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# Synthesis and Pharmacological Profiling of Analogues of Benzyl Quinolone Carboxylic Acid (BQCA) as Allosteric Modulators of the $M_1$ Muscarinic Receptor

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**Abstract**

Established therapy in Alzheimer's disease involves potentiation of the endogenous orthosteric ligand, acetylcholine, at the $M_1$ muscarinic receptors found in higher concentrations in the cortex and hippocampus. Adverse effects, due to indiscriminate activation of other muscarinic receptor subtypes, are common. $M_1$ muscarinic positive allosteric modulators/allosteric agonists such as BQCA offer an attractive solution, being exquisitely $M_1$-selective over other muscarinic subtypes.

A common difficulty with allosteric ligands is interpreting SAR, based on composite potency values derived in the presence of fixed concentration of agonist. In reality these values encompass multiple pharmacological parameters – each potentially, and differentially sensitive to structural modification of the ligand.

We report novel BQCA analogues which appear to augment ligand affinity for the receptor ($pK_B$), intrinsic efficacy ($\tau_B$) and both binding ($\alpha$) and functional ($\beta$) cooperativity with acetylcholine. Ultimately, development of such enriched SAR surrounding allosteric modulators will provide insight into their mode of action.

## INTRODUCTION

Alzheimer's disease is a progressively debilitating, neurodegenerative disorder, primarily affecting the ageing population. Though first described over 100 years ago, the underlying causative factors are yet to be identified. Symptoms include confusion, memory loss and dementia, ultimately leading to death. Progressive degeneration of cholinergic neurons in several brain areas, including the cerebral cortex and hippocampus, is known to occur. In particular, the muscarinic $M_1$ receptor has been found to be more prevalent in these same areas of the brain. [1,2]

Although no disease modifying therapy is currently available, the majority of pharmacotherapy provides symptomatic relief through augmentation of cholinergic function, via administration of anticholinesterase inhibitors. The undesired effects (e.g. nausea, vomiting, bradycardia) of this type of therapy result from indiscriminate augmentation of the endogenous orthosteric ligand; acetylcholine (ACh), and subsequent non-selective overactivation of other muscarinic receptor subtypes ($M_2$-$M_5$).[3] A selective orthosteric agonist of the $M_1$ receptor is likely to offer better therapy, through fewer off-target effects. However, subtype selectivity within the muscarinic receptors is notoriously difficult to achieve, primarily due to the highly conserved amino acid sequence within the orthosteric site of each muscarinic receptor subtype.[4]

More recently, compounds binding to topographically distinct sites on the receptor (allosteric sites), able to potentiate the agonist activity of an orthosteric ligand (positive allosteric modulators, PAMs), or activate the receptor in their own right as well as potentiating agonist activity (allosteric agonists), have been discovered.[5] PAMs have the potential to offer a more desirable therapeutic profile than classic orthosteric agonists, as they would potentiate endogenous ligand activity (via altering affinity, efficacy, or both) under more physiological conditions, rather than the unselective and continuous agonist activity conferred by an orthosteric agonist. In addition, the potential for subtype selectivity is higher, due to less evolutionary pressure to conserve amino acid sequences that are not within the orthosteric site.[5]

The compound 1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (BQCA), has been shown to exhibit unprecedented positive allosteric activity for ACh binding as well as inherent agonist activity at the $M_1$ muscarinic receptor. [6,7] Additionally, BQCA appears to display high subtype selectivity, with no activity detected at concentrations up to 100 μM at any of the other four muscarinic receptor subtypes, in several assays. [6,8]

Among the previously reported structure-activity relationships (SARs), one study especially suggests that the activity of BQCA analogues as $M_1$ muscarinic PAMs is improved by incorporation of fluorine atoms at either or both the 5- and 8-positions of the quinolone ring.[9] However, all of these SAR studies are based on an 'allosteric potency' value, determined from titration of the modulator in the presence of a fixed (e.g., $EC_{20}$) concentration of the endogenous ligand, ACh. The main issue with such titration curves is that the estimated 'allosteric potency' reflects a composite of at least four operational properties,[10] namely; the affinity of the modulator for the free receptor ($K_B$), its allosteric effects on the binding and signalling of the orthosteric ligand (α and β, respectively), and its intrinsic agonist efficacy (if any) in the system ($\tau_B$). In reality, each of these parameters may be separately "tuned" through chemical modification, often in opposite directions such that minimal changes may be observed in the composite allosteric potency parameter. Consequently, use of an all-encompassing allosteric potency value can lead to misinterpretation of SAR data. In fact, a common observation in the field, based predominantly on this approach, is that allosteric ligand SAR is often "flat". [5,11]

Our approach to allosteric ligand SAR is based on the premise that a more thorough profiling of key-selected compounds would allow us to correlate chemical modifications to each of the individual operational parameters of the modulator (affinity, binding/functional cooperativity and efficacy). Therefore, using an operational model of allosterism that we have previously described, [7,10] we sought to quantify the effect of subtle chemical changes on each of the four pharmacological parameters, generating an 'enriched allosteric ligands SAR' study.

With this in mind, we have synthesised over 35 novel compounds based on the BQCA scaffold and introduced four key structural modifications, namely; alternative substitution of the quinolone ring in the 5- and 8-positions, isosteric replacement of the carboxylic acid moiety or amide derivatives of the acid function and finally, replacement of the N-alkyl group. We characterised each of these compounds in radioligand binding experiments to provide affinity and binding cooperativity estimates, then investigated the agonist properties of ten of the most effective modulators by quantifying their efficacy in an intracellular calcium-mobilization assay. Finally, using the same functional assay, we assessed five of the most relevant allosteric agonist analogues of BQCA in full interaction studies with ACh to provide functional allosteric modulation estimates. With this approach we have been able to better define the key moieties triggering the affinity, cooperativity and efficacy of BQCA and its analogues for the $M_1$ muscarinic receptor, in addition to gathering some information about the nature of the allosteric binding pocket implicated. By generating such enriched allosteric SAR, we believe it is possible to rationally design allosteric ligands for GPCRs with tailored characteristics (affinity, cooperativity or agonism). In addition, such SAR provides valuable insight into the nature of the target allosteric binding site.

## RESULTS AND DISCUSSION

**Chemistry.** The 4-oxo-1,4-dihydroquinoline moiety is prevalent in a variety of reported compounds, thus its synthesis is well established. Rapid access to series of analogues substituted at the 5- and 8-positions of the 4-oxo-1,4-dihydroquinoline ring system was achieved using the appropriately substituted aniline as a starting material, utilising Gould-Jacobs chemistry.[12] Two parallel sets of compounds were easily obtained by variation of the alkyl halide at the N-alkylation step, using literature precedent to determine the choice of electrophile.[9]

Initial thermal condensation of a variety of 2- or 2,5-disubstituted anilines with commercially available diethylethoxymethylene malonate gave substituted (phenylamino)methylene malonates **1a-d** in good yield (Scheme 1). Compounds generally solidified from the neat reaction mixture on cooling. Subsequent cyclisation of **1a-d** to quinolones **2a-d** was carried out with varied yield, by heating in the presence of Eaton's reagent (1:10 w/w $P_2O_5$ in methanesulfonic acid). Typically this type of cyclisation has been achieved using diphenyl ether as the solvent, requiring high temperatures (over 200 °C). [9,13] In addition, diphenyl ether can prove difficult to remove when used as a solvent, becoming a viscous liquid or solid on cooling. In comparison, Eaton's reagent[14] offers lower temperatures, easy removal (being water miscible) and higher yields.[15] Subsequent selective N-alkylation proceeded in generally good yield, with crude product precipitating from the reaction mixture after addition of water to afford the ester precursors **3a-d** and **4a-d**. Finally the desired free acids **5a-d** and **6a-d** were obtained after either acidic or basic hydrolysis under standard conditions. It was found more aggressive basic hydrolysis of ester **4a** resulted in displacement of the 5-F group, presumably *via* a $S_NAr$ mechanism, to give phenolic compound **7**.

*<insert Scheme 1 here>*

Amide derivatives of 5,8-difluoro BQCA were obtained through several synthetic routes, starting with either carboxylic acid **5a** (Scheme 2) or the corresponding ethyl ester **3a** (Scheme 3). Although

several amides (**8a, 8c, 8d, 8g-h**) were obtained after condensation of the appropriate amine with the corresponding acid chloride derivative of **5a** (not isolated), this method gave generally poor yields with several side products. In addition, some N-debenzylation was observed, presumably during conversion to the acid chloride, under Vilsmeier-Haack conditions. With the aim of improving yields and using milder conditions, the coupling reagent HCTU was employed, with DIPEA as a base. This afforded amides (**8b, 8e, 8f**) in good to excellent yield, with negligible side product formation.

*<insert Scheme 2 here>*

In the case of amides **10a-b** and **11**, direct aminolysis of ester **3a** proved to be a viable option. Stirring at room temperature in the presence of $MeNH_{2\ (aq)}$, ethanolamine or ammonium hydroxide respectively gave the desired products in excellent yields (Scheme 3).

*<insert Scheme 3 here>*

Compounds bearing established acid isosteric groups were easily accessible *via* common intermediates employed in the synthesis of amide derivatives described above. Condensation of the acid chloride intermediate (Scheme 2) with hydroxylamine, gave the corresponding hydroxamic acid **8i**, whilst using methanesulfonamide in the presence of TEA, gave acylsulfonamide **9**, following previously reported conditions.[16]

Carboxamide **11** was a useful precursor to both the tetrazole **13** and imide **14** (Scheme 3). Initial dehydration of **11** in the presence of $PdCl_2$ in MeCN,[17] gave the corresponding nitrile **12**. This was cyclised with $NaN_3$ to form tetrazole **13** using reported methodology.[18] Acylation of **11** was readily achieved with $Ac_2O$/pyridine to give imide **14**.

*<insert Scheme 4 here>*

Although compounds bearing different N-alkyl groups could be readily accessed through direct N-alkylation of intermediate **2a**, as before (Scheme 4); there were several target compounds that were not accessible through this route. Construction of the 4-oxo-dihydroquinoline core *via* a tandem addition-elimination mechanism, where a substituted amine nucleophile ultimately forms part of the bicyclic core has also been widely reported,[9,19] and was employed to synthesise derivatives **26a-b, 28, 29, 31 and 32** (Scheme 5).

To interrogate the environment surrounding the substituted *N*-benzyl group, positional isomers of the literature compound **6a**, bearing phenyl substituents in the 2- and 3- position of the benzyl pendant were synthesised (**16a** and **16b**, respectively) (Scheme 4). These were readily accessed in the same manner as the corresponding 4-phenylbenzyl compound **6a**. An additional analogue, bearing the 4-(3-aminopropynyl)benzyl group was also made, as this allowed probing of the 4'-position further incorporating both a linear extension to the ring and a polar, charged head group.

Initial *N*-Boc protection of propargyl amine proceeded in excellent yield using mild aqueous conditions to give carbamate **18**. Subsequent Sonogashira coupling[20] in the presence of CuI and $PdCl_2(PPh_3)_2$ afforded the arylalkynyl conjugate **19**. The benzylic alcohol group was converted to the corresponding benzylic bromide **20** in moderate yield, using standard Appel[21] conditions. Compound **20** was then used to alkylate ethyl 5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**2a**) as previously described, giving compound **21**. Under aqueous acidic conditions, with heating, ester hydrolysis and *N*-Boc cleavage of **21** occurred in one step, affording amino acid **22** as the hydrochloride salt.

Although similar compounds are present in the quinolone class of antibiotic therapeutics, to our knowledge, there are no reported examples of the *N*-aryl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid class possessing allosteric activity at the $M_1$ muscarinic receptor. These compounds were of

interest as they differ from the core BQCA-type structure, only by the absence of the methylene group linking the two aromatic moieties. To assess whether such a structure would be viable as an $M_1$ allosteric modulator, compounds **26a** and **26b** were synthesised (Scheme 5).

*<insert Scheme 5 here>*

The final group of compounds of interest incorporated a piperidin-4-ylmethyl group in replacement of the pendant substituted benzyl group (Scheme 5, compounds **28**, **29**, **30**, **31**). Although similar structures have been incorporated into $M_1$ muscarinic allosteric compounds of the quinolizidinone class,[5,22-24] to our knowledge, no such moieties have been reported attached to the 4-oxo-1,4-dihydroquinoline-3-carboxylic acid core.

**Pharmacology.**

According to a previous study utilizing modulator titration curves, the biological activity of BQCA analogues as $M_1$ muscarinic PAMs of ACh activity in a calcium mobilisation assay, was improved by incorporation of fluorine atoms at the 5- and/or 8-positions of the quinolone ring.[9] In order to elucidate the effect engendered by the presence of these electronegative atoms on the BQCA scaffold, we decided to investigate the effects of the fluorine incorporation on each of the four operational parameters describing allosteric modulation by comparing **5a** to BQCA (Figure 1, Table 1). To assess the binding activity of **5a**, we initially performed radioligand binding experiments, using $M_1$-expressing CHO cells in the presence of a $K_D$ concentration of the radiolabelled antagonist [$^3$H]-NMS (0.1 nM), increasing concentrations of ACh with or without increasing concentrations of **5a**. Such binding interaction studies allowed us to estimate the affinity ($pK_B$) of the modulator for the allosteric site of the $M_1$ muscarinic receptor, as well as its binding cooperativity (log$\alpha$) with the endogenous ligand, ACh. Compared to BQCA, the affinity estimate of **5a** ($pK_B = 4.96 \pm 0.13$) was very similar to that of BQCA ($pK_B = 4.72 \pm 0.07$), and the binding cooperativity exerted by **5a** on ACh binding ($\log\alpha = 2.68 \pm 0.16$; $\alpha = 420$) was also not significantly

different from that exerted by BQCA ($\log\alpha$ = 2.60 ± 0.11; $\alpha$ = 400). Next, we investigated the agonist properties of **5a** compared to BQCA, in a calcium mobilization assay. As expected, ACh was a full agonist in the system, with a potency estimate in the nanomolar range ($pEC_{50}$ = 8.75 ± 0.12). Interestingly, both allosteric ligands were full (allosteric) agonists in the $M_1$-expressing CHO cells, albeit with lower potency compared to ACh; $pEC_{50}$ = 6.89 ± 0.11 and 7.50 ± 0.20 for BQCA and **5a**, respectively. We estimated the efficacy of both modulators in the system. Interestingly, **5a** appeared to have significantly greater efficacy ($\log\tau_B$ = 2.18 ± 0.06; $\tau_B$ = 150) compared to BQCA ($\log\tau_B$ = 1.45 ± 0.09; $\tau_B$ = 28). Finally, we interacted either BQCA or **5a** with ACh in a calcium mobilisation assay to estimate the composite cooperativity parameter on binding and function ($\log\alpha\beta$) in this assay, using an operational model of allosteric agonism. Notably, both BQCA and **5a**, exhibited comparable binding/function cooperativity estimates, with $\log\alpha\beta$ = 2.72 ± 0.13 ($\alpha\beta$ = 525) and $\log\alpha\beta$ = 2.31 ± 0.28 ($\alpha\beta$ = 205) for BQCA and **5a**, respectively. As mentioned previously, the 'allosteric potency' estimated by generation of titration curves reflects a composite of affinity, binding/functional cooperativity and efficacy. Therefore, taken together, these findings suggest that the observed improved biological activity of **5a** compared to BQCA is predominantly driven by an improvement in the agonist properties of the modulator ($\tau_B$) rather than an improvement of its affinity or allosteric properties ($pK_B$ or $\alpha$ and $\beta$, respectively).

