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4-Phenylpyridin-2-one Derivatives: A Novel Class of Positive Allosteric Modulator of the M_1 Muscarinic Acetylcholine Receptor

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ABSTRACT: Positive allosteric modulators (PAMs) of the M_1 muscarinic acetylcholine receptor (M_1 mAChR) are a promising strategy for the treatment of the cognitive deficits associated with diseases including Alzheimer's and schizophrenia. Herein, we report the design, synthesis and characterization of a novel family of M_1 mAChR PAMs. The most active compounds of the 4-phenylpyridin-2-one series exhibited comparable binding affinity to the reference compound, 1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (BQCA) (**1**), but markedly improved positive cooperativity with acetylcholine, and retained exquisite selectivity for the M_1 mAChR. Furthermore, our pharmacological characterization revealed ligands with a diverse range of activities, including modulators that displayed both high intrinsic efficacy and PAM activity, those that showed no detectable agonism but robust PAM activity, and ligands that displayed robust allosteric agonism but little modulatory activity. Thus the 4-phenylpyridin-2-one scaffold offers an attractive starting point for further lead optimization.

■ INTRODUCTION

Selective activation of the M_1 mAChR may provide a useful approach for the treatment of the cognitive deficits associated with Alzheimer's disease (AD) and schizophrenia (SZ).[1,2] Evidence of cholinergic loss in the cortex of AD patients and in the striatum of SZ patients suggested a link between mAChR function and disease pathology.[3-5] The M_1 mAChR has received particular attention for its role in cognition. It is expressed predominantly in the hippocampus, striatum and cortex, and activation of the receptor causes cognition-enhancing effects in animal models and M_1 mAChR knock-out mice display a range of cognitive deficits.[6-15] In addition, both acetylcholinesterase (AChE) inhibitors and the M_1/M_4 mAChR preferring agonist, xanomeline, improved cognitive function and/or have antipsychotic efficacy in human patients of AD and SZ.[16-18] Unfortunately, both AChE inhibitors and xanomeline display limiting gastrointestinal side effects, likely due to their lack of selectivity across the muscarinic receptor subtype family and, in particular, action at the M_2 and M_3 mAChRs, which are widely expressed in the periphery.

Unfortunately, the development of subtype-selective drugs that target the orthosteric site (i.e. the ACh binding site) remains challenging because this site is highly conserved across the mAChR receptor family. Therefore, considerable research efforts have focused upon targeting less conserved and spatially distinct allosteric sites with allosteric modulators, allosteric agonists and bitopic ligands.[7-9, 14, 19-21] 1-(4-Methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (BQCA) (**1**) is a prototypical example of a highly selective positive allosteric modulator (PAM) for the M_1 mAChR.[9, 22, 23] Although **1** has a low affinity for the allosteric site on the M_1 mAChR, it nonetheless displays *in vivo* activity in animal models of cognitive deficits, an action most likely driven by very high positive cooperativity with ACh when both molecules are co-bound on the receptor. We have recently published a SAR study of **1** that incorporated analytical modeling into our pharmacological analysis to relate PAM structural features to changes in allosteric ligand binding affinity (pK_B), intrinsic efficacy (τ_B), cooperativity with ACh binding (α) and/or modulatory

effects upon ACh efficacy (β). The standout analogue of this series, 5,8-difluoro-*N*-((1*S*,2*S*)-2-hydroxycyclohexyl)-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**2**), demonstrated higher affinity, intrinsic efficacy and functional cooperativity with ACh, compared to **1**.[20] Furthermore, the studies revealed a strong correlation between the intrinsic efficacy and cooperativity of this series of ligands, whereby the greater the level of allosteric agonism displayed by the modulator, the greater the level of observed cooperativity when combined with ACh.

The findings with **1** and the aforementioned analogues adhere to the classic Monod-Wyman-Changeux (MWC) two-state model of allostery, which predicts a correlation between allosteric agonism and allosteric modulation.[22] As with orthosteric agonists, the degree of allosteric agonism can also be dependent upon the pathway stimulus-response coupling efficiency and/or receptor density. However, in order to truly understand the relationship between in vitro measures of allosteric ligand behaviour and the actual in vivo efficacy of such modulators, one needs a broader suite of M_1 mAChR PAMs that display a range of different allosteric behaviours. Indeed, a recent study revealed that two selective M_1 mAChR agonists could differentially regulate coupling of the M_1 mAChR to specific signalling pathways and lead to selective actions on some, but not all, M_1 mAChR mediated responses in brain circuits important for memory, learning and psychosis – a property not consistent with the MWC model and instead suggestive of pathway-biased allosteric modulation.[14] Such region-specific effects may be therapeutically advantageous.

We have recently combined site-directed mutagenesis and molecular modelling/docking experiments to infer the structural nature of the M_1 mAChR allosteric binding site to which compound **1** binds.[24] In particular, our results highlighted the role of Tyr179 within the second extracellular loop (ECL2) of the M_1 mAChR for binding via formation of hydrophobic/edge-to-face π-π interactions with both the bicyclic 4-oxo-1,2-dihydroquinoline core and the benzylic pendant moiety of **1**. Similarly, Trp400 at the top of transmembrane domain 7 (TM7) was predicted to make a π-π interaction with the benzylic pendant. Herein, we report the design, synthesis and detailed pharmacological characterization of a novel family of positive allosteric modulators at the M_1

mAChR. The compound design was based on the desire to explore simpler heterocyclic cores that can maintain the key receptor-ligand interactions described above, in combination with formalizing the presence of the pseudo third ring present in **1** (due to intramolecular hydrogen bonding between the carboxylic acid and ketone moieties).[25] Accordingly we devised the general scaffold depicted in Figure 1.

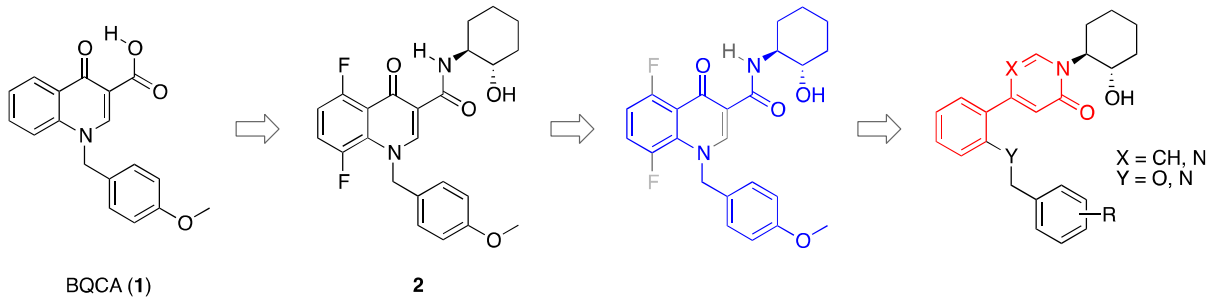


Figure 1. Conceptual development of the novel M_1 mAChR PAM scaffold starting from **1**[9,22,23] via 5,8-difluoro-*N*-((1*S*,2*S*)-2-hydroxycyclohexyl)-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**2**)[20] to evolve to the general structure of the novel 4-phenylpyridin-2-one based compounds reported herein.

■ RESULTS AND DISCUSSION

Chemistry. The commercial availability of both 4-bromo-2-hydroxypyridine (**3**) and 1,2-cyclohexene oxide allowed easy access to both the pyridine derivative **4a** and the pyridin-2-one **4b** (Scheme 1). Initially, we adapted a literature procedure that reported alkylation of **3** in the presence of K_2CO_3 in DMF giving a mixture of the O- and N-alkyl products.[26] More specifically, heating compound **3** in a 2.5-fold excess of both K_2CO_3 and 1,2-cyclohexene oxide in DMF at 120 °C overnight gave a mixture of the 2-alkoxypyridine (**4a**) and the *N*-alkylpyridin-2-one (**4b**). These isomers were easily separated based on their contrasting solubility profiles in EtOAc and the ability to remove remaining impurities through aqueous washing. Whilst the overall yield was good, no selectivity was observed in terms of the site of alkylation with **4a** and **4b** being formed in a ~1:1 ratio. In each case only the *trans*-isomer was observed as a racemic mixture. The target alkylation products arose from the tautomeric nature of compound **3**, with the ability to exist in both the 2-hydroxypyridine (**3a**) and pyridin-2(1*H*)-one (**3b**) forms. Although crystallization studies on 2-pyridone have demonstrated the amide tautomer to predominate in the solid state,[27,28] tautomerism in solution is heavily dependent on solvent nature.[29,30] As a consequence, a solvent screen was performed to evaluate the preferred product formation (**4a** versus **4b**) after addition of 1,2-cyclohexene oxide. The non-polar solvent toluene formed exclusively the 2-alkoxypyridine **4a** within 1 hour at reflux. Polar solvents including DMF, ethanol, DME, DMSO exhibited ratios between 30:70 to 70:30 of compounds **4a** and **4b** and reaction times were generally about 1 day (as assessed by LC-MS). In water, no reaction was observed, while under neat conditions (using 10 equivalents of reagent) after 1 hour reaction time at 120 °C a product ratio of ~30:70 of compound **4a** versus **4b** was obtained. Hence, when the reaction was performed under neat conditions using 5 equivalents of 1,2-cyclohexene oxide the *N*-alkylpyridin-2-one (**4b**) was obtained in an improved isolated yield of 77%.

With the aim of identifying a tractable scaffold, we elected to first synthesize comparable examples incorporating the scaffold of molecules **4a** and **4b**, before embarking on a more in-depth SAR campaign. Initially, we carried out parallel Suzuki coupling of both **4a** and **4b** with 2-hydroxyphenylboronic acid, in the presence of 10% $PdCl_2(PPh_3)_2$ in 1 M $Na_2CO_{3(aq)}$/THF at 100 °C, giving the 2-arylphenol derivatives **5** and **7** in excellent yield after flash column chromatography. A 4-phenylbenzylic pendant was attached to the phenolic group of compounds **5** and **7**, on the basis of such a moiety imparting improvements in affinity (K_B) and binding cooperativity (α) with ACh in our previously reported enriched SAR study of **1**.[20] This was achieved using Finkelstein-type modification of standard alkylation conditions, with catalytic KI, 4-(bromomethyl)biphenyl and K_2CO_3 in DMF at room temperature, to give the desired ethers **6** and **8a**.

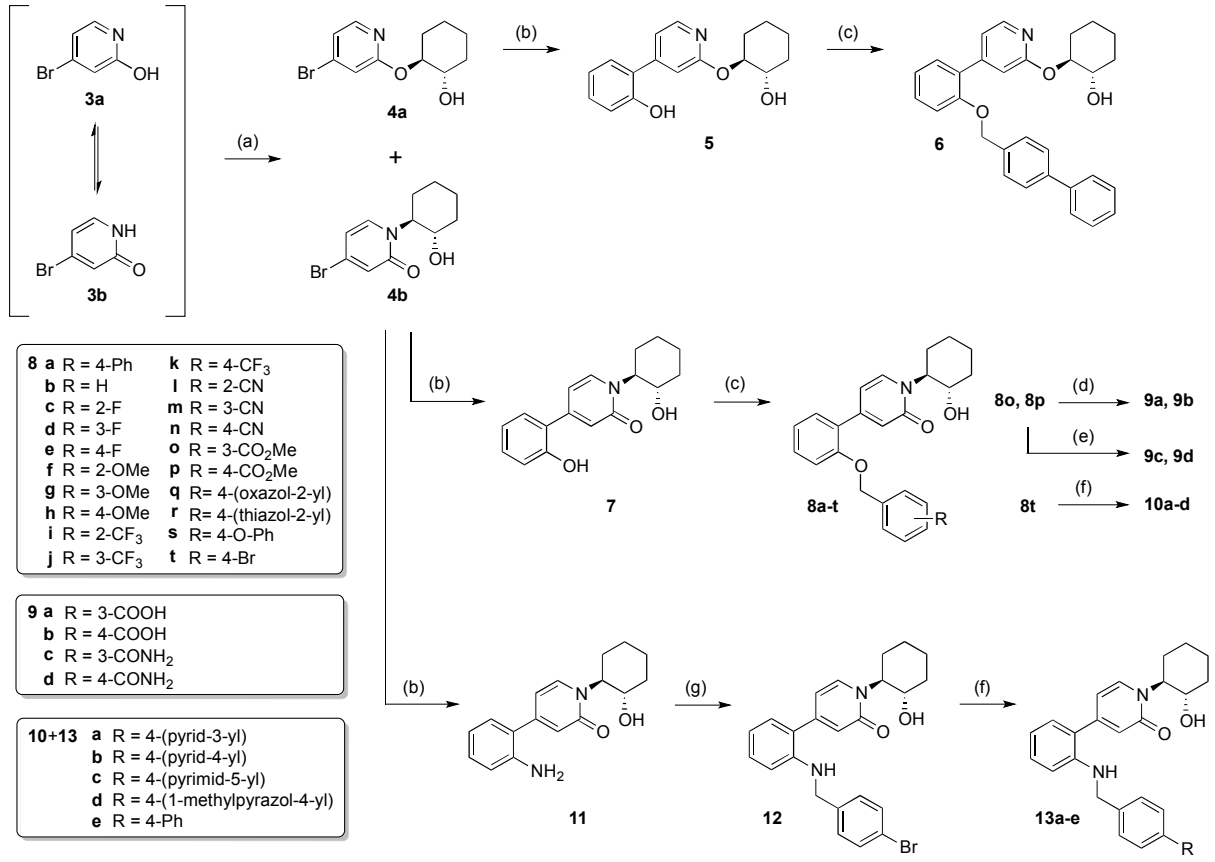
Subsequent analogues **8b-t** were synthesised as a parallel series in the same manner as compound **8a**, by varying the nature of the alkyl halide used to alkylate phenol **7**. The methyl benzoate compounds **8o** and **8p** offered an ideal starting point to generate the corresponding benzoic acid (**9a** and **9b**) and benzamide (**9c** and **9d**) analogues, through basic hydrolysis and direct aminolysis with NH_4OH, respectively.

Whilst the 4-bromobenzyl derivative **8t**, was of interest in building SAR around this series in its own right, it was also an attractive moiety to further elaborate the core structure. This was achieved through a second series of Suzuki couplings, with selected boronic acids and boronate esters, to give 4-arylbenzyl ether compounds **10a-d**.

Finally, a focussed selection of aniline derivatives **13a-e** was also synthesised, to compare directly to the corresponding phenol analogue **8a** as well as **10a-d** and investigate activity in relation to the nature of atom used to link the benzylic pendant to the parent core. Installation of the aniline moiety was carried out using Suzuki chemistry as before, coupling 4-bromo-1-(2-hydroxycyclohexyl)pyridin-2(*1H*)-one (**4b**) with 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline. Selective monoalkylation of the free aniline was achieved through established reductive

alkylation methodology reported by Abdel-Magid *et al.*,[31] employing 4-bromobenzaldehyde to introduce the benzylic pendant, giving 4-bromobenzylamino derivative **12**. Suzuki coupling of compound **12** with the previously selected group of boronic acids and boronate esters gave the desired analogues **13a-e**.

Scheme 1. Synthesis of initial series of 4-phenylpyridin-2-one derivatives [a]

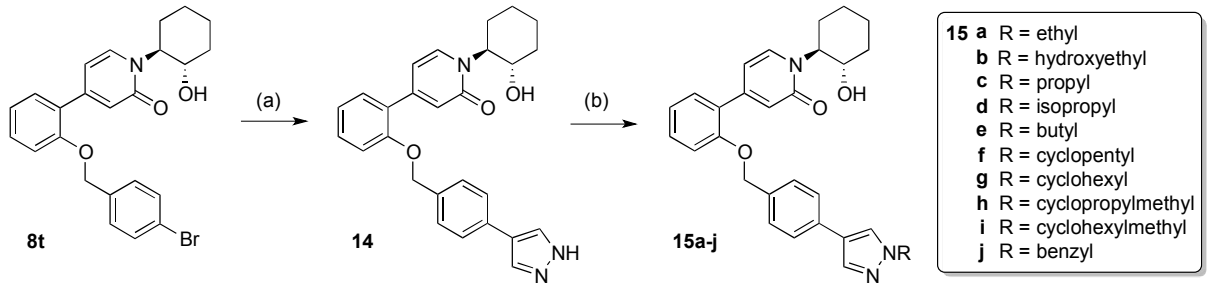


[a]Reagents and conditions: (a) 1,2-cyclohexene oxide, K_2CO_3, 120 °C, 77% (rac-*trans*); (b) 2-hydroxyphenylboronic acid or 2-(4,4,5,5-tetramethyl-1,2,3-dioxaborolan-2-yl)aniline, cat. $PdCl_2(PPh_3)_2$, 1 M $Na_2CO_{3(aq)}$/THF degassed, 100 °C, 83-100%; (c) substituted benzyl halide, K_2CO_3, cat. KI, DMF, rt, 18-93%; (d) NaOH, EtOH/H_2O, 50 °C, 82-93%; (e) NH_4OH/MeOH, rt, 14-52%; (f) boronic acid or boronate ester, cat. $PdCl_2(PPh_3)_2$, 1 M $Na_2CO_{3(aq)}$/THF degassed, 100 °C, 29-77%; (g) 4-bromobenzaldehyde, AcOH, $NaB(OAc)_3H$, 1,2-dichloroethane, rt, 50%.

A smaller second series of compounds was synthesized to investigate changes to the pyrazole moiety of compound **10d** (Scheme 2). Therefore, the unsubstituted pyrazole derivative **14** was obtained via Suzuki reaction of intermediate **8t** with the *N*-Boc protected pyrazole boronic ester.

These reaction conditions afforded the Boc deprotected product **14**, circumventing the need for an additional acid-mediated cleavage procedure to remove the Boc group. It is worth noting that compound **14** could not be synthesized efficiently when using (1*H*-pyrazol-4-yl)boronic acid hence the use of the *N*-Boc protected pyrazole boronate ester. In the next step, alkylation of compound **14** afforded analogues **15a-j** in yields varying from 6-82%. Depending on the steric bulk of the alkyl halide the alkylation reactions proceeded at room temperature or at elevated temperatures of up to 100 °C. The alkylation with bromocyclobutane was unsuccessful even at elevated temperature and extended reaction time.

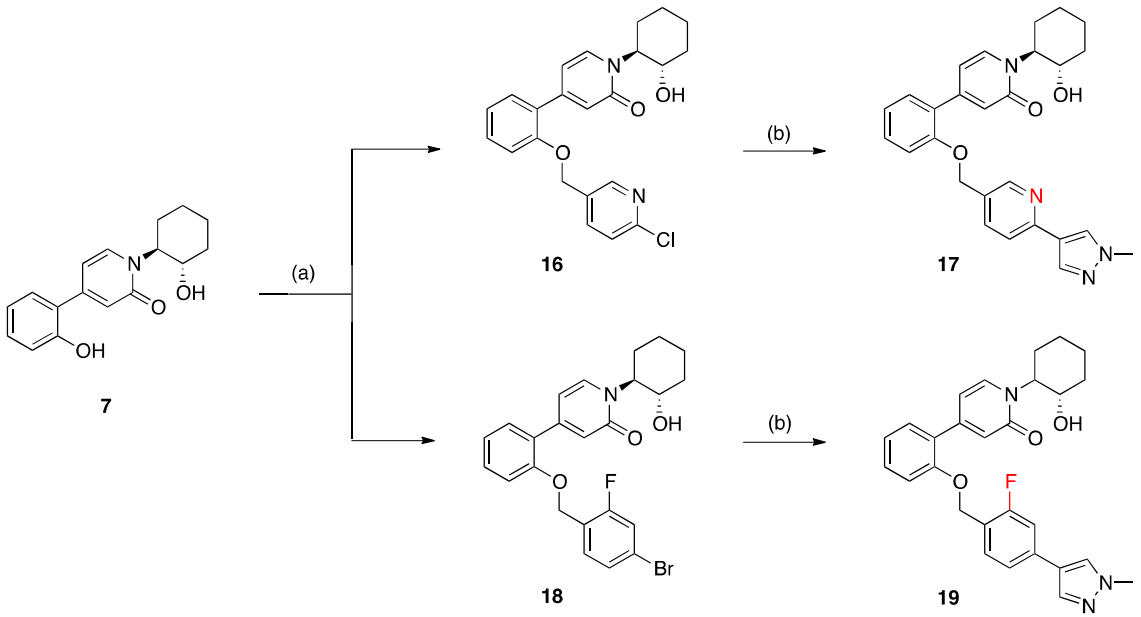
Scheme 2. Synthesis of a series of *N*-substituted pyrazole derivatives[a]



[a]Reagents and conditions: (a) 1-(*tert*-butoxycarbonyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole, cat. $PdCl_2(PPh_3)_2$, 1 M $Na_2CO_{3(aq)}$/THF degassed, 100 °C, 77%; (b) alkyl halide, K_2CO_3, cat. KI, DMF, rt to 100 °C, 6-82%.

We subsequently synthesized two additional compounds, **17** and **19**, incorporating modifications to the benzyl component of the 1-methylpyrazol-4-yl)benzyl pendant of **10d** (Scheme 3). This was achieved through initial alkylation of intermediate **7** with 2-chloro-5-(chloromethyl)pyridine or 4-bromo-1-(bromomethyl)-2-fluorobenzene, giving **16** and **18** respectively. The desired analogues **17** and **19** were subsequently obtained via Suzuki coupling of **16** and **18** with 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole.

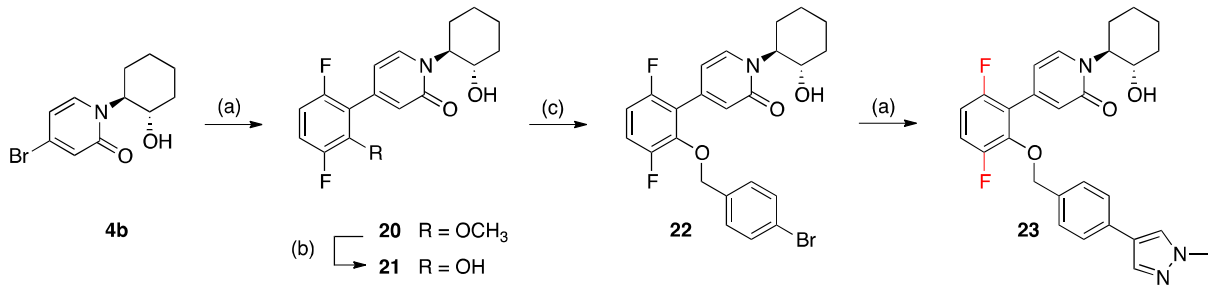
Scheme 3. Synthesis of analogues with modifications to the 4-(1-methylpyrazol-4-yl)benzyl pendant [a]



[a] Reagents and conditions: (a) substituted benzyl halide, K_2CO_3, cat. KI, DMF, rt, 63% for **16**, 62% for **18**; (b) boronic acid / boronate ester, cat. $PdCl_2(PPh_3)_2$, 1 M $Na_2CO_{3(aq)}$, THF degassed, 100 °C, 32% for **17**, 30% for **19**.

Next, we synthesized analogue **23** (Scheme 4), incorporating fluoro substituents in analogous positions to those present in **2** (Figure 1), which were previously shown to influence intrinsic efficacy in compounds of the 4-oxo-1,4-dihydroquinoline class.[20] The intermediate **4b** was coupled with the commercially available (3,6-difluoro-2-methoxyphenyl)boronic acid to afford a 1:1 mixture of **20** and an unidentified side product in an overall yield of 23% (Scheme 4). *O*-Demethylation of intermediate **20** was achieved with BBr_3, to afford the corresponding phenol (**21**), in good yield. Purification of **21** via flash column chromatography (FCC) permitted separation of the unidentified side product from the previous step. Alkylation with 1-bromo-4-(bromomethyl)benzene was performed at room temperature to give ether **22**, followed by a Suzuki coupling reaction with 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole at 100 °C to give **23** in modest yield.

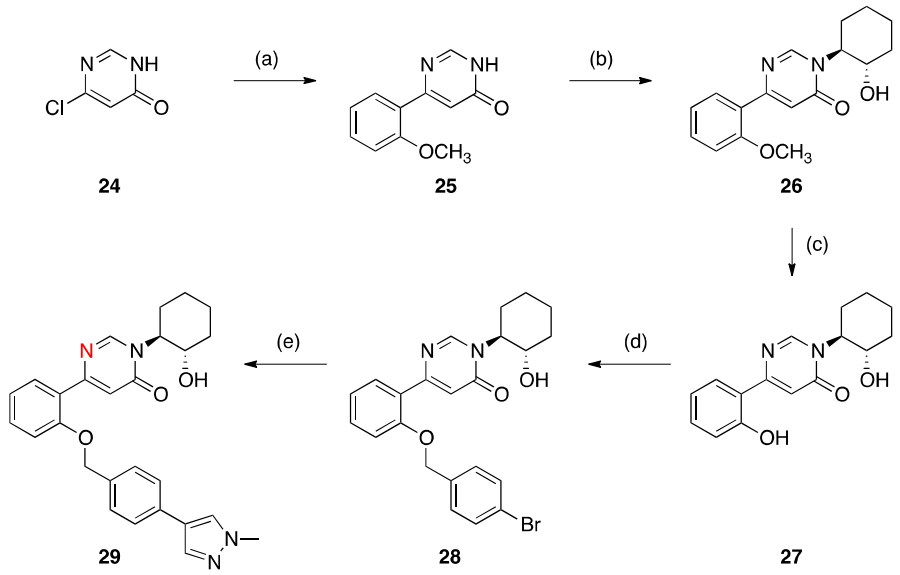
Scheme 4. Synthesis of difluoro-substituted 4-phenylpyridin-2-one **23** [a]



[a]Reagents and conditions: (a) boronate ester, cat. $PdCl_2(PPh_3)_2$, 1 M $Na_2CO_{3(aq)}$/THF degassed, 100 °C, 23% for **20**, 31% for **23**; (b) 1 M BBr_3 in hexane, DCM, 0 °C to rt, 72%; (c) 4-bromobenzyl bromide, K_2CO_3, cat. KI, DMF, rt, 73%.

