: 408.1824; found: 408.1822. LC-MS t_R = 3.46.

***N*-(2-Hydroxyphenyl)-1-(4-(1-methyl-1*H*-pyrazol-3-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6l).** Compound **9l** (35 mg, 0.14 mmol) and **10** (35 mg, 0.14 mmol) were reacted

according to general procedure B. The crude product was purified by preparative HPLC to afford **6l** as a colourless oil (10 mg, 18%). ^{1}H NMR (DMSO-d_6) δ 10.96 (s, 1H), 10.02 (s, 1H), 8.56 (s, 1H), 8.54 (dd, J = 4.6/1.2 Hz, 1H), 8.36 (d, J = 7.6 Hz, 1H), 8.13 (dd, J = 8.3/1.2 Hz, 1H), 8.08 (s, 1H), 7.81 (s, 1H), 7.52 (d, J = 8.2 Hz, 2H), 7.36–7.28 (m, 3H), 6.92–6.88 (m, 2H), 6.84–6.79 (m, 1H), 5.54 (s, 2H), 3.83 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 161.6, 146.9, 144.2, 142.9, 137.2, 136.5, 134.8, 132.8, 129.7, 128.6, 128.3, 128.0, 127.5, 125.7, 121.8, 120.6, 120.0, 119.5, 118.0, 115.2, 110.7, 50.2, 39.1. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{25}H_{22}N_5O_2$ calcd: 424.1773; found: 424.1767. LC-MS t_R = 3.57.

1-(4-(1-Methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6m). Compound **9m** (20 mg, 0.12 mmol) and **10** (24 mg, 0.12 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **6m** as a white solid (32 mg, 82%). ^{1}H NMR (DMSO-d_6) δ 8.48 (dd, J = 4.7/1.2 Hz, 1H), 8.38 (s, 1H), 8.23 (d, J = 3.0 Hz, 1H), 8.10–8.04 (m, 2H), 7.81 (s, 1H), 7.52 (d, J = 8.2 Hz, 2H), 7.40 (d, J = 3.0 Hz, 1H), 7.31 (d, J = 8.2 Hz, 2H), 7.26 (dd, J = 8.3/4.7 Hz, 1H), 5.49 (s, 2H), 3.83 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 164.7, 144.1, 143.3, 136.5, 134.8, 132.8, 129.7, 128.6, 128.3, 125.7, 121.8, 119.8, 117.7, 110.6, 50.0, 39.1. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{19}H_{18}N_5O$ calcd: 332.1511 found: 332.1508. LC-MS t_R = 2.81.

***N*-Methyl-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6n).** Compound **9n** (12 mg, 0.06 mmol) and **10** (16 mg, 0.06 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **6n** as a white solid (16 mg, 72%). ^{1}H NMR (DMSO-d_6) δ 8.59 (q, J = 5.0 Hz, 1H), 8.48 (dd, J = 4.3/0.9 Hz, 1H), 8.39 (s, 1H), 8.07 (m, 2H), 7.80 (s, 1H), 7.51 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 7.25 (dd, J = 8.4/4.3 Hz, 1H), 5.49 (s, 2H), 3.83 (s, 3H), 2.92 (d, J = 5.0 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ 163.8, 143.9, 143.1, 136.5, 136.1, 134.9, 132.7, 129.7, 128.6, 128.3, 125.7, 121.8, 119.8, 117.7, 110.5, 50.0, 39.1, 25.8. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{20}H_{20}N_5O$ calcd: 346.1668 found: 364.1668. LC-MS t_R = 2.87.

***N*-Ethyl-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6o).** Compound **9o** (19 mg, 0.10 mmol) and **10** (21 mg, 0.10 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 20:1) to afford **6o** as a white solid (22 mg, 60%). ^{1}H NMR (DMSO-d_6) δ 8.68 (t, J = 5.7 Hz, 1H), 8.48 (dd, J = 4.7/0.9 Hz, 1H), 8.39 (s, 1H), 8.10–8.06 (m, 2H), 7.80 (s, 1H), 7.51 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.2 Hz, 2H), 7.26 (dd, J = 8.4/4.7 Hz, 1H), 5.49 (s, 2H), 3.83 (s, 3H), 3.45–3.37(m, 2H) 1.71 (t, J = 7.2 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ 163.1, 144.0, 143.1, 136.5, 136.2, 134.9, 132.7, 129.7, 128.6, 128.3, 125.7, 121.8, 119.8, 117.7, 110.4, 50.0, 39.1, 33.5, 15.8. HRMS *m/z* [M+H]+ (Q-TOF/ESI) $C_{21}H_{22}N_5O$ calcd: 360.1824, found: 360.1825. LC-MS t_R= 2.97.

***N*-(2-Hydroxyethyl)-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6p).** Compound **9p** (25 mg, 0.12 mmol) and **10** (25 mg, 0.12 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent EtOAc/MeOH 20:1) to afford **6p** as a colourless oil (31 mg, 69%). ^{1}H NMR (DMSO-d_6) δ 8.86 (t, J = 5.7 Hz, 1H), 8.49 (dd, J = 4.7/0.8 Hz, 1H), 8.41 (s, 1H), 8.09–8.05 (m, 2H), 7.81 (s, 1H), 7.52 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.2 Hz, 2H), 7.25 (dd, J = 8.4/4.7 Hz, 1H), 5.49 (s, 2H), 4.84 (t, J = 5.2 Hz, 1H), 3.83 (s, 3H), 3.59–3.54 (m, 2H), 3.51–3.45 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 163.3, 144.0, 143.1, 136.5, 136.3, 134.9, 132.7, 129.7, 128.5, 128.3, 125.7, 121.8, 119.8, 117.7, 110.5, 60.7, 50.0, 41.5, 39.1. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{21}H_{22}N_5O_2$ calcd: 376.1773, found: 376.1779. LC-MS t_R = 2.80.

***N*-((1*S*,2*S*)-2-Aminocyclohexyl)-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6q).** The *N*-Boc protected intermediate **6d** (80 mg, 0.15 mmol) was dissolved in DCM (2 mL), cooled in an ice bath and TFA (200 µL, 10 equiv) was added dropwise. The reaction was stirred at rt overnight. The mixture was then diluted with 1 M NaOH solution (50 mL) and extracted with EtOAc (5 × 50 mL) and the combined organic layers were washed with brine (100 mL), filtered over anhydrous Na_2SO_4 and dried under reduced pressure. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 20:1) to afford **6q** as a white solid (44 mg,

67%). ^{1}H NMR (DMSO-d_6) δ 8.69 (d, J = 8.5 Hz, 1H), 8.50 (dd, J = 4.7/1.2 Hz, 1H), 8.42 (s, 1H), 8.10 (dd, J = 8.4/1.2 Hz, 1H), 8.08 (s, 1H), 7.81 (s, 1H), 7.52 (d, J = 8.2 Hz, 2H), 7.32 (d, J = 8.2 Hz, 2H), 7.27 (dd, J = 8.4/4.7 Hz, 1H), 5.50 (s, 2H), 3.84 (s, 3H), 3.72–3.71 (m, 1H), 2.65–2.60 (m, 1H), 2.00–1.85 (m, 2H), 1.70–1.60 (m, 2H), 1.34–1.17 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 163.3, 144.0, 143.2, 136.5, 136.3, 134.8, 132.7, 129.7, 128.6, 128.3, 125.7, 121.9, 119.8, 117.8, 110.5, 55.0, 54.5, 50.0, 39.1, 34.5, 32.5, 25.1, 24.6. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{25}H_{29}N_6O$ calcd: 429.2403; found: 429.2404. LC-MS t_R = 2.83.

1-(4-(1-Methyl-1*H*-pyrazol-4-yl)benzyl)-*N*-(piperidin-4-yl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6r). The *N*-Boc protected intermediate **6i** (20 mg, 0.40 mmol) was dissolved in DCM (2 mL), cooled in an ice bath and TFA (300 μL, 10 equiv) was added dropwise. The reaction was stirred at rt overnight. The mixture was then diluted with 1 M NaOH solution (50 mL) and extracted with EtOAc (5 × 50 mL) and the combined organic layers were washed with brine (100 mL), filtered over anhydrous Na_2SO_4 and dried under reduced pressure. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 20:1) to afford **6r** as a yellow solid (14 mg, 86%). ^{1}H NMR (DMSO-d_6) δ 8.73 (d, J = 7.7 Hz, 1H), 8.48 (dd, J = 4.7/1.2 Hz, 1H), 8.38 (s, 1H), 8.09–8.04 (m, 2H), 7.79 (s, 1H), 7.49 (d, J = 8.2 Hz, 2H), 7.28 (d, J = 8.2 Hz, 2H), 7.25 (dd, J = 4.7/8.3 Hz, 1H), 5.48 (s, 2H), 4.02–3.92 (m, 1H), 3.82 (s, 3H), 3.03–2.96 (m, 1H), 2.69–2.59 (m, 1H), 1.93–1.84 (m, 2H), 1.47–1.19 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 163.2, 144.8, 143.9, 137.5, 137.1, 135.6, 133.5, 130.5, 129.3, 129.1, 126.5, 122.6, 120.7, 118.5, 111.1, 50.8, 49.8, 46.5, 45.5, 39.9, 34.0, 30.3. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{24}H_{27}N_6O$ calcd: 415.2246; found: 415.2246. LC-MS t_R = 2.75.

1-(4-(1-Methyl-1*H*-pyrazol-4-yl)benzyl)-*N*-(4-oxocyclohexyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (6s). Cyclohexanol carboxamide **6j** (40 mg, 0.10 mmol) was dissolved in acetone (10 mL), cooled in an ice bath and Jones reagent (20 μL, 0.4 equiv) was added [Jones reagent: 0.5 g CrO_3, 0.5 mL H_2SO_4, 1.5 mL H_2O]. The reaction was allowed to warm to room temperature and stirred overnight. The mixture was then diluted with water (75 mL) and extracted with $CHCl_3$ (3 × 75 mL) and the combined organic layers were washed with brine (75 mL), filtered over anhydrous

Na_2SO_4 and dried under reduced pressure. This crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **6s** as a white solid (5.3 mg, 13%). ^{1}H NMR ($CDCl_3$) δ 9.14 (d, J = 7.0 Hz, 1H), 8.48 (dd, J = 4.6/1.0 Hz, 1H), 8.11 (s, 1H), 7.71 (s, 1H), 7.64 (dd, J = 8.4/1.0 Hz, 1H), 7.57 (s, 1H), 7.42 (d, J = 8.1 Hz, 2H), 7.16–7.12 (m, 3H), 5.32 (s, 2H), 4.60–4.50 (m, 1H), 3.92 (s, 3H), 2.60–2.38 (m, 4H), 2.36–2.28 (m, 2H), 2.07–1.98 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 210.5, 163.3, 144.8, 144.6, 136.7, 135.2, 133.1, 133.0, 129.7, 127.7, 127.0, 126.1, 122.4, 118.3, 117.3, 110.9, 77.2, 76.9, 51.0, 45.2, 39.1, 39.0, 32.8. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{25}H_{26}N_5O_2$ calcd: 428.2087; found: 428.2090. LC-MS t_R = 2.98.