*<insert Figure 1 here>*

The next phase of this study aimed at investigating the effects of four key structural modifications of the BQCA scaffold; alternative substitution of the quinolone ring in the 5- and 8- positions, isosteric replacement of the carboxylic acid moiety or amide derivatives of the acid function and finally the replacement of the *N*-alkyl group. Initially, radioligand binding experiments were carried out for all ligands, as described above for BQCA and **5a**. This allowed us to determine both affinity and binding cooperativity estimates for each of the modulators synthesised in this study. Further

functional characterisation in a $Ca^{2+}$ mobilisation assay was carried out on selected analogues exhibiting binding cooperativity with ACh (α) of more than 50-fold. Finally, a small selection of allosteric agonist analogues of BQCA (where $\tau_B > 100$) were investigated further in order to estimate the binding/functional cooperativity (log αβ) of each analogue with ACh, thus allowing estimates of functional cooperativity (log β) to be extracted.

First, we investigated the importance of the 5- and 8-fluoro groups on the BQCA scaffold, with compounds, **5b-d**, **6b-d** and **7**, as well as the previously reported **5a** and **6a**. The ester precursors **3a-d** and **4a-d** were also incorporated in the study, to determine the role of the carboxylic acid functionality. Interestingly, when comparing **5a** and the corresponding ester analogue **3a**, the replacement of the carboxylic acid function by an ethyl ester group did not affect the affinity of the analogue for the allosteric site, however had a dramatic effect on the binding cooperativity with ACh, such that **3a** was virtually incapable of modulating ACh binding ($\log\alpha = 0.18 \pm 0.09$; $\alpha = 1.5$). Additionally, replacement of the fluorine or hydrogen atoms with methoxy groups, in the ester-modified analogues of BQCA (**3b-d**) did not show any improvement in binding cooperativity with ACh. Similar results were obtained with the *N*-(4-phenyl)benzyl analogues (**4a-d**) with all four analogues exhibiting similar affinity estimates to BQCA or **5a**, but considerably lower binding cooperativity with ACh. In comparison, the corresponding 5- and 8- substituted carboxylic acid derivatives (**5b-d, 6b-d**) exhibited, a different profile of activity. With analogues retaining the *N*-(4-methoxy)benzyl group (**5b-d**), no significant effect was observed on the analogues' affinity for the $M_1$ muscarinic receptor. In contrast, the presence of an 8-OMe group was significantly detrimental for the ligand's binding cooperativity with ACh (compare **5b-c** with **5a**/**5d** and **BQCA**), whereas a 5-OMe group (**5d**) had very little effect on the binding cooperativity estimate (compare **5d** to **BQCA**).

Interestingly, replacement of the methoxy group in the $R^3$ position with a phenyl group (**6a-d**) caused a significant increase in the affinity of each analogue for the allosteric site. Similarly to the *N*-(4-methoxy)benzyl series (**5a-d**), the presence of an 8-OMe group (**6b-c**) was detrimental for the

ligand's binding cooperativity with ACh. As before, a 5-OMe group (**6d**) was better tolerated, having less of an effect on the binding cooperativity estimate, compared to **6b** and **6c**.

Finally, replacement of the 5-F group of **6a** with a phenol moiety in compound **7** resulted in around a 0.75 log unit drop in the binding cooperativity estimate, though still being comparable to other compounds exhibiting high binding cooperativity estimates (**BQCA, 5a, 5d**).

These findings suggest that whilst the carboxylic acid functionality is not essential for ligand affinity at the allosteric site (as masking with an ethyl ester group had no significant effect on p$K_B$), it appears to be an important determinant of binding cooperativity with ACh. Additionally, substitution to a methoxy group in the 8-position is detrimental for the binding cooperativity of the ligand; while the same substituent at the 5-position causes relatively less reduction in binding cooperativity. The 5-position is able to tolerate hydrogen bond donating (phenol), hydrogen bond accepting (fluoro and methoxy) as well as non-hydrogen bonding groups (hydrogen atom). It may be of importance to note that the phenolic group in **7** is ideally situated adjacent to the carbonyl moiety in the 4-position, allowing a possible intramolecular hydrogen bond interaction to take place. This may impact on the ability of the phenolic group to form other interactions (so may only be functioning as a hydrogen bond acceptor).

According to the binding data, apart from BQCA and **5a**, the best modulators were **5d**, **6a, 6d** and **7**, with binding cooperativity (α) equal or higher than 50. We therefore investigated the agonist properties of three of these analogues (**5d**, **6a** and **6d**).

All three modulators were full agonists, as observed for **5a**, in the calcium mobilisation assay. Using an operational model of agonism, we derived estimates of the efficacy of each modulator (**5d**, **6a** and **6d)**, in the system. Interestingly, all three analogues exhibited significantly higher efficacy estimates than BQCA. Incidentally, when comparing the three analogues to **5a**, both **6a** and **6d** exhibited similar efficacy estimates, while **5d** was significantly lower than **5a**. These findings suggest that modifications in the 5- and 8-positions of the BQCA scaffold consistently increase the agonistic properties of the modulator, while having very little effect on their ability to modulate

ACh binding. Finally, only one analogue (**6a**) exhibited an efficacy estimate ($\log\tau_B = 2.37 \pm 0.20$; $\tau_B = 240$) higher than 100. We therefore investigated the combined binding/functional cooperativity of **6a** with ACh in a calcium mobilisation assay. Notably, the combined binding/function cooperativity estimate of **6a**, was not significantly higher than that of BQCA or **5a**, with $\log\alpha\beta = 2.87 \pm 0.24$ ($\alpha\beta = 740$). Overall, these findings suggest that modification at the 5- and 8-positions of the BQCA scaffold increase the efficacy of the modulators in the system, whereas replacement of the *N*-(4-methoxy)benzyl pendant with *N*-(4-phenyl)benzyl), increases the affinity of the modulator for the allosteric site without affecting the binding cooperativity.

We then turned our attention to whether the carboxylic acid moiety of the 4-oxo-1,4-dihydroquinoline-3-carboxylic acid core could be replaced by a group other than an ethyl ester. To start, a series of novel amide derivatives, **8a-h, 10a-b** and **11** were synthesised, incorporating primary amides to bulky tertiary amides. The unsubstituted carboxamide **11** was found to retain affinity, but lost an order of magnitude in binding cooperativity with ACh ($\log\alpha = 1.51 \pm 0.12$; $\alpha = 32$) relative to the parent acid **5a**. As mentioned previously, the corresponding ethyl ester **3a** showed a complete loss of binding cooperativity with ACh, suggesting that the presence of hydrogen bond donor functionality in $R^4$ position may be of importance for binding cooperativity with the endogenous ligand, rather than the size of the group. Supporting this hypothesis, compounds **8d-f** and **10b**, which also possessed this hydrogen bond donor functionality, retained appreciable binding cooperativity with ACh. Of particular interest is **8f** ($\log\alpha = 2.22 \pm 0.14$; $\alpha = 170$) that showed only a small drop in binding cooperativity relative to parent compound **5a**, demonstrating that an acidic functionality is not essential for the transmission of cooperativity with the endogenous ligand. The secondary alcohol group on this compound may be able to make further interactions within the allosteric site. Interestingly, **10b**, which possessed a hydroxyethyl group and should theoretically be able to make a similar type of interaction to **8f,** exhibited significantly lower binding cooperativity with ACh ($\log\alpha = 0.96 \pm 0.12$; $\alpha = 9$) compared to **8f**. This suggests that the cyclohexyl ring in **8f** is able to either make further desirable contacts with the receptor, or restrain

the hydroxyl moiety in a manner that is energetically favourable, holding the compound in a more desirable pose. Notably, the piperidyl analogue **8h** also retained appreciable binding cooperativity with ACh, though lacks the amide proton. Conversely, methylamide **10a** and ethylamide **8a** lacked appreciable positive binding cooperativity with ACh, suggesting that the presence of a simple hydrogen bond moiety alone is not sufficient to explain the effect on binding cooperativity.

Further investigation of the importance of an acidic moiety at the 3-position of the 4-oxo-1,4-dihydroquinoline ring was deemed possible through a series of carboxylic acid isosteres. Many of these isosteres offer advantages in terms of ADMET properties, whilst also varying in strength of acidity. Hydroxamic acid **8i** ($pK_a$ ~ 8-9),[25] acylsulfonamide **9** ($pK_a$ ~ 4.5),[26] tetrazole **13** ($pK_a$ ~ 5.4-7.2)[26] and imide **14** ($pK_a$ ~ 8-9)[27] were synthesised and evaluated as they vary in both size and acidity relative to the parent carboxylic acid **5a** ($pK_a$ ~ 6-7)[28]. In addition, the intermediate nitrile **12** was screened, though not possessing any inherent acidic functionality. All of the acid isostere analogues retained appreciable binding cooperativity with ACh ($\alpha > 10$). Consistently with what we observed in the previous analogues, no significant effect on the analogues affinity estimates was noted. Interestingly, the nitrile intermediate **12** suffered almost complete abolition of positive binding cooperativity with ACh. When both acid isosteres and amide analogues are compared, it is evident that replacement of carboxylic acid is tolerated at the level of the affinity parameter though causes a reduction in binding cooperativity with ACh. The $pK_a$ of the heteroatom-bonded hydrogen does not appear to influence either the ability of binding or the binding cooperativity of the modulators for the allosteric site. The possibility to make such replacements offers potential improvements from a pharmacokinetic perspective, particularly in terms of intrinsic permeability and CNS penetration (generally poor for BQCA-type analogues),[8,9,29-31] especially with the analogue **8f** that appears to retain satisfactory binding affinity and binding cooperativity.

*<insert Table 2 here>*

According to the binding data, three analogues of **5a** exhibit binding cooperativity with ACh, higher than 50. These three analogues, **8d**, **8f** and **13**, were therefore selected to investigate their ability to induce agonist activity at the $M_1$ muscarinic receptor. We performed concentration-response curves with all three analogues and estimated the efficacy parameter of each modulator. Interestingly, compared to **5a**, the substitution of the carboxylic acid with either a benzylamide **8d**, or a tetrazole **13**, had a deleterious effect on the efficacy of the modulator for the receptor with $\log\tau_B = 1.25 \pm 0.20$; ($\tau_B = 18$) and $\log\tau_B = 0.99 \pm 0.07$; ($\tau_B = 10$) for **8d** and **13**, respectively. Comparatively, the substitutions with a 2-hydroxylcyclohexylamide, as in **8f**, had no significant effect on the efficacy of the modulators; with efficacy estimates similar to the one observed for **5a**. **8f** being the only analogue that exhibited good efficacy, we interacted this modulator with ACh in a calcium-mobiization assay and observed that interestingly, **8f** exhibited a combined binding/function cooperativity estimate slightly higher than **5a**, with $\log\alpha\beta = 2.92 \pm 0.06$ ($\alpha\beta = 840$). Thus far **8f** appears to be the only compound in this series that appears to exhibit appreciable cooperativity through binding and function with ACh in the calcium mobilisation assay.

*<insert Table 3 here>*

Probing of the *N*-alkyl pendant seemed prudent, given most of the related compounds reported, focussed on a 4-substituted-benzyl group very early in the structural optimisation programme.[9,22-24,30-33] Though compound **6a**, that exhibits over 1000-fold potentiation of ACh binding, has been previously reported, to our knowledge, the related *N*-(2-phenyl)benzyl and *N*-(3-phenyl)benzyl analogues are unreported. We synthesised the corresponding analogues **16a-b** and an additional compound, **22** which probes the 4-position of the *N*-benzylic pendant in a more linear fashion *via* an aminopropynyl group. Both **16a-b** had similar affinity relative to **6a**. In terms of binding cooperativity, both compounds retain satisfactory cooperativity with ACh, albeit considerably less than that observed with **6a**, such that over a log unit of cooperativity was lost with **16a** and **b**. The

amino compound **22** retained both affinity and exhibited only small reduction in binding cooperativity relative to **5a** (to which it is structurally related). Overall, though the 4-position of the *N*-benzylic pendant is still the favoured substitution, both the 2- and 3-phenyl substituted analogues offer good affinity and appreciable binding cooperativity with ACh. The apparent ability to exchange a variety of groups at the 4-position of the benzylic pendant, whilst retaining good binding cooperativity with ACh, indicates the presence of a potentially open pocket within the receptor. Compounds **16a** and **22** were selected to assess agonist properties. Both exhibited greater than 50-fold binding cooperativity with ACh. In contrast, the 2-phenyl analogue **16a**, lost most of its agonist properties compared to the 4-phenyl analogue **6a** ($\log\tau_B$ = 1.10 ± 0.23; $\tau_B$ = 13). Interestingly, the propargylamine derivative, **22**, exhibited just as good efficacy ($\log\tau_B$ = 2.29 ± 0.07; $\tau_B$ = 195) as **5a**, its closest related analogue. Interacting **22**, the only compound with an appreciable efficacy, with ACh in calcium mobilization assay provided a binding/function cooperativity estimate nearly identical to the one established for **5a** ($\log\alpha\beta$ = 2.35 ± 0.09; $\alpha\beta$ = 225).