Finally, we synthesized the 6-phenylpyrimidin-4-one analogue **29** (Scheme 5). This analogue allowed us to probe if further interaction with the allosteric pocket of the M_1 mAChR can be achieved through the introduction of an additional hydrogen bond acceptor (tertiary nitrogen) to the scaffold. In the case of **29** the order of the reaction steps was altered, since the epoxide ring opening reaction with 1,2-cyclohexene oxide and 6-chloropyrimidin-4(3*H*)-one (**24**) did not afford the desired intermediate. The failure of this reaction may be explained by the preference for polymerization through the 6-chloro group over the epoxide ring opening reaction with 1,2-cyclohexene oxide. As a consequence, starting material **24** was first coupled with (2-methoxyphenyl)boronic acid to afford intermediate **25** in modest yield (20%), before subsequent reaction with 1,2-cyclohexene oxide to give compound **26**. LC-MS indicated the formation of the 4-alkoxypyrimidine and *N*-alkylpyrimidin-4-one (**26**) in an approximately 1:3 ratio. Nonetheless, only the *N*-alkylpyrimidin-4-one (**26**) could be isolated after FCC due to side products interfering with the 4-alkoxypyrimidine product. We then converted the 2-methoxyphenyl moiety to a phenol using boron tribromide to give intermediate **27** in good yield (87%). The last two steps involved alkylation with 1-bromo-4-(bromomethyl)benzene and Suzuki coupling with 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole under the same conditions as reported for the pyridinone analogues, to give the desired 6-phenylpyrimidin-2-one **29**.

Scheme 5. Synthesis of the 6-phenylpyrimidin-4-one analogue **29** [a]



[a]Reagents and conditions: (a) (2-methoxyphenyl)boronic acid, cat. $PdCl_2(PPh_3)_2$, 1 M $Na_2CO_{3(aq)}$, THF degassed, 100 °C, 20%; (b) 1,2-cyclohexene oxide, K_2CO_3, 120 °C, 38%; (c) 1 M BBr_3 in hexane, DCM, 0 °C to rt, 87%; (d) 4-bromobenzyl halide, K_2CO_3, cat. KI, DMF, rt, 55%; (e) boronate ester, cat. $PdCl_2(PPh_3)_2$, 1 M $Na_2CO_{3(aq)}$, THF degassed, 100 °C, 11%.

Pharmacology. Our recent SAR study of **1** allowed us to correlate chemical modifications to changes in parameters that describe allosteric action at the M_1 mAChR. We applied the same approach in this study for selected intermediates and all final compounds. Competition binding studies between ACh and the radiolabelled antagonist [^{3}H]NMS at the M_1 mAChR expressed in FlpIN CHO cells were performed in the absence and presence of increasing concentrations of test compound, and data were analysed with an allosteric ternary complex model to determine the binding affinity of the test compound (K_B) for the unoccupied M_1 mAChR and its cooperativity with ACh (α). To assess the ability of our test compounds to modulate ACh function, we used myo-inositol 1 phosphate (IP_1) accumulation as a measure of M_1 mAChR activation resulting from preferential activation of canonical G_q proteins. Concentration response curves of ACh were generated in the presence of increasing concentrations of test compound, and an operational model of allostery was applied to the data, with the K_B parameter fixed to that determined in the binding

studies, thus allowing an overall estimate of functional cooperativity with ACh ($\alpha\beta$, where β describes the modulatory effect upon ACh efficacy) and any intrinsic efficacy (τ_B) of the allosteric ligand. Values of α or $\beta > 1$ describe a positive modulatory effect upon ACh, whereas values < 1 (but greater than 0) describe a negative allosteric effect. Since it is well established that the logarithms of affinity and cooperativity values are normally distributed, whereas their absolute values (antilogarithms) are not[32], all statistical comparisons for interpretation of the SAR described below (Tables 1-3) were performed on the logarithmic values. For ease of interpretation, however, allosteric parameter antilogarithms are also highlighted in the main text for selected key derivatives.

As described in Figure 1, we devised a general molecular scaffold, distinct from that of **1**, that we hypothesized would maintain key receptor-ligand interactions. As a starting point, we compared the ether analogue **6** with the pyridinone analogue **8a** (both of which incorporate the 4-phenylbenzylic pendant). Compound **8a** displayed an affinity for the M_1 mAChR of approximately 10 μM and positive cooperativity with ACh binding and function. The affinity and cooperativity displayed by **8a** (Table 1) were not significantly different from that of **1** ($p > 0.05$, one way ANOVA), while the intrinsic efficacy of **8a** ($\tau_B = 1.38$) was superior to that of **1** ($\tau_B = 0.22$). In contrast analogue **6** was inactive. As a consequence, compounds based on the 1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one moiety were further investigated (Table 1), whereas the development of analogues containing a 2-(pyridin-2-yloxy)cyclohexan-1-ol moiety was discontinued.

Intermediate **7** exhibited a binding affinity not significantly different from that of **1** but essentially neutral binding cooperativity with ACh ($\alpha = 0.91$), demonstrating that the 4-phenylbenzylic pendant is important for the positive cooperativity of compound **8a** with ACh. Interestingly, analogue **8b**, with an unsubstituted benzylic pendant (i.e. lacking the additional 4-phenyl group), maintained binding affinity and positive cooperativity with ACh comparable to that displayed by compound **8a**. Therefore, we investigated the effect of incorporating a range of

substituted monocyclic ligands (compounds **8c-p** and **9a-d**) instead of the 4-phenylbenzylic pendant present in compound **8a**.

While affinity was unchanged across these compounds, phenyl rings substituted at the *ortho*-position with methoxy, trifluoromethyl or nitrile groups (**8f**, **8i** and **8l**, respectively) exhibited lower binding (α) and functional (*αβ)* cooperativity compared to the respective *meta*- and *para*-substituted analogues (**8g-h**, **8j-k** and **8m-n**). While a similar trend was observed for the fluoro-substituted analogues (**8c-e**), this change was not significant. However, for the ester and carboxylic acid analogues (**8o-p** and **9a-b**) the *meta*-substituted analogue displayed similar activity to the corresponding *para*-substituted compound. The stand out compound of this series was the *para*-substituted carboxamide **9d**, which exhibited a binding affinity comparable to **1**, but significantly improved binding (α = 320) and functional ($\alpha\beta$ = 230) cooperativity with ACh. The observed intrinsic efficacy (τ_B) of compound **9d** was 4-fold greater than that of **1**. While the *meta*-substituted carboxamide analogue **9c** displayed binding and functional cooperativity with ACh comparable to that of **1**, it did not display any detectable allosteric agonism. Due to the promising properties of the carboxamide analogue **9d**, the structurally related oxazole **8q** and thiazole **8r** were also synthesized. However, both bioisosteres (**8q** and **8r**) displayed a significant reduction in binding cooperativity with ACh (α = 27 and α = 25 respectively) as compared to **9d**.

Next, we investigated the effect of replacing the distal phenyl ring of the 4-phenylbenzyl moiety present on **8a**, with a variety of bioisosteres. These included 6-membered heterocycles (**10a-c**), a 5-membered heterocycle (**10d**), and a simple bromo substituent (**8t**) that has previously been used as a phenyl isostere. All the compounds displayed a binding affinity not significantly different from that of **1** and comparable binding and functional cooperativity with ACh and intrinsic efficacy, with the notable exception of the pyrazole analogue **10d**, which displayed significantly improved binding cooperativity (α = 370) as compared to that of **1**. Incorporation of an oxygen atom between the two ring systems, as for the phenoxy analogue **8s**, resulted in total loss of the binding cooperativity with ACh but maintenance of affinity for the M_1 mAChR.

We next investigated modification of the linker atom between the benzylic pendant and the parent core. A comparison of aniline analogues **12** and **13a-e** with the corresponding ether analogues **8t**, **10a-d** and **8a**, respectively, revealed that this modification resulted in a reduction in binding and functional cooperativity with ACh but no change in affinity for the M_1 mAChR. The unsubstituted aniline intermediate **11** exhibited both an 8-fold reduction in binding affinity to that of **1** and neutral binding cooperativity (α = 0.98) with ACh, demonstrating the importance of the presence of the benzylic pendant linked to the parent core for the allosteric action of this series. Furthermore, in terms of binding cooperativity with ACh, the trend seen for the different ligands of the aniline series (**13d** > **13a** = **13c** = **13b** >**13e** = **12**) is similar to the trend observed for the ether analogues (**10d** > **10a** = **10c** = **10b** = **8a** = **8t**), whereby the 4-(1-methylpyrazol-4-yl) analogue was the most active compound in both series.

None of the compounds in Table 1 showed a significant improvement in intrinsic efficacy compared to **1**. However, it is interesting to note that both **8j** and **8k**, which display neutral functional cooperativity with ACh, still displayed robust allosteric agonism comparable to that of **1** (τ_B = 1.02 and 0.77, respectively). Taking compound **10d** as the most active from this initial series, we then tested whether the novel compound displayed selectivity towards the M_1 mAChR. As shown in Figure 2, while both **1** and **10d** display robust modulatory activity at the M_1 mAChR in a [^{3}H]NMS binding assay, they exhibit no activity up to a concentration of 10 μM at the other mAChR subtypes. As such, the novel 4-phenylpyridin-2-one scaffold appears to share the same exquisite selectivity for the M_1 mAChR as the benzyl-4-oxo-1,4-dihydroquinoline scaffold.

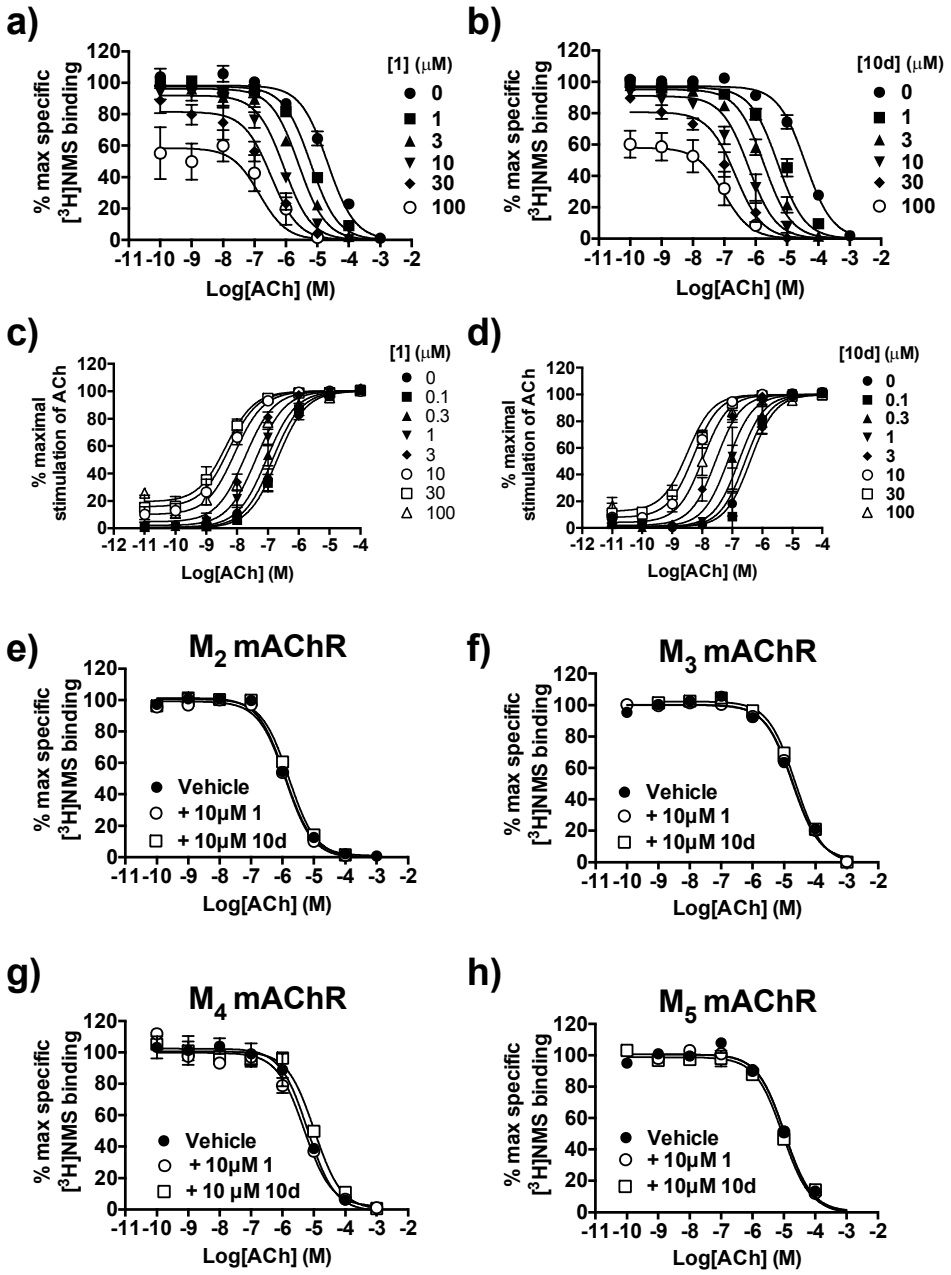
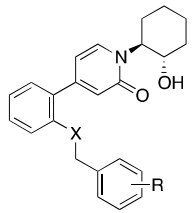


Figure 2. (**a**-**d**) (**a**-**d**) Pharmacological characterization of **1** and **10d** in binding and function at the mAChRs. (**a**-**b**) Radioligand binding experiments were performed using FlpIn-CHO cells expressing the M_1 mAChR, 0.1 nM of the radiolabeled antagonist [^{3}H]NMS, increasing concentrations of ACh, with or without increasing concentrations of either **1** (**a**) or **10d** (**b**). (**c**-**d**) IP_1 accumulation experiments were performed using FlpIn-CHO cells expressing the M_1 mAChR and increasing concentrations of ACh with or without increasing concentrations of either compound **1** (**c**) or **10d** (**d**). 100% represents the maximal stimulation of ACh in the absence of test compound. (**e**-**h**) Activity of **1** and **10d** at the human $M_{2\text{-}5}$ mAChRs in a [^{3}H]NMS binding assay. No detectable effect of these compounds was observed at the other mAChR subtypes up to a concentration of 10 μM.

Table 1: Binding and functional parameters of 4-phenylpyridin-2-one analogues 7-13e at the M_1 mAChR.



	X	R	Radioligand binding ([^{3}H]NMS)			IP_1 accumulation	
			pK_B (K_B, μM)	Logα' [a]	Logα (α) [b]	Log$\alpha\beta$ ($\alpha\beta$) [c]	Logτ_B (τ_B) [d]
1	-	-	4.78 ± 0.06 (17)	-3	1.77 ± 0.13 (58)	1.84 ± 0.08 (69)	-0.60 ± 0.10 (0.22)
7	OH	-	4.10 ± 0.08 (79)*	-3	-0.04 ± 0.11 (0.91)*	n.d.	
8a	O	4-Ph	4.99 ± 0.10 (10)	-0.27 ± 0.06	1.22 ± 0.07 (17)	1.09 ± 0.15 (12)	0.14 ± 0.04 (1.38)
8b	O	H	4.55 ± 0.10 (28)	-3	1.20 ± 0.08 (15)	0.94 ± 0.21 (9)	-0.24 ± 0.13 (0.58)
8c	O	2-F	4.52 ± 0.07 (30)	-3	1.30 ± 0.10 (20)	0.62 ± 0.26 (4)*	-0.36 ± 0.16 (0.44)
8d	O	3-F	4.38 ± 0.07 (41)	-3	1.50 ± 0.07 (31)	0.85 ± 0.26 (7)	-0.11 ± 0.12 (0.78)
8e	O	4-F	4.22 ± 0.01 (60)	-3	1.63 ± 0.07 (43)	1.15 ± 0.10 (14)	0.00 ± 0.24 (1)
8f	O	2-OMe	4.53 ± 0.15 (30)	-3	0.53 ± 0.14 (3)	n.d.	
8g	O	3-OMe	4.19 ± 0.02 (65)	-3	1.37 ± 0.05 (23)	0.84 ± 0.14 (7)	0.16 ± 0.04 (1.45)
8h	O	4-OMe	4.28 ± 0.10 (52)	-3	1.67 ± 0.09 (47)	0.80 ± 0.06 (6)	-1.10 ± 0.17 (0.08)
8i	O	2-CF_3	4.48 ± 0.12 (33)	-3	-0.07 ± 0.22 (1)*	n.d.	
8j	O	3-CF_3	4.53 ± 0.17 (30)	-3	0.90 ± 0.10 (7.9)*	= 0	0.01 ± 0.12 (1.02)
8k	O	4-CF_3	4.44 ± 0.10 (36)	-3	1.36 ± 0.11 (23)	0.40 ± 0.31 (2.5)*	-0.11 ± 0.18 (0.77)
8l	O	2-CN	4.38 ± 0.07 (42)	-0.65 ± 0.04	0.59 ± 0.08 (3.8)*	n.d.	
8m	O	3-CN	4.06 ± 0.14 (87)*	-3	1.55 ± 0.11 (35)	0.88 ± 0.35 (8)	-0.59 ± 0.35(0.25)
8n	O	4-CN	4.85 ± 0.13 (14)*	-0.29 ± 0.05	0.84 ± 0.09 (7)*	0.49 ± 0.16 (3)*	-0.73 ± 0.16 (0.19)
8o	O	3-CO_2Me	3.99 ± 0.08 (100)*	-3	1.49 ± 0.10 (31)	0.52 ± 0.22 (3)*	-0.82 ± 0.28 (0.15)

8p	O	4-CO_2Me	4.06 ± 0.14 (87)*	-3	1.32 ± 0.09 (21)	0.96 ± 0.28 (9)	-0.26 ± 0.18 (0.54)
8q	O	4-(oxazol-2-yl)	4.56 ± 0.08 (28)	-0.68 ± 0.06	1.43 ± 0.09 (27)	1.61 ± 0.18 (41)	-0.24 ± 0.24 (0.57)
8r	O	4-(thiazol-2-yl)	4.96 ± 0.05 (11)	-0.45 ± 0.09	1.40 ± 0.08 (25)	0.80 ± 0.19 (6)	-0.35 ± 0.13 (0.45)
8s	O	4-O-Ph	4.71 ± 0.08 (19)	-0.30 ± 0.02	0.08 ± 0.10 (1)*	n.d.	
8t	O	4-Br	4.24 ± 0.11 (58)	-3	1.26 ± 0.15 (18)	n.d.	
9a	O	3-COOH	4.04 ± 0.10 (91)*	-3	1.38 ± 0.11(24)	1.08 ± 0.06 (12)	-0.69 ± 0.09 (0.20)
9b	O	4-COOH	3.88 ± 0.06 (130)*	-3	1.31 ± 0.09 (20)	0.92 ± 0.08 (8)	-1.24 ± 0.34
9c	O	3-$CONH_2$	4.04 ± 0.05 (91)*	-3	1.46 ± 0.11 (29)	0.87 ± 0.09 (7)	-3
9d	O	4-$CONH_2$	4.66 ± 0.12 (22)	-0.86 ± 0.15	2.51 ± 0.10 (320)*	2.36 ± 0.08 (230)	0.05 ± 0.08 (0.89)
10a	O	4-(pyrid-3-yl)	4.72 ± 0.09 (19)	-3	1.76 ± 0.11 (58)	1.29 ± 0.12 (19)	-0.42 ± 0.37 (0.38)
10b	O	4-(pyrid-4-yl)	4.95 ± 0.14 (11)	-0.76 ± 0.07	1.49 ± 0.09 (31)	1.01 ± 0.29 (10)	-0.37 ± 0.19 (0.43)
10c	O	4-(pyrimid-5-yl)	4.45 ± 0.09 (35)	-0.37 ± 0.05	1.56 ± 0.07 (36)	1.34 ± 0.28 (22)	-0.80 ± 0.35 (0.16)
10d	O	4-(1-methyl pyrazol-4-yl)	4.37 ± 0.07 (43)	-3	2.57 ± 0.17 (370)*	2.30 ± 0.08 (200)	-0.68 ± 0.15 (0.21)
11	NH_2	-	3.86 ± 0.06 (138)*	-3	-0.01 ± 0.14 (1)*	n.d.	
12	NH	4-Br	4.35 ± 0.10 (45)	-3	0.33 ± 0.08 (2)*	n.d.	
13a	NH	4-(pyrid-3-yl)	4.67 ± 0.04 (21)	-0.57 ± 0.02	1.08 ± 0.05 (12)	0.47 ± 0.11 (3)*	-0.13 ± 0.04 (0.74)
13b	NH	4-(pyrid-4-yl)	5.03 ± 0.02 (9.3)	-0.69 ± 0.05	0.90 ± 0.08 (8)*	n.d.	
13c	NH	4-(pyrimid-5-yl)	3.86 ± 0.10 (138)*	-3	0.97 ± 0.19 (9)*	0.68 ± 0.16 (5)*	0.04 ± 0.05 (1.10)
13d	NH	4-(1-methyl pyrazol-4-yl)	4.18 ± 0.07 (66)	-0.58 ± 0.14	1.97 ± 0.04 (93)	1.44 ± 0.09 (28)	-0.36 ± 0.08 (0.44)
13e	NH	4-Ph	4.16 ± 0.15 (69)	-0.30 ± 0.04	0.44 ± 0.11 (2.8)*	n.d.	

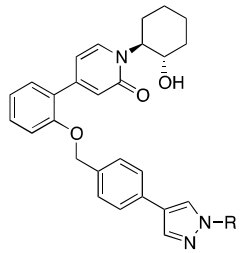
[a] Binding cooperativity with [^{3}H]NMS; for instances where a complete inhibition of [^{3}H]NMS binding by the allosteric modulator was observed (consistent with a high level of negative cooperativity), logα' was fixed to -3; [b]binding cooperativity with ACh; [c]functional cooperativity with ACh; [d]intrinsic efficacy of the modulator; for instances where no intrinsic efficacy was observed, Logτ_B was fixed to -3. * = significant difference ($p < 0.05$) relative to same parameter determined for **1**, one-way ANOVA with Dunnett's post-test. n.d. = inactive at concentrations up to 30 μM; values represent the mean ± SEM from at least three experiments performed in duplicate.

Further studies were performed around the 4-(1-methylpyrazol-4-yl) analogue **10d**, which, together with carboxamide analogue **9d**, displayed the greatest level of positive cooperativity with ACh of the first series of compounds in both binding and function. We initially investigated the exchange of the methyl group with a hydrogen atom (**14**), linear and branched aliphatic carbon chains (**15a-e**) as well as cyclic unsaturated and aromatic substituents (**15f-15j**) (Scheme 2, Table 2). The removal of the methyl group (compound **14**) caused a 120-fold loss in positive binding cooperativity with ACh in comparison to the methylpyrazole analogue **10d**. This dramatic effect could potentially be explained by the loss of an important hydrophobic interaction within the allosteric binding pocket or the change of the tautomeric properties of the pyrazole moiety. Of interest, this loss of binding and functional cooperativity was accompanied by a significant 5-fold increase in intrinsic efficacy (τ_B = 1.10). Elongation of the chain length from the methyl (**10d**) to the ethyl (**15a**) derivative had no effect upon affinity or cooperativity with ACh. However, a loss in binding cooperativity was observed with further extension to the propyl (**15c,** α = 40; 10-fold loss) and isopropyl (**15d,** α = 120; 3-fold loss) and, most dramatically, the *n*-butyl derivate (**15e,** α = 6; 120-fold loss). In contrast, no significant change in intrinsic efficacy was observed. The hydroxethyl derivative **15b** showed comparable cooperativity with ACh compared to that of the methyl and ethyl pyrazole (**10d** and **15a**). The incorporation of cyclic unsaturated and aromatic substituents (**15f-15j**) caused a decrease in binding cooperativity with ACh as compared to **10d** with the most dramatic effect being the 170-fold decrease observed upon incorporation of a benzyl group (**15j**). Interestingly, compounds with larger cyclic aliphatic substituents attached to the pyrazole moiety, such as compounds **15f** and **15g**, exhibited higher ligand binding affinity (K_B < 10 μM), but with no improvement of binding and functional cooperativity. In the case of **15h,** the lack of intrinsic efficacy was also noteworthy.