(3*S*,4*R*)-4-Aminotetrahydro-2*H*-pyran-3-ol (7b) and (3*S*,4*S*)-3-aminotetrahydro-2*H*-pyran-4-ol (7c). Neat aqueous ammonium hydroxide (3 mL, 20 equiv) was added to a solution of 3,7-dioxabicyclo[4.1.0]heptane (200 mg, 0.50 mmol) in methanol (6 mL) and the reaction was stirred overnight at rt. Volatiles were removed under reduced pressure and the crude product was purified by FCC (eluent MeOH 100%) to afford **7c** (first band, R_f = 0.250, colorless viscous oil, 77 mg, 33%) and **7b** (second band, R_f = 0.125, white solid, 90 mg, 38%). Compound **7b**: ^{1}H NMR (CD_3OD) δ 3.92–3.83 (m, 2H), 3.45–3.30 (m, 2H), 3.06 (t, J = 10.6 Hz, 1H), 2.62–2.54 (m, 1H), 1.93–1.86 (m, 1H), 1.60–1.49 (m, 1H); ^{13}C NMR (CD_3OD) δ 72.8, 70.4, 66.4, 53.8, 32.3. Compound **7c**: ^{1}H NMR (CD_3OD) δ 3.91–3.82 (m, 2H), 3.45–3.32 (m, 1H), 3.26–3.18 (m, 1H), 3.03 (t, J = 10.6 Hz, 1H), 2.65–2.56 (m, 1H), 1.87–1.80 (m, 1H), 1.52–1.40 (m, 1H). ^{13}C NMR (CD_3OD) δ 72.4, 70.4, 65.7, 53.8, 33.0.

***tert*-Butyl ((1*R*,2*R*)-2-aminocyclohexyl)carbamate (7d).** A solution of di-*tert*-butyl dicarbonate (0.95 g, 4.13 mmol) in DCM (3 mL) was added dropwise to a cold (0 ˚C) solution of *trans*-1,2-diaminocyclohexane (1 mL, 8.3 mmol) in dioxane (5 mL) under vigorous stirring. After 30 min the reaction media was allowed to warm up to rt and then stirred overnight. The volatiles were removed under reduced pressure. The residue was diluted with 1 M Na_2CO_3 solution (50 mL) and extracted with DCM (5 × 50 mL). The combined organic layers were washed with brine (50 mL), filtered over anhydrous Na_2SO_4 and dried under reduced pressure. The crude product was purified

by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **7d** as a yellow oil (140 mg, 15%). ^{1}H NMR ($CDCl_3$) δ 4.46 (br s, 1H), 3.07 (br s, 1H), 2.29–2.20 (m, 1H), 1.95–1.84 (m, 2H), 1.66–1.60 (m, 2H), 1.45–1.37 (m, 1H), 1.38 (s, 9H), 1.25–0.99 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 156.1, 79.3, 57.6, 55.7, 35.2, 32.9, 28.4, 25.2, 25.0. LRMS *m/z* $[M+H]^+$ (TOF ES^+) 215.1.

***N*-((1*S*,2*S*)-2-Hydroxycyclohexyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (9a).** 4-Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and *trans*-2-aminocyclohexanol (**7a)** (86 mg, 0.74 mmol) were reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 20:1) to afford **9a** as a white solid (73 mg, 46%). ^{1}H NMR (CD_3OD) δ 8.46 (dd, *J* = 4.6/1.3 Hz, 1H), 8.11 (s, 1H), 7.86 (dd, *J* = 8.2/1.3 Hz, 1H), 7.23 (dd, *J* = 8.2/4.6 Hz, 1H), 3.92–3.84 (m, 1H), 3.62–3.53 (m, 1H), 2.18–2.06 (m, 2H), 1.80–1.70 (m, 2H), 1.60–1.32 (m, 4H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 260.0.

***N*-((3*R*,4*S*)-3-Hydroxytetrahydro-2*H*-pyran-4-yl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (9b).** Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and *trans*-4-aminotetrahydropuran-3-ol (**7b**) (86 mg, 0.74 mmol) were reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 20:1) to afford **9b** as a white solid (52 mg, 32%). ^{1}H NMR (CD_3OD) δ 8.48 (dd, *J* = 4.7/1.2 Hz, 1H), 8.13 (s, 1H), 7.88 (dd, *J* = 8.2/1.2 Hz, 1H), 7.26 (dd, *J* = 8.2/4.7 Hz, 1H), 4.12–4.03 (m, 1H), 4.03–3.96 (m, 1H), 3.69–3.63 (m, 1H), 3.58–3.50 (m, 1H), 3.33–3.25 (m, 2H), 2.21–2.14 (m, 1H), 1.76–1.66 (m, 1H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 262.0.

***N*-((3*S*,4*S*)-4-Hydroxytetrahydro-2*H*-pyran-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (9c).** Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and *trans*-3-aminotetrahydropuran-4-ol (**7c**) (86 mg, 0.74 mmol) were reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 20:1) to afford **9c** as a colourless oil (25 mg, 23%). ^{1}H NMR (CD_3OD) δ 8.52–8.48 (m, 1H), 8.14 (s, 1H), 7.89–7.86 (m, 1H), 7.30–7.25 (m, 1H), 4.20–4.08 (m, 1H), 4.08–3.98 (m, 1H), 3.94–3.85 (m, 1H), 3.67–3.57 (m, 1H), 3.46–3.38

(m, 1H), 3.35–3.28 (m, 1H), 2.17–2.08 (m, 1H), 1.76–1.66 (m, 1H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 261.9.

***tert*-Butyl ((1*S*,2*S*)-2-(1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamido)cyclohexyl)carbamate (9d).** Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and *tert*-butyl ((1*R*,2*R*)-2-aminocyclohexyl) carbamate (**7d**) (115 mg, 0.74 mmol) were reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 20:1) to afford **9d** as a yellow oil (90 mg, 41%). ^{1}H NMR (CD_3OD) δ 8.47 (dd, *J* = 4.6/0.8 Hz, 1H), 8.12 (s, 1H), 7.84 (dd, *J* = 8.2/0.8 Hz, 1H), 7.21 (dd, *J* = 8.2/4.6 Hz, 1H), 3.92–3.82 (m, 1H), 3.55–3.45 (m, 1H), 2.79 (s, 9H), 2.18–2.08 (m, 1H), 2.05–1.98 (m, 1H), 1.80–1.70 (m, 2H), 1.50–1.32 (m, 4H). m/z $[M+H]^+$ (TOF ES^+) 359.0

N-((1*S*,2*S*)-2-Hydroxycyclopentyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (9e). Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and *trans*-2-aminocyclopentanol hydrochloride (**7e**) (101 mg, 0.74 mmol) were reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 20:1) to afford **9e** as a white solid (68 mg, 45%). ^{1}H NMR (CD_3OD) δ 8.44 (dd, *J* = 4.7/1.3 Hz, 1H), 8.10 (s, 1H), 7.82 (dd, *J* = 8.3/1.3 Hz, 1H), 7.18 (dd, *J* = 8.3/4.7 Hz, 1H), 4.22–4.14 (m, 2H), 2.30–2.21 (m, 1H), 2.08–1.98 (m, 1H), 1.88–1.82 (m, 2H), 1.75–1.65 (m, 2H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 245.9.

N-((2*S*)-2-((3*R*,4*S*)-3,4-Dihydroxytetrahydrofuran-2-yl)-2-hydroxyethyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (9f). Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and amino-1-deoxy-3,6-L-anhydrogalactose[37] (**7f**) (77 mg mg, 0.74 mmol) were reacted according to general procedure A. The crude extract was purified by two FCC (eluent 1: EtOAc/MeOH 4:1; eluent 2: DCM/MeOH 100:1 to 10:1) to afford **9f** as a colourless oil (18 mg, 29 % yield). ^{1}H NMR (CD_3OD) δ 8.46 (d, *J* = 4.3 Hz, 1H), 8.11 (s, 1H), 7.86 (t, *J* = 8.2 Hz, 1H), 7.23–7.18 (m, 1H), 4.15–4.10 (m, 1H), 4.12–4.04 (m, 1H), 4.00–3.96 (m, 2H), 3.87–3.79 (m, 1H), 3.80–3.70 (m, 2H), 3.61 (dd, *J* = 12.3/7.6 Hz, 1H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 308.1.

***N*-Cyclohexyl-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (9g).** Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and cyclohexylamine (**7g**) (85 μL, 0.74 mmol) were reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 20:1) to afford **9g** as a yellow oil (60 mg, 40%). ^{1}H NMR (CD_3OD) δ 8.47 (dd, *J* = 4.3/1.3 Hz, 1H), 8.10 (s, 1H), 7.87 (dd, *J* = 8.2/1.3 Hz, 1H), 7.25 (dd, *J* = 8.2/4.3 Hz, 1H), 4.05–3.95 (m, 1H), 2.10–1.96 (m, 2H), 1.85–1.75 (m, 2H), 1.55–1.32 (m, 6H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 244.0.

***N*-(Tetrahydro-2*H*-pyran-4-yl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (9h).** Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and 4-aminotetrahydropyranol (**7h**) (83 μL, 0.74 mmol) were reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 20:1) to afford **9h** as a colourless oil (52 mg, 34%). ^{1}H NMR (CD_3OD) δ 8.47 (dd, *J* = 4.7/1.1 Hz, 1H), 8.12 (s, 1H), 7.89 (dd, *J* = 8.2/1.1 Hz, 1H), 7.26 (dd, *J* = 8.2/4.7 Hz, 1H), 4.25–4.35 (m, 1H), 4.04–3.96 (m, 2H), 3.64–3.55 (m, 2H), 2.05–1.97 (m, 2H), 1.80–1.68 (m, 2H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 246.0.