*<insert Table 4 here>*

Finally, to further interrogate the N-substituent of the 4-oxo-1,4-dihydroquinoline core, compounds removing the methylene linker to the pendant aromatic ring (**26a-b**) and those replacing the aromatic ring with an aliphatic heterocycle were synthesized (**28, 29, 31** and **32**). Simple removal of the methylene linker in **26a-b** completely abolished the ability of the analogues to transmit cooperativity with ACh, when compared to BQCA. Similarly, all piperidyl-linked compounds (**28, 29, 31** and **32**), which lack aromaticity, exhibited a heavy reduction in binding cooperativity with ACh, relative to BQCA. In the case of amine salt **29** and *N*-benzyl amine **31**, cooperative binding was completely abolished. However, the bulky urea **32** displayed some remaining binding cooperativity with ACh, compared to **29** and **31**, suggesting that the loss of

cooperativity with ACh would rather be due to the potential protonation of the amine rather than the introduction of a bulky group, though a more extensive set of analogues would need to be investigated to establish this hypothesis. Taken together, the *N*-alkyl and *N*-aryl series suggest the presence of a fairly open pocket removed from the core 4-oxo-1,4-dihydroquinoline structure, which is able to tolerate a variety of groups differing in size and polarity. It would appear however, that access to this proposed pocket is *via* a conformationally sensitive linker region, preferring planar aromatic groups, hinged through a methylene unit. Indeed, literature would suggest that branching or extension of the methylene linker is not tolerated.[9] Taken with our findings that removal of the linker is not tolerated in terms of retaining binding cooperativity, the methylene linker appears thus far to be optimal.

## CONCLUSIONS

The pharmacological activity of the prototypical selective $M_1$-Muscarinic PAM/allosteric agonist BQCA and related compounds, has only previously been described through an all-encompassing 'allosteric potency' estimate. In reality this estimate is comprised at least four operational parameters ($K_B$ - affinity for the receptor; α and β - binding and functional cooperativity factors with the orthosteric ligand, respectively; and $\tau_B$ – intrinsic efficacy). With each of these parameters being independently tuneable through chemical modification, we first sought to define a robust pharmacological profile of BQCA and key related literature compounds **5a** and **6a**. Building on this, we initiated a SAR campaign, synthesising over 35 novel compounds with structural modification in four areas: alternative substitution of the 5- and 8- positions of the quinolone ring, isosteric replacement of the carboxylic acid moiety or amide derivatives and replacement of the pendant *N*-alkyl group.

Evaluation of the literature compounds BQCA, **5a** and **6a** revealed the mechanism through which 5,8-difluoro analogues display improved biological activity. This was found to be due to an increased intrinsic efficacy ($\tau_B$), relative to BQCA. Related analogues bearing varied 5- and 8-substituents (**3a-d**, **4a-d**, **5a-d**, **6a-d** and **7**) on both the carboxylic acid and ester scaffold revealed that though the presence of the carboxylic acid group is not essential for affinity, it is important in determining the binding cooperativity with ACh. Whilst an 8-methoxy substituent is generally detrimental towards binding cooperativity with ACh, the corresponding 5-methoxy substituent is better tolerated. Furthermore, the 5-position is able to tolerate both hydrogen bond donating (phenol) and accepting (fluoro and methoxy) as well as non-hydrogen bonding groups (hydrogen atom), whilst still maintaining appreciable affinity for the receptor and binding cooperativity with ACh. Overall, substituents at the 5- and 8- positions seem to be more important in tuning the intrinsic efficacy, whereas changing the *N*-(4-methoxy)benzyl group to *N*-(4-phenyl)benzyl tended to improve affinity for the receptor without improving cooperative binding with ACh.

Our series of novel amide derivatives (**8a-h, 10a-b** and **11**) revealed that the presence of a hydrogen bond donor group, rather than a carboxylic acid functionality may be of importance for binding cooperativity with ACh, though amide derivatives did suffer a reduction in this parameter compared to **5a**. Notably, **8f** only displayed a small drop in binding cooperativity with ACh, relative to parent compound **5a**. The presence of a secondary alcohol group on the cyclohexylamide substituent may allow for further hydrogen bond or polar interactions. Furthermore, the orientation of the amide substituent seems key as the more flexible **10b** exhibited significantly reduced binding cooperativity with ACh compared to **8f**. Further evaluation of **8f** revealed it to be the only compound in our novel series, which also possessed appreciable functional cooperativity with ACh in the calcium mobilisation assay (i.e. $\log \alpha\beta > \log \alpha$).

Isosteric replacement of the carboxylic acid group was tolerated at the level of affinity for the receptor, but caused a reduction in binding cooperativity with ACh. The p$K_a$ of the acidic hydrogen atom does not seem to correlate with the degree of cooperative binding with ACh.

Though the amide and acid isostere derivatives of **5a** synthesised to date do not improve binding cooperativity with ACh over that observed with **5a**, such groups do allow further elaboration of the core BQCA scaffold, whilst offering potential improvements in terms of ADMET. Furthermore, our findings are in contrast to those recently published,[34] which report that functionalization of the carboxylic acid moiety on the quinolone-based scaffold is not tolerated. Indeed, amide **8f** whilst being comparable to the parent acid **5f** in terms of affinity for the receptor and binding cooperativity with ACh, actually exhibits improved intrinsic efficacy, as well as functional cooperativity with ACh in the calcium mobilisation assay.

Finally, our investigations into the *N*-alkyl pendant showed that substitution in the 4-position of the benzylic group is the most favourable, and seemingly a range of groups can be accommodated. Gratifyingly, propargylamine derivative **22** retained a similar pharmacological profile to **5a**, indicating a potentially open pocket or area able to tolerate a variety of functional groups and polarities. However, access to this pocket appears to be tightly controlled, as removal of the

methylene linker in **26a** and **26b** resulted in complete loss of binding cooperativity with ACh without major effect on affinity. The presence of a more bulky aliphatic heterocycle instead of the planar aromatic ring present in a benzyl group, again caused severe loss of binding cooperativity with ACh over several analogues. This again supports the notion that access to a more open pocket is most optimally achieved via a conformationally sensitive linker region, preferring planar aromatic groups, hinged through a methylene unit.

In summary, our detailed pharmacological profiling approach to allosteric ligands for the $M_1$ muscarinic receptor has demonstrated the subtle tunability of individual ligand parameters through chemical modification, which has previously only been described as an overall 'allosteric potency' estimate. This allows a more enriched understanding of the SAR surrounding allosteric ligands, bringing us closer to delineating some of the 'flat' SAR often encountered in this field.

## EXPERIMENTAL SECTION

**Chemistry.** Chemicals and solvents were purchased from standard suppliers and used without further purification. Davisil® silica gel (40-63 μm), for flash column chromatography (FCC) was supplied by Grace Davison Discovery Sciences (Victoria, Australia) and deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. (USA, distributed by Novachem PTY. Ltd, Victoria, Australia).

Unless otherwise stated, reactions were carried out at ambient temperature. All microwave reactions took place in a Biotage Initiator Microwave Synthesiser. Reactions were monitored by thin layer chromatography on commercially available precoated aluminium-backed plates (Merck Kieselgel 60 $F_{254}$). Visualisation was by examination under UV light (254 and 366 nm). General staining 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 400 MHz 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.

LC-MS were run to verify reaction outcome and purity using an Agilent 6100 Series Single Quad coupled to an Agilent 1200 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 Phenomenex Luna 3μM C8(2) 15 × 4.6 mm column, and a flow rate of 0.5 mL/min and total run time of 12 min; 0–4 min 95% buffer A and 5% buffer B, 4–7 min 0% buffer A and 100% buffer B, 7–12 min 95% buffer A and 5% buffer B. Mass spectra were acquired in positive and negative ion mode with a scan range of 0–1000 *m/z* at 5V. UV detection was carried out at 254 nm. All retention times ($t_R$) are quoted in minutes. All compounds were of > 95% purity.

**General procedure A: Condensation of substituted anilines with diethylethoxymethylene malonate.** Aniline and diethylethoxymethylene malonate (1 eq) were heated at 100-120 °C (oil bath) together until TLC analysis (EtOAc/PE 3:7) or LC-MS analysis indicated conversion was complete (1–7 h). The mixture was cooled to rt, or until precipitation was noted. At this point, the mixture was diluted with excess PE, and the resulting precipitate collected by filtration (vacuum), before washing with further PE, and drying.

**General procedure B: Cyclisation of substituted diethyl 2-((phenylamino)methylene)malonates (1a–1d) in Eaton's reagent, to give corresponding ethyl 4-oxo-1,4-dihydroquinoline-3-carboxylates.** Substituted diethyl 2-((phenylamino)methylene)malonate was dissolved in Eaton's reagent (a solution of $P_2O_5$/methanesulfonic acid 1:10 w/w, 1 mL/mmol) and heated at 90-100 °C (oil bath) under an atmosphere of nitrogen gas until TLC analysis (EtOAc) indicated disappearance of starting material (5 h to overnight). The mixture was cooled to 5 °C, before pouring into sat. $NaHCO_{3\,(aq)}$ and stirring for 15 min. The resulting precipitate was isolated by filtration (vacuum) and washed with water, then PE, before drying.

**General procedure C: N-Alkylation of substituted ethyl 4-oxo-1,4-dihydroquinoline-3-carboxylates (2a–2e).** Substituted ethyl 4-oxo-1,4-dihydroquinoline-3-carboxylate, $K_2CO_3$ (1.1-1.3 eq), KI (0.1 eq) and the appropriate alkyl halide (1.1-1.3 eq) were stirred in DMF (2-4 mL/mmol) at rt overnight. The reaction was monitored by TLC (EtOAc or EtOAc/DCM 8:2). The reaction

mixture was poured onto ice/.water and the resulting precipitate collected by filtration (vacuum), with further washings of water, followed by washings of PE, before drying.

**General procedure D: Basic hydrolysis of esters to give corresponding carboxylic acids.** The appropriate ester was stirred in THF/$H_2O$ (1:1, 4–10 mL/mmol), with the vessel atmosphere being purged with nitrogen gas. To this was added LiOH.$H_2O$ (2-4 eq), and the mixture stirred at rt overnight. Once TLC analysis indicated disappearance of the starting material, the reaction mixture was diluted with water (to 20 mL) and washed with $Et_2O$ (20 mL). The aqueous layer was carefully acidified (~pH 3) with 2 M $HCl_{(aq)}$, before extracting with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (20 mL), before concentration under reduced pressure.

**General procedure E: Synthesis of amide derivatives of 5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5a), through an acid chloride intermediate.** 5,8-Difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(5a)** (1.00 g, 2.90 mmol) was dispersed in anhydrous DCM, under an atmosphere of $N_2$, and cooled to 0 °C, over an ice bath. To this was added oxalyl chloride (0.278 mL, 3.19 mmol, 1.1 eq) and DMF (1 drop). The mixture was stirred over the ice bath for 15 min, before warming to rt, and stirring for 1 h. Oxalyl chloride (0.5 eq) was added, and stirring continued for a further 30 min. One tenth of this batch mixture was then added to a vessel containing the appropriate amine (0.725 mmol, 2.5 eq) in DCM (3 mL). Where the amine was present as the hydrochloride salt, TEA (0.754 mmol, 2.6 eq) were also added. The mixtures were stirred at rt overnight, before diluting with DCM (20 mL), and washing with 2 M $HCl_{(aq)}$ (20 mL). After concentration of the organic layers, the crude products were further purified by FCC (eluent EtOAc/PE or EtOAc/DCM).

**General procedure F: HCTU-mediated coupling of 5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5a) with amines to give the corresponding amide.** 5,8-Difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(5a)** (100 mg, 0.29 mmol), HCTU (144 mg, 0.35 mmol, 1.2 eq) and amine (1.2-1.5 eq) where stirred in DMF (2 mL) at rt. To this mixture, was added DIPEA (1.5-3 eq, depending on whether the amine was used in the

hydrochloride salt form) in DCM (2 mL), before stirring at rt overnight. DCM was removed from the reaction mixture under reduced pressure, before diluting the residue with 1 M $HCl_{(aq)}$ (20 mL), and extracting with EtOAc (3 × 20 mL). The combined organic layers were washed with sat. $NaHCO_3$ $_{(aq)}$ (30 mL), water (30 mL) and brine (30 mL). After concentration of the organic layers, the crude product was further purified by FCC.

**Diethyl 2-(((2,5-difluorophenyl)amino)methylene)malonate (1a).**[35] 2,5-Difluoroaniline (11.79 g, 91.32 mmol) was reacted according to general procedure A to give 22.597 g of off-white crystalline solid (83%). $^1$H NMR (400 MHz, $CDCl_3$) δ 11.06 (d, $J$ = 13.1 Hz, 1H), 8.39 (d, $J$ = 13.3 Hz, 1H), 7.11 (ddd, $J$ = 10.1/9.1/4.8 Hz, 1H), 7.00 (ddd, $J$ = 9.2/6.3/2.9 Hz, 1H), 6.76 (dddd, $J$ = 9.0/7.6/3.7/2.9 Hz, 1H), 4.33 (q, $J$ = 7.1 Hz, 2H), 4.27 (q, $J$ = 7.1 Hz, 2H), 1.38 (t, $J$ = 7.1 Hz, 3H), 1.34 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 168.52, 165.40, 159.33 (dd, $J_{CF}$ = 243.6/2.3 Hz), 150.08, 148.95 (dd, $J_{CF}$ = 242.1/2.8 Hz), 129.02 (dd, $J_{CF}$ = 12.9/10.3 Hz), 117.17 (dd, $J_{CF}$ = 21.4/9.7 Hz), 110.83 (dd, $J_{CF}$ = 24.2/7.4 Hz), 103.38 (dd, $J_{CF}$ = 28.4/1.5 Hz), 96.42, 60.90, 60.58, 14.53, 14.39; *m/z* MS (TOF ES$^-$) $C_{14}H_{14}F_2NO_4$ $[M-H]^-$ calcd 298.1; found 298.0; LC-MS $t_R$: 6.18.