We subsequently investigated compounds with small changes to the parent core of the 4-(1-methylpyrazol-4-yl) analogue **10d** (Scheme 3, Table 3). Compound **17**, which incorporates a 2-(1-methylpyrazol-4-yl)pyridinyl moiety, exhibited comparable binding cooperativity with ACh as

compared to that of **10d** but, of particular interest, displayed no detectable intrinsic efficacy. The 2-fluorobenzyl analogue **19** did not exhibit a change in binding cooperativity or intrinsic efficacy as compared to compound **10d**. Intermediate **18** displayed a substantial reduction in binding cooperativity as compared to **10d**, demonstrating the importance of the 4-(1-methylpyrazol-4-yl) substituent of compound **19**. In comparison, the introduction of fluoro groups to analogues of **1** at the corresponding positions to those in compound **2** (see Figure 1), translated to a small improvement of potency.[33] Our earlier work demonstrated that the introduction of 5,8-difluoro groups to the 4-oxo-1,4-dihydroquinoline core of **1** increased intrinsic activity.[20] The introduction of the corresponding 3,6-difluoro groups to our novel 4-phenylpyridin-2-one scaffold (compound **23**) caused a 60-fold decrease in binding cooperativity (α = 6) but a significant 6-fold increase in intrinsic efficacy (τ_B = 1.34). Finally, we investigated the effect of adding an additional nitrogen atom, therefore changing the pyridine-2(1*H*)-one core to a pyrimidin-4(3*H*)-one core. Although no gain in affinity for the M_1 mAChR was observed, **29** was the standout compound of the series displaying a 4-fold increase in binding cooperativity with ACh and a 11-fold increase in intrinsic efficacy as compared to **10d** (Figure 3). Furthermore, compound **29** displays a similar affinity as reference compound **1**, but a significant 24-fold increase in binding cooperativity with ACh and a 11-fold increase in intrinsic activity.

Table 2: Binding and functional allosteric parameters of pyrazole analogues 14-15j at the M_1 mAChR.



		Radioligand binding ([^{3}H]NMS)			IP_1 accumulation	
	R	pK_B (K_B, μM)	Logα [a]	Logα (α) [b]	Log$\alpha\beta$ ($\alpha\beta$) [c]	Logτ_B (τ_B) [d]
10d	-CH_3	4.37 ± 0.07 (43)	-3	2.57 ± 0.17 (370)	2.30 ± 0.08 (200)	-0.68 ± 0.15 (0.21)
14	-H	4.56 ± 0.26 (28)	-0.10 ± 0.11	0.53 ± 0.22 (3.4)*	0.68 ± 0.16 (4.8)*	0.04 ± 0.05 (1.1)*
15a	-CH_2CH_3	4.38 ± 0.15 (41)	0.03 ± 0.07	2.47 ± 0.12 (295)	2.26 ± 0.11 (181)	-0.05 ± 0.08 (0.89)*
15b	-$(CH_2)_2OH$	4.43 ± 0.09 (37)	-3	2.76 ± 0.13 (575)	2.49 ± 0.11 (310)	-0.11 ± 0.06 (0.77)*
15c	-$(CH_2)_2CH_3$	4.64 ± 0.12 (23)	-0.18 ± 0.07	1.60 ± 0.25 (40)*	1.78 ± 0.08 (60)	-0.52 ± 0.09 (0.3)
15d	-$CH(CH_3)_2$	4.57 ± 0.21 (27)	-0.41 ± 0.21	2.08 ± 0.10 (120)	1.93 ± 0.11 (85)	-0.33 ± 0.07 (0.47)
15e	-$(CH_2)_3CH_3$	4.86 ± 0.11 (14)	-0.10 ± 0.06	0.78 ± 0.23 (6)*	0.75 ± 0.13 (6)*	-0.30 ± 0.08 (0.50)
15f	-cyclopentyl	5.45 ± 0.03 (4)	-0.50 ± 0.02	1.08 ± 0.04 (12)*	1.92 ± 0.10 (83)	-0.42 ± 0.07 (0.38)
15g	-cyclohexyl	5.67 ± 0.07 (2)*	-0.50 ± 0.02	1.05 ± 0.09 (11)*	0.63 ± 0.17 (4)*	-0.29 ± 0.09 (0.51)
15h	-CH_2-cyclopropyl	5.13 ± 0.05 (7)	-0.39 ± 0.04	1.38 ± 0.15 (24)*	0.58 ± 0.12 (3.8)*	-3
15i	-CH_2-cyclohexyl	4.52 ± 0.31 (30)	-0.19 ± 0.05	0.21 ± 0.10 (1.6)*	n.d.	
15j	-CH_2Ph	4.68 ± 0.49 (21)	-0.12 ± 0.03	0.33 ± 0.04 (2.1)*	n.d.	

[a]Binding cooperativity with [^{3}H]NMS; for instances where a complete inhibition of [^{3}H]NMS binding by the allosteric modulator was observed (consistent with a high level of negative cooperativity), logα' was fixed to -3; [b]binding cooperativity with ACh; [c]functional cooperativity with ACh; [d]intrinsic efficacy of the modulator; for instances where no intrinsic efficacy of the modulator was observed Logτ_B was fixed to -3. * = significant difference ($p < 0.05$) relative to same parameter determined for **10d**, one-way ANOVA with Dunnett's post-test. n.d. = inactive at concentrations up to 30 μM; values represent the mean ± SEM from at least three experiments performed in duplicate.

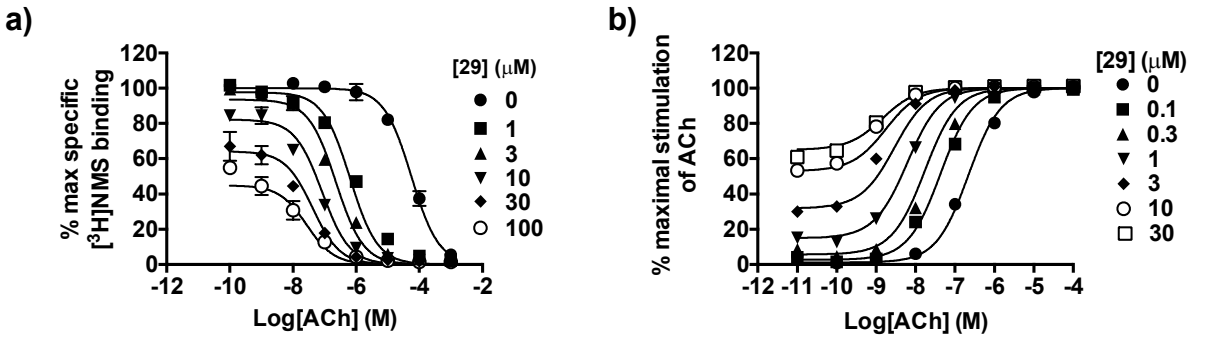


Figure 3. Pharmacological characterization of **29** in binding and function at the mAChRs. (**a**) Radioligand binding experiments were performed using FlpIn-CHO cells stably expressing the M_1 mAChR, 0.1 nM of the radiolabeled antagonist [^{3}H]NMS, increasing concentrations of ACh, with or without increasing concentrations of **29**. (**b**) IP_1 accumulation experiments were performed using FlpIn-CHO cells stably expressing the M_1 mAChR, increasing concentrations of ACh with or without increasing concentrations of **29**. 100% represents the maximal stimulation of ACh in the absence of **29**.

It is interesting to note that compound **1** exhibited equivalent values for both binding (α = 60) and functional ($\alpha\beta$ = 62) cooperativity, suggesting that it is the allosteric effect on ACh binding affinity that underlies the cooperativity measured in our functional assay. Similarly, there was no significant difference between values of binding (α) and functional ($\alpha\beta$) cooperativity determined for any compound in this novel series based around the 4-phenylpyridin-2-one scaffold (Tables 1-3, Student's t-test, $p < 0.05$). Indeed, we observed a strong correlation between Log$\alpha\beta$ and Logα (Figure 4a), demonstrating that binding cooperativity mediates the modulatory effect upon ACh in the functional assay observed for the compounds in this series. Another key observation from our characterization the compounds based around the 4-phenylpyridin-2-one scaffold was the apparent "uncoupling" of the correlation between positive cooperativity with ACh and allosteric ligand intrinsic efficacy, in contrast to our previous SAR study of compound **1** (Figure 4b & c). Although the most active compound of the current series (**29**) displayed both the largest positive cooperativity with ACh and the largest intrinsic efficacy (Figure 4), we also identified analogues ranging from **17**, which displayed no intrinsic efficacy but robust positive cooperativity with ACh (α = 125, Figure

4e), to **14** , which displayed intrinsic efficacy comparable to that of **1** (τ_B = 1.1, Figure 4d) but only very weak positive cooperativity with ACh (α = 3). This range of different ligand activities suggests that the action of some ligands within the current series no longer adhere to a simple two-state allosteric model of action. Further evidence of this is provided by our binding assay. In the majority of cases, a robust inhibition of the orthosteric antagonist, [^{3}H]NMS, by increasing concentrations of test compound was observed, consistent with these ligands displaying positive cooperativity with agonists but high negative cooperativity with the binding of antagonists. This is consistent with a two-state mode of action and our previous observations of the action of **1** or analogues.[20,22] However, in the present series we observed a number of compounds (for example **15a**, **15c-e**) that displayed neutral cooperativity with [^{3}H]NMS but robust positive cooperativity with the agonist ACh. As such, these observations suggest that a simple two-state model can no longer accommodate the action of these ligands.

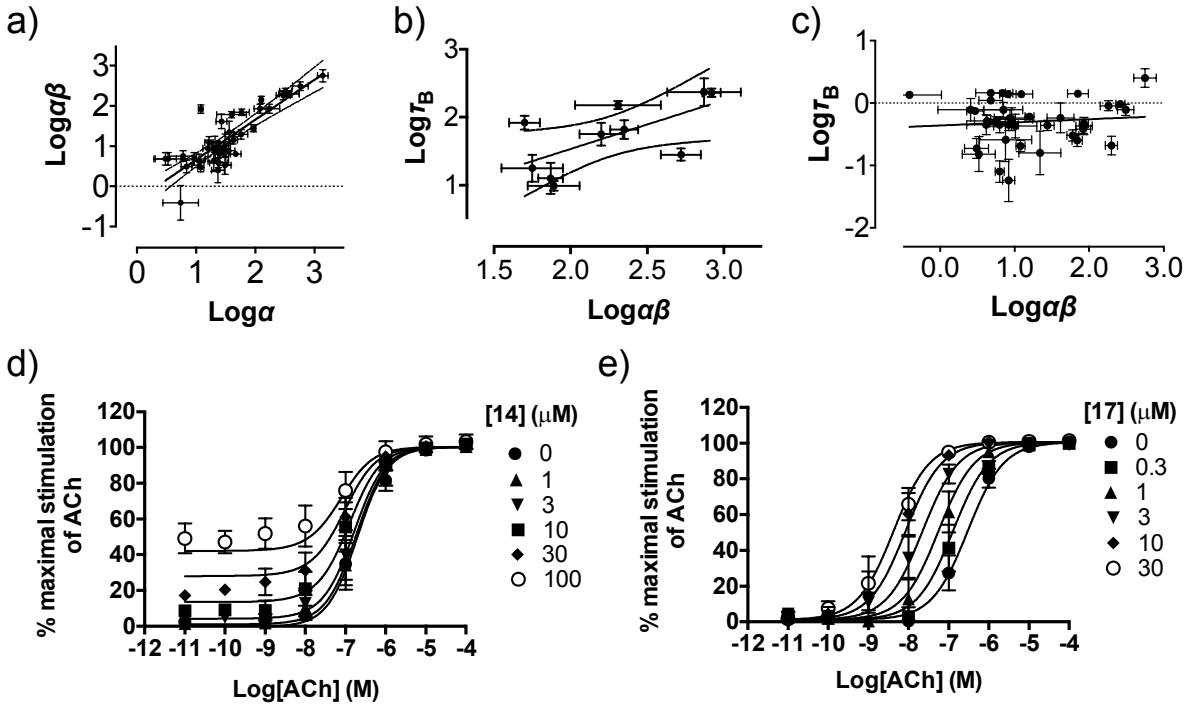
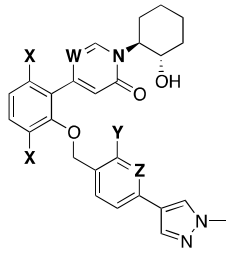


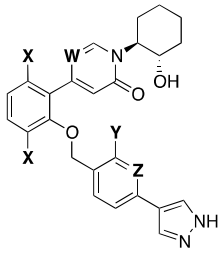
Figure 4. (**a**) Correlation between binding and functional cooperativity for 4-phenylpyridin-2-one derivatives generated in the present study (Pearson's test, r = 0.84, $p < 0.05$, slope = 1.00 ± 0.01. (**b**) Plots of functional cooperativity (Log$\alpha\beta$) versus intrinsic efficacy (Logτ_B) for derivatives of **1** generated and characterized in our previous study reveal a positive correlation (Pearson's test, r = 0.65, $p < 0.05$) between these two parameters. (**c**) A similar plot for 4-phenylpyridin-2-one

derivatives generated in the present study reveals no such correlation. (**d** & **e**) IP_1 accumulation experiments were performed using FlpIn-CHO cells stably expressing the M_1 mAChR, increasing concentrations of ACh with or without increasing concentrations of **14** (**d**) and **17** (**e**). (**d**) **14** displays intrinsic activity comparable to that of **1**, but very weak positive cooperativity with ACh (**e**) **17** displays no intrinsic activity but robust positive cooperativity with ACh. 100% represents the maximal stimulation of ACh in the absence of test compound.

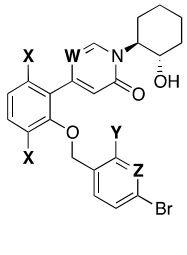
Table 3: Binding and functional allosteric parameters at the M_1 mAChR of 4-(1-methylpyrazol-4-yl) analogues.



10d, 17, 19, 23, 29



14



18

					Radioligand binding ([^{3}H]NMS)			IP$_1$ accumulation	
	W	**X**	**Y**	**Z**	**pK_B (K_B, μM)**	**Logα^{a}**	**Logα (α)^b**	**Log$\alpha\beta$ ($\alpha\beta$)^c**	**Logτ_B (τ_B)^d**
10d	CH	H	H	CH	4.37 ± 0.07 (43)	-3	2.57 ± 0.17 (370)	2.30 ± 0.08 (200)	-0.68 ± 0.15 (0.21)
14	CH	H	H	CH	4.45 ± 0.26 (35)	-0.09 ± 0.11	0.48 ± 0.19 (3)*	0.68 ± 0.16*(5)	0.04 ± 0.05(1.09)*
17	CH	H	H	N	4.50 ± 0.04 (32)	-3	2.10 ± 0.02 (125)	2.15 ± 0.09 (141)	= -3
18	CH	H	F	CH	4.75 ± 0.10 (18)	-3	0.75 ± 0.14 (6)*	n.d.	
19	CH	H	F	CH	4.51 ± 0.08 (31)	-3	2.24 ± 0.17 (174)	1.93 ± 0.11 (85)	-0.37 ± 0.14 (0.43)
23	CH	F	H	CH	4.38 ± 0.12 (42)	-3	0.76 ± 0.30 (6)*	-0.41 ± 0.43 (0.39)*	0.13 ± 0.03 (1.34)*
29	N	H	H	CH	4.88 ± 0.04 (13)	-3	3.14 ± 0.09 (1380)	2.75 ± 0.15 (562)	0.40 ± 0.15 (2.51)*

[a] Binding cooperativity with [^{3}H]NMS; for instances where a complete inhibition of [^{3}H]NMS binding by the allosteric modulator was observed (consistent with a high level of negative cooperativity), logα' was fixed to -3; [b]binding cooperativity with ACh; [c]functional cooperativity with ACh; [d]intrinsic efficacy of the modulator; for instances where no intrinsic efficacy of the modulator was observed Logτ_B was fixed to -3. * = significant difference ($p < 0.05$) relative to same parameter determined for **10d**, one-way ANOVA with Dunnett's post-test. n.d. = inactive at concentrations up to 30 μM; values represent the mean ± SEM from at least three experiments performed in duplicate.

■ CONCLUSIONS

In this study, we report the design and characterization of a structurally novel series of M_1 mAChR PAMs based on the novel 4-phenylpyridin-2-one scaffold, that offer potentially greater scope for future development than the extensively investigated BQCA core. Our previous studies have revealed that, while **1** displays low affinity for the M_1 mAChR, it displays high positive cooperativity with agonist binding and has exquisite subtype selectivity.[22] However, BQCA does contain a carboxylic acid group that is often associated with poor permeability.[34-36]

The majority of active compounds displayed both comparable binding affinities and intrinsic efficacy to those of **1**. Indeed, the lack of significant gains in affinity may be due to the nature of the binding pocket, which if it is indeed the same as that of **1**, lies at the extracellular interface of the M_1 mAChR and is largely defined by aromatic residues. However, we also generated a number of compounds that displayed markedly higher binding and functional cooperativity with ACh than **1**. Furthermore, compound **10d** displayed high selectivity towards the M_1 mAChR over other mAChR subtypes (Figure 1). Bioisosteric replacement of the carboxamide functionality of compound **9d** with an oxazole (**8q**) or thiazole (**8r**) was not well tolerated, whereas small changes to the pyrazole moiety of compound **10d** were generally better accommodated. However, replacement of the *N*-methyl group (compound **14**) with longer chains and bulkier alkyl as well as aromatic-bearing groups (compounds **15e**, **15i** and **15j**) had a detrimental effect on the cooperativity with ACh in comparison to the *N*-methylpyrazole analogue **10d**. The standout PAM in terms of binding cooperativity with ACh was the 6-phenylpyrimid-4-one analogue **29** with an α value of 1380, i.e., a 1380-fold potentiation of ACh affinity. Consequently, compound **29** represents a promising lead for future investigations of the previously unreported 6-phenylpyrimidin-4-one scaffold.

We have previously shown that **1** adheres to a two-state mode of action, whereby the degree of agonism of **1** is dependent upon the pathway stimulus-response coupling efficiency and/or receptor density.[22] Furthermore, in an SAR exploration of analogues of **1**, we have shown a strong positive correlation between the degree of cooperativity and the level of allosteric agonism.[20] We have also

made similar observations in an SAR study of M_4 mAChR allosteric modulators.[37] However, in the case of the present series of 6-phenylpyrimidin-4-one derivatives we observe no such correlation. Instead, our characterization revealed a range of behaviours from ligands that displayed little or weak positive cooperativity with ACh but robust allosteric agonism to those that displayed no agonism but a high level of positive cooperativity with ACh (Tables 1-3, Figure 3). This range of behaviours may, in the future, allow us to explore the relationship between *in vivo* efficacy and *in vitro* parameters that describe the functional cooperativity and intrinsic efficacy of allosteric M_1 mAChR ligands. This is particularly relevant given recent observations of M_1 mAChR agonists displaying signalling-pathway and brain-region specific effects that may be of therapeutic relevance.[14]

■ 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 was supplied by Grace Davison Discovery Sciences (Victoria, Australia) and deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. (USA, distributed by Novachem PTY. Ltd, Victoria, Australia).

Unless otherwise stated, reactions were carried out at ambient temperature. Reactions were monitored by thin layer chromatography on commercially available pre-coated aluminium-backed plates (Merck Kieselgel 60 F_{254}). Visualisation was by examination under UV light (254 and 366 nm). General staining was carried out with $KMnO_4$ or phosphomolybdic acid. A solution of ninhydrin (in ethanol) was used to visualize primary and secondary amines. All organic extracts collected after aqueous work-up procedures were dried over anhydrous $MgSO_4$ or Na_2SO_4 before gravity filtering and evaporation to dryness. Organic solvents were evaporated *in vacuo* at ≤ 40°C (water bath temperature). Purification using preparative layer chromatography (PLC) was carried out on Analtech preparative TLC plates (200 mm × 200 mm × 2 mm).

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance Nanobay III 400MHz Ultrashield Plus spectrometer at 400.13 MHz and 100.62 MHz respectively. Chemical shifts (δ) are recorded in parts per million (ppm) with reference to the chemical shift of the deuterated solvent. Coupling constants (J) and carbon-fluorine coupling constants (J_{CF}) are 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), doublet of triplets (dt). Spectra were assigned using appropriate COSY, distortionless enhanced polarisation transfer (DEPT), HSQC and HMBC sequences.

LCMS were run to verify reaction outcome and purity using an Agilent 6120 Series Single Quad coupled to an Agilent 1260 Series HPLC. The following buffers were used; buffer A: 0.1% formic

acid in H_2O; buffer B: 0.1% formic acid in MeCN. The following gradient was used with a Poroshell 120 EC-C18 50 × 3.0 mm 2.7 micron column, and a flow rate of 0.5 mL/min and total run time of 5 min; 0–1 min 95% buffer A and 5% buffer B, from 1-2.5 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. 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. All retention times (t_R) are quoted in minutes.

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 Altima 5µM C8 22 × 250 mm column. The following buffers were used; buffer A: H_2O; buffer B: MeCN, with sample being run at a gradient of 5% buffer B to 100% buffer B over 20 min, at a flow rate of 20 mL/min. All screening compounds were of > 95% purity unless specified in the individual monologue.

All NMR experiments were performed in d_6-DMSO allowing comparison of the spectra of the various analogues. It is important to point out that the ^{13}C NMR signals of the hydroxycyclohexane moiety were often difficult to be obtained in d_6-DMSO. Especially one tertiary carbon of the hydroxycyclohexane moiety was not observed in the ^{13}C NMR spectra of all the respective analogues even with extended relaxation time and another tertiary aromatic carbon was only ever observed by using HSQC experiments. However, additional experiments were performed on selected compounds to confirm the integrity of the presented NMR data. These results have shown that the all the signals can be observed when the NMR solvent was changed to $CDCl_3$.[38] Furthermore, the quaternary carbon of the pyrazole moiety was not always observed depending on individual analogue.

General Procedure A: Suzuki coupling of aryl halides and boronic acids. A mixture of the aryl halide (1.0 eq) and boronic acid (1.5 eq) in degassed (by sonication followed by a stream of

nitrogen) THF/1 M $Na_2CO_{3(aq)}$ (3:1 4 mL/100 mg) was evacuated and flushed with nitrogen. $PdCl_2(PPh_3)_2$ (0.1 eq) was added and the reaction mixture boiled under reflux for 3 h. THF was evaporated under reduced pressure. The mixture was diluted with water (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous $MgSO_4$ and filtered, before concentration under reduced pressure. The crude product was purified by flash column chromatography.

General Procedure B: O-Alkylation of 1-(2-hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (7). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxy-phenyl)pyridin-2(1*H*)-one (**7**) (1.0 eq), K_2CO_3 (1.1 eq), KI (0.1 eq) and the appropriately substituted benzyl halide (1.1 eq) were stirred in DMF (2 mL/100 mg) at rt overnight. Reaction progress was monitored through TLC analysis, with further addition of K_2CO_3 (0.5 eq) and substituted benzyl halide (0.5 eq) every 24 h until the reaction appeared complete. The reaction mixture was poured onto ice/water and stirred for 30 min, before extraction with EtOAc (3 × 20 mL). The combined organic layers were washed with 2 M $NaOH_{(aq)}$ (20 mL), water (20 mL) and brine (20 mL), before concentration under reduced pressure. The resulting crude product was purified by flash column chromatography.

General Procedure C: Ester hydrolysis with NaOH. To a solution of the ester (1.0 eq) in EtOH/H_2O (1:1, 2 mL/0.1 mmol) was added NaOH (4.0 eq). The reaction mixture was heated at 50 °C for 2 h. EtOH was evaporated under reduced pressure, before acidifying with 1 M $HCl_{(aq)}$ to pH 2. The resulting precipitate was filtered (vacuum) to give the desired product as the free acid.

General Procedure D: Ester aminolysis with ammonium hydroxide. A mixture of ester (1.0 eq) in MeOH/NH_4OH (1:1, 1.0 mL/100 mg) in a sealed tube was stirred at rt for 3 d. The resulting precipitate was collected by filtration (vacuum) and washed with EtOAc to give the desired carboxamide product.

General Procedure E: N-Alkylation of 4-(2-((4-(1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (14). 4-(2-((4-(1*H*-Pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (1.0 eq), K_2CO_3 (1.1 eq), KI (0.1 eq) and the appropriate organohalide (1.1 eq) were stirred in DMF (2 mL/100 mg) at the indicated temperature. Reaction progress was monitored through TLC analysis, with further addition of K_2CO_3 and organohalide until the reaction appeared complete or conversion remained stagnant. The reaction mixture was poured onto ice/water and stirred for 30 min, before extraction with EtOAc (3 × 20 mL). The combined organic layers were washed with 2 M $NaOH_{(aq)}$ (20 mL), water (20 mL) and brine (20 mL), before concentration under reduced pressure. The resulting crude product was purified by flash column chromatography.