***tert*-Butyl 4-(1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamido)piperidine-1-carboxylate (9i).** Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and *1-N*-Boc-4-aminopiperidine (**7i**) (148 mg 0.74 mmol) were reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 20:1) to afford **9i** as a brown oil (86 mg, 40%). ^{1}H NMR (CD_3OD) δ 8.51–8.45 (m, 1H), 8.16–8.12 (m, 1H), 7.92–7.86 (m, 1H), 7.29–7.22 (m, 1H), 4.24–4.14 (m, 1H), 4.05–3.96 (m, 2H), 3.19–3.07 (m, 2H), 2.09–2.00 (m, 2H), 1.67–1.54 (m, 2H), 1.48 (s, 9H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 345.1.

***N*-((1*R*,4*R*)-4-Hydroxycyclohexyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (9j).** Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and *trans*-4-aminocyclohexanol hydrochloride (**7j**) (112 mg, 0.74 mmol) were reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 20:1) to afford **9j** as a white solid (42 mg, 26%). ^{1}H NMR (CD_3OD) δ 8.45 (dd, *J* = 4.7/1.3 Hz, 1H), 8.10 (s, 1H), 7.85 (dd, *J* = 8.2/1.3 Hz, 1H), 7.22

(dd, *J* = 8.2/4.7 Hz, 1H), 3.94–3.84 (m, 1H), 3.69–3.59 (m, 1H), 2.15–2.05 (m, 2H), 2.06–1.98 (m, 2H), 1.55–1.32 (m, 4H). LRMS *m/z* $[M-H]^-$ (TOF ES^+) 258.0.

***N*-Phenyl-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (9k).** Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and aniline (**7k**) (86 µL, 1.5 equiv) were reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 20:1) to afford **9k** as a white solid (58 mg, 40%). ^1H NMR (CD_3OD) δ 8.46 (dd, *J* = 4.6/1.2 Hz, 1H), 8.12 (s, 1H), 7.83 (dd, *J* = 8.3/1.3 Hz, 1H), 7.66 (d, *J* = 8.2 Hz, 2H), 7.28 (dt, *J* = 7.8 Hz, 2H), 7.19 (dd, *J* = 8.3/4.7 Hz, 1H), 7.03 (t, *J* = 7.8 Hz, 1H). LRMS *m/z* $[M-H]^+$ (TOF ES^+) 238.1.

***N*-(2-Hydroxyphenyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (9l).** Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and 2-aminophenol (**7l**) (101 mg, 1.5 equiv) were reacted according to general procedure A. The crude extract was purified by FCC (eluent $CHCl_3$/MeOH 100:2) to afford **9l** as a white solid (40 mg, 26%). ^1H NMR (CD_3OD) δ 8.45 (br s, 1H), 8.12 (s, 1H), 7.97 (dd, *J* = 8.0/1.5 Hz, 1H), 7.81 (d, *J* = 8.2 Hz, 1H), 7.17 (dd, *J* = 8.0/4.2 Hz, 1H), 6.89–6.77 (m, 3H). LRMS *m/z* $[M-H]^+$ (TOF ES^+) 253.9.

1*H*-Pyrrolo[3,2-*b*]pyridine-3-carboxamide (9m). A mixture of azaindole-3-carboxylic acid **8** (81 mg, 0.50 mmol) and HCTU (265 mg, 1.2 equiv) in DMF (1 mL) was stirred for 5 min until a fine homogenous suspension was observed. Concentrated ammonium hydroxide aqueous solution (28%, 3 equiv) was added dropwise and the reaction was stirred overnight at rt. The reaction media was diluted with 1 M Na_2CO_3 solution (80 mL) and extracted with EtOAc (3 x 80 mL) and the combined organic layers were washed with brine (80 mL), filtered over anhydrous Na_2SO_4 and dried under reduced pressure. The crude product was purified by FCC (eluent EtOAc/MeOH 20:1) to afford **9m** as a white solid (19 mg, 23%). ^1H NMR (CD_3OD) δ 8.47 (dd, *J* = 4.0/0.6 Hz, 1H), 8.14 (s, 1H), 7.86 (dd, *J* = 8.1/0.6 Hz, 1H), 7.23 (dd, *J* = 8.1/4.0 Hz, 1H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 162.0.

***N*-Methyl-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (9n).** Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and methylamine hydrochloride (**7n**) (50 mg, 0.74 mmol) reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 20:1) to afford **9n** as a white solid (20 mg, 22%). ^{1}H NMR (CD_3OD) δ 8.46 (dd, *J* = 4.0/0.7 Hz, 1H), 8.10 (s, 1H), 7.86 (dd, *J* = 8.2/0.7 Hz, 1H), 7.24 (dd, *J* = 8.2/4.0 Hz, 1H), 3.03 (s, 3H). m/z $[M+H]^+$ (TOF ES^+) 176.0.

***N*-Ethyl-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (9o).** Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and ethylamine (**7o**) (30% in ethanol) (150 µL, 1.6 equiv) reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 20:1) to afford the **9o** as a yellow oil (25 mg, 22%). ^{1}H NMR (CD_3OD) δ 8.47 (dd, *J* = 4.0/1.0 Hz, 1H), 8.10 (s, 1H), 7.87 (dd, *J* = 8.2/1.0 Hz, 1H), 7.23 (dd, *J* = 8.2/4.0 Hz, 1H), 3.54 (q, *J* = 7.2 Hz, 2H), 1.30 (t, *J* = 7.2 Hz, 3H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 190.1.

***N*-(2-Hydroxyethyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (9p).** Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and ethanolamine (**7p**) (60 µL, 1.6 equiv) reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 10:1) to afford **9p** as a colourless oil (27 mg, 21%). ^{1}H NMR (CD_3OD) δ 8.45 (dd, *J* = 4.0/1.0 Hz, 1H), 8.10 (s, 1H), 7.85 (dd, *J* = 8.2/1.0 Hz, 1H), 7.22 (dd, *J* = 8.2/4.0 Hz, 1H), 3.79 (t, *J* = 5.6 Hz, 2H), 3.63 (t, *J* = 5.6 Hz, 2H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 206.1.

4-(4-(Chloromethyl)phenyl)-1-methyl-1*H*-pyrazole (10). A solution of **27a** (1.00 g, 5.30 mmol) in DCM (100 mL) was placed in an ice bath. Thionyl chloride (2 mL, 5.0 equiv) was added dropwise under vigorous stirring. The reaction mixture was allowed to warm to rt and stirred for 1 h. Saturated $NaHCO_3$ solution (100 mL) was added to the reaction mixture and the product was extracted with chloroform (3 × 100 mL). The combined organic layers were washed with brine (150 mL), filtered over anhydrous Na_2SO_4 and dried under reduced pressure. The crude product was purified by FCC (eluent $CHCl_3$) to afford **10** as a white solid (840 mg, 76%). ^{1}H NMR ($CDCl_3$)

7.75 (s, 1H), 7.60 (s, 1H), 7.44 (d, *J* = 8.2 Hz, 2H), 7.38 (d, *J* = 8.2 Hz, 2H), 4.59 (s, 2H), 3.93 (s, 3H). ^{13}C NMR ($CDCl_3$) 136.9, 135.4, 132.9, 129.2, 127.0, 125.8, 122.6, 46.2, 39.1. LRMS *m/z* $[M+H]^+$ (TOF ES^+) 207.0.

***N*,*N*-Dimethyl-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (14a).** Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and dimethylamine hydrochloride (76 mg, 1.5 equiv) were reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 9:1) to afford **14a** as a white solid (22 mg, 18%). ^{1}H NMR (CD_3OD) δ 8.46 (br s, 1H), 7.94 (br s, 1H), 7.86 (s, 1H), 7.30 (br s, 1H), 3.15 (s, 6H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 190.1.

***N*,*N*-Diethyl-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (14b).** Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and diethylamine (100 µL, 1.5 equiv) were reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 9:1) to afford **14b** as a white solid (30 mg, 22%). ^{1}H NMR (CD_3OD) δ 8.41 (d, *J* = 4.2 Hz, 1H), 7.88 (d, *J* = 8.2 Hz, 1H), 7.82 (s, 1H), 7.22 (dd, *J* = 4.2/8.2 Hz, 1H), 3.60 (br s, 4H), 1.40–1.10 (m, 6H). LRMS *m/z* $[M-H]^-$ (TOF ES^+) 216.0.

***N*,*N*-Diallyl-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (14c).** Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and diallylamine (115 µL, 1.5 equiv) were reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 9:1) to afford **14c** as a light yellow oil (25 mg, 18%). ^{1}H NMR (CD_3OD) δ 8.47 (dd, *J* = 5.2/1.1 Hz, 1H), 8.09 (dd, *J* = 8.3/1.1 Hz, 1H), 8.00 (s, 1H), 7.39 (dd, *J* = 8.3/5.2 Hz, 1H), 6.00–5.90 (m, 2H), 5.23 (d, *J* = 10.0 Hz, 4H), 4.20 (d, *J* = 5.6 Hz, 4H). LCMS *m/z* $[M+H]^+$ (TOF ES^+) 242.0.

***N*-Cyclohexyl-*N*-methyl-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (14d).** A mixture of azaindole-3-carboxylic acid (**8**) (300 mg, 1.85 mmol) and *N*-methylcyclohexanamine (**13d**) (314 mL, 2.22 mmol) reacted according to general procedure A. The crude product was purified by FCC (eluent EtOAC 100% → EtOAc: MeOH 8:2) to afford **14d** as a white solid (82 mg, 17%). ^{1}H NMR (DMSO-d_6) δ 11.68 (s, 1H), 8.38 (d, *J* = 3.8 Hz, 1H), 7.86 (s, 1H), 7.82 (dd, *J* = 8.2/1.5 Hz, 1H),

7.16 (dd, J = 8.2/4.6 Hz, 1H), 4.45–4.21 (m, 0.5H, rotamer), 4.01–3.75 (m, 0.5H, rotamer), 2.90 (s, 3H), 1.84–1.64 (m, 4H), 1.62–1.47 (m, 3H), 1.43–0.77 (m, 3H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 258.0.