**Diethyl 2-(((5-fluoro-2-methoxyphenyl)amino)methylene)malonate (1b).** 5-Fluoro-2-methoxyaniline (2.17 g, 15.37 mmol) was reacted according to general procedure A to give 3.387 g of off-white solid (71%). $^1$H NMR (400 MHz, $CDCl_3$) δ 11.09 (d, $J$ = 13.5 Hz, 1H), 8.44 (d, $J$ = 13.9 Hz, 1H), 6.97 (dd, $J$ = 9.4/2.8 Hz, 1H), 6.85 (dd, $J$ = 9.0, 4.8 Hz, 1H), 6.76 (ddd, $J$ = 8.9/8.0/2.8 Hz, 1H), 4.33 (q, $J$ = 7.1 Hz, 2H), 4.26 (q, $J$ = 7.1 Hz, 2H), 3.92 (s, 3H), 1.38 (t, $J$ = 7.1 Hz, 3H), 1.34 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 168.45, 165.82, 157.56 (d, $J_{CF}$ = 239.4 Hz), 149.78, 145.16 (d, $J_{CF}$ = 2.3 Hz), 129.85 (d, $J_{CF}$ = 10.0 Hz), 112.06 (d, $J_{CF}$ = 9.2 Hz), 110.13 (d, $J_{CF}$ = 23.0 Hz), 102.04 (d, $J_{CF}$ = 27.9 Hz), 95.09, 60.58, 60.37, 56.58, 14.57, 14.44; LC-MS $t_R$: 6.15.

**Diethyl 2-(((2-methoxyphenyl)amino)methylene)malonate (1c).**[36] *ortho*-Anisidine (2.00 g, 16.24 mmol) was reacted according to general procedure A, After reaction completion, addition of PE on cooling caused formation of a dark red oily layer at the bottom of the flask. This was stirred

in PE for 5 min, before allowing full separation to occur, and decanting the top layer. The red oil was washed with PE in this manner a further two times before drying under vacuum, causing a solidification to occur. Filtration (vacuum) gave 1.155 g of light red solid. The decanted PE washings began to show crystal formation. These were collected by filtration (vacuum) and washed with PE to give 2.958 g of off-white crystals. Spectral comparison of the two solids indicated both were the desired product. Total yield: 4.113 g (86%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 11.11 (d, *J* = 13.9 Hz, 1H), 8.57 (d, *J* = 14.0 Hz, 1H), 7.29–7.21 (m, 1H), 7.12–7.07 (m, 1H), 6.99 (ddd, *J* = 7.6/7.6/ 0.9 Hz, 1H), 6.94 (dd, *J* = 8.1/1.2 Hz, 1H), 4.33 (q, *J* = 7.1 Hz, 2H), 4.25 (q, *J* = 7.1 Hz, 2H), 3.94 (s, 3H), 1.39 (t, *J* = 7.1 Hz, 3H), 1.33 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 168.67, 166.17, 150.55, 149.08, 128.90, 124.94, 121.33, 114.47, 111.38, 93.90, 60.40, 60.19, 56.08, 14.59, 14.48; *m/z* MS (TOF ES$^+$) $C_{15}H_{19}NNaO_5$ $[M+Na]^+$ calcd 316.3; found 316.2; LC-MS $t_R$: 6.08.

**Diethyl 2-(((2-chloro-5-methoxyphenyl)amino)methylene)malonate (1d).** 6-Chloro-*m*-anisidine hydrochloride (3.00 g, 15.46 mmol), $Et_3N$ (2.37 mL, 17.00 mmol, 1.1 eq) and diethylethoxymethylene malonate (3.34 g, 15.46 mmol, 1eq) were stirred together under a reflux condenser at 100 °C for 1 h. TLC analysis (EtOAc/PE 3:7) indicated disappearance of starting aniline. The mixture was cooled to rt overnight, before partitioning between water (100 mL) and $Et_2O$ (100 mL). The aqueous layer was extracted with EtOAc (100 mL), and the combined organic layers washed with water (100 mL) then brine (50 mL), before drying over $MgSO_4$ and concentrating to give a crude purple solid. This was dispersed in PE, sonicated, then filtered (vacuum), with washings of PE, to give 4.024 g (79%) of purple solid. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 11.24 (d, *J* = 13.1 Hz, 1H), 8.47 (d, *J* = 13.2 Hz, 1H), 7.31 (d, *J* = 8.8 Hz, 1H), 6.82 (d, *J* = 2.7 Hz, 1H), 6.62 (dd, *J* = 8.9/2.7 Hz, 1H), 4.34 (q, *J* = 7.1 Hz, 2H), 4.26 (q, *J* = 7.1 Hz, 2H), 3.83 (s, 3H), 1.38 (t, *J* = 7.1 Hz, 3H), 1.34 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 168.48, 165.85, 159.62, 150.24, 137.13, 130.80, 115.35, 110.25, 102.05, 95.82, 60.78, 60.49, 55.90, 14.54, 14.46; *m/z* MS (TOF ES$^-$) $C_{15}H_{17}ClNO_5$ $[M-H]^-$ calcd 326.8; found 326.0; LC-MS $t_R$: 6.33.

**Ethyl 5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (2a).**[35] Diethyl 2-(((2,5-difluorophenyl)amino)methylene)malonate **(1a)** (26.07 g, 87.12 mmol) was cyclised according to general procedure B to give 18.54 g of yellow solid (84%). $^{1}$H NMR (400 MHz, DMSO) δ 8.62 (s, 1H), 7.27 (ddd, $J$ = 10.4/8.8/4.2 Hz, 1H), 6.79 (ddd, $J$ = 12.3/8.7/3.9 Hz, 1H), 4.17 (q, $J$ = 7.1 Hz, 2H), 1.27 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, DMSO) δ 173.24, 166.79, 156.87 (dd, $J_{CF}$ = 256.0/2.8 Hz), 153.47, 152.94 (dd, $J_{CF}$ = 247.2/3.7 Hz), 140.68 (d, $J_{CF}$ = 12.5 Hz), 119.64 (d, $J_{CF}$ = 4.6 Hz), 114.04 (dd, $J_{CF}$ = 21.5/11.1 Hz), 108.79, 106.88 (dd, $J_{CF}$ = 24.5/7.7 Hz), 58.59, 14.46; *m/z* MS (TOF ES$^-$) $C_{12}H_8F_2NO_3$ $[M-H]^-$ calcd 252.1; found 252.1; MS (TOF ES$^+$) $C_{12}H_{10}F_2NO_3$ $[MH]^+$ calcd 254.1; found 254.1; LC-MS $t_R$: 4.55.

**Ethyl 5-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (2b).** Diethyl 2-(((5-fluoro-2-methoxyphenyl)amino)methylene)malonate **(1b)** (3.303 g, 10. 61 mmol) was cyclised according to general procedure B. On pouring the cooled reaction mixture on to sat. $NaHCO_{3\,(aq)}$, precipitation did not occur. The aqueous mixture was extracted with EtOAc (3 x 100 mL), and the combined organic extracts washed with brine (120 mL). The organic layers were then concentrated under reduced pressure to give a crude orange solid. This was redissolved in the minimum amount of EtOAc, before adding enough PE to effect precipitation. The resultant solid was collected by filtration (vacuum), to give 1.226 g of brown solid (44%). $^{1}$H NMR (400 MHz, DMSO) δ 11.81 (d, $J$ = 6.0 Hz, 1H), 8.25 (d, $J$ = 6.8 Hz, 1H), 7.27 (dd, $J$ = 8.9/3.8 Hz, 1H), 7.04 (dd, $J$ = 11.9/8.9 Hz, 1H), 4.20 (q, $J$ = 7.1 Hz, 2H), 3.97 (s, 3H), 1.26 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, DMSO) δ 172.12, 164.34, 154.32 (d, $J_{CF}$ = 252.6 Hz), 144.55 (d, $J_{CF}$ = 3.5 Hz), 143.62, 130.73 (d, $J_{CF}$ = 3.7 Hz), 117.11 (d, $J_{CF}$ = 9.6 Hz), 112.47 (d, $J_{CF}$ = 9.6 Hz), 111.70, 109.93 (d, $J_{CF}$ = 22.9 Hz), 59.72, 56.59, 14.26; *m/z* MS (TOF ES$^+$) $C_{13}H_{13}FNO_4$ $[MH]^+$ calcd 266.1; found 266.1; LC-MS $t_R$: 4.75.

**Ethyl 8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (2c).** Diethyl 2-(((2-methoxyphenyl)amino)methylene)malonate **(1c)** (3.97 g, 13.53 mmol) was cyclised according to general procedure B. The resulting crude solid was recrystallised from EtOH/water, giving two crops: 994 mg of pale brown solid and 944 mg of brown solid. Total yield = 1.938 g (58%). $^{1}$H

NMR (400 MHz, DMSO) δ 11.91 (s, 1H), 8.34 (d, *J* = 5.6 Hz, 1H), 7.71 (d, *J* = 6.2 Hz, 1H), 7.44 – 7.26 (m, 2H), 4.21 (q, *J* = 7.0 Hz, 2H), 4.00 (s, 3H), 1.27 (t, *J* = 7.0 Hz, 3H); $^{13}$C NMR (101 MHz, DMSO) δ 173.20, 164.66, 148.71, 144.03, 129.34, 128.17, 124.66, 116.78, 112.24, 109.99, 59.61, 56.36, 14.33; *m/z* MS (TOF ES$^+$) $C_{13}H_{14}NO_4$ $[MH]^+$ calcd 248.3; found 248.1; LC-MS $t_R$: 4.79.

**Ethyl 8-chloro-5-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (2d).**[37] Diethyl 2-(((2-chloro-5-methoxyphenyl)amino)methylene)malonate **(1d)** (3.983 g, 12.15 mmol) was cyclised according to general procedure B. On pouring the cooled reaction mixture on to sat. $NaHCO_{3\,(aq)}$, precipitation did not occur. The aqueous mixture was extracted with EtOAc (3 × 50 mL). TLC analysis (EtOAc) of the aqueous and organic layers indicated product was still present in the aqueous layer. This was concentrated, and saturated with NaCl, before re-extraction with EtOAc (3 x 50 mL). Concentration of both organic layers found them to be impure, so they were combined, concentrated and further purified by FCC (eluent MeOH/EtOAc/PE 0:50:50 to 0:100:0, then up to 2:98:0) to give 1.213 g of brown solid (35%). $^1$H NMR (400 MHz, DMSO) δ 11.36 (s, 1H), 8.23 (s, 1H), 7.75 (d, *J* = 8.9 Hz, 1H), 6.90 (d, *J* = 8.9 Hz, 1H), 4.19 (q, *J* = 7.1 Hz, 2H), 3.83 (s, 3H), 1.26 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, DMSO) δ 172.71, 164.49, 159.09, 149.13, 143.24, 132.62, 121.34, 115.93, 112.29, 106.82, 59.75, 56.09, 14.23; *m/z* MS (TOF ES$^+$) $C_{13}H_{13}ClNO_4$ $[MH]^+$ calcd 282.7; found 282.1; LC-MS $t_R$: 4.88.

**Ethyl 5-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (2e).**[37] Ethyl 8-chloro-5-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate **(2d)** (500 mg, 1.78 mmol) was dispersed in EtOH (10 mL), before sonication to degas the solvent. The vessel was evacuated and filled with nitrogen, before addition of 10% Pd/C (50 mg), with care. After resealing, the vessel underwent three cycles of evacuation and filling with hydrogen gas (balloon), before stirring at rt overnight. TLC analysis (MeOH/DCM 1:9) indicated only partial progression, so EtOH (10 mL) was added to aid dissolution of the starting material, and hydrogenation continued for a further 2 nights. After this time 10% Pd/C (50 mg) was added with care (after evacuation of the vessel and filling with nitrogen gas), and hydrogenation continued. After a total of 5 nights of stirring, no starting material

was evident by TLC. The mixture was diluted with DCM, and filtered through a bed of celite, with further washings of DCM. Concentration of the filtrate gave 457 mg of yellow solid (quantitative yield). $^1$H NMR (400 MHz, DMSO) δ 8.36 (s, 1H), 7.58 (dd, *J* = 8.2/8.2 Hz, 1H), 7.33 (dd, *J* = 8.3/0.8 Hz, 1H), 7.15 (dd, *J* = 8.2/0.6 Hz, 1H), 6.88 (d, *J* = 8.1 Hz, 1H), 4.19 (q, *J* = 7.1 Hz, 2H), 3.82 (s, 3H), 1.27 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, DMSO) δ 173.26, 165.01, 159.78, 143.15, 141.64, 133.04, 116.86, 111.40, 110.48, 106.35, 59.64, 55.86, 14.28; *m/z* MS (TOF ES$^+$) $C_{13}H_{14}NO_4$ $[MH]^+$ calcd 248.3; found 248.2; LC-MS $t_R$: 4.53.

**Ethyl 5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (3a).**[8] Ethyl 5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **(2a)** (3.00 g, 11.85 mmol), was alkylated with 4-methoxybenzyl chloride, according to general procedure C. After the initial period of overnight stirring, further $K_2CO_3$ (0.5 eq) and 4-methoxybenzyl chloride (0.5 eq) were added and stirring continued for a further overnight period. Due to difficulty filtering the product after pouring onto ice water, EtOAc (500 mL) was added to redissolve the wet crude material, and this was washed with water (100 mL) and brine (100 mL), before concentration under reduced pressure. Crude residue was redissolved in the minimum amount of EtOAc, and precipitation effected by addition of PE. The desired product was isolated by filtration (vacuum) to give 3.074 g of brown solid (69%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.32–7.15 (m, 1H), 7.08 (d, *J* = 8.4 Hz, 2H), 6.96 (ddd, *J* = 10.4/9.0/3.4 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 2H), 5.43 (d, *J* = 2.8 Hz, 2H), 4.39 (q, *J* = 7.1 Hz, 2H), 3.78 (s, 3H), 1.40 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 174.20, 165.33, 161.99 (d, $J_{CF}$ = 233.7 Hz), 159.88, 151.22, 150.53 (d, $J_{CF}$ = 231.7 Hz), 127.89, 127.88, 127.17 (d, $J_{CF}$ = 1.0 Hz), 125.16 (d, $J_{CF}$ = 7.5 Hz), 120.04, 114.64, 113.09, 112.30 (d, $J_{CF}$ = 25.2 Hz), 61.39, 60.64 (d, $J_{CF}$ = 17.3 Hz), 55.46, 14.52; *m/z* MS (TOF ES$^-$) $C_{20}H_{17}F_2NNaO_4$ $[M+Na]^+$ calcd 396.1; found 396.1; LC-MS $t_R$: 5.62.