(±)-trans-2-((4-Bromopyridin-2-yl)oxy)cyclohexan-1-ol (4a) and (±)-trans-4-bromo-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (4b). A mixture of 4-bromo-2-hydroxypyridine (**1**) (10.0 g, 57.5 mmol), 1,2-cyclohexene oxide (29.1 mL, 287 mmol, 5.0 eq) and K_2CO_3 (19.9 g, 144 mmol, 2.5 eq) was heated at 120 °C for 4 h. The reaction mixture was cooled to rt and concentrated to dryness under reduced pressure. The remaining residue was diluted with EtOAc (50 mL) and sonicated for 15 min at rt before the resulting suspension was collected by filtration (vacuum), and washed with EtOAc (filter cake 1). The EtOAc washings were concentrated under reduced pressure, then taken up in DCM (15 mL) and sonicated for 15 min at rt before the resulting suspension was collected by filtration (vacuum) (filter cake 2). Filter cake 2 was washed with further DCM, to give 2-((4-bromopyridin-2-yl)oxy)cyclohexan-1-ol (**4a**) as 1.11 g of a beige solid (7%). ^{1}H NMR δ 8.15–7.89 (m, 1H), 7.17 (dd, J = 5.5/1.7 Hz, 1H), 7.10–6.93 (m, 1H), 4.93–4.66 (m, 2H), 3.67–3.42 (m, 1H), 2.11–1.94 (m, 1H), 1.94–1.75 (m, 1H), 1.74–1.47 (m, 2H), 1.44–1.07 (m, 4H); ^{13}C NMR δ 164.2, 147.9, 133.3, 119.8, 114.0, 78.6, 70.5, 33.1, 29.2, 23.3, 23.2; *m/z* MS (TOF ES$^+$) $C_{11}H_{15}BrNO_2$ $[M+H]^+$ calcd 272.0; found 272.1; LC-MS t_R: 3.72 min.

Filter cake 1, was taken up in water (70 mL) and stirred for 15 min at rt. Any remaining solid was collected by filtration (vacuum) and washed with water to give 4-bromo-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**4b**) as 12.1 g of a white solid (77%). ^{1}H NMR δ 7.68 (d, *J* = 7.4 Hz, 1H), 6.67 (d, *J* = 2.2 Hz, 1H), 6.44 (dd, *J* = 7.4/2.3 Hz, 1H), 4.79 (d, *J* = 5.8 Hz, 1H), 4.44 (br s, 1H), 3.74 (br s, 1H), 2.04–1.88 (m, 1H), 1.76–1.60 (m, 3H), 1.51 (m, 1H), 1.40–1.18 (m, 3H); ^{13}C NMR δ 160.8, 136.6, 134.1, 121.0, 108.9, 69.1, 35.2, 30.8, 24.9, 23.9; resonance at δ 136.6 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{11}H_{15}BrNO_2$ $[M+H]^+$ calcd 272.0; found 272.1; LC-MS t_R: 3.33 min.

2-(2-((2-Hydroxycyclohexyl)oxy)pyridin-4-yl)phenol (5). 2-((4-Bromopyridin-2-yl)oxy)cyclohexan-1-ol (**4a**) (500 mg, 1.84 mmol) and 2-hydroxyphenylboronic acid (380 mg, 2.76 mmol, 1.5 eq) were dispersed in 1 M $Na_2CO_{3(aq)}$ (5 mL) and THF (10 mL) in a 30 mL microwave vial. The mixture was sonicated at rt, then degassed with a stream of nitrogen for 10 min. $PdCl_2(PPh_3)_2$ (129 mg, 0.18 mmol, 0.1 eq) was added and the vial sealed, before heating at 100 °C for 2.5 h. The mixture was cooled, before adding water (30 mL) and extracting with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (30 mL) before concentration under reduced pressure. The resulting residue was purified by FCC (eluent EtOAc/PE 10:90 to 100:0, wet load in DCM), to give 609 mg of a pale yellow solid (quantitative). ^{1}H NMR δ 9.80 (s, 1H), 8.11 (dd, *J* = 5.4/0.5 Hz, 1H), 7.33 (dd, *J* = 7.7/1.7 Hz, 1H), 7.23 (ddd, *J* = 8.2/7.4/1.7 Hz, 1H), 7.12 (dd, *J* = 5.4/1.5 Hz, 1H), 6.97 (dd, *J* = 8.2/1.0 Hz, 1H), 6.94 (d, *J* = 0.8 Hz, 1H), 6.90 (ddd, *J* = 7.5/7.5/1.1 Hz, 1H), 4.83 (td, *J* = 8.5/4.6 Hz, 1H), 3.56 (td, *J* = 9.0/4.2 Hz, 1H), 2.19–2.01 (m, 1H), 1.96–1.83 (m, 1H), 1.70–1.50 (m, 2H), 1.43–1.20 (m, 4H); ^{13}C NMR (101 MHz, DMSO) δ 163.5, 154.7, 149.0, 146.0, 129.9, 129.9, 124.8, 119.6, 117.2, 116.3, 110.8, 77.6, 70.6, 33.2, 29.3, 23.3, 23.2; *m/z* MS (TOF ES$^+$) $C_{17}H_{20}NO_3$ $[MH]^+$ calcd 286.1; found 286.2; LC-MS t_R: 3.23 min.

2-((4-(2-([1,1'-Biphenyl]-4-ylmethoxy)phenyl)pyridin-2-yl)oxy)cyclohexan-1-ol (6). 2-(2-((2-Hydroxycyclohexyl)oxy)pyridin-4-yl)phenol (**5**) (100 mg, 0.35 mmol), K_2CO_3 (53 mg, 0.39 mmol,

1.1 eq), KI (7 mg, 0.04 mmol, 0.1 eq) and 4-(bromomethyl)biphenyl (95 mg, 0.39 mmol, 1.1 eq) were dispersed in DMF (2 mL). The mixture was stirred at rt overnight. The mixture was diluted with water/ice and stirred for 10 min, before collecting the resulting precipitate by filtration (vacuum) and washing with water. The crude solid product was further purified by FCC (eluent MeOH/DCM 0:100 to 10:90, with a plateau at 5:95). Mixed fractions were combined and repurified by FCC (eluent EtOAc/PE 0:100 to 30:70). A combined total of 87 mg of glassy solid (55%) was obtained. ^{1}H NMR ($CDCl_3$) δ 8.11 (d, J = 5.4 Hz, 1H), 7.69–7.51 (m, 4H), 7.51–7.29 (m, 7H), 7.19 (dd, J = 5.4/1.3 Hz, 1H), 7.13–6.99 (m, 3H), 5.16 (s, 2H), 4.78 (ddd, J = 10.9/8.9/4.7 Hz, 1H), 3.70 (ddd, J = 11.0/8.9/4.7 Hz, 1H), 2.24–2.08 (m, 2H), 1.85–1.62 (m, 2H), 1.59–1.14 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 164.1, 155.8, 150.6, 144.8, 141.0, 140.8, 135.8, 130.6, 130.5, 128.9, 128.1, 127.6, 127.5, 127.4, 127.2, 121.7, 118.8, 113.5, 112.6, 81.5, 74.2, 70.5, 33.7, 31.0, 24.3, 24.0; *m/z* MS (TOF ES$^+$) $C_{30}H_{30}NO_3$ $[MH]^+$ calcd 452.2; found 452.3; LC-MS t_R: 4.18 min.

1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (7). 4-Bromo-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**4b**) (2.00 g, 7.35 mmol) was coupled to 2-hydroxyphenyl boronic acid (1.52 g, 11.0 mmol) according to General Procedure A. After the THF was evaporated under reduced pressure, the resulting brown precipitate was filtered (vacuum). The precipitate was taken up in MeOH and adsorbed onto Telos bulk sorbent and dry loaded onto the column (no extractive workup was necessary). FCC purification (eluent MeOH/DCM 0:100 to 20:80) gave 1.75 g of a white solid (83%). ^{1}H NMR δ 9.84 (s, 1H), 7.65 (d, J = 7.3 Hz, 1H), 7.30 (dd, J = 7.7/1.7 Hz, 1H), 7.22 (ddd, J = 8.2/7.4/1.7 Hz, 1H), 6.94 (dd, J = 8.1/1.0 Hz, 1H), 6.87 (td, J = 7.5/1.1 Hz, 1H), 6.52 (d, J = 1.9 Hz, 1H), 6.45 (dd, J = 7.2/2.0 Hz, 1H), 4.75 (d, J = 6.0 Hz, 1H), 4.54 (br s, 1H), 3.79 (br s, 1H), 2.07–1.93 (m, 1H), 1.80–1.63 (m, 3H), 1.53 (br s, 1H), 1.44–1.22 (m, 3H); ^{13}C NMR δ 161.9, 154.8, 148.5, 134.0, 130.0, 129.6, 124.5, 119.5, 118.0, 116.3, 106.8, 69.2, 35.4, 31.1, 25.0, 24.0; resonance at δ 134.0 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{17}H_{20}NO_3$ $[M+H]^+$ calcd 286.1; found 286.2; LC-MS t_R: 3.33 min.

4-(2-([1,1'-Biphenyl]-4-ylmethoxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (8a). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (100 mg, 0.35 mmol) was alkylated with 4-(bromomethyl)biphenyl (95 mg, 0.39 mmol) according to General Procedure B. A precipitate formed after pouring the reaction mixture into ice/water. The precipitate was collected by filtration (vacuum), then purified by FCC (eluent EtOAc/PE 30:70 to 100:0, wet load in DCM) to give 97 mg of a white solid (61%). ^{1}H NMR δ 7.74–7.60 (m, 5H), 7.58–7.43 (m, 4H), 7.43–7.31 (m, 3H), 7.22 (d, *J* = 8.2 Hz, 1H), 7.11–6.99 (m, 1H), 6.52 (d, *J* = 1.9 Hz, 1H), 6.46 (dd, *J* = 7.2/2.0 Hz, 1H), 5.25 (s, 2H), 4.75 (d, *J* = 6.0 Hz, 1H), 4.54 (s, 1H), 3.80 (s, 1H), 2.09–1.87 (m, 1H), 1.85–1.43 (m, 4H), 1.43–1.21 (m, 3H); ^{13}C NMR δ 161.8, 155.3, 148.3, 139.8, 139.6, 136.2, 134.3, 130.3, 129.9, 129.0, 127.9, 127.5, 127.2, 126.8, 126.7, 121.2, 118.5, 113.4, 107.0, 69.3, 69.2, 35.4, 31.1, 25.0, 24.0; *m/z* MS (TOF ES$^+$) $C_{30}H_{30}NO_3$ $[MH]^+$ calcd 452.2; found 452.30; LC-MS t_R: 3.60 min.

4-(2-(Benzyloxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (8b). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (80 mg, 0.28 mmol) was alkylated with benzyl bromide (37 μL, 0.31 mmol) according to General Procedure B. After a total of 72 h of stirring with two further additions of K_2CO_3 and benzyl bromide, the mixture was heated at 90 °C for 2 h, cooled then worked up. The crude product was purified by FCC (eluent EtOAc/PE 50:50 to 100:0) to give 19.4 mg of a colourless oil (18%). ^{1}H NMR δ 7.67 (d, *J* = 7.2 Hz, 11H), 7.47–7.26 (m, 7H), 7.19 (d, *J* = 8.3 Hz, 1H), 7.04 (ddd, *J* = 7.6/7.6/0.7 Hz, 1H), 6.50 (d, *J* = 1.9 Hz, 1H), 6.44 (dd, *J* = 7.2/2.0 Hz, 1H), 5.19 (s, 2H), 4.53 (br s, 1H), 3.79 (br s, 1H), 2.08–1.91 (m, 1H), 1.81–1.62 (m, 3H), 1.53 (m, 1H), 1.43–1.11 (m, 3H); ^{13}C NMR δ 161.8, 155.3, 148.3, 137.0, 134.1, 130.3, 129.9, 128.5, 127.7, 127.3, 127.1, 121.2, 118.5, 113.4, 107.0, 69.6, 69.2, 35.4, 31.1, 25.0, 24.0; resonance at δ 134.1 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{17}H_{20}NO_3$ $[M+H]^+$ calcd 376.2; found 376.2; LC-MS t_R: 3.74 min.

4-(2-((2-Fluorobenzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (8c). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (80 mg, 0.28 mmol) was alkylated

with 2-fluorobenzyl bromide (37 μL, 0.31 mmol) according to General Procedure B. After a total of 72 h of stirring with two further additions of K_2CO_3 and 2-fluorobenzyl bromide, the mixture was heated at 90 °C for 2 h, cooled then worked up. The crude product was purified by FCC (eluent EtOAc/PE 30:70 to 100:0) to give 47 mg of a pale yellow solid (43%). ^{1}H NMR δ 7.64 (d, *J* = 7.3 Hz, 1H), 7.50 (ddd, *J* = 7.6/7.6/1.7 Hz, 1H), 7.44–7.35 (m, 3H), 7.28–7.17 (m, 3H), 7.06 (ddd, *J* = 7.5/7.5/0.9 Hz, 1H), 6.47 (d, *J* = 1.9 Hz, 1H), 6.40 (dd, *J* = 7.2/2.0 Hz, 1H), 5.22 (s, 2H), 4.52 (br s, 1H), 3.79 (br s, 1H), 2.06–1.92 (m, 1H), 1.80–1.61 (m, 3H), 1.54 (m, 1H), 1.41–1.20 (m, 3H); ^{13}C NMR δ 161.8, 160.2 (d, J_{CF} = 245.9 Hz), 155.1, 148.2, 134.1, 130.4, 130.3 (d, J_{CF} = 6.2 Hz), 130.3 (d, J_{CF} = 6.0 Hz), 129.9, 127.1, 124.6 (d, J_{CF} = 3.4 Hz), 123.7 (d, J_{CF} = 14.4 Hz), 121.4, 118.4, 115.4 (d, J_{CF} = 20.9 Hz), 113.3, 106.8, 69.2, 64.1 (d, J_{CF} = 3.8 Hz), 35.4, 31.0, 25.0, 24.0; resonance at δ 134.1 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{24}H_{25}FNO_3$ $[M+H]^+$ calcd 394.2; found 394.2; LC-MS t_R: 3.73 min.

4-(2-((3-Fluorobenzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (8d). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (80 mg, 0.28 mmol) was alkylated with 3-fluorobenzyl bromide (38 μL, 0.31 mmol) according to General Procedure B. The crude product was purified by FCC (eluent EtOAc/PE 50:50 to 100:0) to give 82 mg of a yellow oil (74%). ^{1}H NMR δ 7.68 (d, *J* = 7.2 Hz, 2H), 7.46–7.34 (m, 3H), 7.28–7.10 (m, 4H), 7.09–7.02 (m, 1H), 6.50 (d, *J* = 1.9 Hz, 1H), 6.44 (dd, *J* = 7.2/2.0 Hz, 1H), 5.21 (s, 2H), 4.74 (br s, 1H), 4.54 (br s, 1H), 3.80 (br s, 1H), 2.07–1.92 (m, 1H), 1.82–1.62 (m, 3H), 1.54 (m, 1H), 1.43–1.12 (m, 3H); ^{13}C NMR δ 162.2 (d, J_{CF} = 243.7 Hz), 161.8, 155.1, 148.3, 139.9 (d, J_{CF} = 7.4 Hz), 134.2, 130.5 (d, J_{CF} = 8.3 Hz), 130.3, 129.9, 127.2, 123.1 (d, J_{CF} = 2.7 Hz), 121.3, 118.5, 114.5 (d, J_{CF} = 20.9 Hz), 113.9 (d, J_{CF} = 22.0 Hz), 113.4, 107.0, 69.2, 68.8 (d, J_{CF} = 1.4 Hz), 35.4, 31.1, 25.0, 24.0; resonance at δ 134.2 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{24}H_{25}FNO_3$ $[M+H]^+$ calcd 394.2; found 394.2; LC-MS t_R: 3.75 min.

4-(2-((4-Fluorobenzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (8e). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (100 mg, 0.35 mmol) was alkylated with 4-fluorobenzyl chloride (46 µL, 0.39 mmol) according to General Procedure B, with the reaction temperature changed to 90 °C. Purification by FCC (eluent EtOAc/PE 50:50 to 100:0) gave 90 mg of a yellow solid (65%). ^{1}H NMR δ 7.66 (d, J = 7.3 Hz, 1H), 7.49–7.42 (m, 2H), 7.42–7.34 (m, 2H), 7.23–7.15 (m, 3H), 7.08–7.01 (m, 1H), 6.47 (d, J = 1.7 Hz, 1H), 6.42 (dd, J = 7.2/1.8 Hz, 1H), 5.16 (s, 2H), 4.75 (d, J = 6.0 Hz, 1H), 4.52 (br s, 1H), 3.79 (br s, 1H), 2.06–1.93 (s, 1H), 1.79–1.62 (m, 3H), 1.53 (m, 1H), 1.42–1.23 (m, 3H); ^{13}C NMR δ 161.7 (d, J_{CF} = 243.4 Hz), 161.8, 155.2, 148.3, 134.1, 133.1 (d, J_{CF} = 3.0 Hz), 130.3, 129.8, 129.6 (d, J_{CF} = 8.3 Hz), 127.2, 121.2, 118.4, 115.3 (d, J_{CF} = 21.4 Hz), 113.5, 107.0, 69.2, 69.0, 35.4, 30.8, 25.0, 24.0; resonances at δ 134.1 and 30.8 ppm were taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{24}H_{25}FNO_3$ $[M+H]^+$ calcd 394.2; found 394.2; LC-MS t_R: 3.73 min.

1-(2-Hydroxycyclohexyl)-4-(2-((2-methoxybenzyl)oxy)phenyl)pyridin-2(1*H*)-one (8f). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (7) (80 mg, 0.28 mmol) was alkylated with 2-methoxybenzyl chloride (43 µL, 0.31 mmol) according to General Procedure B. After a total of 48 h of stirring with one further addition of K_2CO_3 and 2-methoxybenzyl chloride, the mixture was worked up. Purification by FCC (eluent EtOAc/PE 30:70 to 80:20) gave 66 mg of a white solid (58%). ^{1}H NMR δ 7.64 (d, J = 7.1 Hz, 1H), 7.43–7.28 (m, 4H), 7.18 (d, J = 8.1 Hz, 1H), 7.09–6.99 (m, 2H), 6.93 (t, J = 7.3 Hz, 1H), 6.52 (s, 1H), 6.44 (d, J = 6.9 Hz, 1H), 5.12 (s, 2H), 4.73 (d, J = 5.7 Hz, 1H), 4.52 (br s, 1H), 3.84 (s, 3H), 3.80 (br s, 1H), 1.98 (br s, 1H), 1.80–1.61 (m, 3H), 1.54 (m, 1H), 1.42–1.18 (m, 3H); ^{13}C NMR δ 161.8, 157.0, 155.5, 148.2, 134.0, 130.3, 129.8, 129.4, 129.0, 127.0, 124.3, 121.0, 120.2, 118.5, 113.2, 110.9, 106.9, 69.2, 65.4, 55.4, 35.4, 31.1, 25.0, 24.0; resonance at δ 134.0 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{25}H_{28}NO_4$ $[M+H]^+$ calcd 406.2; found 406.2; LC-MS t_R: 3.76 min.

1-(2-Hydroxycyclohexyl)-4-(2-((3-methoxybenzyl)oxy)phenyl)pyridin-2(1*H*)-one (8g). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (100 mg, 0.35 mmol) was alkylated with 3-methoxybenzyl chloride (56 μL, 0.39 mmol) according to General Procedure B with the reaction temperature changed to 90 °C. Purification by FCC (eluent EtOAc/PE 50:50 to 100:0) gave 110 mg of a yellow oil (77%). ^{1}H NMR δ 7.68 (d, *J* = 7.2 Hz, 1H), 7.41–7.35 (m, 2H), 7.27 (t, *J* = 7.9 Hz, 1H), 7.21–7.17 (m, 1H), 7.04 (ddd, *J* = 7.6/7.6/0.9 Hz, 1H), 7.01–6.94 (m, 2H), 6.88–6.83 (m, 1H), 6.53 (d, *J* = 1.9 Hz, 1H), 6.45 (dd, *J* = 7.2/2.0 Hz, 1H), 5.17 (s, 2H), 4.54 (br s, 1H), 3.77 (br s, 1H), 3.74 (s, 3H), 2.05–1.94 (m, 1H), 1.79–1.63 (m, 3H), 1.54 (m, 1H), 1.41–1.23 (m, 3H); ^{13}C NMR δ 161.8, 159.4, 155.2, 148.3, 138.6, 134.2, 130.3, 129.9, 129.5, 127.1, 121.1, 119.1, 118.5, 113.7, 113.3, 112.0, 107.0, 69.4, 69.2, 55.0, 35.4, 31.1, 25.0, 24.0; resonance at δ 134.2 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES^{+}) $C_{25}H_{28}NO_4$ $[M+H]^{+}$ calcd 406.2; found 406.3; LC-MS t_R: 3.72 min.

1-(2-Hydroxycyclohexyl)-4-(2-((4-methoxybenzyl)oxy)phenyl)pyridin-2(1*H*)-one (8h). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (80 mg, 0.28 mmol) was alkylated with 4-methoxybenzyl chloride (42 μL, 0.31 mmol) according to General Procedure B. Purification by FCC (eluent EtOAc/PE 50:50 to 100:0) gave 66 mg of a beige solid (58%). ^{1}H NMR δ 7.65 (d, *J* = 7.2 Hz, 1H), 7.40–7.31 (m, 4H), 7.21 (d, *J* = 8.0 Hz, 1H), 7.06–7.00 (m, 1H), 6.95–6.8 (m, 2H), 6.48 (d, *J* = 1.9 Hz, 1H), 6.41 (dd, *J* = 7.2/2.0 Hz, 1H), 5.10 (s, 2H), 4.74 (d, *J* = 5.9 Hz, 1H), 4.52 (br s, 1H), 3.79 (br s, 1H), 3.74 (s, 3H), 2.05–1.92 (m, 1H), 1.78–1.52 (m, 3H), 1.54 (m, 1H), 1.41–1.20 (m, 3H); ^{13}C NMR δ 161.8, 158.9, 155.4, 148.3, 134.1, 130.3, 129.9, 129.2, 128.7, 127.1, 121.1, 118.4, 113.8, 113.5, 106.9, 69.4, 69.2, 55.1, 35.4, 31.1, 25.0, 24.0; resonance at δ 134.1 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES^{+}) $C_{25}H_{28}NO_4$ $[M+H]^{+}$ calcd 406.2; found 406.2; LC-MS t_R: 3.74 min.

1-(2-Hydroxycyclohexyl)-4-(2-((2-(trifluoromethyl)benzyl)oxy)phenyl)pyridin-2(1*H*)-one (8i). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (80 mg, 0.28 mmol) was

alkylated with 2-(trifluoromethyl)benzyl chloride (45 μL, 0.31 mmol) according to General Procedure B. After a total of 48 h of stirring with one further addition of K_2CO_3 and 2-(trifluoromethyl)benzyl chloride, the mixture was worked up. Purification by FCC (eluent EtOAc/PE 50:50 to 100:0) gave 85 mg of a white solid (68%). ^{1}H NMR δ 7.78 (d, J = 7.8 Hz, 1H), 7.73–7.62 (m, 3H), 7.60–7.52 (m, 1H), 7.45–7.36 (m, 2H), 7.15 (d, J = 8.0 Hz, 1H), 7.11–7.06 (m, 1H), 6.48 (d, J = 1.9 Hz, 7H), 6.41 (dd, J = 7.2/2.0 Hz, 7H), 5.30 (s, 2H), 4.74 (d, J = 5.7 Hz, 1H), 4.53 (br s, 1H), 3.79 (br s, 1H), 2.04–1.93 (m, 1H), 1.78–1.62 (m, 3H), 1.53 (m, 1H), 1.41–1.20 (m, 3H); ^{13}C NMR δ 161.8, 155.0, 148.2, 134.9 (app. d, J_{CF} = 1.5 Hz), 134.2, 132.9, 130.4, 130.0, 129.6, 128.5, 127.3, 126.3 (q, J_{CF} = 30.4 Hz), 126.1 (q, J_{CF} = 5.6 Hz), 124.3 (q, J_{CF} = 274.0 Hz), 121.6, 118.5, 113.2, 106.9, 69.2, 66.5 (d, J_{CF} = 2.6 Hz), 35.4, 31.0, 25.0, 24.0; resonance at δ 134.2 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{25}H_{25}F_3NO_3$ $[M+H]^+$ calcd 444.2; found 444.3; LC-MS t_R: 3.89 min.