***N*,*N*-Dimethyl-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (15a).** A mixture of **14a** (22 mg, 0.12 mmol) and **10** (24 mg, 0.12 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **15a** as a white solid (22 mg, 53%). ^{1}H NMR (DMSO-d_6) δ 8.42 (dd, J = 4.6/1.3 Hz, 1H), 8.13 (s, 1H), 8.07 (s, 1H), 8.00 (dd, J = 8.1/1.3 Hz, 1H), 7.81 (s, 1H), 7.52 (d, J = 8.2 Hz, 2H), 7.29 (d, J = 8.2 Hz, 2H), 7.19 (dd, J = 8.1/4.6 Hz, 1H), 5.46 (s, 2H), 3.83 (s, 3H), 3.04 (s, 6H). ^{13}C NMR (DMSO-d_6) δ 165.8, 143.9, 142.9, 136.5, 135.1, 135.0, 132.7, 128.7, 128.5, 128.3, 125.7, 121.9, 118.9, 117.3, 112.5, 49.8, 39.1, 38.6 (HSQC), 35.2 (HSQC). HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{21}H_{22}N_5O$ calcd: 360.1824 found: 360.1828. LCMS t_R = 2.80.

***N*,*N*-Diethyl-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (15b).** A mixture of **14b** (19 mg, 0.09 mmol) and **10** (18 mg, 0.09 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **15b** as a white solid (19 mg, 55%). ^{1}H NMR (CD_3OD) δ 8.41 (d, J = 4.3 Hz, 1H), 7.90–7.86 (m, 3H), 7.79 (s, 1H), 7.52 (d, J = 8.2 Hz, 2H), 7.24–7.18 (m, 3H), 5.46 (s, 2H), 3.90 (s, 3H), 3.60 (br s, 4H), 1.34–1.04 (m, 6H). ^{13}C NMR (CD_3OD) δ 166.6, 143.3, 142.5, 136.0, 134.5, 132.7, 132.4, 129.2, 127.8, 127.5, 125.4, 122.4, 118.9, 117.1, 111.6, 49.8, 39.9 (HSQC), 37.5, 16.6 (HSQC). HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{23}H_{26}N_5O$ calcd: 388.2137, found: 388.2133. LCMS t_R = 2.87.

***N*,*N*-Diallyl-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (15c).** A mixture of **14c** (19 mg, 0.08 mmol) and **10** (16 mg, 0.08 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **15c** as a white solid (15 mg, 46%). ^{1}H NMR (CD_3OD) δ 8.44 (dd, J

= 4.8/1.2 Hz, 1H), 7.94 (s, 1H), 7.91(s, 1H), 7.90 (dd, *J* = 8.4/1.2 Hz, 1H), 7.79 (s, 1H), 7.52 (d, *J* = 8.2 Hz, 2H), 7.23–7.19 (m, 3H), 5.92 (br s, 2H), 5.45 (s, 2H), 5.18 (br s, 4H), 4.18 (br s, 4H), 3.90 (s, 3H). ^{13}C NMR (CD_3OD) δ 167.1, 143.5, 142.6, 136.0, 134.4, 133.3, 132.4, 130.2 (HSQC), 129.3, 127.8, 127.6, 125.4, 122.4, 118.9, 117.2, 116.6 (HSQC), 110.9, 49.8, 49.5 (HSQC), 37.5. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{25}H_{26}N_5O$ calcd: 412.2137, found: 412.2137. LC-MS t_R = 2.94.

***N*-Cyclohexyl-*N*-methyl-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (15d).** A mixture of **14d** (50 mg, 0.19 mmol) and **10** (40 mg, 0.19 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent DCM 100% → DCM: MeOH 9:1), followed by trituration with PET to afford **15d** as a colourless oil (38 mg, 46%). ^{1}H NMR (DMSO-d_6) δ 8.40 (d, *J* = 3.9 Hz, 1H), 8.15–8.04 (m, 2H), 7.99 (dd, *J* = 8.3/1.3 Hz, 1H), 7.81 (s, 1H), 7.54–7.45 (m, 2H), 7.27 (d, *J* = 8.0 Hz, 2H), 7.18 (dd, *J* = 8.3/4.6 Hz, 1H), 5.46 (s, 2H), 4.54–4.11 (m, 1H), 3.84 (s, 3H), 2.91 (s, 3H), 1.85–1.64 (m, 4H), 1.62–1.44 (m, 3H), 1.28–0.79 (m, 3H). ^{13}C NMR (d_3-MeOD) δ 170.8, 147.2, 146.6, 139.9, 138.5, 137.7, 136.3, 133.2, 131.8, 131.5, 129.4, 126.4, 122.8, 121.1, 115.6, 63.0, 58.0, 53.8, 41.5, 34.4, 33.3, 29.4; HRMS *m/z* $[M+H]^+$ $C_{26}H_{30}N_5O$ calcd: 428.2450; found 428.2444. LCMS t_R = 2.92.

Piperidin-1-yl(1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)methanone (17a). Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and piperidine (91 μL, 1.5 equiv) were reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 10:1) to afford **17a** as a white solid (72 mg, 51%). ^{1}H NMR (CD_3OD) δ 8.40 (dd, *J* = 4.8/1.2 Hz, 1H), 7.90 (dd, *J* = 8.1/1.2 Hz, 1H), 7.85 (s, 1H), 7.23 (dd, *J* = 4.8/8.1 Hz, 1H), 3.80–3.45 (m, 4H), 1.80–1.50 (m, 6H). LRMS *m/z* $[M-H]^-$ (TOF ES^+) 230.0.

Pyrrolidin-1-yl(1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)methanone (17b). Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and pyrrolidine (76 μL, 1.5 equiv) reacted according to general procedure A. The crude product was purified by FCC (eluent EtOAc/MeOH 8:1) to afford **17b** as a white solid (53 mg, 40%). ^{1}H NMR (CD_3OD) δ 8.41 (dd, *J* = 4.6/1.2 Hz, 1H), 7.88 (dd, *J* = 8.1/1.2

Hz, 1H), 7.88 (s, 1H), 7.22 (dd, *J* = 4.6/8.1 Hz, 1H), 3.70–3.60 (m, 4H), 2.05–1.98 (m, 2H), 1.96–1.88 (m, 2H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 216.0.

Azetidin-1-yl(1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)methanone (17c). Azaindole-3-carboxylic acid (**8**) (100 mg, 0.62 mmol) and azetidine hydrochloride (87 mg, 1.5 equiv) were reacted according to general procedure A. The crude product was purified by FCC (eluent EtOAc/MeOH 5:1) to afford **17c** as a white solid (57 mg, 45%). ^{1}H NMR (CD_3OD) δ 8.48 (d, *J* = 4.4 Hz, 1H), 7.99 (s, 1H), 7.92 (dd, *J* = 8.4/1.3 Hz, 1H), 7.27 (dd, *J* = 8.3/4.4 Hz, 1H), 4.45–4.38 (m, 2H), 4.28–4.20 (m, 2H), 2.43–2.34 (m, 2H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 202.0.

Morpholino(1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)methanone (17d). Azaindole-3-carboxylic acid (**8**) (80 mg, 0.50 mmol) and morpholine (100 μL, 1.2 equiv) were reacted according to general procedure A. The crude product was purified by FCC (eluent EtOAc/MeOH 10:1) to afford **17d** as a white solid (34 mg, 30%). ^{1}H NMR (CD_3OD) δ 8.42 (d, *J* = 4.3 Hz, 1H), 7.93 (s, 1H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.25 (dd, *J* = 8.1/4.3 Hz, 1H), 3.72 (br s, 8H). LRMS *m/z* $[M+H]^-$ (TOF ES^+) 232.0.

***tert*-Butyl 4-(1*H*-pyrrolo[3,2-*b*]pyridine-3-carbonyl)piperazine-1-carboxylate (17e).** Azaindole-3-carboxylic acid (**8**) (80 mg, 0.5 mmol) and *N*-Boc piperazine (140 μL, 1.5 equiv) were reacted according to general procedure A. The crude extract was purified by FCC (eluent EtOAc/MeOH 10:1) to afford **17e** as a white solid (85 mg, 51%). ^{1}H NMR (CD_3OD) δ 8.43 (d, *J* = 4.2 Hz, 1H), 7.94 (s, 1H), 7.90 (d, *J* = 8.1 Hz, 1H), 7.26 (dd, *J* = 8.1/4.2 Hz, 1H), 3.71 (br s, 4H), 3.55 (br s, 4H), 1.48 (s, 9H). LRMS *m/z* $[M+H]^-$ (TOF ES^+) 331.0.

(1-(4-(1-Methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)(piperidin-1-yl)methanone (18a). Compound **17a** (38 mg, 0.17 mmol) and **10** (36 mg, 0.17 mmol) were reacted according to general procedure B. The crude was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **18a** as a white solid (59 mg, 88%). ^{1}H NMR (DMSO-d_6) δ 8.42 (dd, *J* = 4.6/1.3 Hz, 1H), 8.12 (s, 1H), 8.07 (s, 1H), 8.00 (dd, *J* = 8.3/1.3 Hz, 1H), 7.81 (s, 1H), 7.52 (d, *J* = 8.2 Hz, 2H) 7.29 (d, *J* = 8.2 Hz, 2H), 7.16 (dd, *J* = 8.3/4.6 Hz, 1H), 5.45 (s, 2H), 3.83 (s, 3H), 3.54 (br s, 4H), 1.60–

1.40 (m, 6H). ^{13}C NMR (DMSO-d_6) δ 164.2, 143.9, 142.9, 136.5, 135.1, 135.0, 132.7, 128.6, 128.5, 126.3, 125.6, 121.8, 118.8, 117.3, 112.4, 49.8, 39.1, 31.1, 26.2, 24.6. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{24}H_{26}N_5O$ calcd: 400.2137 found: 400.2136. LC-MS t_R = 2.90.

(1-(4-(1-Methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)(pyrrolidin-1-yl)methanone (18b). Compound **17b** (36 mg, 0.17 mmol) and **10** (36 mg, 0.17 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **18b** as a white solid (64 mg, 98%). ^{1}H NMR (DMSO-d_6) δ 8.42 (dd, *J* = 4.6/1.3 Hz, 1H), 8.16 (s, 1H), 8.07 (s, 1H), 7.98 (dd, *J* = 8.3/1.3 Hz, 1H), 7.81 (s, 1H), 7.50 (d, *J* = 8.2 Hz, 2H), 7.29 (d, *J* = 8.2 Hz, 2H), 7.16 (dd, *J* = 8.3/4.6 Hz, 1H), 5.46 (s, 2H), 3.83 (s, 3H), 3.70–3.61 (m, 2H), 3.53–3.47 (m, 2H), 1.90–1.76 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 164.1, 143.9, 142.9, 136.5, 135.1, 135.0, 132.7, 128.8, 128.5, 128.2, 125.6, 121.9, 118.8, 117.2, 113.6, 49.8, 48.2, 48.3, 39.1, 26.1, 24.5. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{23}H_{24}N_5O$ calcd: 386.1981 found: 386.1980. LC-MS t_R = 2.80.