**Ethyl 5-fluoro-8-methoxy-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (3b).** Ethyl 5-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate **(2b)** (200 mg, 0.75 mmol) was alkylated with 4-methoxybenzyl chloride, according to general procedure C. After the

initial period of overnight stirring, further $K_2CO_3$ (0.3 eq) and 4-methoxybenzyl chloride (0.3 eq) were added and stirring continued for a further overnight period. The desired product was isolated by filtration as described, to give 160 mg of yellow solid (58%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.06–6.88 (m, 4H), 6.87–6.75 (m, 2H), 5.61 (s, 2H), 4.37 (q, $J$ = 7.1 Hz, 2H), 3.76 (s, 3H), 3.72 (s, 3H), 1.39 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 173.47, 165.68, 159.41, 156.53 (d, $J_{CF}$ = 258.3 Hz), 151.84, 145.93 (d, $J_{CF}$ = 3.9 Hz), 131.71, 128.78, 127.47, 121.11 (d, $J_{CF}$ = 7.1 Hz), 115.03 (d, $J_{CF}$ = 10.2 Hz), 114.41, 112.67, 112.13 (d, $J_{CF}$ = 23.8 Hz), 61.41, 61.15, 56.79, 55.42, 14.54; *m/z* MS (TOF ES$^+$) $C_{21}H_{21}FNO_5$ $[MH]^+$ calcd 386.4; found 386.2; LC-MS $t_R$: 5.62.

**Ethyl 8-methoxy-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (3c).** Ethyl 8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate **(2c)** (500 mg, 2.02 mmol) was alkylated with 4-methoxybenyl chloride, according to general procedure C, to give 578 mg of pale brown solid. This was further purified by FCC (eluent EtOAc/PE 0:100 to 100:0) to give 529 mg of light brown solid (71%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 8.16 (dd, $J$ = 8.1/1.3 Hz, 1H), 7.31 (t, $J$ = 8.0 Hz, 1H), 7.06 (dd, $J$ = 7.9/1.0 Hz, 1H), 6.99 (d, $J$ = 8.7 Hz, 2H), 6.81 (d, $J$ = 8.7 Hz, 2H), 5.66 (s, 2H), 4.38 (q, $J$ = 7.1 Hz, 2H), 3.75 (s, 6H), 1.40 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 174.06, 165.96, 159.29, 152.08, 150.02, 131.96, 130.27, 129.12, 127.36, 125.67, 120.06, 114.82, 114.33, 110.83, 61.50, 60.99, 56.37, 55.38, 14.55; *m/z* MS (TOF ES$^+$) $C_{21}H_{22}NO_5$ $[MH]^+$ calcd 368.4; found 368.2; LC-MS $t_R$: 5.65.

**Ethyl 5-methoxy-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (3d).** Ethyl 5-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate **(2e)** (100 mg, 0.40 mmol), was alkylated with 4-methoxybenzyl chloride, according to general procedure C, to give 66 mg of off-white solid (45%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.41 (dd, $J$ = 8.4/8.4 Hz, 1H), 7.08 (d, $J$ = 8.7 Hz, 2H), 6.93 – 6.82 (m, 3H), 6.79 (d, $J$ = 8.2 Hz, 1H), 5.24 (s, 2H), 4.38 (q, $J$ = 7.1 Hz, 2H), 3.95 (s, 3H), 3.78 (s, 3H), 1.39 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 174.91, 166.36, 161.75, 159.80, 148.37, 142.00, 132.85, 127.64, 126.42, 119.63, 114.82, 113.30, 108.49, 106.98,

60.98, 57.61, 56.49, 55.47, 14.61; *m/z* MS (TOF ES$^+$) $C_{21}H_{22}NO_5$ $[MH]^+$ calcd 368.4; found 368.2; LC-MS $t_R$: 5.46.

**Ethyl 1-([1,1'-biphenyl]-4-ylmethyl)-5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (4a).**[9] Ethyl 5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **(2a)** (500 mg, 1.97 mmol), was alkylated with 4-phenylbenzyl bromide, according to general procedure C, to give 420 mg of yellow solid (51%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.64–7.49 (m, 4H), 7.48–7.39 (m, 2H), 7.38–7.30 (m, 1H), 7.29–7.22 (m, 1H), 7.20 (d, $J$ = 8.1 Hz, 2H), 6.97 (ddd, $J$ = 10.3/9.1/3.4 Hz, 1H), 5.54 (d, $J$ = 2.8 Hz, 2H), 4.40 (q, $J$ = 7.1 Hz, 2H), 1.41 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 165.25, 156.74 (d, $J_{CF}$ = 244.7 Hz), 151.39, 147.77 (dd, $J_{CF}$ = 241.9/4.0 Hz), 141.67, 140.25, 134.33, 130.16, 129.02, 127.96, 127.83, 127.19, 126.65, 120.72 (d, $J_{CF}$ = 7.8 Hz), 120.06 (dd, $J_{CF}$ = 25.8/10.6 Hz), 113.31, 112.41 (dd, $J_{CF}$ = 24.6, 8.2 Hz), 61.43, 60.83 (d, $J_{CF}$ = 17.2 Hz), 14.53; *m/z* MS (TOF ES$^+$) $C_{25}H_{20}F_2NO_3$ $[MH]^+$ calcd 420.4; found 420.2; LC-MS $t_R$: 6.08.

**Ethyl 1-([1,1'-biphenyl]-4-ylmethyl)-5-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (4b).** Ethyl 5-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate **(2b)** (500 mg, 1.89 mmol) was alkylated with 4-phenylbenzyl bromide, according to general procedure C. After the initial period of overnight stirring, further $K_2CO_3$ (0.3 eq) and 4-phenylbenzyl chloride (0.3 eq) were added and stirring continued for a further overnight period. The desired product was isolated by filtration as described, to give 258 mg of pale yellow solid (32%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.62–7.49 (m, 4H), 7.43 (dd, $J$ = 10.3/4.8 Hz, 2H), 7.34 (ddd, $J$ = 7.3/3.8/1.2 Hz, 1H), 7.12 (d, $J$ = 8.3 Hz, 2H), 7.05 – 6.91 (m, 2H), 5.72 (s, 2H), 4.39 (q, $J$ = 7.1 Hz, 2H), 3.69 (s, 3H), 1.40 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 173.44, 165.65, 156.58 (d, $J_{CF}$ = 258.4 Hz), 152.00, 145.89 (d, $J_{CF}$ = 3.9 Hz), 140.94, 140.34, 135.92, 131.75 (d, $J_{CF}$ = 1.6 Hz), 129.00, 127.72, 127.69, 127.12, 126.31, 121.08 (d, $J_{CF}$ = 7.2 Hz), 115.14 (d, $J_{CF}$ = 10.1 Hz), 112.83, 112.22 (d, $J_{CF}$ = 23.7 Hz), 61.74, 61.21, 56.82, 14.55; *m/z* MS (TOF ES$^+$) $C_{26}H_{23}FNO_4$ $[MH]^+$ calcd 432.5; found 432.2; LC-MS $t_R$: 6.08.

**Ethyl 1-([1,1'-biphenyl]-4-ylmethyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (4c).** Ethyl 8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate **(2c)** (500 mg, 2.02 mmol) was alkylated with 4-phenylbenzyl bromide, according to general procedure C. After the initial period of overnight stirring, further $K_2CO_3$ (0.3 eq) and 4-phenylbenzyl chloride (0.3 eq) were added and stirring continued for a further overnight period. The desired product was isolated by filtration as described, to give 718 mg of yellow solid (86%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 1H), 8.20 (dd, *J* = 8.1/1.3 Hz, 1H), 7.62–7.47 (m, 4H), 7.42 (dd, *J* = 7.5/7.5 Hz, 2H), 7.38–7.29 (m, 2H), 7.12 (d, *J* = 8.2 Hz, 2H), 7.08 (dd, *J* = 8.0/1.1 Hz, 1H), 5.77 (s, 2H), 4.41 (q, *J* = 7.1 Hz, 2H), 3.73 (s, 3H), 1.42 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 174.08, 165.99, 152.30, 150.02, 140.82, 140.40, 136.27, 131.97, 130.36, 128.98, 127.66, 127.64, 127.11, 126.25, 125.79, 120.19, 114.95, 111.05, 61.86, 61.10, 56.43, 14.59; *m/z* MS (TOF ES$^+$) $C_{26}H_{24}NO_4$ $[MH]^+$ calcd 414.5; found 414.2; LC-MS $t_R$: 6.12.

**Ethyl 1-([1,1'-biphenyl]-4-ylmethyl)-5-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (4d).** Ethyl 5-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate **(2e)** (250 mg, 1.01 mmol) was alkylated with 4-phenylbenzyl bromide, according to general procedure C. The filtered crude solid was further purified by FCC (EtOAc/PE 0:100 to 100:0) to give 201 mg of pale yellow solid (48%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 7.66–7.49 (m, 4H), 7.49–7.39 (m, 3H), 7.39–7.29 (m, 1H), 7.22 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.2 Hz, 1H), 6.81 (d, *J* = 8.2 Hz, 1H), 5.35 (s, 2H), 4.39 (q, *J* = 7.1 Hz, 2H), 3.96 (s, 3H), 1.40 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 174.88, 166.28, 161.79, 148.51, 141.99, 141.58, 140.24, 133.59, 132.96, 129.02, 128.09, 127.82, 127.17, 126.58, 119.59, 113.48, 108.50, 107.06, 61.01, 57.81, 56.51, 14.62; *m/z* MS (TOF ES$^+$) $C_{26}H_{24}NO_4$ $[MH]^+$ calcd 414.5; found 414.2; LC-MS $t_R$: 5.98.

**5,8-Difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5a).** Ethyl 5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **(3a)** (2.37 g, 6.34 mmol) was dissolved in 1,4-dioxane (15 mL), before adding 2 M $HCl_{(aq)}$. The mixture was heated under reflux for 2 h. TLC analysis (EtOAc) indicated complete conversion had taken place. The

mixture was cooled to rt, dilute with water (50 mL) and the precipitate filtered, then washed with water and petroleum ether 40-60. The crude collected precipitate was found to contain a mixture of the desired acid, as well as some debenzylated derivative. The desired compound was obtained by washing the solid with DCM, and concentration of the filtrate. This gave 336 mg (24%) of the debenzylated side-product as a white solid (precipitate), and 1.417g (65%) of the desired compound as a pale yellow solid (DCM washings). $^{1}$H NMR (400 MHz, DMSO) δ 14.76 (s, 1H), 9.11 (s, 1H), 7.77 (ddd, $J$ = 13.8/9.1/4.4 Hz, 1H), 7.39 (ddd, $J$ = 11.1/9.1/3.2 Hz, 1H), 7.12 (d, $J$ = 8.5 Hz, 2H), 6.99–6.78 (m, 2H), 5.78 (d, $J$ = 3.7 Hz, 2H), 3.71 (s, $J$ = 4.7 Hz, 3H); $^{13}$C NMR (101 MHz, DMSO) δ 176.96, 165.21, 158.89, 157.12 (dd, $J_{CF}$ = 261.1/3.1 Hz), 152.20, 147.61 (dd, $J_{CF}$ = 248.1/4.0 Hz), 130.14 (dd, $J_{CF}$ = 9.0/2.8 Hz), 127.91 (d, $J_{CF}$ = 1.9 Hz), 127.59, 121.86 (dd, $J_{CF}$ = 26.0/10.8 Hz), 117.51 (d, $J_{CF}$ = 9.1 Hz), 114.17, 113.27 (dd, $J_{CF}$ = 23.6/8.4 Hz), 109.24, 60.22 (d, $J_{CF}$ = 16.5 Hz), 55.06; *m/z* MS (TOF ES$^-$) $C_{18}H_{12}F_2NO_4$ [M-H]$^-$ calcd 344.3; found 344.1; LC-MS $t_R$: 5.61.

**5-Fluoro-8-methoxy-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5b).** Ethyl 5-fluoro-8-methoxy-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **(3b)** (121 mg, 0.31 mmol) was hydrolysed according to general procedure D. After initial overnight stirring, further LiOH.$H_2O$ (1eq) was added, and stirring continued for a further hour before workup. The product was isolated as 58 mg of pale brown solid (59%)**.** $^{1}$H NMR (400 MHz, DMSO) δ 15.07 (s, 1H), 9.04 (s, 1H), 7.45 (dd, $J$ = 9.1, 4.5 Hz, 1H), 7.33 (dd, $J$ = 11.2, 9.1 Hz, 1H), 7.04 (d, $J$ = 8.7 Hz, 2H), 6.86 (t, $J$ = 5.8 Hz, 2H), 5.94 (s, 2H), 3.79 (s, 3H), 3.69 (s, 3H); $^{13}$C NMR (101 MHz, DMSO) δ 177.48, 165.54, 158.64, 154.52 (d, $J_{CF}$ = 256.2 Hz), 152.27, 146.40 (d, $J_{CF}$ = 3.5 Hz), 131.22 (d, $J_{CF}$ = 1.5 Hz), 128.95, 127.65, 117.48 (d, $J_{CF}$ = 8.6 Hz), 117.11 (d, $J_{CF}$ = 9.9 Hz), 113.99, 113.15 (d, $J_{CF}$ = 22.6 Hz), 108.71, 61.32, 56.89, 55.00; *m/z* MS (TOF ES$^-$) $C_{19}H_{15}FNO_5$ [M-H]$^-$ calcd 356.1; found 356.0; LC-MS $t_R$: 5.87.

**8-Methoxy-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5c).** Ethyl 8-methoxy-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **(3c)** (105 mg, 0.29 mmol) was hydrolysed according to general procedure D. After initial overnight stirring, further

LiOH.$H_2O$ (1.5 eq) was added, and stirring continued for a further overnight period before workup. In this case, the acidifed aqueous layer was extracted with DCM (3 x 20 mL) to obtain the free acid. The product was isolated as 109 mg of pale brown solid (98%). $^1$H NMR (400 MHz, $CDCl_3$) δ 14.95 (s, 1H), 8.74 (s, 1H), 8.16 (dd, *J* = 8.1/1.4 Hz, 1H), 7.47 (dd, *J* = 8.1 Hz, 1H), 7.21 (dd, *J* = 8.0/1.3 Hz, 1H), 7.06–6.90 (m, 2H), 6.88–6.72 (m, 2H), 5.80 (s, 2H), 3.83 (s, 3H), 3.77 (s, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 178.35, 167.12, 159.63, 151.30, 150.46, 130.88, 129.22, 128.23, 127.53, 126.98, 119.17, 115.89, 114.55, 108.76, 62.54, 56.55, 55.44; *m/z* MS (TOF $ES^+$) $C_{19}H_{18}NO_5$ $[MH]^+$ calcd 340.1; found 340.2; LC-MS $t_R$: 5.67.