1-(2-Hydroxycyclohexyl)-4-(2-((3-(trifluoromethyl)benzyl)oxy)phenyl)pyridin-2(1*H*)-one (8j). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (80 mg, 0.28 mmol) was alkylated with 3-(trifluoromethyl)benzyl bromide (47 μL, 0.31 mmol) according to General Procedure B. After a total of 72 h of stirring with two further additions of K_2CO_3 and 3-(trifluoromethyl)benzyl bromide, the mixture was worked up. Purification by FCC (eluent EtOAc/PE 50:50 to 100:0) gave 79 mg of a yellow solid (64%). ^{1}H NMR δ 7.77 (s, 1H), 7.73–7.58 (m, 4H), 7.45–7.35 (m, 2H), 7.20 (d, J = 7.9 Hz, 1H), 7.07 (ddd, J = 7.5/7.5/0.8 Hz, 1H), 6.48 (d, J = 1.8 Hz, 1H), 6.45 (dd, J = 7.1/2.0 Hz, 1H), 5.30 (s, 2H), 4.54 (br s, 1H), 3.79 (br s, 1H), 3.38 (br s, 1H), 2.05–1.94 (m, 1H), 1.79–1.64 (m, 3H), 1.53 (m, 1H), 1.41–1.20 (m, 3H); ^{13}C NMR δ 161.8, 155.0, 148.3, 138.6, 134.2, 131.1, 130.4, 129.9, 129.6, 129.2 (app. d, J_{CF} = 31.6 Hz), 127.3, 124.4 (q, J_{CF} = 3.8 Hz), 124.2 (q, J_{CF} = 272.3 Hz), 123.5 (q, J_{CF} = 3.9 Hz), 121.4, 118.5, 113.4, 107.0, 69.3, 68.8, 35.4, 31.0, 25.0, 24.0; resonance at δ 134.2 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{25}H_{25}F_3NO_3$ $[M+H]^+$ calcd 444.2; found 444.3; LC-MS t_R: 3.82 min.

1-(2-Hydroxycyclohexyl)-4-(2-((4-(trifluoromethyl)benzyl)oxy)phenyl)pyridin-2(1*H*)-one (8k). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (80 mg, 0.28 mmol) was alkylated with 4-(trifluoromethyl)benzyl bromide (48 µL, 0.31 mmol) according to General Procedure B. After a total of 48 h of stirring with one further addition of K_2CO_3 and 4-(trifluoromethyl)benzyl chloride, the mixture was worked up. Purification by FCC (eluent EtOAc/PE 50:50 to 100:0) gave 41 mg of a white solid (33%). ^{1}H NMR δ 7.74 (d, J = 8.2 Hz, 2H), 7.69 (d, J = 7.2 Hz, 1H), 7.62 (d, J = 8.1 Hz, 2H), 7.42–7.35 (m, 2H), 7.19–7.15 (m, 1H), 7.06 (ddd, J = 7.5/7.5/0.8 Hz, 1H), 6.50 (d, J = 1.9 Hz, 1H), 6.45 (dd, J = 7.2/2.0 Hz, 1H), 5.31 (s, 2H), 4.54 (br s, 1H), 3.81 (br s, 1H), 3.43 (br s, 1H), 2.06–1.94 (m, 1H), 1.80–1.64 (m, 3H), 1.54 (m, 1H), 1.42–1.12 (m, 3H); ^{13}C NMR δ 161.8, 155.0, 148.3, 141.9, 134.2, 130.3, 129.9, 128.2 (app. d, J_{CF} = 31.7 Hz), 127.6, 127.2, 125.3 (q, J_{CF} = 3.7 Hz), 124.2 (q, J_{CF} = 271.9 Hz), 121.4, 118.5, 113.4, 107.0, 69.2, 68.8, 35.4, 29.0, 25.0, 24.0; *m/z* MS (TOF ES$^+$) $C_{25}H_{25}F_3NO_3$ $[M+H]^+$ calcd 444.2; found 444.2; LC-MS t_R: 3.83 min.

2-((2-(1-(2-Hydroxycyclohexyl)-2-oxo-1,2-dihydropyridin-4-yl)phenoxy)methyl) benzonitrile (8l). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (100 mg, 0.35 mmol) was alkylated with 2-(bromomethyl)benzonitrile (76 mg, 0.39 mmol) according to General Procedure B. A precipitate formed after pouring the reaction mixture into ice/water. The precipitate was collected by filtration (vacuum) to give 125 mg of beige solid (89%). ^{1}H NMR δ 7.91 (dd, J = 7.7/0.8 Hz, 1H), 7.73 (ddd, J = 7.6/7.6/1.2 Hz, 1H), 7.69–7.61 (m, 2H), 7.57 (ddd, J = 7.6/7.6/1.2 Hz, 1H), 7.47–7.37 (m, 2H), 7.26 (d, J = 8.1 Hz, 1H), 7.13–7.08 (m, 1H), 6.47 (d, J = 1.8 Hz, 1H), 6.45 (dd, J = 7.1/2.0 Hz, 1H), 5.32 (s, 2H), 4.73 (d, J = 6.0 Hz, 1H), 4.52 (br s, 1H), 3.79 (br s, 1H), 2.06–1.92 (m, 1H), 1.8–1.62 (m, 3H), 1.53 (m, 1H), 1.42–1.22 (m, 3H); ^{13}C NMR δ 161.7, 155.0, 148.1, 139.9, 134.1, 133.5, 133.2, 130.4, 130.0, 129.3, 129.1, 127.3, 121.7, 118.5, 117.2, 113.4, 111.0, 106.9, 69.2, 68.1, 35.4, 31.0, 25.0, 24.0; resonance at δ 134.1 ppm was taken

from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{25}H_{25}N_2O_3$ $[M+H]^+$ calcd 401.2; found 401.2; LC-MS t_R: 3.60 min.

3-((2-(1-(2-Hydroxycyclohexyl)-2-oxo-1,2-dihydropyridin-4-yl)phenoxy)methyl) benzonitrile (8m). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (100 mg, 0.35 mmol) was alkylated with 3-(bromomethyl)benzonitrile (76 mg, 0.39 mmol) according to General Procedure B. Purification by FCC (eluent EtOAc/PE 50:50 to 100:0) gave 130 mg of a white foam (93%). ^{1}H NMR δ 7.88–7.85 (m, 1H), 7.80 (ddd, *J* = 7.7/1.3/1.3 Hz, 1H), 7.77–7.73 (m, 1H), 7.68 (d, *J* = 7.2 Hz, 1H), 7.60 (dd, *J* = 7.7/7.7 Hz, 1H), 7.45–7.36 (m, 2H), 7.19 (d, *J* = 7.9 Hz, 1H), 7.08 (ddd, *J* = 7.5/7.5/0.9 Hz, 1H), 6.49 (d, *J* = 1.8 Hz, 1H), 6.45 (dd, *J* = 7.2/2.0 Hz, 1H), 5.25 (s, 2H), 4.75 (d, *J* = 6.0 Hz, 1H), 4.55 (br s, 1H), 3.80 (br s, 1H), 2.10–1.93 (m, 1H), 1.85–1.63 (m, 3H), 1.54 (m, 1H), 1.44–1.22 (m, 3H); ^{13}C NMR δ 161.8, 155.0, 148.2, 138.7, 134.1, 132.0, 131.6, 130.7, 130.4, 129.9, 129.8, 127.3, 121.5, 118.7, 118.5, 113.4, 111.4, 107.0, 69.2, 68.6, 35.4, 31.1, 25.0, 24.0; resonance at δ 134.1 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{25}H_{25}N_2O_3$ $[M+H]^+$ calcd 401.2; found 401.2; LC-MS t_R: 3.64 min.

4-((2-(1-(2-Hydroxycyclohexyl)-2-oxo-1,2-dihydropyridin-4-yl)phenoxy)methyl) benzonitrile (8n). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (100 mg, 0.35 mmol) was alkylated with 4-(bromomethyl)benzonitrile (76 mg, 0.39 mmol) according to General Procedure B. A precipitate formed after pouring the reaction mixture into ice/water. Filtration (vacuum) of the precipitate gave 122 mg of a beige solid (87%). ^{1}H NMR δ 7.87–7.81 (m, 2H), 7.68 (d, *J* = 7.2 Hz, 1H), 7.58 (d, *J* = 8.5 Hz, 1H), 7.44–7.34 (m, 2H), 7.15 (dd, *J* = 8.8/0.8 Hz, 1H), 7.06 (ddd, *J* = 7.5/7.5/0.9 Hz, 1H), 6.48 (d, *J* = 1.8 Hz, 1H), 6.44 (dd, *J* = 7.2/2.0 Hz, 1H), 5.30 (s, 2H), 4.77 (br s, 1H), 4.54 (br s, 1H), 3.80 (br s, 1H), 2.07–1.95 (m, 1H), 1.80–1.63 (m, 3H), 1.55 (m, 1H), 1.43–1.24 (m, 3H); ^{13}C NMR δ 161.8, 154.9, 148.3, 142.9, 134.2, 132.4, 130.3, 129.9, 127.7, 127.3, 121.5, 118.8, 118.5, 113.4, 110.4, 107.0, 69.3, 68.8, 35.4, 31.1, 25.0, 24.0;

resonance at δ 134.2 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{25}H_{25}N_2O_3$ $[M+H]^+$ calcd 401.2; found 401.3; LC-MS t_R: 3.63 min.

Methyl 3-((2-(1-(2-hydroxycyclohexyl)-2-oxo-1,2-dihydropyridin-4-yl)phenoxy)methyl)benzoate (8o). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (200 mg, 0.70 mmol) was alkylated with methyl 3-(bromomethyl)benzoate (177 mg, 0.77 mmol) according to General Procedure B. Purification by FCC (eluent EtOAc/PE 50:50 to 100:0) gave 256 mg of a white foam (84%). ^{1}H NMR δ 8.03–8.01 (m, 1H), 7.92–7.87 (m, 1H), 7.71–7.64 (m, 2H), 7.53 (dd, *J* = 7.7/7.7 Hz, 1H), 7.42–7.35 (m, 2H), 7.19 (dd, *J* = 8.8/0.7 Hz, 1H), 7.05 (ddd, *J* = 7.5/7.5/0.9 Hz, 1H), 6.49 (d, *J* = 1.9 Hz, 1H), 6.45 (dd, *J* = 7.2/2.0 Hz, 1H), 5.28 (s, 2H), 4.75 (d, *J* = 6.0 Hz, 1H), 4.54 (br s, 1H), 3.85 (s, 3H), 3.80 (br s, 1H), 2.06–1.92 (m, 1H), 1.79–1.64 (m, 3H), 1.54 (m, 1H), 1.42–1.21 (m, 3H); ^{13}C NMR δ 166.1, 161.8, 155.1, 148.3, 137.9, 134.1, 131.9, 130.3, 129.9, 129.8, 129.0, 128.4, 127.7, 127.3, 121.3, 118.5, 113.5, 107.0, 69.2, 69.0, 52.2, 35.4, 31.1, 25.0, 24.0; resonance at δ 134.1 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{26}H_{28}NO_5$ $[M+H]^+$ calcd 434.2; found 434.2; LC-MS t_R: 3.73 min.

Methyl 4-((2-(1-(2-hydroxycyclohexyl)-2-oxo-1,2-dihydropyridin-4-yl)phenoxy)methyl)benzoate (8p). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (400 mg, 1.40 mmol) was alkylated with methyl 4-(bromomethyl)benzoate (354 mg, 1.54 mmol) according to General Procedure B. Purification by FCC (eluent EtOAc/PE 50:50 to 100:0) gave 329 mg of a white foam (54%). ^{1}H NMR δ 8.00–7.93 (m, 1H), 7.69 (d, *J* = 7.2 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.41–7.36 (m, 2H), 7.17 (dd, *J* = 8.7/0.8 Hz, 1H), 7.06 (ddd, *J* = 7.5/7.5/0.9 Hz, 1H), 5.28 (s, 1H), 4.76 (d, *J* = 6.1 Hz, 1H), 4.54 (br s, 1H), 3.85 (s, 3H), 3.81 (br s, 1H), 2.06–1.95 (m, 1H), 1.80–1.63 (m, 3H), 1.55 (m, 1H), 1.42–1.23 (m, 3H); ^{13}C NMR δ 166.0, 161.8, 155.1, 148.2, 142.6, 134.2, 130.3, 129.9, 129.3, 128.9, 127.2, 127.2, 121.4, 118.5, 113.3, 107.0, 69.2, 69.0, 52.2, 35.4, 31.1, 25.0, 24.0; resonance at δ 134.2 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{26}H_{28}NO_5$ $[M+H]^+$ calcd 434.2; found 434.3; LC-MS t_R: 3.72 min.

1-(2-Hydroxycyclohexyl)-4-(2-((4-(oxazol-2-yl)benzyl)oxy)phenyl)pyridin-2(1*H*)-one (8q). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (100 mg, 0.35 mmol) was alkylated with 2-(4-(bromomethyl)phenyl)oxazole (92 mg, 0.39 mmol) according to General Procedure B. Purification by FCC (eluent MeOH/DCM 0:100 to 10:90) gave 69 mg of a white solid (45%). ^{1}H NMR δ 8.23 (d, *J* = 0.8 Hz, 1H), 8.02–7.96 (m, 2H), 7.69 (d, *J* = 7.2 Hz, 1H), 7.58 (d, *J* = 8.5 Hz, 2H), 7.43–7.36 (m, 3H), 7.23–7.16 (m, 1H), 7.06 (td, *J* = 7.5/0.9 Hz, 1H), 6.53 (d, *J* = 1.9 Hz, 1H), 6.48 (dd, *J* = 7.2/2.0 Hz, 1H), 5.27 (s, 2H), 4.78 (d, *J* = 6.1 Hz, 1H), 4.55 (br s, 1H), 3.83 (br s, 1H), 2.07–1.95 (m, 1H), 1.80–1.64 (m, 3H), 1.62–1.44 (m, 1H), 1.43–1.24 (m, 3H); ^{13}C NMR δ 162.3, 161.1, 155.6, 148.8, 140.6, 140.0, 134.7, 130.8, 130.3, 129.0, 128.3, 127.6, 126.8, 126.5, 121.8, 119.0, 113.8, 107.5, 69.7, 69.6, 35.9, 31.5, 25.5, 24.5; resonance at δ 134.7 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{27}H_{27}N_2O_4$ $[M+H]^+$ calcd 443.2; found 443.3; LC-MS t_R: 3.58 min.

1-(2-Hydroxycyclohexyl)-4-(2-((4-(thiazol-2-yl)benzyl)oxy)phenyl)pyridin-2(1*H*)-one (8r). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (100 mg, 0.35 mmol) was alkylated with 2-(4-(chloromethyl)phenyl)thiazole (81 mg, 0.39 mmol) according to General Procedure B. Purification by FCC (eluent MeOH/DCM 0:100 to 10:90) gave 154 mg of a white solid (96%). ^{1}H NMR δ 8.02–7.90 (m, 3H), 7.80 (d, *J* = 3.2 Hz, 1H), 7.70 (d, *J* = 7.2 Hz, 1H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.46–7.34 (m, 2H), 7.21 (dd, *J* = 8.8/0.7 Hz, 1H), 7.06 (td, *J* = 7.5/0.9 Hz, 1H), 6.52 (d, *J* = 1.9 Hz, 1H), 6.47 (dd, *J* = 7.2/2.0 Hz, 1H), 5.26 (s, 2H), 4.77 (d, *J* = 6.0 Hz, 1H), 4.56 (s, 1H), 3.81 (s, 1H), 2.07–1.94 (m, 1H), 1.84–1.64 (m, 3H), 1.61–1.47 (m, 1H), 1.42–1.22 (m, 3H); ^{13}C NMR δ 167.2, 162.3, 155.7, 148.8, 144.3, 139.6, 132.9, 130.8, 130.4, 128.5, 127.6, 126.8, 121.7, 121.0, 118.9, 113.9, 107.5, 69.6, 69.5, 35.9, 31.3, 25.5, 24.5; resonances at δ 134.7, 69.5 and 31.3 ppm were taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{27}H_{27}N_2O_3S$ $[M+H]^+$ calcd 459.2; found 459.3; LC-MS t_R: 3.66 min.

1-(2-Hydroxycyclohexyl)-4-(2-((4-phenoxybenzyl)oxy)phenyl)pyridin-2(1*H*)-one (8s). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (60 mg, 0.21 mmol) was alkylated

with 1-(bromomethyl)-4-phenoxybenzene (61 mg, 0.23 mmol) according to General Procedure B. After a total of 4 d of stirring with one further addition of K_2CO_3 and 1-(bromomethyl)-4-phenoxybenzene, the mixture was worked up. Purification by FCC (eluent MeOH/DCM 0:100 to 8:92) gave 43 mg of a white solid (43%). ^{1}H NMR δ 7.67 (d, *J* = 7.2 Hz, 1H), 7.47–7.34 (m, 6H), 7.22 (d, *J* = 8.2 Hz, 1H), 7.15 (t, *J* = 7.4 Hz, 1H), 7.09–6.96 (m, 5H), 6.50 (d, *J* = 1.1 Hz, 1H), 6.45 (d, *J* = 7.1 Hz, 1H), 5.16 (s, 2H), 4.77 (d, *J* = 5.9 Hz, 1H), 4.54 (br s, 1H), 3.80 (br s, 1H), 2.06–1.94 (m, 1H), 1.80–1.64 (m, 3H), 1.62–1.43 (m, 1H), 1.42–1.24 (m, 3H); ^{13}C NMR δ 162.3, 156.9, 156.7, 155.8, 148.8, 134.7, 132.3, 130.8, 130.5, 130.3, 129.8, 127.7, 124.0 (2×), 121.7, 119.2, 118.9, 114.0, 107.5, 69.7, 69.7, 35.9, 31.5, 25.5, 24.5; resonance at δ 134.7 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{30}H_{30}NO_4$ $[M+H]^+$ calcd 468.2; found 468.3; LC-MS t_R: 3.87 min.

4-(2-((4-Bromobenzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (8t). 1-(2-hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (759 mg, 2.66 mmol) was alkylated with 4-bromobenzyl bromide (731 mg, 2.93 mmol) according to General Procedure B. A precipitate formed after pouring the reaction mixture into ice/water, with 2 M NaOH $_{(aq)}$ (15 mL) being added. This was stirred at room temperature for 10 min, before collecting the precipitate by filtration (vacuum). Further purification by FCC (eluent EtOAc/PE 10:90 to 100:0) gave 1.12 g of a white solid (93%). ^{1}H NMR δ 7.67 (d, *J* = 7.2 Hz, 1H), 7.59–7.54 (m, 2H), 7.41–7.34 (m, 4H), 7.17 (d, *J* = 8.1 Hz, 1H), 7.05 (ddd, *J* = 7.6/7.6/0.9 Hz, 1H), 6.48 (d, *J* = 1.9 Hz, 1H), 6.43 (dd, *J* = 7.2/2.0 Hz, 1H), 5.16 (s, 2H), 4.76 (d, *J* = 6.0 Hz, 1H), 4.54 (br s, 1H), 3.80 (br s, 1H), 2.08–1.93 (m, 1H), 1.81–1.62 (m, 3H), 1.54 (m, 1H), 1.43–1.23 (m, 3H); ^{13}C NMR δ 161.8, 155.1, 148.3, 136.4, 134.1, 131.4, 130.3, 129.9, 129.5, 127.2, 121.3, 120.8, 118.5, 113.4, 107.0, 69.2, 68.9, 35.4, 31.0, 25.0, 24.0; resonance at δ 134.1 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{24}H_{25}BrNO_3$ $[M+H]^+$ calcd 454.1; found 454.2; LC-MS t_R: 3.84 min.

3-((2-(1-(2-Hydroxycyclohexyl)-2-oxo-1,2-dihydropyridin-4-yl)phenoxy)methyl)benzoic acid (9a). Methyl 3-((2-(1-(2-hydroxycyclohexyl)-2-oxo-1,2-dihydropyridin-4-yl)phenoxy) methyl)benzoate (**8o**) (92 mg, 0.21 mmol) was hydrolysed according to General Procedure C to give 73 mg of a white solid (82%). ^{1}H NMR δ 13.04 (br s, 1H), 8.01 (s, 1H), 7.88 (d, *J* = 7.7 Hz, 1H), 7.65 (d, *J* = 7.3 Hz, 2H), 7.50 (t, *J* = 7.7 Hz, 1H), 7.43–7.34 (m, 2H), 7.19 (d, *J* = 8.6 Hz, 1H), 7.05 (t, *J* = 7.4 Hz, 1H), 6.51–6.44 (m, 2H), 5.27 (s, 2H), 4.54 (br s, 1H), 3.79 (br s, 1H), 3.60 (s, 6H), 2.04–1.93 (m, 1H), 1.80–1.63 (m, 3H), 1.55 (m, 1H), 1.42–1.20 (m, 3H); ^{13}C NMR δ 167.1, 161.8, 155.2, 148.4, 137.6, 134.0, 131.5, 131.0, 130.4, 129.9, 128.8, 128.6, 127.9, 127.2, 121.3, 118.4, 113.4, 107.1, 69.2, 69.1, 35.4, 31.0, 25.0, 24.0; resonance at δ 134.1 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES^{+}) $C_{25}H_{26}NO_5$ $[M+H]^{+}$ calcd 420.2; found 420.3; LC-MS t_R: 3.54 min.

4-((2-(1-(2-Hydroxycyclohexyl)-2-oxo-1,2-dihydropyridin-4-yl)phenoxy)methyl)benzoic acid (9b). Methyl 4-((2-(1-(2-hydroxycyclohexyl)-2-oxo-1,2-dihydropyridin-4-yl)phenoxy) methyl)benzoate (**8p**) (100 mg, 0.23 mmol) was hydrolysed according to General Procedure C to give 90 mg of a white solid (93%). ^{1}H NMR δ 12.98 (br s, 1H), 7.94 (d, *J* = 8.3 Hz, 2H), 7.69 (d, *J* = 7.2 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.45–7.34 (m, 2H), 7.18 (d, *J* = 8.1 Hz, 1H), 7.06 (ddd, *J* = 7.5/7.5/0.8 Hz, 1H), 6.51 (d, *J* = 1.9 Hz, 1H), 6.46 (dd, *J* = 7.2/2.0 Hz, 1H), 5.28 (s, 2H), 4.54 (br s, 1H), 3.80 (br s, 1H), 2.06–1.93 (m, 1H), 1.81–1.63 (m, 3H), 1.55 (m, 1H), 1.43–1.21 (m, 3H); ^{13}C NMR δ 167.1, 161.8, 155.1, 148.3, 142.1, 134.2, 130.3, 130.1, 129.9, 129.5, 127.2, 127.0, 121.3, 118.5, 113.3, 107.0, 69.2, 69.1, 35.4, 31.1, 25.0, 24.0; resonance at δ 134.2 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES^{+}) $C_{25}H_{26}NO_5$ $[M+H]^{+}$ calcd 420.2; found 420.3; LC-MS t_R: 3.50 min.

3-((2-(1-(2-Hydroxycyclohexyl)-2-oxo-1,2-dihydropyridin-4-yl)phenoxy)methyl) benzamide (9c). Methyl 3-((2-(1-(2-hydroxycyclohexyl)-2-oxo-1,2-dihydropyridin-4-yl)phenoxy)methyl)benzoate (**8o**) (90 mg, 0.21 mmol) was treated with ammonium hydroxide

according to General Procedure D to give 45 mg of a white solid (52%). ^{1}H NMR δ 7.98 (s, 1H), 7.94 (s, 1H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.67 (d, *J* = 7.2 Hz, 1H), 7.56 (d, *J* = 7.7 Hz, 1H), 7.46 (dd, *J* = 7.7/7.7 Hz, 1H), 7.43–7.35 (m, 3H), 7.23–7.17 (m, 1H), 7.06 (ddd, *J* = 7.5/7.5/0.8 Hz, 1H), 6.52 (d, *J* = 1.9 Hz, 1H), 6.47 (dd, *J* = 7.2/2.0 Hz, 1H), 5.24 (s, 2H), 4.77 (d, *J* = 6.0 Hz, 1H), 4.54 (br s, 1H), 3.80 (br s, 1H), 2.07–1.94 (m, 1H), 1.81–1.64 (m, 3H), 1.54 (m, 1H), 1.42–1.21 (m, 3H); ^{13}C NMR δ 167.8, 161.8, 155.3, 148.3, 137.1, 134.6, 134.1, 130.4, 130.0, 129.9, 128.5, 127.1, 126.8, 126.5, 121.3, 118.5, 113.4, 107.0, 69.5, 69.2, 35.4, 31.0, 25.0, 24.0; resonance at δ 134.1 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{25}H_{27}N_2O_4$ $[M+H]^+$ calcd 419.2; found 419.3; LC-MS t_R: 3.44 min.

4-((2-(1-(2-Hydroxycyclohexyl)-2-oxo-1,2-dihydropyridin-4-yl)phenoxy)methyl)benzamide (9d). Methyl 4-((2-(1-(2-hydroxycyclohexyl)-2-oxo-1,2-dihydropyridin-4-yl)phenoxy)methyl)benzoate (**8p**) (105 mg, 0.24 mmol) was treated with ammonium hydroxide according to General Procedure D to give 15 mg of a white solid (14%). ^{1}H NMR δ 7.97 (s, 1H), 7.86 (d, *J* = 8.3 Hz, 2H), 7.68 (d, *J* = 7.2 Hz, 1H), 7.47 (d, *J* = 8.3 Hz, 2H), 7.42–7.33 (m, 3H), 7.20–7.15 (m, 1H), 7.05 (ddd, *J* = 7.5/7.5/0.8 Hz, 1H), 6.51 (d, *J* = 1.9 Hz, 1H), 6.45 (dd, *J* = 7.2/2.0 Hz, 1H), 5.25 (s, 2H), 4.76 (d, *J* = 6.0 Hz, 1H), 4.54 (br s, 1H), 3.80 (br s, 1H), 2.05–1.94 (m, 1H), 1.80–1.63 (m, 3H), 1.55 (m, 1H), 1.42–1.25 (m, 3H); ^{13}C NMR δ 167.6, 161.8, 155.2, 148.3, 140.2, 134.2, 133.6, 130.3, 129.9, 127.7, 127.1, 126.9, 121.3, 118.5, 113.4, 107.0, 69.2, 69.1, 35.4, 31.1, 25.0, 24.0; resonance at δ 134.1 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{25}H_{27}N_2O_4$ $[M+H]^+$ calcd 419.2; found 419.3; LC-MS t_R: 3.40 min.