Azetidin-1-yl(1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)methanone (18c). Compound **17c** (13 mg, 0.06 mmol) and **10** (12 mg, 0.06 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:2) to afford **18c** as a white solid (21 mg, 95%). ^{1}H NMR (DMSO-d_6) δ 8.43 (d, *J* = 4.1 Hz, 1H), 8.25 (s, 1H), 8.07 (s, 1H), 7.98 (d, *J* = 8.0 Hz, 1H), 7.81 (s, 1H), 7.50 (d, *J* = 8.2 Hz, 2H), 7.30 (d, *J* = 8.2 Hz, 2H), 7.18 (dd, *J* = 8.0/4.1 Hz, 1H), 5.46 (s, 2H), 4.37 (br s, 2H), 4.02 (br s, 2H), 3.83 (s, 3H), 2.27–2.18 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 165.7, 144.1, 142.9, 136.5, 136.2, 135.0, 132.7, 129.2, 128.6, 128.3, 125.7, 121.8, 118.9, 117.3, 110.8, 52.3, 49.8, 48.2, 39.1, 15.8. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{22}H_{22}N_5O$ calcd: 372.1824; found: 372.1824. LC-MS t_R = 2.75.

(1-(4-(1-Methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)(morpholino)methanone (18d). Compound **17d** (34 mg, 0.14 mmol) and **10** (20 mg, 0.14 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent

$CHCl_3$/MeOH 100:1) to afford **18d** as a white solid (32 mg, 58%). ^{1}H NMR (DMSO-d_6) δ 8.42 (d, J = 4.4 Hz, 1H), 8.20 (s, 1H), 8.08 (s, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.81 (s, 1H), 7.52 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.2 Hz, 2H), 7.19 (dd, J = 8.2/4.4 Hz, 1H), 5.46 (s, 2H), 3.83 (s, 3H), 3.69–3.54 (m, 8H). ^{13}C NMR (DMSO-d_6) δ 164.5, 144.2, 142.6, 136.5, 136.0, 135.0, 132.7, 128.8, 128.5, 128.3, 125.7, 121.8, 119.0, 117.4, 111.5, 79.6, 66.9, 49.8, 39.1. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{23}H_{24}N_5O_2$ calcd: 402.1930 found: 402.1931. LC-MS t_R = 3.02.

***tert*-Butyl 4-(1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carbonyl) piperazine-1-carboxylate (18e).** Compound **17e** (83 mg, 0.25 mmol) and **10** (52 mg, 0.25 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **18e** as a white solid (93 mg, 75%). ^{1}H NMR (DMSO-d_6) δ 8.42 (d, J = 4.4 Hz, 1H), 8.20 (s, 1H), 8.08 (s, 1H), 8.01 (d, J = 8.5 Hz, 1H), 7.81 (s, 1H), 7.52 (d, J = 8.2 Hz, 2H), 7.31 (d, J = 8.2 Hz, 2H), 7.20 (dd, J = 8.5/4.4 Hz, 1H), 5.47 (s, 2H), 3.83 (s, 3H), 3.57 (br s, 4H), 3.42 (br s, 4H), 1.41 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 164.5, 154.3, 144.2, 142.7, 136.5, 136.1, 135.0, 132.7, 128.8, 128.6, 128.3, 125.7, 121.8, 119.0, 117.4, 111.6, 79.5, 65.4, 49.8, 39.1, 28.5, 15.6. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{28}H_{33}N_6O_3$ calcd: 501.2614 found: 501.2617. LC-MS t_R = 2.83.

(1-(4-(1-Methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)(piperazin-1-yl)methanone (18f). *N*-Boc protected intermediate **18e** (20 mg, 0.04 mmol) was dissolved in DCM (2 mL), cooled in an ice bath and TFA (30 µL, 10 equiv) was added dropwise. The reaction was stirred overnight at rt. The mixture was then diluted with 1 M NaOH solution (50 mL) and extracted with EtOAc (5 × 50 mL) and the combined organic layers were washed with brine (50 mL), filtered over anhydrous Na_2SO_4 and dried under reduced pressure. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 20:1 to 1:1) to afford **18f** as a colourless oil (9 mg, 56%). ^{1}H NMR (CD_3OD) δ 8.42 (d, J = 4.4 Hz, 1H), 8.00 (s, 1H), 7.91 (s, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.77 (s, 1H), 7.51 (d, J = 8.2 Hz, 2H), 7.26 (d, J = 8.2 Hz, 2H), 7.25–7.18 (m, 1H), 5.46 (s, 2H), 3.89 (s, 3H), 3.69 (br s, 4H), 2.88 (br s, 4H). ^{13}C NMR (CD_3OD) δ 165.6, 143.6, 142.3, 136.0,

134.4, 134.3, 132.4, 129.3, 127.8, 127.6, 125.4, 122.4, 119.0, 117.2, 110.5, 49.9, 48.4, 45.1, 37.5. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{23}H_{25}N_6O$ calcd: 401.2090 found: 401.2092. LC-MS $t_R = 2.75$.

4,5-Dimethyl-2-(1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)oxazole (20). To a solution of azaindole-3-carboxylic acid (**8**) (1.00 g, 6.20 mmol) and 2-hydroxy-3-butanone (1.20 g, 2.2 equiv) in DMF (8 mL) was added EDC·HCl (1.78 g, 1.5 equiv) and DMAP (80 mg, 0.1 equiv). The reaction mixture was stirred at rt for 3 d. The reaction mixture was diluted with saturated $NaHCO_3$ (200 mL) and extracted with EtOAc (3 × 200 mL) and the combined organic layers were washed with brine (200 mL), filtered over anhydrous Na_2SO_4 and dried under reduced pressure. The residue containing **19** (100 mg) was diluted with acidic acid (1 mL) and ammonium acetate was added (100 mg). The reaction was conducted under reflux for 24 h. The crude productwas diluted with saturated $NaHCO_3$ (50 mL) and extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine (200 mL), filtered over anhydrous Na_2SO_4 and dried under reduced pressure. The crude extract was purified by FCC (eluent $CHCl_3$/MeOH 10:1) to afford **20** as a yellow oil (26 mg, 2%). ^{1}H NMR (CD_3OD) 8.50–8.40 (m, 1H), 8.12 (s, 1H), 7.94–7.89 (m, 1H), 7.30–7.22 (m, 1H), 2.36 (s, 3H), 2.14 (s, 3H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 214.0.

4,5-Dimethyl-2-(1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-(4-pyrrolo[3,2-*b*]pyridin-3-yl)oxazole (21). Compound **20** (16 mg, 0.08 mmol) and **10** (16 mg, 0.08 mmol) were reacted according to general procedure B. The crude product was purified by preparative HPLC to afford **21** as a yellow oil (5 mg, 19%). ^{1}H NMR (CD_3OD) δ 8.79 (d, J = 8.8 Hz, 1H), 8.74–8.70 (m, 2H), 7.94 (s, 1H), 7.85–7.78 (m, 1H), 7.79 (s, 1H), 7.55 (d, J = 8.2 Hz, 2H), 7.36 (d, J = 8.2 Hz, 2H), 5.70 (s, 2H), 3.90 (s, 3H), 2.38 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (CD_3OD) δ 154.4, 142.6, 141.1, 137.7, 136.5, 134.4, 134.3, 132.4, 128.7, 127.8, 127.6, 125.7, 122.4, 121.4, 119.0, 117.2, 102.8, 50.5, 39.1, 11.9, 10.4. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{23}H_{22}N_5O$ calcd: 384.1824 found: 384.1825. LC-MS $t_R = 3.01$.

N-((1*S*,2*S*)-2-Hydroxycyclohexyl)-1*H*-indazole-3-carboxamide (23a). Indazole-3-carboxylic acid (**22a**) (100 mg, 0.62 mmol) and *trans*-aminocyclohexanol **7a** (86 mg, 0.74 mmol) were reacted

according to general procedure A. The crude product was purified by FCC (eluent EtOAc) to afford **23a** as a white solid (106 mg, 66%). ^{1}H NMR (CD_3OD) δ 8.20 (dt, *J* = 8.3/0.9 Hz, 1H), 7.54 (dt, *J* = 8.5/0.9 Hz, 1H), 7.36 (td, *J* = 8.5/1.1 Hz, 1H), 7.19 (dt, *J* = 8.0/0.9, 1H), 3.86–3.80 (m, 1H), 3.57–3.48 (m, 1H), 2.10–2.00 (m, 2H), 1.75–1.65 (m, 2H), 1.45–1.25 (m, 4H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 260.1.

N-((1*S*,2*S*)-2-Hydroxycyclohexyl)-1*H*-indole-3-carboxamide (23b). Indole-3-carboxylic acid (**22b**) (100 mg, 0.62 mmol) and *trans*-aminocyclohexanol **7a** (86 mg, 0.74 mmol) were reacted according to general procedure A. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:2) to afford **23b** as a white solid (55 mg, 34%). ^{1}H NMR (CD_3OD) δ 8.14–8.08 (m, 1H), 7.95–7.91 (m, 1H), 7.46–7.39 (m, 1H), 7.20–7.12 (m, 2H), 3.85–3.79 (m, 1H), 3.56–3.48 (m, 1H), 2.10–2.02 (m, 2H), 1.79–1.71 (m, 2H), 1.48–1.30 (m, 4H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) found: 259.1.

4-Fluoro-*N*-((1*S*,2*S*)-2-hydroxycyclohexyl)-1*H*-indole-3-carboxamide (23c). 4-Fluoro-1*H*-indole-3-carboxylic acid (**22c**) (100 mg, 0.62 mmol) and *trans*-2-aminocyclohexanol (**7a**) (86 mg, 0.74 mmol) were reacted according to general procedure A. The crude extract was purified by FCC (eluent $CHCl_3$/MeOH 100:2) to afford **23c** as a white solid (85 mg, 49%). ^{1}H NMR (CD_3OD) δ 7.92 (s, 1H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.16 (dd, *J* = 8.0/5.0 Hz, 1H), 6.89 (dd, *J* = 13.1/8.0 Hz, 1H), 3.85–3.79 (m, 1H), 3.52–3.44 (m, 1H), 2.20–2.10 (m, 1H), 2.06–1.98 (m, 1H), 1.79–1.71 (m, 2H), 1.48–1.30 (m, 4H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) $C_{15}H_{18}FN_2O_2$ calcd: 277.13; found: 277.1.