**5-Methoxy-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5d).** Ethyl 5-methoxy-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **(3d)** (49 mg, 0.13 mmol) was hydrolysed according to general procedure D. After initial overnight stirring, further LiOH.$H_2O$ (1 eq) was added, and stirring continued for a further hour before workup. The product was isolated as 22 mg of yellow solid (49%). $^1$H NMR (400 MHz, $CD_3CN$) δ 8.83 (s, 1H), 7.65 (dd, *J* = 8.5 Hz, 1H), 7.30 – 7.12 (m, 3H), 7.02 (d, *J* = 8.3 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 2H), 5.48 (s, 2H), 3.93 (s, 3H), 3.75 (s, 3H); $^{13}$C NMR (101 MHz, $CD_3CN$) δ 180.60, 167.96, 162.24, 160.73, 149.65, 143.38, 135.54, 129.30, 127.57, 117.63, 115.37, 110.63, 110.28, 108.69, 58.64, 57.00, 55.99; *m/z* MS (TOF $ES^+$) $C_{19}H_{18}NO_5$ $[MH]^+$ calcd 340.1; found 340.2; LC-MS $t_R$: 5.34.

**1-([1,1'-Biphenyl]-4-ylmethyl)-5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6a).**[38] Ethyl 1-([1,1'-biphenyl]-4-ylmethyl)-5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **(4a)** (100 mg, 0.24 mmol) was dispersed in 1,4-dioxane/1M $HCl_{(aq)}$ (1:1, 2 mL) and heated at 80 °C for 3.5 h, before stirring at rt overnight. The mixture was then refluxed for a further 2.5 h, before TLC analysis (MeOH/DCM 2:98) indicated starting material had been consumed. The mixture was diluted with water, and the resultant precipitate collected by filtration (vacuum), before washing with further water and PE, then drying. This gave 82 mg of pale yellow solid (88%). $^1$H NMR (400 MHz, DMSO) δ 14.79 (s, 1H), 9.19 (s, 1H), 7.79 (ddd, *J* = 13.8, 9.1, 4.4 Hz, 1H), 7.70–7.52 (m, 4H), 7.50– 7.31 (m, 4H), 7.26 (d, *J* = 8.2 Hz, 2H), 5.92 (d, *J* = 3.9 Hz, 2H); $^{13}$C NMR (101 MHz,

DMSO) δ 177.04, 165.23, 157.18 (d, $J_{CF}$ = 262.1 Hz), 152.57, 147.37 (d, $J_{CF}$ = 293.5 Hz), 139.60, 139.47, 135.62 (d, $J_{CF}$ = 2.1 Hz), 130.29, 128.93, 127.56, 127.06, 126.61, 126.37, 122.04 (dd, $J_{CF}$ = 22.5, 11.6 Hz), 117.55 (d, $J_{CF}$ = 9.0 Hz), 113.31 (dd, $J_{CF}$ = 23.3, 8.5 Hz), 109.40, 60.44 (d, $J_{CF}$ = 15.9 Hz); *m/z* MS (TOF ES$^+$) $C_{23}H_{16}F_2NO_3$ $[MH]^+$ calcd 392.3; found 392.1; LC-MS $t_R$ 6.05.

**1-([1,1'-Biphenyl]-4-ylmethyl)-5-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6b).** Ethyl 1-([1,1'-biphenyl]-4-ylmethyl)-5-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate **(4b)** (214 mg, 0.50 mmol) was hydrolysed according to general procedure D. After initial overnight stirring, further $LiOH.H_2O$ (1 eq) was added, and stirring continued for a further hour before workup. The isolated product was found to contain a small amount of starting material, so the crude product was dispersed in 1,4-dioxane/$H_2O$ (1:1, 2 mL), and heated at 80 °C for 3.5 hour, followed stirring at rt overnight, then reflux for 8.5 h. The desired product was isolated by filtration, after dilution of the reaction mixture with water, to give 156 mg of pink solid (77%). $^1$H NMR (400 MHz, DMSO) δ 15.08 (s, 1H), 9.09 (s, 1H), 7.69–7.55 (m, 4H), 7.51 – 7.40 (m, 3H), 7.39 – 7.28 (m, 2H), 7.18 (d, *J* = 8.3 Hz, 2H), 6.06 (s, 2H), 3.75 (s, 3H); $^{13}$C NMR (101 MHz, DMSO) δ 177.54, 165.55, 154.60 (d, $J_{CF}$ = 256.2 Hz), 152.58, 146.37 (d, $J_{CF}$ = 3.4 Hz), 139.46, 139.20, 136.60, 131.36, 128.91, 127.50, 126.85, 126.54, 126.48, 117.53, 117.29 (d, $J_{CF}$ = 10.1 Hz), 113.20 (d, $J_{CF}$ = 22.9 Hz), 108.81, 61.67, 56.95; *m/z* MS (TOF ES$^+$) $C_{24}H_{19}FNO_4$ $[MH]^+$ calcd 404.1; found 404.2; LC-MS $t_R$: 6.06.

**1-([1,1'-Biphenyl]-4-ylmethyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6c).** Ethyl 1-([1,1'-biphenyl]-4-ylmethyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate **(4c)** (310 mg, 075 mmol), was hydrolysed by the same procedure, as described for the hydrolysis of ethyl 1-([1,1'-biphenyl]-4-ylmethyl)-5-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate **(4b)** in the synthesis of 1-([1,1'-biphenyl]-4-ylmethyl)-5-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6b)** above. The crude product after acidic hydrolysis required washings of DCM/PE (1:1) to remove residual starting material. The title compound was isolated as 180 mg of white solid (62%). $^1$H NMR (400 MHz, DMSO) δ 15.16 (s, 1H), 9.14 (s, 1H), 8.01 (dd, *J*

= 8.0, 1.4 Hz, 1H), 7.67 – 7.53 (m, 5H), 7.48 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.43 (dd, *J* = 7.6, 7.6 Hz, 2H), 7.38–7.30 (m, 1H), 7.18 (d, *J* = 8.3 Hz, 2H), 6.10 (s, 2H), 3.80 (s, 3H); $^{13}$C NMR (101 MHz, DMSO) δ 177.50, 165.82, 152.15, 150.35, 139.47, 139.21, 136.78, 130.28, 128.90, 128.06, 127.49, 127.27, 126.86, 126.54, 126.50, 117.59, 116.71, 107.65, 61.54, 56.60; *m/z* MS (TOF ES$^+$) $C_{24}H_{20}NO_4$ $[MH]^+$ calcd 386.1; found 386.2; LC-MS $t_R$: 6.15.

**1-([1,1'-Biphenyl]-4-ylmethyl)-5-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6d).** Ethyl 1-([1,1'-biphenyl]-4-ylmethyl)-5-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate **(4d)** (140 mg, 0.34 mmol), was hydrolysed by the same procedure, as described for the hydrolysis of ethyl 1-([1,1'-biphenyl]-4-ylmethyl)-5-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate **(4b)** in the synthesis of 1-([1,1'-biphenyl]-4-ylmethyl)-5-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6b)** above. The title compound was isolated as 118 mg of pale yellow solid (90%). $^1$H NMR (400 MHz, DMSO) δ 9.22 (s, 1H), 7.74 (dd, *J* = 8.5 Hz, 1H), 7.68–7.55 (m, 4H), 7.44 (dd, *J* = 7.6 Hz, 2H), 7.39–7.24 (m, 4H), 7.10 (d, *J* = 8.3 Hz, 1H), 5.84 (s, 2H), 3.90 (s, 3H); $^{13}$C NMR (101 MHz, DMSO) δ 178.84, 166.37, 160.71, 149.46, 141.88, 139.74, 139.44, 134.89, 134.70, 128.93, 127.59, 127.17, 127.04, 126.61, 115.96, 109.86, 108.68, 107.90, 56.73, 56.29; *m/z* MS (TOF ES$^+$) $C_{24}H_{20}NO_4$ $[MH]^+$ calcd 386.1; found 386.2; LC-MS $t_R$: 5.82.

**1-([1,1'-Biphenyl]-4-ylmethyl)-8-fluoro-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (7).** Ethyl 1-([1,1'-biphenyl]-4-ylmethyl)-5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **(4a)** (102 mg, 0.24 mmol) was dispersed in 1 M NaOH $_{(aq)}$/1,4-dioxane (1:1, 2mL) and heated at 80 °C for 3.5 h, followed by stirring at rt overnight. After this time, complete conversion to the acid had been achieved. The mixture was heated under reflux for a further 30 h, with no appreciable progress noted. LiOH.$H_2O$ (41 mg, 1.22 mmol, 5.1 eq) were added and reflux continued overnight, followed by stirring at rt for a further week. After this time, the entire mixture was transferred to a microwave vial, and LiOH.$H_2O$ (5 eq) added. The vessel was sealed, and heated at 160 °C for 1 h. LC-MS analysis after this time indicated complete conversion to the title compound. The mixture was diluted with water (20 mL) and washed with $Et_2O$ (20 mL), before

acidification with 2 M HCl $_{(aq)}$. The resultant precipitate was collected by filtration (vacuum), to give 75 mg of yellow/brown solid. This was recrystallised from EtOAc, to give 49 mg of off-white solid (53%). $^1$H NMR (400 MHz, DMSO) δ 13.37 (s, 1H), 13.29 (s, 1H), 9.17 (s, 1H), 7.78–7.54 (m, 5H), 7.45 (dd, $J$ = 7.6 Hz, 2H), 7.35 (dd, $J$ = 7.3 Hz, 1H), 7.25 (d, $J$ = 8.1 Hz, 2H), 6.87 (dd, $J$ = 9.0, 3.2 Hz, 1H), 5.89 (d, $J$ = 3.0 Hz, 2H); $^{13}$C NMR (101 MHz, DMSO) δ 180.38, 164.38, 157.74 (d, $J_{CF}$ = 1.8 Hz), 153.29, 143.26 (d, $J_{CF}$ = 240.5 Hz), 139.62, 139.47, 135.58 (d, $J_{CF}$ = 1.6 Hz), 128.92, 128.47, 127.55, 127.05, 126.61, 126.42, 123.44 (d, $J_{CF}$ = 24.7 Hz), 114.04, 111.99 (d, $J_{CF}$ = 7.7 Hz), 107.97 (d, $J_{CF}$ = 1.9 Hz), 60.38 (d, $J_{CF}$ = 14.9 Hz); *m/z* MS (TOF ES$^+$) $C_{23}H_{17}FNO_4$ $[MH]^+$ calcd 390.1; found 390.1; LC-MS $t_R$: 5.93.

***N*-Ethyl-5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (8a).** The title compound was obtained through condensation of 2M ethylamine in THF with the acid chloride intermediate described in general procedure E, to give 40 mg of white solid (37%). $^1$H NMR (400 MHz, $CDCl_3$) δ 9.77 (s, 1H), 8.80 (s, 1H), 7.35–7.26 (m, 1H), 7.08 (d, $J$ = 8.3 Hz, 2H), 7.00 (ddd, $J$ = 10.8, 9.0, 3.4 Hz, 1H), 6.87–6.79 (m, 2H), 5.52 (d, $J$ = 2.9 Hz, 2H), 3.77 (s, 3H), 3.49 (qd, $J$ = 7.3, 5.6 Hz, 2H), 1.26 (t, $J$ = 7.3 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 175.71, 163.89, 159.90, 158.69 (dd, $J_{CF}$ = 262.2, 2.7 Hz), 150.44, 147.81 (d, $J_{CF}$ = 283.9 Hz), 129.35, 127.98, 127.19, 120.14 (d, $J_{CF}$ = 11.5 Hz), 119.83, 114.60, 113.82, 111.97 (dd, $J_{CF}$ = 24.4, 8.3 Hz), 61.00 (d, $J_{CF}$ = 17.1 Hz), 55.44, 34.35, 14.98; *m/z* MS (TOF ES$^+$) $C_{20}H_{18}F_2N_2NaO_3$ $[MH]^+$ calcd 395.1; found 395.1; LC-MS $t_R$: 5.70.

**5,8-Difluoro-1-(4-methoxybenzyl)-*N*,*N*-dimethyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (8b).** The title compound was obtained through coupling of dimethylamine hydrochloride with 5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(5a)**, as described in general procedure F. FCC purification (eluent MeOH/DCM 1:99 to 2:98) gave 112 mg of pale yellow oil that solidified on standing to a yellow solid (quantitative yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.23 (ddd, $J$ = 13.6, 6.6, 2.8 Hz, 1H), 7.09 (d, $J$ = 8.4 Hz, 2H), 6.91 (ddd, $J$ = 10.5, 9.0, 3.3 Hz, 1H), 6.87 – 6.78 (m, 2H), 5.39 (d, $J$ = 2.6 Hz, 2H), 3.75 (s, 3H), 3.08 (s, 3H), 3.03

(s, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.80, 166.31, 159.75, 158.44 (dd, $J_{CF}$ = 260.5, 1.7 Hz), 147.82 (dd, $J_{CF}$ = 246.0, 4.4 Hz), 147.18, 130.60 (dd, $J_{CF}$ = 8.2, 2.8 Hz), 127.98 (d, $J_{CF}$ = 1.4 Hz), 127.52 (d, $J_{CF}$ = 1.0 Hz), 121.17, 119.59 (d, $J_{CF}$ = 10.7 Hz), 119.33 (d, $J_{CF}$ = 8.6 Hz), 114.54, 111.08 (dd, $J_{CF}$ = 24.6, 8.2 Hz), 60.01 (d, $J_{CF}$ = 17.2 Hz), 55.38, 38.77, 35.61; *m/z* MS (TOF ES$^+$) $C_{20}H_{19}F_2N_2O_3$ $[MH]^+$ calcd 373.1; found 373.2; LC-MS $t_R$: 4.92.

**5,8-Difluoro-1-(4-methoxybenzyl)-4-oxo-*N*-phenyl-1,4-dihydroquinoline-3-carboxamide (8c).** The title compound was obtained through condensation of aniline with the acid chloride intermediate described in general procedure E, to give 47 mg of white solid (39%). $^1$H NMR (400 MHz, $CDCl_3$) δ 11.97 (s, 1H), 8.89 (s, 1H), 7.77 (dd, *J* = 8.6, 1.0 Hz, 2H), 7.41–7.28 (m, 3H), 7.16–7.00 (m, 4H), 6.90–6.80 (m, 2H), 5.56 (d, *J* = 2.8 Hz, 2H), 3.77 (s, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 175.78, 162.07, 160.01, 158.73 (dd, $J_{CF}$ = 266.7, 4.2 Hz), 150.79, 148.03 (dd, $J_{CF}$ = 247.2, 3.6 Hz), 138.61, 130.27, 129.08, 128.09, 126.95, 124.22, 120.68, 120.36 (dd, $J_{CF}$ = 26.2, 10.7 Hz), 119.71 (d, $J_{CF}$ = 7.4 Hz), 114.69, 113.79, 112.37 (dd, $J_{CF}$ = 24.9, 8.2 Hz), 61.24 (d, $J_{CF}$ = 17.5 Hz), 55.45; *m/z* MS (TOF ES$^+$) $C_{24}H_{19}F_2N_2O_3$ $[MH]^+$ calcd 421.1; found 421.1; LC-MS $t_R$: 6.12.