1-(2-Hydroxycyclohexyl)-4-(2-((4-(pyridin-3-yl)benzyl)oxy)phenyl)pyridin-2(1*H*)-one (10a). 4-(2-((4-Bromobenzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**8t**) (100 mg, 0.22 mmol) was coupled to pyridine-3-boronic acid (41 mg, 0.33 mmol) according to General Procedure A. Purification by FCC (eluent MeOH/DCM 0:100 to 7.5:92.5) gave 48 mg of a white solid (48%). ^{1}H NMR δ 8.90 (d, *J* = 2.1 Hz, 1H), 8.57 (dd, *J* = 4.7/1.5 Hz, 1H), 8.10–8.05 (m, 1H), 7.74 (d, *J* =

8.2 Hz, 2H), 7.68 (d, *J* = 7.2 Hz, 1H), 7.54 (d, *J* = 8.2 Hz, 2H), 7.49 (dd, *J* = 7.9/4.8 Hz, 1H), 7.43–7.94 (m, 2H), 7.22 (d, *J* = 8.2 Hz, 1H), 7.05 (t, *J* = 7.4 Hz, 1H), 6.51 (d, *J* = 1.9 Hz, 1H), 6.46 (dd, *J* = 7.2/1.9 Hz, 1H), 5.26 (s, 2H), 4.77 (d, *J* = 6.0 Hz, 1H), 4.54 (br s, 1H), 3.80 (br s, 1H), 2.05–1.93 (m, 1H), 1.81–1.61 (m, 3H), 1.53 (m, 1H), 1.41–1.25 (m, 3H); ^{13}C NMR δ 161.8, 155.3, 148.6, 148.4, 147.7, 137.0, 136.5, 135.2, 134.2, 134.1, 130.3, 129.9, 128.1, 127.2, 127.0, 123.9, 121.2, 118.5, 113.5, 107.0, 69.2, 69.0, 35.4, 31.1, 25.0, 24.0; resonances at δ 134.2 and 69.0 ppm were taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{29}H_{29}N_2O_3$ $[M+H]^+$ calcd 453.2; found 453.3; LC-MS t_R: 3.39 min.

1-(2-Hydroxycyclohexyl)-4-(2-((4-(pyridin-4-yl)benzyl)oxy)phenyl)pyridin-2(1*H*)-one (10b). 4-(2-((4-Bromobenzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**8t**) (100 mg, 0.22 mmol) was coupled to pyridine-4-boronic acid (41 mg, 0.33 mmol) according to General Procedure A. Purification by FCC (eluent MeOH/DCM 0:100 to 7.5:92.5) gave 43 mg of a white solid (43%). ^{1}H NMR δ 8.63 (d, *J* = 5.5 Hz, 2H), 7.81 (d, *J* = 8.1 Hz, 2H), 7.75–7.65 (m, 3H), 7.56 (d, *J* = 8.1 Hz, 2H), 7.43–7.34 (m, 2H), 7.21 (d, *J* = 8.5 Hz, 1H), 7.05 (t, *J* = 7.4 Hz, 1H), 6.53–6.50 (m, 1H), 6.46 (dd, *J* = 7.1/1.3 Hz, 1H), 5.27 (s, 2H), 4.77 (d, *J* = 5.9 Hz, 1H), 4.54 (br s, 1H), 3.80 (br s, 1H), 2.05–1.93 (m, 1H), 1.81–1.62 (m, 3H), 1.54 (m, 1H), 1.42–1.24 (m, 3H); ^{13}C NMR δ 161.8, 155.2, 150.3, 148.4, 146.6, 138.2, 136.5, 134.2, 130.3, 129.9, 128.0, 127.2, 127.0, 121.3, 121.2, 118.5, 113.4, 107.0, 69.2, 69.2, 35.4, 31.1, 25.0, 24.0; resonance at δ 134.2 was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{29}H_{29}N_2O_3$ $[M+H]^+$ calcd 453.2; found 453.3; LC-MS t_R: 3.31 min.

1-(2-Hydroxycyclohexyl)-4-(2-((4-(pyrimidin-5-yl)benzyl)oxy)phenyl)pyridin-2(1*H*)-one (10c). 4-(2-((4-Bromobenzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**8t**) (100 mg, 0.22 mmol) was coupled to pyrimidine-5-boronic acid (41 mg, 0.33 mmol) according to General Procedure A. Purification by FCC (eluent MeOH/DCM 0:100 to 5:95) gave 38 mg of a white solid (38%). ^{1}H NMR δ 9.19 (s, 1H), 9.15 (s, 2H), 7.85–7.79 (m, 2H), 7.68 (d, *J* = 7.2 Hz,

1H), 7.57 (d, *J* = 8.3 Hz, 2H), 7.42–7.35 (m, 2H), 7.21 (d, *J* = 8.0 Hz, 1H), 7.05 (ddd, *J* = 7.6/7.6/0.9 Hz, 1H), 6.51 (d, *J* = 1.9 Hz, 1H), 6.46 (dd, *J* = 7.2/2.0 Hz, 1H), 5.28 (s, 2H), 4.77 (d, *J* = 5.9 Hz, 1H), 4.54 (br s, 1H), 3.80 (br s, 1H), 2.05–1.94 (m, 1H), 1.80–1.62 (m, 3H), 1.54 (m, 1H), 1.42–1.24 (m, 3H); ^{13}C NMR δ 161.8, 157.3, 155.2, 155.2, 148.3, 137.8, 134.2, 133.1, 132.9, 130.3, 129.9, 128.1, 127.2, 127.1, 121.3, 118.5, 113.5, 107.0, 69.2, 69.1, 35.4, 31.1, 25.0, 24.0; resonance at δ 134.2 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{28}H_{28}N_3O_3$ $[M+H]^+$ calcd 454.2; found 454.3; LC-MS t_R: 3.56 min.

1-(2-Hydroxycyclohexyl)-4-(2-((4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)pyridin-2(1*H*)-one (10d). 4-(2-((4-Bromobenzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**8t**) (100 mg, 0.22 mmol) was coupled to 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (69 mg, 0.33 mmol) according to General Procedure A. Purification by FCC (eluent MeOH/DCM 0:100 to 5:95) gave 43 mg of a white solid (43%). ^{1}H NMR δ 8.12 (s, 1H), 7.85 (s, 1H), 7.67 (d, *J* = 7.2 Hz, 1H), 7.55 (d, *J* = 8.1 Hz, 2H), 7.42–7.34 (m, 2H), 7.21 (d, *J* = 8.3 Hz, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 6.51 (d, *J* = 1.9 Hz, 1H), 6.44 (dd, *J* = 7.2/1.9 Hz, 1H), 5.16 (s, 2H), 4.75 (d, *J* = 6.0 Hz, 1H), 4.53 (br s, 1H), 3.85 (s, 3H), 3.80 (br s, 1H), 2.04–1.94 (m, 1H), 1.82–1.67 (m, 3H), 1.54 (m, 1H), 1.41–1.25 (m, 3H); ^{13}C NMR δ 161.8, 155.4, 148.3, 136.1, 134.4, 134.2, 132.2, 130.3, 129.9, 128.1, 127.9, 127.1, 125.0, 121.5, 121.1, 118.5, 113.4, 107.0, 69.5, 69.2, 38.9, 35.4, 31.1, 25.0, 24.0; resonance at δ 134.2 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{28}H_{30}N_3O_3$ $[M+H]^+$ calcd 456.2; found 456.3; LC-MS t_R: 3.61 min.

4-(2-Aminophenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (11). 4-Bromo-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**4b**) (200 mg, 0.73 mmol) was coupled to 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (244 g, 1.10 mmol) according to General Procedure A. Purification by FCC (eluent MeOH/DCM 0:100 to 20:80) gave 181 mg of a brown solid (87%). ^{1}H NMR δ 7.71 (d, *J* = 7.2 Hz, 1H), 7.11–7.00 (m, 2H), 6.75 (dd, *J* = 8.1/0.9 Hz, 1H), 6.62 (td, *J* = 7.5/1.1 Hz, 1H), 6.37 (d, *J* = 1.8 Hz, 1H), 6.30 (dd, *J* = 7.1/2.0 Hz, 1H), 4.98 (s, 2H), 4.73 (d, *J* =

5.6 Hz, 1H), 4.54 (br s, 1H), 3.80 (br s, 1H), 2.05–1.94 (m, 1H), 1.80–1.65 (m, 3H), 1.55 (m, 1H), 1.42–1.24 (m, 3H); ^{13}C NMR δ 162.0, 150.0, 145.2, 135.2, 129.4, 129.0, 122.5, 117.5, 116.6, 115.6, 106.2, 69.3, 35.3, 31.0, 25.0, 24.0; resonance at δ 135.2 ppm was taken from HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{17}H_{21}N_2O_2$ $[M+H]^+$ calcd 285.2; found 285.2; LC-MS t_R: 3.35 min.

4-(2-((4-Bromobenzyl)amino)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (12). To a mixture of 4-(2-aminophenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**11**) (100 mg, 0.35 mmol) and 4-bromobenzaldehyde (65 mg, 0.35 mmol, 1.0 eq) in 1,2-dichloroethane (3.5 mL) was added AcOH (0.20 mL) and $NaB(OAc)_3H$ (149 mg, 0.70 mmol, 2.0 eq). After 2 h, the reaction mixture was diluted with DCM (20 mL) washed with 10% $K_2CO_{3(aq)}$ (20 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by FCC (eluent EtOAc/PE 50:50 to 100:0) gave 81 mg of a yellow oil (50%). ^{1}H NMR δ 7.75 (d, *J* = 7.2 Hz, 1H), 7.53–7.47 (m, 2H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.11–7.01 (m, 2H), 6.63 (ddd, *J* = 7.4/7.4/0.9 Hz, 1H), 6.46–6.40 (m, 2H), 6.31 (dd, *J* = 7.1/2.0 Hz, 1H), 5.72 (t, *J* = 5.9 Hz, 1H), 4.73 (d, *J* = 5.6 Hz, 1H), 4.56 (br s, 1H), 4.30 (d, *J* = 5.9 Hz, 2H), 3.82 (br s, 1H), 2.05–1.96 (m, 1H), 1.82–1.65 (m, 3H), 1.56 (m, 1H), 1.43–1.25 (m, 3H); ^{13}C NMR δ 162.0, 149.7, 144.4, 139.7, 135.4, 131.2, 129.5, 129.1, 129.1, 123.9, 119.5, 118.2, 116.4, 111.0, 106.6, 69.3, 45.8, 35.3, 30.7, 25.1, 24.0; resonances at δ 135.4 and 30.7 ppm were taken from HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{24}H_{26}BrN_2O_2$ $[M+H]^+$ calcd 453.1; found 453.2; LC-MS t_R: 3.89.

1-(2-Hydroxycyclohexyl)-4-(2-((4-(pyridin-3-yl)benzyl)amino)phenyl)pyridin-2(1*H*)-one (13a). 4-(2-((4-Bromobenzyl)amino)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**12**) (80 mg, 0.18 mmol) was coupled to pyridine-3-boronic acid (33 mg, 0.26 mmol) according to General Procedure A. Purification by FCC (eluent MeOH/DCM 0:100 to 5:95) gave 33 mg of a yellow oil (41%). ^{1}H NMR δ 8.87 (d, *J* = 1.8 Hz, 1H), 8.55 (dd, *J* = 4.7/1.5 Hz, 1H), 8.05 (ddd, *J* = 8.0/2.4/1.6 Hz, 1H), 7.77 (d, *J* = 7.2 Hz, 1H), 7.69 (d, *J* = 8.3 Hz, 2H), 7.50–7.44 (m, 3H), 7.12–7.06 (m, 1H), 7.05 (dd, *J* = 7.5/1.5 Hz, 1H), 6.63 (ddd, *J* = 7.4/7.4/0.9 Hz, 1H), 6.52 (d, *J* = 8.2 Hz, 1H), 6.45 (d, *J*

= 1.9 Hz, 1H), 6.34 (dd, *J* = 7.1/2.0 Hz, 1H), 5.74 (t, *J* = 6.0 Hz, 1H), 4.74 (d, *J* = 5.6 Hz, 1H), 4.57 (br s, 1H), 4.40 (d, *J* = 5.9 Hz, 2H), 3.83 (br s, 1H), 2.07–1.95 (m, 1H), 1.84–1.65 (m, 3H), 1.57 (m, 1H), 1.44–1.26 (m, 3H); ^{13}C NMR δ 162.0, 149.8, 148.3, 147.6, 144.6, 140.3, 135.4, 135.4, 134.0, 129.5, 129.1, 127.6, 126.9, 123.9, 123.9, 118.1, 116.3, 111.1, 106.6, 69.3, 46.0, 35.3, 31.1, 25.0, 24.0; resonance at δ 135.4 ppm was taken from HSQC experiment. Missing an aromatic quaternary carbon resonance not observed in 2D NMR experiments; *m/z* MS (TOF ES$^+$) $C_{29}H_{30}N_3O_2$ $[M+H]^+$ calcd 452.2; found 452.3; LC-MS t_R: 3.44 min.

1-(2-Hydroxycyclohexyl)-4-(2-((4-(pyridin-4-yl)benzyl)amino)phenyl)pyridin-2(1*H*)-one (13b). 4-(2-((4-Bromobenzyl)amino)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**12**) (80 mg, 0.18 mmol) was coupled to pyridine-4-boronic acid (33 mg, 0.26 mmol) according to General Procedure A. Purification by FCC (eluent MeOH/DCM 0:100 to 5:95) followed by preparative HPLC (30% to 100% buffer B, 20 minutes) gave 19 mg of a yellow oil (29%). ^{1}H NMR δ 8.63–8.59 (m, 2H), 7.77 (d, *J* = 8.2 Hz, 3H), 7.69 (dd, *J* = 4.6/1.6 Hz, 2H), 7.49 (d, *J* = 8.3 Hz, 1H), 7.11–7.02 (m, 2H), 6.63 (ddd, *J* = 7.4/7.4/0.8 Hz, 1H), 6.50 (d, *J* = 8.2 Hz, 1H), 6.45 (d, *J* = 1.9 Hz, 1H), 6.34 (dd, *J* = 7.1/2.0 Hz, 1H), 5.76 (t, *J* = 5.9 Hz, 1H), 4.74 (d, *J* = 5.6 Hz, 1H), 4.57 (br s, 1H), 4.41 (d, *J* = 5.9 Hz, 2H), 3.83 (br s, 1H), 2.06–1.95 (m, 1H), 1.84–1.66 (m, 3H), 1.57 (m, 1H), 1.43–1.26 (m, 3H); ^{13}C NMR δ 162.0, 150.2, 149.8, 146.8, 144.5, 141.6, 135.5, 135.4, 129.5, 129.1, 127.6, 126.9, 123.9, 121.1, 118.1, 116.3, 111.1, 106.6, 69.3, 46.1, 35.3, 31.1, 25.1, 24.0; resonance at δ 135.5 ppm was taken from HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{29}H_{30}N_3O_2$ $[M+H]^+$ calcd 452.2; found 452.3; LC-MS t_R: 3.33 min.

1-(2-Hydroxycyclohexyl)-4-(2-((4-(pyrimidin-5-yl)benzyl)amino)phenyl)pyridin-2(1*H*)-one (13c). 4-(2-((4-Bromobenzyl)amino)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**12**) (80 mg, 0.18 mmol) was coupled to pyrimidine-5-boronic acid (33 mg, 0.26 mmol) according to General Procedure A. Purification by FCC (eluent MeOH/DCM 0:100 to 5:95) followed by preparative HPLC (MeCN:H_2O 40:60 to 100:0) gave 28 mg of a yellow oil (35%). ^{1}H NMR δ 9.16

(s, 1H), 9.12 (s, 2H), 7.77 (d, *J* = 8.2 Hz, 3H), 7.51 (d, *J* = 8.3 Hz, 2H), 7.11–7.02 (m, 2H), 6.63 (ddd, *J* = 7.4/7.4/0.9 Hz, 1H), 6.51 (d, *J* = 8.0 Hz, 1H), 6.45 (d, *J* = 1.9 Hz, 1H), 6.34 (dd, *J* = 7.1/2.0 Hz, 1H), 5.77 (t, *J* = 5.9 Hz, 1H), 4.74 (d, *J* = 5.6 Hz, 1H), 4.57 (br s, 1H), 4.41 (d, *J* = 5.9 Hz, 2H), 3.82 (br s, 1H), 2.06–1.96 (m, 1H), 1.83–1.65 (m, 3H), 1.58 (m, 1H), 1.44–1.25 (m, 3H); ^{13}C NMR δ 162.0, 157.1, 154.6, 149.8, 144.5, 141.3, 135.6, 133.1, 132.1, 129.5, 129.1, 127.7, 127.0, 123.9, 118.1, 116.3, 111.1, 106.6, 69.3, 46.0, 35.3, 31.1, 25.1, 24.0; resonance at δ 135.6 ppm was taken from HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{28}H_{29}N_4O_2$ $[M+H]^+$ calcd 453.2; found 453.3; LC-MS t_R: 3.55 min.

1-(2-Hydroxycyclohexyl)-4-(2-((4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)amino)phenyl)pyridin-2(1*H*)-one (13d). 4-(2-((4-Bromobenzyl)amino)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**12**) (80 mg, 0.18 mmol) was coupled to 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-*1H*-pyrazole (55 mg, 0.26 mmol) according to General Procedure A. Purification by FCC (eluent MeOH/DCM 0:100 to 5:95) followed by preparative HPLC (40% to 100% buffer B, 20 minutes) gave 30 mg of a yellow oil (37%). ^{1}H NMR δ 8.07 (s, 1H), 7.80 (d, *J* = 0.7 Hz, 1H), 7.75 (d, *J* = 7.2 Hz, 1H), 7.49 (d, *J* = 8.3 Hz, 2H), 7.31 (d, *J* = 8.3 Hz, 2H), 7.11–7.05 (m, 1H), 7.03 (dd, *J* = 7.5/1.6 Hz, 1H), 6.62 (ddd, *J* = 7.4/7.4/0.9 Hz, 1H), 6.52 (d, *J* = 8.2 Hz, 1H), 6.43 (d, *J* = 1.9 Hz, 1H), 6.33 (dd, *J* = 7.1/2.0 Hz, 1H), 5.62 (t, *J* = 5.9 Hz, 1H), 4.73 (d, *J* = 5.6 Hz, 1H), 4.56 (br s, 1H), 4.31 (d, *J* = 5.8 Hz, 2H), 3.90–3.76 (m, 4H), 2.06–1.95 (m, 1H), 1.83–1.64 (m, 3H), 1.56 (br s, 1H), 1.43–1.25 (m, 3H); ^{13}C NMR δ 162.0, 149.8, 144.7, 137.7, 135.9, 135.5, 131.0, 129.5, 129.1, 127.6, 127.3, 125.0, 123.9, 121.8, 118.1, 116.2, 111.1, 106.5, 69.1, 46.2, 38.6, 35.3, 31.1, 25.0, 24.0; resonances at δ 135.5 and 69.1 ppm were taken from HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{28}H_{31}N_4O_2$ $[M+H]^+$ calcd 455.2; found 455.3; LC-MS t_R: 3.22 min.

4-(2-(([1,1'-Biphenyl]-4-ylmethyl)amino)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (13e). 4-(2-((4-Bromobenzyl)amino)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**12**) (80 mg, 0.18 mmol) was coupled to phenylboronic acid (32 mg, 0.26 mmol) according to General

Procedure A. Purification by FCC (eluent MeOH/DCM 0:100 to 5:95) followed by preparative HPLC (40% to 100% buffer B, 20 minutes) gave 36 mg of a yellow oil (46%). ^{1}H NMR δ 7.76 (d, *J* = 7.2 Hz, 1H), 7.68–7.58 (m, 4H), 7.49–7.40 (m, 4H), 7.34 (ddd, *J* = 8.5/4.5/1.2 Hz, 1H), 7.12–7.06 (m, 1H), 7.04 (dd, *J* = 7.5/1.5 Hz, 1H), 6.63 (ddd, *J* = 7.4/7.4/0.9 Hz, 1H), 6.53 (d, *J* = 8.1 Hz, 1H), 6.44 (d, *J* = 1.9 Hz, 1H), 6.34 (dd, *J* = 7.1/2.0 Hz, 1H), 5.71 (t, *J* = 5.9 Hz, 1H), 4.74 (d, *J* = 5.6 Hz, 1H), 4.57 (br s, 1H), 4.38 (d, *J* = 5.9 Hz, 2H), 3.82 (br s, 1H), 2.06–1.96 (m, 1H), 1.83–1.65 (m, 3H), 1.57 (m, 1H), 1.43–1.25 (m, 3H); ^{13}C NMR δ 162.0, 149.8, 144.6, 140.0, 139.5, 138.6, 135.5, 129.6, 129.1, 128.9, 127.4, 127.3, 126.7, 126.6, 123.9, 118.1, 116.3, 111.1, 106.6, 69.1, 46.1, 35.3, 30.8, 25.1, 24.0; resonances at δ 135.5, 69.1 and 30.8 ppm were taken from HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{30}H_{31}N_2O_2$ $[M+H]^+$ calcd 451.2; found 451.3; LC-MS t_R: 4.00 min.

4-(2-((4-(1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (14). 4-(2-((4-Bromobenzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**8t**) (200 mg, 0.44 mmol) was coupled to 1-(*tert*-butoxycarbonyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (194 mg, 0.66 mmol) according to General Procedure A. LCMS analysis of the crude residue indicated loss of the Boc group during the reaction. Purification by FCC (eluent MeOH/DCM 0:100 to 10:90), gave 150 mg of a white solid (77%). ^{1}H NMR δ 12.94 (s, 1H), 8.19 (s, 1H), 7.92 (s, 1H), 7.67 (d, *J* = 7.3 Hz, 1H), 7.63–7.53 (m, 2H), 7.47–7.33 (m, 4H), 7.21 (d, *J* = 8.2 Hz, 1H), 7.04 (ddd, *J* = 7.6/7.6/0.9 Hz, 1H), 6.51 (d, *J* = 1.9 Hz, 1H), 6.44 (dd, *J* = 7.2, 2.0 Hz, 1H), 5.17 (s, 2H), 4.75 (d, *J* = 6.0 Hz, 1H), 4.53 (s, 1H), 3.80 (s, 1H), 1.99 (s, 1H), 1.81–1.62 (m, 3H), 1.61–1.42 (m, 1H), 1.42–1.22 (m, 3H); ^{13}C NMR δ 161.8, 155.3, 148.3, 136.2, 134.6, 134.3, 132.5, 130.3, 129.9, 128.0, 127.1, 125.5, 125.1, 121.1, 120.8, 118.5, 113.5, 106.9, 69.5, 69.2, 35.4, 31.0, 25.0, 24.0; *m/z* MS (TOF ES$^+$) $C_{27}H_{28}N_3O_3$ $[MH]^+$ calcd 442.2; found 442.3; LC-MS t_R: 3.47 min.

4-(2-((4-(1-Ethyl-1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (15a). 14-(2-((4-(1*H*-Pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**14**) (65mg, 147 μmol) was alkylated with 1-bromoethane (12 μL, 161 μmol) according to General Procedure B. Reaction progress was monitored via LC-MS analysis, additional K_2CO_3 (22 mg, 162 μmol) and 1-bromoethane (61 μL, 805 μmol) was added over 44 h at rt until the reaction appeared complete. The crude product was dried on the freeze dryer to give 57 mg of white solid (82%). No further purification was required. ^{1}H NMR δ 8.19 (d, *J* = 0.5 Hz, 1H), 7.87 (d, *J* = 0.7 Hz, 1H), 7.67 (d, *J* = 7.2 Hz, 1H), 7.59–7.55 (m, 2H), 7.42–7.36 (m, 4H), 7.24–7.19 (m, 1H), 7.05 (td, *J* = 7.6/0.9 Hz, 1H), 6.52 (d, *J* = 1.9 Hz, 1H), 6.45 (dd, *J* = 7.2/2.0 Hz, 1H), 5.17 (s, 2H), 4.75 (d, *J* = 5.9 Hz, 1H), 4.54 (br s, 1H), 4.15 (q, *J* = 7.3 Hz, 2H), 3.81 (br s, 1H), 2.05–1.95 (m, 1H), 1.80–1.65 (m, 3H), 1.65–1.45 (m, 1H), 1.40 (t, *J* = 7.3 Hz, 3H), 1.33–1.22 (m, 3H); ^{13}C NMR δ 162.3, 155.8, 148.8, 136.4, 134.8, 134.8, 132.7, 130.8, 130.3, 128.5, 127.5, 126.8, 125.4, 121.8, 121.6, 118.9, 113.9, 107.4, 70.0, 69.6, 46.8, 35.9, 31.5, 25.5, 24.5, 15.9; resonance at δ 134.8 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{29}H_{32}N_3O_3$ $[M+H]^+$ calcd 470.2; found 470.3; LC-MS t_R: 3.58 min.