4-Chloro-*N*-((1*S*,2*S*)-2-hydroxycyclohexyl)-1*H*-indole-3-carboxamide (23d). 4-Chloro-1*H*-indole-3-carboxylic acid (**22d**) (100 mg, 0.62 mmol) and *trans*-2-aminocyclohexanol (**7a**) (86 mg, 0.74 mmol) were reacted according to general procedure A. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:2) to afford **23d** as a white solid (87 mg, 48%). ^{1}H NMR (CD_3OD) δ 7.72 (s, 1H), 7.37 (t, *J* = 4.8 Hz, 1H), 7.14–7.10 (m, 2H), 3.83–3.75 (m, 1H), 3.50–3.44 (m, 1H), 2.18–2.09 (m, 2H), 1.79–1.71 (m, 2H), 1.43–1.28 (m, 4H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 293.1.

N-((1*S*,2*S*)-2-Hydroxycyclohexyl)-1*H*-pyrrole-3-carboxamide (23e). Pyrrole-3-carboxylic acid (**22e**) (69 mg, 0.62 mmol) and *trans*-2-aminocyclohexanol (**7a**) (86 mg, 0.74 mmol) were reacted according to general procedure A. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:2) to afford **23e** as a white solid (56 mg, 43%). ^{1}H NMR (CD_3OD) δ 7.38 (d, *J* = 1.5 Hz, 1H), 6.73 (dd, *J* = 2.3 Hz, 1H), 6.58 (dd, *J* = 2.3/1.5 Hz, 1H), 3.76–3.72 (m, 1H), 3.49–3.43 (m, 1H), 2.08–1.96 (m, 2H), 1.75–1.65 (m, 2H), 1.41–1.26 (m, 4H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 209.1.

***N*-((1*S*,2*S*)-2-Hydroxycyclohexyl)-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-indazole-3-carboxamide (24a).** Compound **23a** (50 mg, 0.20 mmol) and **10** (40 mg, 0.20 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **24a** as a white solid (60 mg, 73%). ^{1}H NMR (DMSO-d_6) δ 8.23 (d, *J* = 8.1 Hz, 1H), 8.07 (s, 1H), 7.90 (d, *J* = 8.2 Hz, 1H), 7.82 (d, *J* = 0.7 Hz, 1H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.51 (d, *J* = 8.2 Hz, 2H), 7.45–7.40 (m, 1H), 7.28–7.21 (m, 3H), 5.74 (s, 2H), 3.84 (s, 3H), 3.71–3.62 (m, 1H), 3.52–3.45 (m, 1H), 1.99–1.87 (m, 2H), 1.69–1.58 (m, 2H), 1.39–1.18 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 162.1, 141.1, 138.5, 136.4, 134.8, 132.6, 128.3, 128.2, 127.2, 125.6, 123.0, 122.8, 122.5, 121.9, 110.9, 71.4, 55.2, 52.6, 39.1, 35.1, 31.7, 25.0, 24.6. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{25}H_{28}N_5O_2$ calcd: 430.2246; found: 430.2248. LC-MS t_R = 3.26.

***N*-((1*S*,2*S*)-2-Hydroxycyclohexyl)-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-indole-3-carboxamide (24b).** Compound **23b** (26 mg, 0.10 mmol) and **10** (20 mg, 0.10 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **24b** as a white solid (35 mg, 82%). ^{1}H NMR (DMSO-d_6) δ 8.20–8.16 (m, 2H), 8.07 (s, 1H), 7.81 (s, 1H), 7.62–7.49 (m, 4H), 7.24 (d, *J* = 8.2 Hz, 2H), 7.21–7.11 (m, 2H), 5.42 (s, 2H), 4.66 (d, *J* = 5.0 Hz, 1H), 3.84 (s, 3H), 3.72–3.63 (m, 1H), 3.45–3.38 (m, 1H), 1.97–1.88 (m, 2H), 1.70–1.60 (m, 2H), 1.32–1.18 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 164.8, 136.6, 136.5, 135.5, 132.5, 131.4, 128.3, 128.2, 127.3, 125.6, 122.5, 121.9, 121.8, 121.0, 111.2, 111.0, 72.1, 55.0, 49.7,

39.1, 35.0, 32.0, 25.0, 24.6. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{26}H_{29}N_4O_2$ calcd: 429.2291; found: 429.2289. LC-MS t_R = 3.23.

4-Fluoro-*N*-((1*S*,2*S*)-2-hydroxycyclohexyl)-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-indole-3-carboxamide (24c). Compound **23c** (85 mg, 0.30 mmol) and **10** (63 mg, 0.30 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **24c** as a white solid (72 mg, 54%). ^{1}H NMR (DMSO-d_6) δ 8.13 (s, 1H), 8.07 (s, 1H), 7.82 (s, 1H), 7.54–7.48 (m, 3H), 7.42 (d, *J* = 7.3 Hz, 1H), 7.26 (d, *J* = 8.2 Hz, 2H), 7.22–7.15 (m, 1H), 6.99–6.92 (m, 1H), 5.46 (s, 2H), 4.77 (d, *J* = 4.8 Hz, 1H), 3.84 (s, 3H), 3.69–3.62 (m, 1H), 3.46–3.35 (m, 1H), 2.07–1.98 (m, 1H), 1.96–1.87 (m, 1H), 1.72–1.58 (m, 2H), 1.36–1.17 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 163.4, 157.0 (d, J_{CF} = 245 Hz), 139.5 (d, J_{CF} = 10.9 Hz), 136.5, 135.0, 135.2, 132.5, 128.3, 128.2, 125.6, 123.3 (d, J_{CF} = 8.1 Hz), 121.9, 113.7 (d, J_{CF} = 19.3 Hz), 111.0 (d, J_{CF} = 2.9 Hz), 108.2 (d, J_{CF} = 2.5 Hz), 106.8 (d, J_{CF} = 21.7 Hz), 71.9, 55.2, 49.9, 39.1, 34.5, 31.4, 24.5, 24.2. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{26}H_{28}FN_4O_2$ calcd: 447.2196; found: 447.2201. LC-MS t_R = 3.29.

4-Chloro-*N*-((1*S*,2*S*)-2-hydroxycyclohexyl)-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-indole-3-carboxamide (24d). Compound **23d** (30 mg, 0.10 mmol) and **10** (20 mg, 0.10 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **24d** as a white solid (21 mg, 47%). ^{1}H NMR (DMSO-d_6) δ 8.07 (s, 1H), 7.92 (s, 1H), 7.83 (d, *J* = 7.6 Hz, 1H), 7.81 (s, 1H), 7.53–7.48 (m, 3H), 7.26 (d, *J* = 8.1 Hz, 2H), 7.15–7.11 (m, 2H), 5.43 (s, 2H), 4.61 (d, *J* = 4.6 Hz, 1H), 3.84 (s, 3H), 3.54–3.66 (m, 1H), 3.40–3.33 (m, 1H), 2.01–1.85 (m, 2H), 1.67–1.58 (m, 2H), 1.30–1.15 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 164.6, 137.5, 136.5, 135.5, 133.3, 132.6, 132.1, 128.4, 128.3, 125.6, 123.5, 123.1, 121.9, 121.6, 113.5, 110.3, 71.8, 55.6, 49.7, 39.1, 34.5, 31.2, 24.6, 24.3. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{26}H_{28}ClN_4O_2$ calcd: 463.1901; found: 463.1896. LC-MS t_R = 3.25.

N-((1*S*,2*S*)-2-Hydroxycyclohexyl)-1-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)-1*H*-pyrrole-3-carboxamide (24e). Compound **23e** (52 mg, 0.25 mmol) and **10** (51 mg, 0.25 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **24e** as a white solid (51 mg, 54%). ^{1}H NMR (DMSO-d_6) δ 8.07 (s, 1H), 7.83 (s, 1H), 7.52 (d, J = 8.2 Hz, 2H), 7.40–7.35 (m, 2H), 7.20 (d, J = 8.2 Hz, 2H), 6.68 (dd, J = 2.6/0.8 Hz, 1H), 6.50 (dd, J = 2.6/1.6 Hz, 1H), 5.08 (s, 2H), 4.57 (d, J = 5.1 Hz, 1H), 3.85 (s, 3H), 3.58–3.49 (m, 1H), 3.35–3.29 (m, 1H), 1.90–1.77 (m, 2H), 1.65–1.56 (m, 2H), 1.25–1.14 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 164.3, 136.5, 136.2, 132.5, 128.5, 128.3, 125.6, 123.8, 122.0, 121.9, 120.9, 108.5, 72.1, 54.9, 52.7, 39.1, 35.0, 32.0, 25.0, 24.6. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{22}H_{27}N_4O_2$ calcd: 379.2063; found: 379.2137. LC-MS t_R = 3.09.

(4-(1-Methyl-1*H*-pyrazol-4-yl)phenyl)methanol (27a). A solution of 4-bromobenzyl alcohol (**25**) (1.0 g, 5.30 mmol) and 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (**26a**) (8.00 mmol) in 40 mL degassed THF/1M Na_2CO_3 3:1 was flushed with nitrogen. $PdCl_2(PPh_3)_2$ (373 mg, 0.1 equiv) was added and the reaction was stirred under reflux for 6 h. The THF was evaporated under reduced pressure and the mixture was diluted with water (150 mL) and extracted with EtOAc (3 x 150 mL). The combined organic layers were washed with brine (150 mL), filtered over anhydrous Na_2SO_4 and dried under reduced pressure. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **27a** as a pale yellow solid (710 mg, 70%). ^{1}H NMR ($CDCl_3$) δ 7.72 (s, 1H), 7.59 (s, 1H), 7.44 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 8.2 Hz, 2H), 4.67 (s, 2H), 3.92 (s, 3H). LRMS *m/z* $[M+H]^+$ (TOF ES$^+$) 189.1.

(4-(1-Methyl-1*H*-pyrazol-3-yl)phenyl)methanol (27b). 4-Bromobenzyl alcohol (**25**) (125 mg, 0.60 mmol) and 1-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (**26b**) (208 mg, 1.00 mmol) were coupled according to procedures used for the synthesis of compound **10**. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **27b** as a pale white solid (90 mg, 72%). ^{1}H NMR ($CDCl_3$) δ 7.61 (d, J = 8.23 Hz, 2H), 7.24 (d, J = 2.2 Hz, 1H), 7.22 (d, J

= 8.23 Hz, 2H), 6.41 (d, *J* = 2.2 Hz, 1H), 4.54 (d, *J* = 3.9 Hz, 2H), 3.79 (s, 3H). LRMS *m/z* $[M+H]^+$ (TOF ES^+) 189.1.