***N*-Benzyl-5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (8d).** The title compound was obtained through condensation of benzylamine with the acid chloride intermediate described in general procedure E, to give 49 mg of white solid (39%). $^1$H NMR (400 MHz, $CDCl_3$) δ 10.18 (t, *J* = 5.4 Hz, 1H), 8.83 (s, 1H), 7.47–7.19 (m, 7H), 7.08 (d, *J* = 8.4 Hz, 2H), 7.00 (ddd, *J* = 10.8, 9.0, 3.4 Hz, 1H), 6.91–6.76 (m, 2H), 5.52 (d, *J* = 2.9 Hz, 2H), 4.65 (d, *J* = 5.8 Hz, 2H), 3.77 (s, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 175.69, 164.13, 159.93, 158.76 (dd, $J_{CF}$ = 244.7, 2.6 Hz), 150.63, 147.88 (d, $J_{CF}$ = 263.1 Hz), 138.62, 129.50 (d, $J_{CF}$ = 10.1 Hz), 128.75, 128.00, 127.99, 127.34, 127.12 (d, $J_{CF}$ = 1.2 Hz), 120.12 (dd, $J_{CF}$ = 25.8, 10.7 Hz), 119.84 (d, $J_{CF}$ = 7.4 Hz), 114.62, 113.64, 112.08 (dd, $J_{CF}$ = 24.6, 8.6 Hz), 61.06 (d, $J_{CF}$ = 17.3 Hz), 55.44, 43.61; *m/z* MS (TOF ES$^+$) $C_{25}H_{21}F_2N_2O_3$ $[MH]^+$ calcd 435.2; found 435.1; LC-MS $t_R$: 5.99.

**5,8-Difluoro-1-(4-methoxybenzyl)-4-oxo-*N*-(piperidin-1-yl)-1,4-dihydroquinoline-3-carboxamide (8e).** The title compound was obtained through coupling of 1-aminopiperidine with

5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(5a)**, as described in general procedure F. FCC purification (eluent MeOH/DCM 0:100 to 5:95), followed PLC purification (MeOH/DCM 2:98, plate run 3 times) gave 77 mg of off-white solid (62%). $^1$H NMR (400 MHz, $CDCl_3$) δ 10.58 (s, 1H), 8.81 (s, 1H), 7.36–7.26 (m, 1H), 7.14–6.94 (m, 3H), 6.89–6.76 (m, 2H), 5.53 (d, $J$ = 2.9 Hz, 2H), 3.77 (s, 3H), 3.09–2.68 (m, 4H), 1.88–1.65 (m, 4H), 1.47 (d, $J$ = 5.2 Hz, 2H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 175.34, 161.54, 159.92, 158.73 (d, $J_{CF}$ = 257.5 Hz), 150.65, 147.95 (d, $J_{CF}$ = 242.2 Hz), 128.66 (d, $J_{CF}$ = 5.6 Hz), 127.97, 127.13, 120.05 (d, $J_{CF}$ = 5.2 Hz), 119.76 (d, $J_{CF}$ = 6.7 Hz), 114.61, 113.78, 112.07 (dd, $J_{CF}$ = 24.7, 8.3 Hz), 61.08 (d, $J_{CF}$ = 16.9 Hz), 57.18, 55.44, 25.25, 23.64; *m/z* MS (TOF ES$^+$) $C_{23}H_{24}F_2N_3O_3$ $[MH]^+$ calcd 428.2; found 428.2; LC-MS $t_R$: 5.21.

**5,8-Difluoro-*N*-((*1S*,*2S*)-2-hydroxycyclohexyl)-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (8f).** The title compound was obtained through coupling of (*1S*,*2S*)-2-aminocyclohexanol hydrochloride with 5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(5a)**, as described in general procedure F. FCC purification (eluent EtOAc/PE 0:100 to 100:0) gave 148 mg of off-white solid (quantitative yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 9.99 (d, $J$ = 6.9 Hz, 1H), 8.80 (s, 1H), 7.39–7.17 (m, 1H), 7.14–6.94 (m, 3H), 6.93 – 6.73 (m, 2H), 5.52 (d, $J$ = 2.7 Hz, 2H), 4.07 (s, 1H), 3.91 – 3.67 (m, 4H), 3.58–3.37 (m, 1H), 2.19–1.95 (m, 2H), 1.83–1.68 (m, 2H), 1.53–1.27 (m, 4H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 175.63, 165.92, 159.94, 156.41 (d, $J_{CF}$ = 238.8 Hz), 150.80, 145.47 (d, $J_{CF}$ = 255.7 Hz), 130.24 (dd, $J_{CF}$ = 8.7, 1.9 Hz), 127.97, 127.03, 120.26 (dd, $J_{CF}$ = 25.4, 10.5 Hz), 119.71 (d, $J_{CF}$ = 7.5 Hz), 114.64, 113.06, 112.25 (dd, $J_{CF}$ = 24.9, 8.2 Hz), 76.11, 61.14 (d, $J_{CF}$ = 17.1 Hz), 55.97, 55.44, 38.75, 34.48, 31.49, 24.83, 24.10; *m/z* MS (TOF ES$^+$) $C_{24}H_{25}F_2N_2O_4$ $[MH]^+$ calcd 443.2; found 443.2; LC-MS $t_R$: 5.32; $[\alpha]_D^{24}$ = + 498° (0.0105, DCM).

**5,8-Difluoro-1-(4-methoxybenzyl)-3-(morpholine-4-carbonyl)quinolin-4(*1H*)-one (8g).** 5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(5a)** (100 mg, 0.29 mmol) was dispersed in dry DCM (1 mL) under an atmosphere of $N_2$ and cooled to 0 °C, over an

ice bath. To this was added oxalyl chloride (0.028 mL, 0.32 mmol, 1.1 eq) and DMF (1 drop) and the mixture stirred on the ice bath for 10 min, before warming to rt and stirring for a further 20 min. TLC analysis (EtOAc) indicated starting material had been consumed. Morpholine (0.053 mL, 0.61 mmol, 2.1 eq) was added, and the mixture stirred for 10 min at rt, before diluting with DCM (20 mL) and washing with 0.5 M $HCl_{(aq)}$ (20 mL) and sat. $NaHCO_{3\ (aq)}$ (20 mL). After concentration of the organic layer under reduced pressure, the crude was further purified by FCC (eluent EtOAc 10:90 to 100:0) to give 100 mg of colourless glassy solid (83%). $^1$H NMR (400 MHz, DMSO) δ 8.35 (s, 1H), 7.66–7.49 (m, 1H), 7.22–7.00 (m, 3H), 6.95–6.80 (m, 2H), 5.52 (d, $J$ = 3.2 Hz, 2H), 3.70 (s, 3H), 3.67–3.53 (m, 6H), 3.40–3.19 (m, 2H); $^{13}$C NMR (101 MHz, DMSO) δ 170.90, 164.13, 158.74, 158.71 (d, $J_{CF}$ = 235.1 Hz), 146.88, 146.86 (d, $J_{CF}$ = 260.9 Hz), 130.37 (d, $J_{CF}$ = 3.4 Hz), 128.82, 127.30, 120.50 (d, $J_{CF}$ = 6.2 Hz), 119.91 (d, $J_{CF}$ = 2.4 Hz), 114.15, 111.51, 110.76 (dd, $J_{CF}$ = 22.5/9.0 Hz), 66.47, 66.05, 58.74 (d, $J_{CF}$ = 15.3 Hz), 55.04, 47.10, 41.99; *m/z* MS (TOF ES$^-$) $C_{22}H_{21}F_2N_2O_4$ $[M+H]^+$ calcd 415.2; found 415.2; LC-MS $t_R$: 5.27.

**5,8-Difluoro-1-(4-methoxybenzyl)-3-(piperidine-1-carbonyl)quinolin-4(*1H*)-one (8h).** The title compound was obtained through condensation of piperidine with the acid chloride intermediate described in general procedure E, to give 45 mg of off-white semisolid (38%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.26–7.16 (m, 1H), 7.09 (d, $J$ = 8.5 Hz, 2H), 6.91 (ddd, $J$ = 10.5, 9.0, 3.3 Hz, 1H), 6.87 – 6.80 (m, 2H), 5.39 (d, $J$ = 2.5 Hz, 2H), 3.77 (s, 3H), 3.69 (s, 2H), 3.36 (s, 2H), 1.83–1.43 (m, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.91, 164.51, 159.77, 158.51 (dd, $J_{CF}$ = 261.8, 2.9 Hz), 147.84 (dd, $J_{CF}$ = 246.0, 4.4 Hz), 146.82, 130.66 (dd, $J_{CF}$ = 8.1, 2.8 Hz), 128.00, 127.99, 127.62, 121.38, 119.41 (dd, $J_{CF}$ = 25.6, 10.7 Hz), 114.56, 111.24, 111.00 (dd, $J_{CF}$ = 24.6, 8.2 Hz), 60.01 (d, $J_{CF}$ = 17.2 Hz), 55.41, 48.75, 43.45, 26.64, 25.69, 24.62; *m/z* MS (TOF ES$^+$) $C_{23}H_{23}F_2N_2O_3$ $[MH]^+$ calcd 413.2; found 413.2; LC-MS $t_R$: 5.55.

**5,8-Difluoro-*N*-hydroxy-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (8i).** The title compound was obtained through condensation of hydroxylamine hydrochloride with the acid chloride intermediate described in general procedure E. The crude product was dissolved in

the minimum amount of MeOH, before addition of EtOAc, causing precipitation of a pink solid to occur. This was collected by filtration (vacuum) to give 19 mg of the desired compound (18%, > 90% pure). $^{1}$H NMR (400 MHz, DMSO) δ 11.46 (s, 1H), 9.28 (s, 1H), 8.89 (s, 1H), 7.75 – 7.54 (m, 1H), 7.33 – 7.18 (m, 1H), 7.07 (d, $J$ = 8.5 Hz, 2H), 6.89 (d, $J$ = 8.7 Hz, 2H), 5.71 (d, $J$ = 3.1 Hz, 2H), 3.70 (s, 3H); $^{13}$C NMR (101 MHz, DMSO) δ 173.59, 161.53, 160.37 (d, $J_{CF}$ = 250.8 Hz), 158.80, 150.21, 147.49 (d, $J_{CF}$ = 244.2 Hz), 129.96 (dd, $J_{CF}$ = 6.6, 1.7 Hz), 128.48, 127.45, 120.54 (dd, $J_{CF}$ = 26.3, 9.9 Hz), 118.58 (d, $J_{CF}$ = 7.9 Hz), 114.19, 112.56, 112.04 (dd, $J_{CF}$ = 23.3, 8.1 Hz), 59.55 (d, $J_{CF}$ = 16.2 Hz), 55.05; *m/z* MS (TOF ES$^{+}$) $C_{18}H_{15}F_2N_2O_4$ $[MH]^{+}$ calcd 361.1; found 361.1; LC-MS $t_R$: 5.51.

**5,8-Difluoro-1-(4-methoxybenzyl)-*N*-(methylsulfonyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (9).** 5,8-Difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(5a)** (100 mg, 0.29 mmol) was dispersed in dry DCM (1 mL) under an atmosphere of $N_2$ and cooled to 0 °C, over an ice bath. To this were added oxalyl chloride (0.028 mL, 0.32 mmol, 1.1 eq) and DMF (1 drop) and the mixture stirred on the ice bath for 10 min, before stirring at rt for 30 min. Oxalyl chloride (0.2 eq) was added, and stirring continued at rt for 10 min. The mixture was concentrated under reduced pressure, before redissolving in dry DCM (1 mL) and methanesulfonamide (28 mg, 0.29 mmol, 1 eq). The mixture was cooled over an ice bath, under an atmosphere of $N_2$, before adding TEA (0.121 mL, 0.87 mmol, 3 eq). The mixture was warmed to rt, and left to stir overnight, before diluting with DCM (20 mL) and washing with 1 M $HCl_{(aq)}$ (20 mL). The organic layer was concentrated and the residue purified by FCC (eluent EtOAc/PE 0:100 to 100:0) to give 48 mg of white crystalline solid (39%) $^{1}$H NMR (400 MHz, $CDCl_3$) δ 12.61 (s, 1H), 8.72 (s, 1H), 7.39 (ddd, $J$ = 13.5, 9.0, 4.3 Hz, 1H), 7.18–7.01 (m, 3H), 6.95–6.77 (m, 2H), 5.56 (d, $J$ = 2.8 Hz, 2H), 3.79 (s, 3H), 3.36 (s, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 175.36, 163.08, 160.28, 159.30 (d, $J_{CF}$ = 240.6 Hz), 151.09, 147.11 (d, $J_{CF}$ = 278.8 Hz), 129.65, 128.35, 126.04, 121.39 (dd, $J_{CF}$ = 13.6, 10.8 Hz), 121.15, 114.89, 113.63 (d, $J_{CF}$ = 12.6 Hz), 111.19, 61.62 (d, $J_{CF}$ =

17.6 Hz), 55.49, 41.97; *m/z* MS (TOF ES$^+$) $C_{19}H_{16}F_2N_2NaO_5S$ $[M+Na]^+$ calcd 445.1; found 445.1; LC-MS $t_R$: 5.32.