1-(2-Hydroxycyclohexyl)-4-(2-((4-(1-(2-hydroxyethyl)-1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)pyridin-2(1*H*)-one (15b). 14-(2-((4-(1*H*-Pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**14**) (80 mg, 181 μmol) was alkylated with bromoethanol (14 μL, 199 μmol) according to General Procedure B. Reaction progress was monitored via LC-MS analysis, the reaction was stopped after 10 d at 100 °C. Purification by FCC (eluent MeOH/DCM 0:100 to 10:90) gave 19 mg of a white solid (22%). ^{1}H NMR δ 8.15 (s, 1H), 7.88 (d, *J* = 0.5 Hz, 1H), 7.67 (d, *J* = 7.2 Hz, 1H), 7.57 (d, *J* = 8.2 Hz, 2H), 7.43–7.35 (m, 4H), 7.22 (d, *J* = 8.2 Hz, 1H), 7.08–7.01 (m, 1H), 6.52 (d, *J* = 1.9 Hz, 1H), 6.45 (dd, *J* = 7.2/2.0 Hz, 1H), 5.17 (s, 2H), 4.94 (t, *J* = 5.3 Hz, 1H), 4.76 (d, *J* = 6.0 Hz, 1H), 4.55 (br s, 1H), 4.16 (t, *J* = 5.6 Hz, 2H), 3.86–3.72 (m, 3H), 2.05–1.96 (m, 1H), 1.78–1.65 (m, 3H), 1.62–1.45 (m, 1H), 1.40–1.26 (m, 3H).

^{13}C NMR δ 162.3, 155.8, 148.8, 136.5, 134.8, 134.6, 132.7, 130.7, 130.3, 128.5, 128.1, 127.5, 125.4, 121.6, 121.6, 118.9, 113.9, 107.4, 70.0, 69.7, 60.5, 55.4, 35.9, 31.5, 25.5, 24.5; resonance at δ 134.6 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{29}H_{32}N_3O_4$ $[M+H]^+$ calcd 486.2; found 486.3; LC-MS t_R: 3.42 min.

1-(2-Hydroxycyclohexyl)-4-(2-((4-(1-propyl-1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)pyridin-2(1*H*)-one (15c). 14-(2-((4-(1*H*-Pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**14**) (60 mg, 136 μmol) was alkylated with 1-bromopropane (14 μL, 149 μmol) according to General Procedure B. Reaction progress was monitored via LC-MS analysis, additional 1-bromopropane (54 μL, 596 μmol) was added over 48 h at rt until the reaction appeared complete. The crude product was dried on the freeze dryer to give 51 mg of the desired product as of a white solid (77%). No further purification was required. ^{1}H NMR δ 8.18 (d, *J* = 0.5 Hz, 1H), 7.87 (d, *J* = 0.6 Hz, 1H), 7.67 (d, *J* = 7.3 Hz, 1H), 7.57 (d, *J* = 8.3 Hz, 2H), 7.43–7.35 (m, 4H), 7.22 (d, *J* = 8.2 Hz, 1H), 7.05 (td, *J* = 7.6/0.8 Hz, 1H), 6.52 (d, *J* = 1.9 Hz, 1H), 6.45 (dd, *J* = 7.2/2.0 Hz, 1H), 5.17 (s, 2H), 4.76 (d, *J* = 6.0 Hz, 1H), 4.54 (br s, 1H), 4.07 (t, *J* = 7.0 Hz, 2H), 3.80 (br s, 1H), 2.05–1.95 (m, 1H), 1.85–1.73 (m, 2H), 1.76–1.65 (m, 3H), 1.60–1.48 (m, 1H), 1.41–1.19 (m, 3H), 0.85 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR δ 162.2, 155.8, 148.8, 136.4, 134.7, 134.8, 132.7, 130.8, 130.3, 128.5, 127.5, 125.4, 121.6, 121.6, 118.9, 113.9, 107.4, 70.0, 69.6, 53.5, 35.9, 31.5, 25.5, 24.5, 23.7, 11.4; resonance at δ 134.7 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{30}H_{34}N_3O_3$ $[M+H]^+$ calcd 484.3; found 484.3; LC-MS t_R: 3.67 min.

1-(2-Hydroxycyclohexyl)-4-(2-((4-(1-isopropyl-1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)pyridin-2(1*H*)-one (15d). 14-(2-((4-(1*H*-Pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**14**) (60 mg, 136 μmol) was alkylated with 2-bromopropane (14 μL, 149 μmol) according to General Procedure B. Reaction progress was monitored via LC-MS analysis, additional K_2CO_3 (21 mg, 149 μmol) and 2-bromopropane (56 μL, 596 μmol) was added over 48 h at rt, then the reaction mixture was heated to 40 °C for another 5 d until the reaction appeared

complete. Purification by FCC (eluent MeOH/DCM 0:100 to 8:92) gave 28 mg of a white solid (42%). ^{1}H NMR δ 8.22 (d, *J* = 0.5 Hz, 1H), 7.87 (d, *J* = 0.6 Hz, 1H), 7.67 (d, *J* = 7.3 Hz, 1H), 7.59 (d, *J* = 8.3 Hz, 2H), 7.44–7.33 (m, 4H), 7.26–7.18 (m, 1H), 7.05 (td, *J* = 7.5/0.8 Hz, 1H), 6.54 (d, *J* = 1.9 Hz, 1H), 6.46 (dd, *J* = 7.2/2.0 Hz, 1H), 5.17 (s, 2H), 4.78 (d, *J* = 6.0 Hz, 1H), 4.63–4.43 (m, 2H), 3.80 (br s, 1H), 2.05–1.95 (m, 1H), 1.78–1.65 (m, 3H), 1.62–1.50 (m, 1H), 1.45 (d, *J* = 6.7 Hz, 6H), 1.39–1.25 (m, 3H); ^{13}C NMR δ 162.3, 155.8, 148.8, 136.0, 134.7, 134.7, 132.8, 130.8, 130.3, 128.5, 127.5, 125.4, 125.2, 121.6, 121.5, 119.0, 113.9, 107.4, 70.0, 69.7, 53.6, 35.9, 31.6, 25.5, 24.5, 23.1; resonance at δ 134.7 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{30}H_{34}N_3O_3$ $[M+H]^+$ calcd 484.3; found 484.3; LC-MS t_R: 3.63 min.

4-(2-((4-(1-Butyl-1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (15e). 14-(2-((4-(1*H*-Pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**14**) (60 mg, 136 μmol) was alkylated with 1-bromobutane (16 μL, 149 μmol) according to General Procedure B. Reaction progress was monitored via LC-MS analysis, additional K_2CO_3 (21 mg, 149 μmol) and 1-bromobutane (54 μL, 600 μmol) was added over 72 h at rt, then the reaction mixture was heated to 40 °C for another 4 d until the reaction appeared complete. Purification by FCC (eluent MeOH/DCM 0:100 to 8:92) gave 42 mg of a white solid (63%). ^{1}H NMR δ 8.18 (d, *J* = 0.5 Hz, 1H), 7.87 (d, *J* = 0.7 Hz, 1H), 7.67 (d, *J* = 7.3 Hz, 1H), 7.57 (d, *J* = 8.3 Hz, 2H), 7.44–7.34 (m, 4H), 7.25–7.19 (m, 1H), 7.04 (td, *J* = 7.5/0.8 Hz, 1H), 6.54 (d, *J* = 1.9 Hz, 1H), 6.46 (dd, *J* = 7.2/2.0 Hz, 1H), 5.17 (s, 2H), 4.78 (d, *J* = 6.0 Hz, 1H), 4.55 (br s, 1H), 4.11 (t, *J* = 7.0 Hz, 2H), 3.81 (br s, 1H), 2.05–1.96 (m, 1H), 1.83–1.64 (m, 5H), 1.61–1.45 (m, 1H), 1.41–1.19 (m, 5H), 0.89 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR δ 162.3, 155.8, 148.8, 136.4, 134.8, 134.7, 132.7, 130.7, 130.3, 128.5, 127.5, 127.5, 125.4, 121.7, 121.6, 118.9, 113.9, 107.4, 70.0, 69.7, 51.5, 35.9, 32.3, 31.5, 25.5, 24.5, 19.7, 13.9; resonance at δ 134.7 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{31}H_{36}N_3O_3$ $[M+H]^+$ calcd 498.3; found 498.4; LC-MS t_R: 3.73 min.

4-(2-((4-(1-Cyclopentyl-1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (15f). 14-(2-((4-(1*H*-Pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**14**) (72 mg, 163 μmol) was alkylated with bromocyclopentane (19 μL, 179 μmol) according to General Procedure B. Reaction progress was monitored via LC-MS analysis, additional K_2CO_3 (25 mg, 179 μmol) and bromocyclopentane (58 μL, 537 μmol) was added over 24 h at rt, then the reaction mixture was heated to 40 °C for another 5 d until the reaction appeared complete. Purification by FCC (eluent MeOH/DCM 0:100 to 8:92) gave 33 mg of a white solid (40%). ^{1}H NMR δ 8.22 (d, *J* = 0.5 Hz, 1H), 7.87 (d, *J* = 0.6 Hz, 1H), 7.67 (d, *J* = 7.3 Hz, 1H), 7.58 (d, *J* = 8.3 Hz, 2H), 7.45–7.33 (m, 4H), 7.28–7.18 (m, 1H), 7.05 (td, *J* = 7.5/0.8 Hz, 1H), 6.53 (d, *J* = 1.9 Hz, 1H), 6.45 (dd, *J* = 7.2/2.0 Hz, 1H), 5.17 (s, 2H), 4.77 (d, *J* = 6.0 Hz, 1H), 4.73–4.62 (m, 1H), 4.55 (br s, 1H), 3.82 (br s, 1H), 2.16–2.05 (m, 2H), 2.04–1.89 (m, 3H), 1.88–1.44 (m, 8H), 1.41–1.23 (m, 3H); ^{13}C NMR δ 162.2, 155.8, 148.8, 136.3, 134.74, 134.7, 132.8, 130.7, 130.3, 128.5, 127.5, 126.2, 125.4, 121.6, 118.9, 113.9, 107.4, 70.0, 69.6, 62.7, 35.9, 33.1, 31.5, 25.5, 24.5, 24.2; resonance at δ 134.7 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{32}H_{36}N_3O_3$ $[M+H]^+$ calcd 510.3; found 510.4; LC-MS t_R: 3.80 min.

4-(2-((4-(1-Cyclohexyl-1H-pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (15g). 14-(2-((4-(1*H*-Pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**14**) (72 mg, 163 μmol) was alkylated with bromocyclohexane (22 μL, 179 μmol) according to General Procedure B. Reaction progress was monitored via LC-MS analysis, additional K_2CO_3 (25 mg, 179 μmol), KI (3 mg, 16 μmol) and bromocyclohexane (22 μL, 179 μmol) was added over 24 h at rt, then the reaction mixture was heated to 40 °C for another 5 d, then to 60 °C for 24 h, and 80 °C for another 24 h until the reaction appeared complete. Purification by FCC (eluent MeOH/DCM 0:100 to 8:92) gave 6 mg of a white solid (6%). ^{1}H NMR δ 8.22 (s, 1H), 7.86 (d, *J* = 0.6 Hz, 1H), 7.67 (d, *J* = 7.2 Hz, 1H), 7.58 (d, *J* =

8.3 Hz, 2H), 7.38 (dd, *J* = 10.4/4.5 Hz, 4H), 7.22 (d, *J* = 8.2 Hz, 1H), 7.05 (td, *J* = 7.6/0.8 Hz, 1H), 6.52 (d, *J* = 1.9 Hz, 1H), 6.45 (dd, *J* = 7.2/2.0 Hz, 1H), 5.17 (s, 2H), 4.75 (d, *J* = 6.0 Hz, 1H), 4.54 (br s, 1H), 4.20–4.05 (m, 1H), 3.82 (br s, 1H), 2.08–1.96 (m, 3H), 1.86–1.66 (m, 7H), 1.64–1.48 (m, 1H), 1.47–1.16 (m, 7H); ^{13}C NMR δ 162.2, 155.8, 148.7, 135.9, 134.7, 132.8, 130.7, 130.3, 128.5, 127.5, 125.4, 125.3, 121.6, 118.9, 113.9, 107.4, 70.0, 69.8, 60.7, 35.9, 33.4, 25.5, 25.4, 25.3, 24.5; resonances at δ 134.7, 69.8 and 31.7 ppm were taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{33}H_{38}N_3O_3$ $[M+H]^+$ calcd 524.3; found 524.4; LC-MS t_R: 3.89 min.

4-(2-((4-(1-(Cyclopropylmethyl)-1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (15h). Compound (**15h**) was the main "side" product when alkylating 14-(2-((4-(1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**14**) with bromocyclobutane. The titled product was synthesized and isolated when the reaction was performed at rt or at 40 °C. Purification by FCC (eluent MeOH/DCM 0:100 to 8:92) gave 10 mg of the titled compound as a white solid (12%). ^{1}H NMR δ 8.21 (d, *J* = 0.6 Hz, 1H), 7.87 (d, *J* = 0.7 Hz, 1H), 7.67 (d, *J* = 7.3 Hz, 1H), 7.61–7.55 (m, 2H), 7.47–7.33 (m, 4H), 7.26–7.18 (m, 1H), 7.05 (td, *J* = 7.5/0.9 Hz, 1H), 6.53 (d, *J* = 1.9 Hz, 1H), 6.45 (dd, *J* = 7.2/2.0 Hz, 1H), 5.18 (s, 2H), 4.76 (d, *J* = 6.0 Hz, 1H), 4.54 (br s, 1H), 3.98 (d, *J* = 7.1 Hz, 2H), 3.82 (br s, 1H), 2.06–1.96 (m, 1H), 1.81–1.65 (m, 3H), 1.63–1.45 (m, 1H), 1.42–1.19 (m, 4H), 0.62–0.49 (m, 2H), 0.45–0.35 (m, 2H); ^{13}C NMR δ 162.2, 155.8, 148.8, 136.3, 134.8, 134.8, 132.7, 130.8, 130.3, 128.5, 127.5, 127.1, 125.4, 121.8, 121.6, 118.9, 113.9, 107.4, 70.0, 69.6, 56.3, 35.9, 31.4, 25.5, 24.5, 12.0, 4.1; resonances at δ 134.8 and 31.4 ppm were taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{31}H_{34}N_3O_3$ $[M+H]^+$ calcd 496.3; found 496.3; LC-MS t_R: 3.70 min.

4-(2-((4-(1-(Cyclohexylmethyl)-1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (15i). 14-(2-((4-(1*H*-Pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**14**) (80 mg, 181 μmol) was alkylated with bromomethylcyclohexane (29 μL, 199 μmol) according to General Procedure B. Reaction progress

was monitored via LC-MS analysis, additional bromocyclopentane (199 μL, 597 μmol) was added over 4 days at rt, then the reaction mixture was heated to 40 °C for another 2 d until the reaction appeared complete. Purification by FCC (eluent MeOH/DCM 0:100 to 8:92) gave 65 mg of a white solid (67%). ^{1}H NMR δ 8.14 (s, 1H), 7.87 (s, 1H), 7.67 (d, *J* = 7.3 Hz, 1H), 7.57 (d, *J* = 8.2 Hz, 2H), 7.45–7.33 (m, 4H), 7.25–7.17 (m, 1H), 7.04 (dd, *J* = 10.9/4.1 Hz, 1H), 6.53 (d, *J* = 1.9 Hz, 1H), 6.45 (dd, *J* = 7.2/2.0 Hz, 1H), 5.17 (s, 2H), 4.76 (d, *J* = 6.0 Hz, 1H), 4.54 (s, 1H), 3.95 (d, *J* = 7.2 Hz, 2H), 3.88–3.74 (m, 1H), 2.06–1.95 (m, 1H), 1.90–1.78 (m, 1H), 1.77–1.47 (m, 9H), 1.41–1.26 (m, 3H), 1.26–1.05 (m, 3H), 1.03–0.87 (m, 2H); ^{13}C NMR δ 162.2, 155.8, 148.8, 136.5, 134.8, 134.7, 132.7, 130.7, 130.3, 128.5, 128.0, 127.5, 125.4, 121.6, 121.5, 118.9, 113.9, 107.4, 70.0, 69.7, 58.0, 38.7, 35.9, 31.5, 30.4, 26.4, 25.6, 25.5, 24.5; resonance at δ 134.7 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{34}H_{40}N_3O_3$ $[M+H]^+$ calcd 538.4; found 538.3; LC-MS t_R: 4.31 min.

4-(2-((4-(1-Benzyl-1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (15j). 14-(2-((4-(1*H*-Pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**14**) (80 mg, 181 μmol) was alkylated with benzyl bromide (24 μL, 199 μmol) according to General Procedure B. Reaction progress was monitored via LC-MS analysis, after 21 h at rt the reaction appeared complete. Purification by FCC (eluent MeOH/DCM 0:100 to 8:92) gave 61 mg of a white solid (63%). ^{1}H NMR δ 8.30 (d, *J* = 0.5 Hz, 1H), 7.93 (d, *J* = 0.6 Hz, 1H), 7.67 (d, *J* = 7.3 Hz, 1H), 7.58 (d, *J* = 8.3 Hz, 2H), 7.43–7.25 (m, 9H), 7.21 (d, *J* = 8.2 Hz, 1H), 7.09–7.01 (m, 1H), 6.52 (d, *J* = 1.9 Hz, 1H), 6.45 (dd, *J* = 7.2/2.0 Hz, 1H), 5.35 (s, 2H), 5.17 (s, 2H), 4.75 (d, *J* = 6.0 Hz, 1H), 4.54 (br s, 1H), 3.80 (br s, 1H), 2.04–1.96 (m, 1H), 1.79–1.65 (m, 3H), 1.62–1.44 (m, 1H), 1.41–1.25 (m, 3H); ^{13}C NMR δ 162.2, 155.8, 148.8, 138.0, 137.1, 135.0, 134.7, 132.5, 130.7, 130.3, 129.0, 128.5, 128.1, 128.0, 127.9, 127.5, 125.5, 122.3, 121.6, 118.9, 113.9, 107.4, 70.0, 69.6, 55.5, 35.9, 31.5, 25.5, 24.5; resonance at δ 134.7 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{34}H_{34}N_3O_3$ $[M+H]^+$ calcd 532.3; found 532.4; LC-MS t_R: 3.74 min.

4-(2-((6-Chloropyridin-3-yl)methoxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (16). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (**7**) (200 mg, 0.70 mmol) was alkylated with 2-chloro-5-(chloromethyl)pyridine (125 mg, 0.77 mmol) according to General Procedure B. After a total of 40 h of stirring the mixture was worked up. Purification by FCC (eluent MeOH/DCM 0:100 to 8:92) gave 181 mg of a yellow oil (63%). ^{1}H NMR δ 8.48 (d, *J* = 1.9 Hz, 1H), 7.89 (dd, *J* = 8.2/2.5 Hz, 1H), 7.67 (d, *J* = 7.2 Hz, 1H), 7.54 (dd, *J* = 8.2/0.6 Hz, 1H), 7.46–7.35 (m, 2H), 7.23 (d, *J* = 7.7 Hz, 1H), 7.08 (td, *J* = 7.5/0.9 Hz, 1H), 6.48 (d, *J* = 1.9 Hz, 1H), 6.43 (dd, *J* = 7.2/2.0 Hz, 1H), 5.23 (s, 2H), 4.77 (d, *J* = 6.0 Hz, 1H), 4.54 (br s, 1H), 3.82 (br s, 1H), 2.10–1.92 (m, 1H), 1.83–1.64 (m, 3H), 1.61–1.46 (m, 1H), 1.44–1.24 (m, 3H); ^{13}C NMR δ 162.2, 155.4, 150.1, 149.4, 148.6, 139.5, 132.6, 134.7, 130.8, 130.4, 127.7, 124.7, 122.0, 118.9, 113.9, 107.4, 69.7, 67.1, 35.9, 31.5, 25.5, 24.5; resonance at δ 134.7 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{23}H_{24}ClN_2O_3$ $[M+H]^+$ calcd 411.1; found 411.2; LC-MS t_R: 3.74 min.

1-(2-Hydroxycyclohexyl)-4-(2-((6-(1-methyl-1*H*-pyrazol-4-yl)pyridin-3-yl)methoxy)phenyl)pyridin-2(1*H*)-one (17). 4-(2-((6-Chloropyridin-3-yl)methoxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**16**) (145 mg, 353 μmol) was coupled to 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (110 mg, 529 μmol) according to General Procedure A. Purification by FCC (eluent MeOH/DCM 0:100 to 8:92) gave 51 mg of a white solid (32%). ^{1}H NMR δ 8.56 (d, *J* = 1.7 Hz, 1H), 8.28 (s, 1H), 8.00 (d, *J* = 0.6 Hz, 1H), 7.79 (dd, *J* = 8.2/2.3 Hz, 1H), 7.71–7.61 (m, 2H), 7.44–7.35 (m, 2H), 7.26 (d, *J* = 7.9 Hz, 1H), 7.07 (td, *J* = 7.5/0.8 Hz, 1H), 6.52 (d, *J* = 1.9 Hz, 1H), 6.44 (dd, *J* = 7.2/2.0 Hz, 1H), 5.19 (s, 2H), 4.78 (d, *J* = 6.0 Hz, 1H), 4.55 (br s, 1H), 3.89 (s, 3H), 3.80 (br s, 1H), 2.06–1.96 (m, 1H), 1.79–1.64 (m, 3H), 1.63–1.41 (m, 1H), 1.41–1.22 (m, 3H); ^{13}C NMR δ 162.2, 155.6, 151.8, 149.2, 148.7, 137.6, 136.9, 134.7, 130.8, 130.4, 130.1, 129.8, 127.6, 123.0, 121.8, 119.4, 118.9, 114.0, 107.3, 69.7, 68.0, 39.2,

35.9, 31.5, 25.5, 24.5; resonance at δ 134.7 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{27}H_{29}N_4O_3$ $[M+H]^+$ calcd 457.2; found 457.3; LC-MS t_R: 3.34 min.

4-(2-((4-Bromo-2-fluorobenzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (18). 1-(2-Hydroxycyclohexyl)-4-(2-hydroxyphenyl)pyridin-2(1*H*)-one (7) (300 mg, 1.05 mmol) was alkylated with 4-bromo-1-(bromomethyl)-2-fluorobenzene (310 mg, 1.16 mmol) according to General Procedure B. After a total of 64 h of stirring the mixture was worked up. Purification by FCC (eluent MeOH/DCM 0:100 to 6:94) gave 310 mg of a yellow oil (62%). ^{1}H NMR δ 7.65 (d, *J* = 7.2 Hz, 1H), 7.63–7.57 (m, 1H), 7.49–7.36 (m, 4H), 7.24 (d, *J* = 7.8 Hz, 1H), 7.08 (td, *J* = 7.5/0.9 Hz, 1H), 6.47 (d, *J* = 1.9 Hz, 1H), 6.40 (dd, *J* = 7.2/2.0 Hz, 1H), 5.19 (s, 2H), 4.76 (d, *J* = 5.9 Hz, 1H), 4.53 (br s, 1H), 3.84 (br s, 1H), 2.06–1.94 (m, 1H), 1.78–1.64 (m, 3H), 1.62–1.43 (m, 1H), 1.41–1.24 (m, 3H); ^{13}C NMR δ 162.2, 161.8, 160.5 (d, J_{CF} = 252 Hz), 148.6, 134.8, 132.2 (d, J_{CF} = 4.7 Hz), 130.8, 130.4, 128.2 (d, J_{CF} = 3.5 Hz), 127.6, 127.6, 123.9 (d, J_{CF} = 14.5 Hz), 122.1, 122.0, 119.3 (d, J_{CF} = 24.7 Hz), 118.9, 113.8, 107.3, 69.6, 64.1 (d, J_{CF} = 3.1 Hz), 35.9, 31.5, 25.5, 24.5; resonance at δ 134.8 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{24}H_{24}BrFNO_3$ $[M+H]^+$ calcd 472.1; found 472.2; LC-MS t_R: 3.79 min.