3-(4-(Chloromethyl)phenyl)-1-methyl-1*H*-pyrazole (28b). Benzyl alcohol (**27b**) (90 mg, 0.48 mmol) was reacted according to procedures used for the synthesis of compound **10**. The crude product was purified by FCC (eluent $CHCl_3$) to afford **28b** as a white solid (93 mg, 95%). ^{1}H NMR ($CDCl_3$) δ 7.75 (s, 1H), 7.60 (s, 1H), 7.44 (d, *J* = 8.2 Hz, 2H), 7.38 (d, *J* = 8.2 Hz, 2H), 4.59 (s, 2H), 3.93 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 136.9, 135.4, 132.9, 129.2, 127.0, 125.8, 122.6, 46.2, 39.1. LRMS *m/z* $[M+H]^+$ (TOF ES^+) 207.0.

***N*-((1*S*,2*S*)-2-Hydroxycyclohexyl)-1-(4-(1-methyl-1*H*-pyrazol-3-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (29b).** Compound **9a** (28 mg, 0.11 mmol) and **28b** (22 mg, 0.11 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **29b** as a white solid (12 mg, 26%). ^{1}H NMR (DMSO-d_6) δ 8.77 (d, *J* = 7.7 Hz, 1H), 8.49 (dd, *J* = 4.6/1.0 Hz, 1H), 8.40 (s, 1H), 8.06 (dd, *J* = 8.3/1.0 Hz, 1H), 7.74 (d, *J* = 8.2 Hz, 2H), 7.69 (d, *J* = 2.1, 1H), 7.32 (d, *J* = 8.2 Hz, 2H), 7.27 (dd, *J* = 8.3/4.6 Hz, 1H), 6.63 (d, *J* = 2.1 Hz, 1H), 5.53 (s, 2H), 4.81 (d, *J* = 5.0 Hz, 1H), 3.85 (s, 3H), 3.78–3.70 (m, 1H), 3.46–3.37 (m, 1H), 2.06–1.99 (m, 1H), 1.92–1.84 (m, 1H), 1.69–1.58 (m, 2H), 1.37–1.21 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 163.1, 155.7, 149.9, 144.0, 143.2, 136.3, 133.5, 132.8, 129.7, 128.2, 125.7, 119.8, 117.7, 110.7, 103.0, 71.8, 54.0, 50.0, 39.1, 34.4, 31.5, 24.4, 23.8. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{25}H_{28}N_5O_2$ calcd: 430.2243; found: 430.2248. LC-MS t_R = 3.04.

***N*-((1*S*,2*S*)-2-Hydroxycyclohexyl)-1-(4-(oxazol-2-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (29c).** A mixture of **9a** (15 mg, 0.06 mmol) and the substituted benzyl chloride **28c** (15 mg, 0.06 mmol) was reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **29c** as a white solid (15 mg, 63%). ^{1}H NMR (DMSO-d_6) δ 8.75 (d, *J* = 7.6 Hz, 1H), 8.50 (dd, *J* = 4.6/1.0 Hz, 1H), 8.42 (s, 1H), 8.18 (s, 1H), 8.03 (dd, *J* = 8.3/1.0 Hz, 1H), 7.92 (d, *J* = 8.2 Hz, 2H), 7.42 (d, *J* = 8.2 Hz, 2H), 7.35 (s, 1H), 7.26

(dd, J = 8.3/4.6 Hz, 1H), 5.62 (s, 2H), 4.80 (br s, 1H), 3.78–3.69 (m, 1H), 3.45–3.38 (m, 1H), 2.07–1.99 (m, 1H), 1.93–1.85 (m, 1H), 1.68–1.60 (m, 2H), 1.39–1.22 (m, 4H). ^{13}C NMR (CD_3OD) δ 163.2, 160.9, 144.1, 143.2, 140.6, 139.9, 136.4, 129.8, 129.0, 128.5, 126.9, 126.8, 119.7, 117.8, 110.9, 71.8, 53.9, 49.8, 34.4, 31.5, 24.4, 23.8. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{24}H_{25}N_4O_3$ calcd: 417.1927; found: 417.1923. LC-MS t_R = 3.01.

***N*-((1*S*,2*S*)-2-Hydroxycyclohexyl)-1-(4-(thiazol-2-yl)benzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (29d).** Compound **9a** (15 mg, 0.06 mmol) and the substituted benzyl chloride **28d** (12 mg, 0.06 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **29d** as a white solid (20 mg, 80%). ^{1}H NMR (DMSO-d_6) δ 8.76 (d, J = 7.6 Hz, 1H), 8.50 (dd, J = 4.6/1.2 Hz, 1H), 8.43 (s, 1H), 8.05 (dd, J = 8.4/1.2 Hz, 1H), 7.93–7.89 (m, 3H), 7.76 (d, J = 3.2 Hz, 1H), 7.40 (d, J = 8.2 Hz, 2H), 7.26 (dd, J = 8.4/4.6 Hz, 1H), 5.60 (s, 2H), 4.81 (d, J = 5.0 Hz, 1H), 3.78–3.70 (m, 1H), 3.46–3.38 (m, 1H), 2.07–1.99 (m, 1H), 1.92–1.85 (m, 1H), 1.69–1.58 (m, 2H), 1.37–1.22 (m, 4H). ^{13}C NMR (CD_3OD) δ 167.0, 163.2, 144.3, 144.1, 143.2, 139.5, 136.4, 133.0, 129.7, 128.6, 127.0, 121.0, 119.7, 117.8, 110.9, 71.9, 55.0, 49.8, 34.4, 31.5, 24.4, 23.8. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{24}H_{25}N_4O_2S$ calcd: 433.1698; found: 433.1695. LC-MS t_R = 3.07.

1-Benzyl-*N*-((1*S*,2*S*)-2-hydroxycyclohexyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (29e). Compound **9a** (28 mg, 0.11 mmol) and benzyl bromide (**28e**) (13 μL, 0.11 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **29e** as a white solid (31 mg, 83%). ^{1}H NMR (DMSO-d_6) δ 8.76 (d, J = 7.6 Hz, 1H), 8.50 (dd, J = 4.5/1.3 Hz, 1H), 8.38 (s, 1H), 8.06 (dd, J = 8.3/1.1 Hz, 1H), 7.35–7.23 (m, 6H), 5.53 (s, 2H), 4.78 (d, J = 5.1 Hz, 1H), 3.78–3.68 (m, 1H), 3.46–3.37 (m, 1H), 2.06–1.99 (m, 1H), 1.93–1.85 (m, 1H), 1.69–1.58 (m, 2H), 1.38–1.21 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 163.2, 144.0, 143.2, 137.5, 136.3, 129.8, 129.2, 128.3, 127.8, 119.7, 117.7, 110.7, 71.8, 53.9, 50.7, 34.4, 31.4, 24.4, 23.8. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{21}H_{24}N_3O_2$ calcd: 350.1869; found: 350.1872. LC-MS t_R = 2.98.

***N*-((1*S*,2*S*)-2-Hydroxycyclohexyl)-1-(4-methoxybenzyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (29f).** Compound **9a** (28 mg, 0.11 mmol) and 4-methoxybenzyl chloride (**28f**) (12 μL, 0.11 mmol) were reacted according to general procedure B. The crude product was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **29f** as a white solid (32 mg, 80%). ^{1}H NMR (DMSO-d_6) δ 8.75 (d, *J* = 7.6 Hz, 1H), 8.48 (dd, *J* = 4.7/1.3 Hz, 1H), 8.34 (s, 1H), 8.08 (dd, *J* = 8.4/1.3 Hz, 1H), 7.31–7.22 (m, 3H), 6,89 (d, *J* = 8.5 Hz, 2H), 5.43 (s, 2H), 4.79 (d, *J* = 5.1 Hz, 1H), 3.78–3.72 (m, 1H), 3.71 (s, 3H), 3.45–3.37 (m, 1H), 2.06–1.99 (m, 1H), 1.91–1.85 (m, 1H), 1.68–1.57 (m, 2H), 1.36–1.21 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 163.2, 159.3, 143.9, 143.2, 136.0, 129.6, 129.5, 129.3, 119.8, 117.6, 114.5, 110.6, 71.9, 55.5, 53.9, 49.7, 34.4, 31.4, 24.4, 23.8. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{22}H_{26}N_3O_3$ calcd: 380.1974; found: 380.1961. LC-MS t_R = 2.97.

Methyl 4-((3-(((1*S*,2*S*)-2-hydroxycyclohexyl)carbamoyl)-1*H*-pyrrolo[3,2-*b*]pyridin-1-yl)methyl) benzoate (29g). Compound **9a** (28 mg, 0.11 mmol) and methyl 4-(chloromethyl)benzoate (**28g**) (25 mg, 0.11 mmol) were reacted according to general procedure B. The crude was purified by FCC (eluent $CHCl_3$/MeOH 100:1) to afford **29g** as a white solid (22 mg, 50%). ^{1}H NMR (DMSO-d_6) δ 8.78 (d, *J* = 7.6 Hz, 1H), 8.58 (dd, *J* = 4.6/1.2 Hz, 1H), 8.42 (s, 1H), 8.00 (dd, *J* = 8.4/1.2 Hz, 1H), 7,92 (d, *J* = 8.3 Hz, 2H), 7.38 (d, *J* = 8.3 Hz, 2H), 7.26 (dd, *J* = 8.4/4.6 Hz, 1H), 5.64 (s, 2H), 4.79 (d, *J* = 5.1 Hz, 1H), 3.81 (s, 3H), 3.78–3.71 (m, 1H), 3.47–3.39 (m, 1H), 2.06–1.99 (m, 1H), 1.93–1.86 (m, 1H), 1.70–1.59 (m, 2H), 1.37–1.24 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 163.2, 163.1, 144.1, 143.1, 142.9, 136.5, 130.1, 129.8, 129.5, 127.9, 119.7, 117.8, 110.9, 71.9, 53.9, 52.6, 49.9, 34.4, 31.4, 24.4, 23.8. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{23}H_{26}N_3O_4$ calcd: 408.1923; found: 408.1926. LC-MS t_R = 3.04.