4-(2-((2-Fluoro-4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (19). 4-(2-((4-Bromo-2-fluorobenzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**18**) (150 mg, 318 μmol) was coupled to 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (99 mg, 476 μmol) according to General Procedure A. Purification by FCC (eluent MeOH/DCM 0:100 to 8:92), followed freeze drying of the combined product fractions to remove the remaining pinacol, gave 45 mg of a white solid (30%). ^{1}H NMR δ 8.22 (s, 1H), 7.94 (s, 1H), 7.64 (d, *J* = 7.2 Hz, 1H), 7.51–7.36 (m, 5H), 7.27 (d, *J* = 8.2 Hz, 1H), 7.07 (t, *J* = 7.4 Hz, 1H), 6.49 (d, *J* = 1.6 Hz, 1H), 6.41 (dd, *J* = 7.2/1.8 Hz, 1H), 5.19 (s, 2H), 4.74 (d, *J* = 6.0 Hz, 1H), 4.52 (br s, 1H), 3.87 (s, 3H), 3.78 (br s, 1H), 2.04–1.92 (m, 1H), 1.79–1.62 (m, 3H), 1.60–1.39 (m, 1H), 1.39–1.19 (m, 3H); ^{13}C NMR δ 162.2, 161.3 (d, J_{CF} = 246

Hz), 155.7, 148.6, 136.9, 135.6 (d, J_{CF} = 10.0 Hz), 134.6, 131.4 (d, J_{CF} = 4.7 Hz), 130.8, 130.4, 128.9, 121.8, 121.2, 121.1, 121.0 (d, J_{CF} = 2.2 Hz), 118.9, 113.8, 112.0 (d, J_{CF} = 4.7 Hz), 107.2, 69.6, 64.5, 39.2, 35.9, 31.3, 25.5, 24.4; resonances at δ 134.6 ppm and 31.3 ppm were taken from the HSQC experiment; *m/z* MS (TOF ES^+) $C_{28}H_{29}FN_3O_3$ $[M+H]^+$ calcd 474.2; found 474.3; LC-MS t_R: 3.55 min.

4-(3,6-Difluoro-2-methoxyphenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (20). 4-Bromo-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**4b**) (1.00 g, 3.67 mmol) was coupled to (3,6-difluoro-2-methoxyphenyl)boronic acid (1.04 g, 5.51 mmol) according to General Procedure A. The reaction was stirred for 5 h before work up. Purification by FCC (eluent MeOH/DCM 0:100 to 10:90) and (eluent EtOAC 100%) gave 285 mg of the desired compound as a white solid (23%) in a 1:1 mixture of the desired product and an unidentified impurity. ^{1}H NMR δ 7.77 (d, *J* = 7.2 Hz, 1H), 7.41 (ddd, *J* = 11.3/9.3/5.3 Hz, 1H), 7.11 (td, *J* = 9.2/3.9 Hz, 1H), 6.39 (s, 1H), 6.26–6.20 (m, 1H), 4.82 (d, *J* = 6.1 Hz, 1H), 4.55 (br s, 1H), 3.82 (d, *J* = 1.9 Hz, 3H), 3.75 (br s, 1H), 2.06–1.90 (m, 1H), 1.81–1.64 (m, 3H), 1.62–1.46 (m, 1H), 1.42–1.21 (m, 3H).

4-(3,6-Difluoro-2-hydroxyphenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (21). Boron tribromide in hexane (1 M, 3.88 mL, 3.88 mmol) was added at 0 °C to a solution of 4-(3,6-difluoro-2-methoxyphenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**20**) (260 mg, 775 μmol) (1:1 mixture with unidentified impurity) in dichloromethane (8 mL). The mixture was stirred at rt under N_2 for 1 h, and then poured into ice-water. The pH of the solution was adjusted to pH 6 by addition of sat. $NaHCO_3$. Dichloromethane (20 mL) was added and the layers were separated. The organic layer was washed with water (2 × 20 mL) and brine (20 mL) and then dried with Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The desired compound was obtained as a white solid (89 mg, 72%). No further purification was required. ^{1}H NMR δ 10.33 (br s, 1H), 7.73 (d, *J* = 7.2 Hz, 1H), 7.27 (ddd, *J* = 10.4/9.2/5.3 Hz, 1H), 6.78 (td, *J* = 9.3/3.8 Hz, 1H), 6.38 (s, 1H), 6.27–6.20 (m, 1H), 4.80 (d, *J* = 6.0 Hz, 1H), 4.51 (br s, 1H), 3.81 (br s, 1H), 2.07–1.95 (m, 1H),

1.82–1.66 (m, 3H), 1.63–1.45 (m, 1H), 1.43–1.25 (m, 3H); ^{13}C NMR δ 162.0, 155.8 (dd, J_{CF} = 240.9/1.6 Hz), 148.8 (dd, J_{CF} = 234.9/2.7 Hz), 143.6 (dd, J_{CF} = 17.1/7.0 Hz), 142.0 (d, J_{CF} = 2.1 Hz), 135.1, 121.1, 117.0 (dd, J_{CF} = 19.0/2.8 Hz), 116.4 (dd, J_{CF} = 21.1/11.0 Hz), 107.9, 106.2 (dd, J_{CF} = 25.5/7.3 Hz), 69.6, 35.9, 31.5, 25.5, 24.5; resonance at δ 135.1 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{17}H_{18}F_2NO_3$ $[M+H]^+$ calcd 322.1; found 322.1; LC-MS t_R: 3.35 min.

4-(2-((4-Bromobenzyl)oxy)-3,6-difluorophenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (22). 4-(3,6-Difluoro-2-hydroxyphenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**21**) (75 mg, 233 μmol, 1.0 eq), K_2CO_3 (36 mg, 257 μmol, 1.1 eq), KI (4 mg, 23 μmol, 0.1 eq) and 1-bromo-4-(bromomethyl)benzene (64 mg, 257 mmol, 1.1 eq) were stirred in DMF (3 mL) at rt for 3 h. The reaction mixture was poured onto water and stirred for 30 min, before extraction with EtOAc (2 × 20 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. Purification by FCC (eluent MeOH/DCM 0:100 to 10:90) gave 83 mg of the desired product as a white solid (73%). ^{1}H NMR δ 7.75 (d, *J* = 7.2 Hz, 1H), 7.51–7.46 (m, 2H), 7.46–7.39 (m, 1H), 7.18–7.10 (m, 3H), 6.40–6.30 (m, 1H), 6.24–6.16 (m, 1H), 4.96 (s, 2H), 4.83 (d, *J* = 6.0 Hz, 1H), 4.56 (br s, 1H), 3.86 (br s, 1H), 2.11 – 1.97 (m, 1H), 1.83–1.67 (m, 3H), 1.67–1.48 (m, 1H), 1.44–1.29 (m, 3H); ^{13}C NMR (101 MHz, DMSO) δ 161.7, 155.2 (dd, J_{CF} = 242.8/1.9 Hz), 152.4 (dd, J_{CF} = 242.1/3.0 Hz), 144.1 (dd, J_{CF} = 13.4/5.8 Hz), 141.5 (d, J_{CF} = 2.0 Hz), 135.9, 135.5, 131.7, 130.8, 122.7 (dd, J_{CF} = 18.5/2.3 Hz), 122.0, 121.1, 117.8 (dd, J_{CF} = 21.8/10.6 Hz), 112.0 (dd, J_{CF} = 25.1/8.1 Hz), 107.6, 75.3 (d, *J* = 5.3 Hz), 69.7, 35.8, 31.5, 25.5, 24.5; resonance at δ 135.5 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{24}H_{23}BrF_2NO_3$ $[M+H]^+$ calcd 490.1; found 490.2; LC-MS t_R: 3.79 min.

4-(3,6-Difluoro-2-((4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)-1-(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (23). 4-(2-((4-Bromobenzyl)oxy)-3,6-difluorophenyl)-1-

(2-hydroxycyclohexyl)pyridin-2(1*H*)-one (**22**) (77 mg, 157 μmol) was coupled to 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (49 mg, 236 μmol) according to General Procedure A. The reaction was stirred at reflux for 2 h before work up. Purification by FCC (eluent MeOH/DCM 0:100 to 10:90) gave 24 mg of a white solid (31%). ^{1}H NMR δ 8.11 (s, 1H), 7.83 (d, J = 0.6 Hz, 1H), 7.74 (d, J = 7.2 Hz, 1H), 7.50–7.45 (m, 2H), 7.45–7.39 (m, 1H), 7.18–7.11 (m, 3H), 6.37 (d, J = 1.6 Hz, 1H), 6.26–6.15 (m, 1H), 4.94 (s, 2H), 4.83 (d, J = 6.0 Hz, 1H), 4.60 (br s, 1H), 3.86 (s, 3H), 3.81 (br s, 1H), 2.11–1.99 (m, 1H), 1.86–1.68 (m, 3H), 1.65–1.47 (m, 1H), 1.45–1.28 (m, 3H); ^{13}C NMR δ 161.7, 155.2 (dd, J_{CF} = 243.1/2.0 Hz), 152.5 (dd, J_{CF} = 242.0/2.9 Hz), 144.2 (dd, J_{CF} = 13.6/5.9 Hz), 141.6, 136.5, 135.4, 133.9, 133.1, 129.5, 128.4, 125.2, 122.8 (dd, J_{CF} = 18.2/2.2 Hz), 121.9, 121.1, 117.7 (dd, J_{CF} = 21.8/10.3 Hz), 111.9 (dd, J_{CF} = 25.1/8.1 Hz), 107.6, 76.0 (d, J_{CF} = 5.0 Hz), 69.7, 39.1, 35.9, 31.6, 25.5, 24.5; resonance at δ 135.4 ppm was taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{28}H_{28}F_2N_3O_3$ $[M+H]^+$ calcd 492.2; found 492.3; LC-MS t_R: 3.59 min.

6-(2-Methoxyphenyl)pyrimidin-4(3*H*)-one (25). 6-Chloropyrimidin-4(3*H*)-one (**24**) (1.00 g, 7.66 mmol) was coupled to (2-methoxyphenyl)boronic acid (1.85 g, 11.5 mmol) according to General Procedure A. The reaction was stirred for 48 h before work up. Purification by FCC (eluent MeOH/DCM 0:100 to 10:90) gave 315 mg of a white solid (20%). ^{1}H NMR δ 12.48 (br s, 1H), 8.24 (d, J = 1.0 Hz, 1H), 7.92 (dd, J = 7.8/1.8 Hz, 1H), 7.50–7.40 (m, 1H), 7.21–7.13 (m, 1H), 7.06 (td, J = 7.7/1.0 Hz, 1H), 6.90 (d, J = 1.0 Hz, 1H), 3.87 (s, 3H); ^{13}C NMR δ 162.0, 159.1, 158.1, 149.6, 131.9, 130.7, 125.4, 120.9, 115.0, 112.5, 56.1; *m/z* MS (TOF ES$^+$) $C_{11}H_{11}N_2O_2$ $[M+H]^+$ calcd 203.2; found 203.1; LC-MS t_R: 3.25 min.

3-(2-Hydroxycyclohexyl)-6-(2-methoxyphenyl)pyrimidin-4(3*H*)-one (26). A mixture of 6-(2-methoxyphenyl)pyrimidin-4(3*H*)-one (**25**) (274 mg, 1.36 mmol, 1.0 eq), 1,2-cyclohexene oxide (686 μL, 6.78 mmol, 5.0 eq), K_2CO_3 (468 mg, 3.39 mmol, 2.5 eq) was heated at 120 °C for 5 h. The reaction mixture was cooled to rt and concentrated to dryness under reduced pressure. The

remaining residue was taken up in EtOAc (50 mL) and washed with water. The organic layer was dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. Purification by FCC (eluent MeOH/DCM 0:100 to 10:90) and FCC eluent MeOH/DCM 0:100 to 6:94) yielded 153 mg of the desired compound as a colourless oil (38%). ^{1}H NMR δ 8.54 (br s, 1H), 7.95 (dd, *J* = 7.8/1.8 Hz, 1H), 7.68–7.53 (m, 1H), 7.16 (dd, *J* = 8.4/0.7 Hz, 1H), 7.07 (td, *J* = 7.7/1.0 Hz, 1H), 6.96 (d, *J* = 0.4 Hz, 1H), 4.98 (d, *J* = 5.7 Hz, 1H), 4.33 (br s, 1H), 3.97 (br s, 1H), 3.88 (s, 3H), 2.08–1.97 (m, 1H), 1.84–1.61 (m, 4H), 1.40–1.22 (m, 3H); ^{13}C NMR δ 161.5, 158.1, 157.0, 150.1, 131.8, 130.6, 125.2, 120.9, 113.9, 112.5, 69.2, 56.1, 35.7, 31.0, 25.4, 24.4; resonances at δ 150.1 and 31.0 ppm were taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{17}H_{21}N_2O_3$ $[M+H]^+$ calcd 301.2; found 301.2; LC-MS t_R: 3.42 min.

3-(2-Hydroxycyclohexyl)-6-(2-hydroxyphenyl)pyrimidin-4(3*H*)-one (27). A 1 M solution boron tribromide in hexane (2.37 mL, 2.37 mmol, 5.0 eq) was added at 0 °C to a solution of 3-(2-hydroxycyclohexyl)-6-(2-methoxyphenyl)pyrimidin-4(3*H*)-one (**26**) (90 mg, 475 μmol, 1.0 eq) in dichloromethane (4 mL). The mixture was stirred at rt under N_2 for 30 min, and then poured into ice-water. The pH of the solution was adjusted to pH 6 by addition of sat. $NaHCO_3$. Dichloromethane (20 mL) was added and the layers were separated. The organic layer was washed with water (2 × 20 mL) and brine (20 mL) and then dried with Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The desired compound was obtained as a white solid (75 mg, 87%). No further purification was required. ^{1}H NMR δ 12.80–10.80 (br s, 1H), 8.61 (br s, 1H), 7.84 (dd, *J* = 7.9/1.6 Hz, 1H), 7.24 (ddd, *J* = 8.5/7.2/1.6 Hz, 1H), 6.99 (s, 1H), 6.87–6.77 (m, 2H), 4.78 (s, 1H), 4.25 (br s, 1H), 3.92–3.77 (br s, 1H), 1.96–1.88 (m, 1H), 1.79–1.53 (m, 4H), 1.31–1.16 (m, 3H); ^{13}C NMR δ 161.1, 158.8, 158.5, 150.4, 132.8, 128.8, 119.8, 118.2, 118.2, 109.3, 69.2, 35.6, 30.8, 25.4, 24.4; resonances at δ 150.4, 69.2 and 30.8 ppm were taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{16}H_{19}N_2O_3$ $[M+H]^+$ calcd 287.1; found 287.2; LC-MS t_R: 3.46 min.

6-(2-((4-Bromobenzyl)oxy)phenyl)-3-(2-hydroxycyclohexyl)pyrimidin-4(3*H*)-one (28). 3-(2-Hydroxycyclohexyl)-6-(2-hydroxyphenyl)pyrimidin-4(3*H*)-one (**27**) (76 mg, 265 μmol, 1.0 eq), K_2CO_3 (40 mg, 292 μmol, 1.1 eq), KI (4 mg, 27 μmol, 0.1 eq) and 1-bromo-4-(bromomethyl)benzene (73 mg, 292 mmol, 1.1 eq) were stirred in DMF (3 mL) at rt for 4 h. The reaction mixture was poured onto water and stirred for 30 min, before extraction with EtOAc (2 × 20 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. Purification by FCC (eluent MeOH/DCM 0:100 to 10:90) gave 66 mg of the desired product as a beige solid (55%). ^{1}H NMR δ 8.54 (s, 1H), 7.96 (dd, *J* = 7.8/1.8 Hz, 1H), 7.62–7.58 (m, 2H), 7.47–7.39 (m, 3H), 7.21 (d, *J* = 7.7 Hz, 1H), 7.12–7.06 (m, 1H), 6.98 (d, *J* = 0.6 Hz, 1H), 5.24 (s, 2H), 4.96 (d, *J* = 5.8 Hz, 1H), 4.27 (br s, 1H), 3.95 (br s, 1H), 2.06–1.95 (m, 1H), 1.85–1.62 (m, 4H), 1.40–1.23 (m, 3H); ^{13}C NMR δ 161.4, 157.0, 156.9, 150.1, 136.7, 131.9, 131.4, 130.8, 130.2, 125.6, 121.5, 121.3, 114.0, 113.9, 69.5, 69.1, 35.7, 31.0, 25.4, 24.4; resonances at δ 150.1, 69.1 and 31.0 ppm were taken from the HSQC experiment; *m/z* MS (TOF ES$^+$) $C_{23}H_{24}BrN_2O_3$ $[M+H]^+$ calcd 455.1; found 455.2; LC-MS t_R: 3.77 min.

3-(2-Hydroxycyclohexyl)-6-(2-((4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)oxy)phenyl)pyrimidin-4(3*H*)-one (29). 6-(2-((4-Bromobenzyl)oxy)phenyl)-3-(2-hydroxycyclohexyl)pyrimidin-4(3*H*)-one (**28**) (66 mg, 145 μmol) was coupled to 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (45 mg, 217 μmol) according to General Procedure A. The reaction was stirred at reflux for 25 h before the work up. Purification by FCC (eluent MeOH/DCM 0:100 to 8:92) gave 7 mg of a white solid (11%). ^{1}H NMR δ 8.54 (br s, 1H), 8.15 (s, 1H), 7.99 (dd, *J* = 7.8/1.8 Hz, 1H), 7.87 (d, *J* = 0.7 Hz, 1H), 7.61–7.56 (m, 2H), 7.48–7.40 (m, 3H), 7.26 (d, *J* = 7.8 Hz, 1H), 7.13–7.06 (m, 1H), 7.05 (t, *J* = 3.1 Hz, 1H), 5.24 (s, 2H), 4.95 (d, *J* = 5.7 Hz, 1H), 4.39–4.22 (m, 1H), 3.99–3.91 (m, 1H), 3.87 (br s, 3H), 2.06–2.00 (m, 1H), 1.88–1.63 (m, 4H), 1.37–1.28 (m, 3H); ^{13}C NMR δ 161.4, 157.2, 156.9, 150.1, 136.5, 134.6, 132.8, 131.7, 130.8, 128.7, 128.3, 125.5, 122.0, 121.2,

120.8, 114.0, 113.9, 68.9, 39.1, 35.7, 29.4, 25.4, 21.2; resonances at δ 150.1, 114.0 and 69.9 ppm were taken from the HSQC experiment; *m/z* MS (TOF ES^+) $C_{27}H_{29}N_4O_3$ $[M+H]^+$ calcd 457.2; found 457.3; LC-MS t_R: 3.53 min.

Pharmacology. *Intact cell radioligand binding assays.* Flp-In™ Chinese hamster ovary (CHO) cells expressing the human muscarinic acetylcholine $M_{1\text{-}5}$ receptor ($hM_{1\text{-}5}$ mAChR) were grown in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA) supplemented with foetal bovine serum (FBS) (ThermoTrace (Melbourne, Australia) and 0.2 mg/mL hygromycin-B (Roche, Mannheim, Germany). The cells were plated at 10^4 cells per well in 96-well Isoplates (Perkin Elmer). Prior to assay the growth medium was removed and the attached cells were used to perform radioligand binding studies in the presence of 0.2 nM [^{3}H]NMS and varying concentrations of acetylcholine (Sigma, St. Loius, MI) and PAMs in a total volume of 200 µL of binding buffer (10 mM HEPES, 145 mM NaCl, 1 mM $MgSO_4 \cdot 7H_2O$, 10 mM glucose, 5 mM KCl, 2 mM $CaCl_2$, 1.5 mM $NaHCO_3$, pH 7.4). The binding reaction mixtures were incubated for 1 h at 37°C, in a humidified incubator and 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 Microscint scintillation liquid (PerkinElmer Life Sciences) to each well and counting in a MicroBeta plate reader (PerkinElmer Life Sciences).

IP-One accumulation assays. The IP-One assay kit (Cisbio, France) was used for the direct quantitative measurement of myo-Inositol 1 phosphate (IP_1) in FlpIn CHO cells stably expressing the hM_1 mAChR. The cells were detached and resuspended in IP_1 stimulation buffer (10 mM Hepes, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 4.2 mM KCl, 146 mM NaCl, 5.5 mM glucose, 50 mM LiCl, pH 7.4). The stimulations were performed in 384-well Proxy-plates (PerkinElmer) in a total volume of 14 µL, in the absence or presence of increasing concentrations of ACh and the PAMs, at cell density of 10^6 million cells/ml for 1 h at 37 °C, 5% CO_2. The reactions were terminated by addition

of 6 μL lysis buffer containing HTRF reagents (the anti-IP1 Tb cryptate conjugate and the IP1-D2 conjugate), followed by incubation for 1 h at room temperature. The emission signals were measured at 590 and 665 nm after excitation at 340 nm using an Envision multi-label plate reader (PerkinElmer) and the signal was expressed as the HTRF ratio: F= ((fluorescence$_{665\ nm}$/fluorescence$_{590\ nm}$) ×10^4).

Data Analysis. All data were analyzed using Prism 6.01 (GraphPad Software, San Diego, CA). Binding-interaction studies with allosteric ligands were fitted to the following allosteric ternary complex model (equation 1):[39]

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

Where Y is percentage (vehicle control) binding, B_{max} is the total number of receptors, [A], [B] and [I] are the concentrations of radioligand, allosteric modulator and the orthosteric ligand, respectively, K_A and K_B and K_I are the equilibrium dissociation constants of the radioligand, allosteric modulator orthosteric ligand, respectively. α' and α are the binding cooperativities between the allosteric ligand and [^{3}H]NMS and the allosteric modulator and the agonist acetylcholine, respectively. Saturation binding experiments were used to determine the value of pK_A for [^{3}H]NMS (pK_A = 9.70 ± 0.01, K_A =0.2 nM).Values of α (or α') > 1 denote positive cooperativity; values < 1 (but > 0) denote negative cooperativity, and value = 1 denotes neutral cooperativity. For the majority of compounds an unlimited displacement of [^{3}H]NMS by the allosteric modulator was observed consistent with a high level of negative cooperativity. In these cases to allow fitting of the data logα' was fixed to -3 to reflect this high negative cooperativity. The dissociation constant of ACh (K_I) was not fixed in these analyses but rather determined for each separate experiment. No difference was observed in the value of K_I between experiments (mean pK_I = 4.56 ± 0.02, K_I = 28 μM).

Concentration-response curves for the interaction between the allosteric ligand and the orthosteric ligand in the IP-One accumulation assays were globally fitted to the following operational model of allosterism and agonism (equation 2):[40]

$$E = \frac{E_m(\tau_A[A](K_B+\alpha\beta[B])+\tau_B[B]K_A)^n}{([A]K_B+K_AK_B+[B]K_A+\alpha[A][B])^n+(\tau_A[A](K_B+\alpha\beta[B])+\tau_B[B]K_A)^n} \quad (2)$$

Where E_m is the maximum possible cellular response, [A] and [B] are the concentrations of orthosteric and allosteric ligands, respectively, K_A and K_B are the equilibrium dissociation constant of the orthosteric and allosteric ligands, respectively, τ_A and τ_B are operational measures of orthosteric and allosteric ligand efficacy, respectively, α is the binding cooperativity parameter between the orthosteric and allosteric ligand, β denotes the magnitude of the allosteric effect of the modulator on the efficacy of the orthosteric agonist and n denotes the transducer slope that describes the underlying stimulus-response coupling of the ligand-occupied receptor to the signal pathway. This parameter was constrained to be shared between all curves within a fitted dataset for each interaction study, and in all instances was not significantly different from unity (average across entire series, n = 1.04 ± 0.04). In many instances, the individual model parameters of equation 2 could not be directly estimated via the nonlinear regression algorithm by analysis of the functional data alone due to parameter redundancy. To facilitate model convergence, therefore, we fixed the equilibrium dissociation constant of each ligand to that determined from the whole cell binding assays. For compounds **9c**, **15h** and **17**, no agonism was observed and therefore logτ_B was fixed to -3.

All affinity, potency, and cooperativity values were estimated as logarithms and statistical comparisons between values were by one-way analysis of variance using a Dunnett's multiple comparison post test to determine significant differences between mutant receptors and the WT M_1 mAChR. A value of $p < 0.05$ was considered statistically significant.

■ ASSOCIATED CONTENT

Supporting Information

The provided document comprises of representative NMR experiments of the compound **8p** in d_6-DMSO and $CDCl_3$.

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■ ABBREVIATIONS

ACh, acetylchloline; AD, Alzheimer's disease, Boc, *tert*-Butyloxycarbonyl; cat, catalytic; DCM, dichloromethane; DMF, dimethylformamide; eq, equivalent; EtOAc, ethyl acetate; FCC, flash column chromatography; M_1 mAChR, M_1 muscarinic acetylcholine receptor; PE, petroleum spirits 40-60; rt, room temperature; SZ, schizophrenia; THF, tetrahydrofuran.

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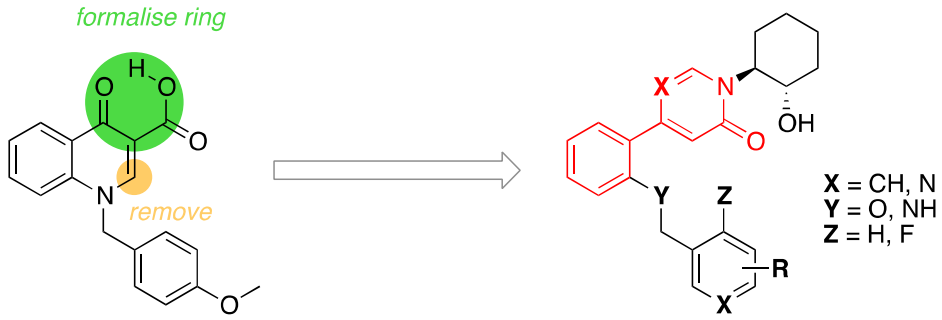

4-Oxo-1,4-dihydroquinolines (BQCA)

4-Phenylpyridin-2-ones/ 6-Phenylpyrimidin-4-ones