4-((3-(((1*S*,2*S*)-2-Hydroxycyclohexyl)carbamoyl)-1*H*-pyrrolo[3,2-*b*]pyridin-1-yl)methyl)benzoic acid (29h). A mixture of methyl ester **29g** (33 mg, 0.08 mmol), EtOH/H_2O (1:1) (2 mL) and 1 M NaOH aqueous solution (1 mL) was stirred overnight under reflux. The reaction media was diluted with 1 M HCl solution until pH 3.0, concentrated under reduced pressure and filtered. The crude product was dried and purified by FCC (eluent $CHCl_3$/MeOH 2:1) to afford **29h**

as a white solid (14 mg, 45%). ^{1}H NMR (DMSO-d_6) δ 8.77 (d, J = 7.6 Hz, 1H), 8.50 (dd, J = 4.7/1.2 Hz, 1H), 8.40 (s, 1H), 8.02 (dd, J = 8.4/1.2 Hz, 1H), 7,88 (d, J = 8.0 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 7.25 (dd, J = 8.4/4.7 Hz, 1H), 5.62 (s, 2H), 4.80 (br s, 1H), 3.77–3.69 (m, 1H), 3.45–3.38 (m, 1H), 2.06–1.99 (m, 1H), 1.93–1.85 (m, 1H), 1.69–1.59 (m, 2H), 1.36–1.22 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 187.9, 163.1, 144.1, 143.1, 141.9, 136.4, 130.2, 130.1, 129.7, 127.6, 119.7, 117.8, 110.9, 71.9, 53.9, 49.9, 34.4, 31.4, 24.4, 23.8. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{22}H_{24}N_3O_4$ calcd: 394.1767; found: 394.1766. LC-MS t_R = 2.88.

1-(4-Carbamoylbenzyl)-*N*-((1*S*,2*S*)-2-hydroxycyclohexyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (29i). Methyl ester **29g** (94 mg, 0.23 mmol), methanol (3 mL) and NH_4OH (28% solution, 3 mL) were stirred under reflux for 3 d. The reaction media was concentrated under reduced pressure, diluted with 1 M Na_2CO_3 (80 mL) and extracted with EtOAc (3 × 80 mL). The combined organic layers were dried under reduced pressure. The crude product was purified by FCC ($CHCl_3$/MeOH 20:1) to afford **29i** as a white solid (29 mg, 32%). ^{1}H NMR (DMSO-d_6) δ 8.77 (d, J = 7.7 Hz, 1H), 8.50 (dd, J = 4.6/1.2 Hz, 1H), 8.41 (s, 1H), 8.03 (dd, J = 8.3/1.2 Hz, 1H), 7.92 (br s, 1H), 7,83 (d, J = 8.2 Hz, 2H), 7.35–7.31 (m, 3H), 7.27 (dd, J = 8.3/4.6 Hz, 1H), 5.29 (s, 2H), 4.80 (d, J = 5.2 Hz, 1H), 3.78–3.68 (m, 1H), 3.46–3.39 (m, 1H), 2.06–1.99 (m, 1H), 1.92–1.85 (m, 1H), 1.68–1.58 (m, 2H), 1.37–1.19 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 168.0, 163.1, 144.1, 143.1, 140.6, 136.4, 134.3, 129.7, 128.4, 127.6, 119.7, 117.8, 110.8, 71.9, 53.9, 49.9, 34.4, 31.4, 24.4, 23.8. HRMS *m/z* $[M+H]^+$ (Q-TOF/ESI) $C_{22}H_{25}N_4O_3$ calcd: 393.1927; found: 393.1921. LC-MS t_R = 2.79.

Whole Cell Radioligand Binding Assays. FlpIn Chinese hamster ovary (CHO) cells stably expressing the human M_1 mAChR were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Carlsbad, CA) supplemented with 5% fetal bovine serum (FBS) (ThermoTrace, Melbourne, Australia) and 300 μg/mL G418 (Invitrogen, Carlsbad, CA). The cells were plated at 50,000 cells per well in 96-well Isoplates (PerkinElmer), and incubated overnight. The following day, cells were washed twice with Phosphate Buffered Saline (PBS), and incubated with varying concentrations of ACh (Sigma, St. Loius, MI) or PAMs or PAMs in the presence of EC_{20}

concentration of ACh, and 0.2 nM [^{3}H]NMS in binding buffer (20 mM HEPES, 100 mM NaCl, 10 mM $MgCl_2$, pH 7.4) for 5 h at room temperature. Non-specific binding was determined using atropine at the final concentration of 100 μM. The assays were terminated by rapid removal of radioligand followed by two 100 μL washes with ice-cold 0.9% NaCl buffer. Radioactivity was determined by addition of 100 μL of Microscint scintillation liquid (PerkinElmer Life Sciences) to each well and counting in a MicroBeta plate reader (PerkinElmer Life Sciences).

IP_1 Accumulation Assays. The IP_1 assay kit (Cisbio) was used for the direct quantitative measurement of myo-inositol 1 phosphate (IP_1). FlpIn CHO cells stably expressing the hM_1 mAChR were seeded at 25,000 per well in 96-well transparent cell culture plates, and incubated overnight. The following day, cells were pre-incubated with IP_1 stimulation buffer (1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 4.2 mM KCl, 146 mM NaCl, 5.5 mM D-Glucose, 10 mM HEPES and 50 mM LiCl, pH 7.4) for 1 h at 37 °C, 5% CO_2. Cells were then incubated with varying concentration of ACh and the PAMs for 1 h at 37 °C, 5% CO_2. The reactions were terminated by removal of the stimulation buffer and addition of 50 μL of lysis buffer (50 mM HEPES pH 7.0, 15 mM KF, 1.5% V/V Triton-X-100, 3% V/V FBS, 0.2% W/V BSA). Seven μL of cell lysates were transferred to wells of 384-well Proxy-plates (PerkinElmer), and IP_1 levels were measured by incubating the lysates with 1.5 μL of the anti-IP_1 Tb cryptate conjugate and 1.5 μL of the IP_1-D2 conjugate for 1 h at 37 °C. The emission signals were measured at 620 and 665 nm after excitation at 340 nm using an Envision multilabel plate reader (PerkinElmer). The signal was expressed as the HTRF ratio: [(fluorescence 665 nm/fluorescence 620 nm) × 10^4], interpolated from the standard curve, and normalized to the maximum response to ACh.

Data Analysis. All data were analyzed using Prism 7 (GraphPad Software, San Diego, CA). Binding interaction studies with allosteric ligands were fitted to the following allosteric ternary complex model (eq. 1)[36]

$$Y = \frac{B_{max}[A]}{[A]+\left[\frac{K_A K_B}{\alpha'[B]+K_B}\right]\left[1+\frac{[I]}{K_I}+\frac{[B]}{K_B}+\frac{\alpha[I][B]}{K_I K_B}\right]} \quad (1)$$

where B_{max} is the total number of receptors, [A], [B] and [I] denote the concentrations of radioligand, allosteric modulator, and orthosteric ligand, respectively; K_A, K_B and K_I are their respective equilibrium dissociation constants. α' and α are the cooperativities between the allosteric ligand and radioligand or the allosteric modulator and orthosteric ligand, respectively. Values of α or α' > 1 denote positive cooperativity, values< 1 but >0 denote negative cooperativity, and a value of 1 indicates neutral cooperativity. α' values were determined from interaction studies between radioligand and allosetric modulator in absence of the orthosteric agonist (i.e., [I] = 0).

Functional interaction studies between orthosteric agonists and allosteric modulators in IP_1 assays were analysed according to a three-parameter logistic equation or the following operational model of allosterism and agonism (Eq. 2)[27,38]

$$E = \text{Basal} + \frac{(E_m - \text{Basal})\,([A](K_B + \alpha\,\beta\,[B]) + \tau_B[B]EC_{50})^n}{EC_{50}{}^n(K_B + [B])^n + ([A](K_B + \alpha\,\beta\,[B]) + \tau_B[B]EC_{50})^n} \quad (2)$$

where E_m is the maximal possible system response, and Basal is the response in the absence of agonist. [A] and [B] are concentrations of orthosteric and allosteric ligands, respectively. K_B is the equilibrium dissociation constant of allosteric ligand, and EC_{50} is the concentration of orthosteric agonist required to achieve half maximal response. α and β represent the magnitude of the allosteric effects on orthosteric ligand affinity and efficacy, respectively; τ_B is the efficacy of allosteric ligand, and n is the slope factor of the transducer function that links occupancy to response. The application of this simplified equation is only valid if the orthosteric agonist is a full agonist both in the absence and presence of all concentrations of modulator,[38] which was the case in all studies conducted herein.

All potency, affinity, efficacy, and cooperativity values were estimated as logarithms,[39] and unpaired t-test or one-way analysis of variance with a Dunnett's multiple comparison post test was used to determine statistical differences, where appropriate. A value of $p < 0.05$ was considered statistically significant.

■ ASSOCIATED CONTENT

Supporting Information

Table with shift values of ACh potency (ΔpEC_{50}) and baseline (Δbaseline) of the graphs in Figures 3 and 4 at 1 and 10 μM; binding and functional response curves for compounds **1**, **2**, **3**, **6a**, **6o**, **15b**, **15d**, **24e**, **29h**;Spectral data for all tested derivatives (^{1}H and ^{13}C NMR, HRMS and LCMS retention times);

Molecular formula strings.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ACh, acetylchloline; AD, Alzheimer's disease; AE, adverse effects; Boc, tertbutyloxycarbonyl; DCM, dichloromethane; DMF, dimethylformamide; equiv, equivalent; EtOAc, ethyl acetate; FCC, flash column chromatography; M_1 mAChR, M_1 muscarinic acetylcholine receptor; PAM, positive allosteric modulator; TFA, trifluoroacetic acid.

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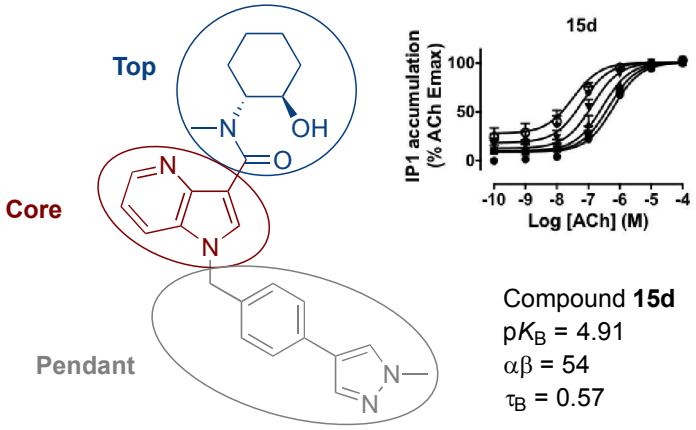
TABLE OF CONTENTS GRAPHIC



Compound **15d**
pK_B = 4.91
$\alpha\beta$ = 54
τ_B = 0.57