**5,8-Difluoro-1-(4-methoxybenzyl)-*N*-methyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (10a).** Ethyl 5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **(3a)** (100 mg, 0.27 mmol) was stirred in a mixture of EtOH/40% $MeNH_{2\ (aq)}$ (1:1, 2 mL) at rt overnight. TLC analysis (EtOAc/DCM 1:1) indicated complete conversion had taken place. The mixture was poured on to ice water, and the resulting precipitate collected by filtration (vacuum), before drying to give 80 mg of pale yellow solid (83%). $^1$H NMR (400 MHz, $CDCl_3$) δ 9.72 (d, $J$ = 3.7 Hz, 1H), 8.80 (s, 1H), 7.38–7.19 (m, 1H), 7.07 (d, $J$ = 8.4 Hz, 2H), 7.00 (ddd, $J$ = 10.8, 9.1, 3.3 Hz, 1H), 6.83 (d, $J$ = 8.7 Hz, 2H), 5.52 (d, $J$ = 2.7 Hz, 2H), 3.77 (s, 3H), 2.99 (d, $J$ = 4.9 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 175.64, 164.76, 159.91, 158.68 (dd, $J_{CF}$ = 262.7, 2.8 Hz), 150.35, 147.88 (d, $J_{CF}$ = 259.7 Hz), 130.33 (dd, $J_{CF}$ = 8.0, 2.4 Hz), 127.98 (d, $J_{CF}$ = 1.5 Hz), 127.18 (d, $J_{CF}$ = 1.0 Hz), 120.03 (dd, $J_{CF}$ = 25.7, 11.0 Hz), 119.86 (d, $J_{CF}$ = 7.4 Hz), 114.61, 113.79, 111.98 (dd, $J_{CF}$ = 24.8, 8.2 Hz), 60.98 (d, $J_{CF}$ = 17.2 Hz), 55.43, 26.10; *m/z* MS (TOF ES$^+$) $C_{19}H_{16}F_2N_2NaO_3$ $[M+Na]^+$ calcd 381.1; found 381.2; LC-MS $t_R$: 5.16.

**5,8-Difluoro-*N*-(2-hydroxyethyl)-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (10b).** Ethyl 5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **(3a)** (100 mg, 0.27 mmol) was stirred in a mixture of EtOH/ethanolamine (2:1, 3 mL) at rt for 3.75 h. TLC analysis (EtOAc) indicated only partial progression, so EtOH (2 mL) was added to aid further dissolution. After overnight stirring, complete conversion had taken place. The mixture was poured on to ice/2 M $HCl_{\ (aq)}$, and the resulting precipitate collected by filtration (vacuum), with washings of water. After drying, 91 mg of yellow solid was obtained (88%). $^1$H NMR (400 MHz, $CDCl_3$) δ 10.17 (s, 1H), 8.79 (s, 1H), 7.31 (ddd, $J$ = 13.6, 9.0, 4.3 Hz, 1H), 7.14–6.96 (m, 3H), 6.91–6.78 (m, 2H), 5.52 (d, $J$ = 2.8 Hz, 2H), 3.83 (s, 2H), 3.77 (s, 3H), 3.63 (dd, $J$ = 10.0, 5.6 Hz, 2H), 3.37 (d, $J$ = 4.5 Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 175.60, 165.93, 159.96, 157.29 (d, $J_{CF}$ = 273.1 Hz), 150.58, 147.96 (d, $J_{CF}$ = 245.2 Hz), 130.28 (d, $J_{CF}$ = 8.5 Hz), 127.98 (d,

$J_{CF}$ = 1.5 Hz), 127.02 (d, $J_{CF}$ = 1.1 Hz), 120.25 (dd, $J_{CF}$ = 25.6, 11.0 Hz), 119.74 (d, $J_{CF}$ = 7.7 Hz), 114.65, 113.21, 112.25 (dd, $J_{CF}$ = 24.7, 8.1 Hz), 63.43, 61.11 (d, $J_{CF}$ = 17.1 Hz), 55.44, 43.16; *m/z* MS (TOF $ES^+$) $C_{20}H_{19}F_2N_2O_4$ $[MH]^+$ calcd 389.1; found 389.2; LC-MS $t_R$: 4.90.

**5,8-Difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (11).** Ethyl 5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **(3a)** (520 mg, 1.39 mmol) was dispersed in 28-30% $NH_{3\ (aq)}$/EtOH (1:1, 8 mL) and stirred at rt in a closed vessel for 60 h. TLC analysis (EtOAc) indicated complete conversion had taken place. The mixture was diluted with water and stirred for 10 min, before collecting the resulting precipitate by filtration (vacuum), and drying to give 423 mg (88%) of white solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.50 (s, 1H), 8.79 (s, 1H), 7.30 (ddd, *J* = 13.6/9.0/4.3 Hz, 1H), 7.08 (d, *J* = 8.2 Hz, 2H), 7.02 (ddd, *J* = 10.7/9.0/3.4 Hz, 1H), 6.88 – 6.80 (m, 2H), 5.75 (s, 1H), 5.51 (d, *J* = 2.9 Hz, 2H), 3.77 (s, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 175.41, 165.80, 159.92, 158.69 (d, $J_{CF}$ = 262.4 Hz), 151.03, 147.98 (d, $J_{CF}$ = 246.8 Hz), 130.37 (dd, $J_{CF}$ = 8.6/2.9 Hz), 127.92, 127.90, 127.08, 120.26 (dd, $J_{CF}$ = 25.8/10.7 Hz), 119.87 (d, $J_{CF}$ = 7.2 Hz), 114.62, 113.42, 112.30 (dd, $J_{CF}$ = 24.8/8.4 Hz), 61.10 (d, $J_{CF}$ = 16.9 Hz), 55.44; *m/z* MS (TOF $ES^+$) $C_{18}H_{14}F_2N_2NaO_3$ $[M+Na]^+$ calcd 367.3; found 367.1; LC-MS $t_R$: 5.38.

**5,8-Difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carbonitrile (12).** 5,8-Difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide **(11)** (104 mg, 0.30 mmol) was stirred in MeCN/$H_2O$/MeOH (3:3:1, 7 mL) at rt, before adding $PdCl_2$ (20 mg, 0.12 mmol, 0.4 eq). The mixture was stirred at rt for 2 h, before adding THF (2 mL) and continuing stirring at rt overnight. TLC analysis (EtOAc) indicated partial reaction progression, so stirring was continued at 50 °C for a further overnight period, before adding in $PdCl_2$ (0.1 eq) and continuing stirring at 50 °C for a final overnight period. After this, organic solvents were removed under reduced pressure, and the residue diluted with water (20 mL), before extraction with DCM (3 x 20 mL). The combined organic extracts were washed with brine (30 mL), before concentration under reduced pressure. The crude product was further purified by FCC (eluent EtOAc/PE 0:100 to 100:0) to give 34 mg of off-white solid (35%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.35 (ddd, *J* =

13.7, 9.1, 4.4 Hz, 1H), 7.12 (d, $J$ = 8.4 Hz, 2H), 7.05 (ddd, $J$ = 10.0, 9.1, 3.4 Hz, 1H), 6.96–6.82 (m, 2H), 5.44 (d, $J$ = 2.9 Hz, 2H), 3.81 (s, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.03, 160.35, 160.02 (dd, $J_{CF}$ = 257.5, 6.9 Hz), 150.26, 147.99 (dd, $J_{CF}$ = 247.6, 4.3 Hz), 130.12 (dd, $J_{CF}$ = 6.7, 3.5 Hz), 128.58, 125.91, 121.20 (dd, $J_{CF}$ = 26.0, 10.4 Hz), 118.47 (d, $J_{CF}$ = 8.5 Hz), 115.01, 114.68, 113.24 (dd, $J_{CF}$ = 24.0, 8.4 Hz), 98.65, 60.87 (d, $J_{CF}$ = 18.3 Hz), 55.52; *m/z* MS (TOF $ES^+$) $C_{18}H_{12}F_2N_2NaO_2$ $[M+Na]^+$ calcd 349.1; found 349.1; LC-MS $t_R$: 5.20.

**5,8-Difluoro-1-(4-methoxybenzyl)-3-(*1H*-tetrazol-5-yl)quinolin-4(*1H*)-one (13).** 5,8-Difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carbonitrile **(12)** (34 mg, 0.20 mmol), $NaN_3$ (20 mg, 0.31 mmol, 3 eq) and triethylammonium chloride (43 mg, 0.31 mmol, 3eq) were heated with stirring in toluene (5 mL) at 100 °C overnight. TLC analysis (EtOAc) indicated no progress, so further triethylammonium chloride (143 mg, 10 eq) and $NaN_3$ (67 mg, 10 eq) were added, and heating continued under reflux for a further overnight period. TLC analysis indicated almost complete conversion, so heating under reflux was continued for a further 7 h, then reduced to 70 °C over the weekend. The mixture was cooled, then diluted with $Et_2O$ (10 mL) and extracted with water (2 x 10 mL). The aqueous layer was washed with $Et_2O$ (10 mL), before acidifying with 2M $HCl_{(aq)}$ and extracting with EtOAc (2 x 10 mL). Concentration of the EtOAc layers followed by PLC purification (MeOH/DCM 5:95, plate run twice) gave 14 mg of yellow solid (38%). $^1$H NMR (400 MHz, $CDCl_3$) δ 14.22 (s, 1H), 9.00 (s, 1H), 7.40 (ddd, $J$ = 13.6, 9.0, 4.3 Hz, 1H), 7.20–7.01 (m, 3H), 6.96–6.72 (m, 2H), 5.61 (d, $J$ = 2.4 Hz, 2H), 3.78 (s, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 176.71, 160.18, 158.50 (dd, $J_{CF}$ = 266.5, 3.1 Hz), 150.66, 148.30 (d, $J_{CF}$ = 271.9 Hz), 146.61, 130.12 (d, $J_{CF}$ = 11.1 Hz), 128.13, 126.55, 120.80 (dd, $J_{CF}$ = 26.1, 10.9 Hz), 118.73 (d, $J_{CF}$ = 8.7 Hz), 114.83, 113.01 (dd, $J_{CF}$ = 23.9, 7.6 Hz), 106.86, 61.34 (d, $J_{CF}$ = 17.8 Hz), 55.48; *m/z* MS (TOF $ES^-$) $C_{18}H_{12}F_2N_5O_2$ $[M-H]^-$ calcd 368.1; found 368.0; LC-MS $t_R$: 5.10.

***N*-Acetyl-5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (14).** 5,8-Difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide **(11)** (50 mg, 0.15 mmol) was dissolved in pyridine (1 mL), before adding $Ac_2O$ (0.021 mL, 0.22 mmol, 1.5 eq) and

stirring at rt overnight. TLC analysis (EtOAc) indicated no progress, so $Ac_2O$ (1 mL) and DCM (1 mL) were added, and stirring continued at rt overnight. The temperature was then increased to 100 °C, and after 2 d the starting material had disappeared. The mixture was cooled, diluted with water, and stirred for 30 min at rt. The resulting precipitate was collected by filtration (vacuum) and washed with water and PE. The crude solid was further purified by FCC (eluent EtOAc/PE 0:100 to 100:0) to give 37 mg of off-white solid (64%). $^1$H NMR (400 MHz, $CDCl_3$) δ 12.27 (s, 1H), 8.77 (s, 1H), 7.36 (ddd, $J$ = 13.5, 9.0, 4.3 Hz, 1H), 7.16–7.01 (m, 3H), 6.95–6.80 (m, 2H), 5.55 (d, $J$ = 2.8 Hz, 2H), 3.78 (s, 3H), 2.46 (s, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 175.41, 171.69, 162.89, 160.16, 158.71 (dd, $J_{CF}$ = 264.1, 3.1 Hz), 151.64, 147.98 (d, $J_{CF}$ = 247.4 Hz), 130.10 (dd, $J_{CF}$ = 9.3, 2.5 Hz), 128.18, 126.45, 120.95 (dd, $J_{CF}$ = 26.0, 10.5 Hz), 119.72 (d, $J_{CF}$ = 7.7 Hz), 114.80, 113.14 (dd, $J_{CF}$ = 25.0, 8.4 Hz), 112.28, 61.45 (d, $J_{CF}$ = 17.5 Hz), 55.47, 26.39; *m/z* MS (TOF ES$^+$) $C_{20}H_{16}F_2N_2NaO_4$ $[M+Na]^+$ calcd 409.1; found 409.1; LC-MS $t_R$: 5.26.

**Ethyl 1-([1,1'-biphenyl]-2-ylmethyl)-5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (15a).** Ethyl 5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **(2a)** (500 mg, 1.97 mmol), was alkylated with 2-(bromomethyl)biphenyl, according to general procedure C. The resulting crude product was further purified by FCC (eluent EtOAc/PE 0:100 to 20:80) to give 480 mg (73%) of pale yellow solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.58–7.21 (m, 8H), 7.16 (ddd, $J$ = 13.5/9.0/4.3 Hz, 1H), 7.02–6.83 (m, 2H), 5.42 (d, $J$ = 2.7 Hz, 2H), 4.35 (q, $J$ = 7.1 Hz, 2H), 1.37 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.35, 164.99, 158.52 (dd, $J_{CF}$ = 263.1/2.7 Hz), 151.17, 147.66 (dd, $J_{CF}$ = 246.9/4.3 Hz), 141.06, 139.89, 133.07 (d, $J_{CF}$ = 2.1 Hz), 130.75, 130.24 (d, $J_{CF}$ = 5.7 Hz), 128.82, 128.78, 128.32, 128.23, 127.90, 125.61, 120.52 (d, $J_{CF}$ = 7.7 Hz), 119.83 (dd, $J_{CF}$ = 25.6/10.5 Hz), 113.04, 112.13 (dd, $J_{CF}$ = 24.7/8.1 Hz), 61.22, 59.55 (d, $J_{CF}$ = 16.9 Hz), 14.48; *m/z* MS (TOF ES$^+$) $C_{25}H_{20}F_2NO_3$ $[M+H]^+$ calcd 420.1; found 420.2; LC-MS $t_R$: 6.06.

**Ethyl 1-([1,1'-biphenyl]-3-ylmethyl)-5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (15b).** Ethyl 5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **(2a)** (500 mg, 1.97 mmol), was alkylated with 3-phenylbenzyl bromide, according to general procedure C. The resulting crude

product was further purified by FCC (eluent EtOAc/PE 0:100 to 20:80) to give 619 mg (75%) of pale yellow solid. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.58–7.47 (m, 3H), 7.47–7.31 (m, 5H), 7.29–7.17 (m, 1H), 7.07 (d, $J$ = 7.7 Hz, 1H), 6.96 (ddd, $J$ = 10.0, 9.3, 3.4 Hz, 1H), 5.56 (d, $J$ = 2.9 Hz, 2H), 4.39 (q, $J$ = 7.1 Hz, 2H), 1.40 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.48, 165.17, 158.68 (dd, $J_{CF}$ = 263.2/2.6 Hz), 151.38, 147.76 (dd, $J_{CF}$ = 246.5/4.5 Hz), 142.40, 140.39, 136.04 (d, $J_{CF}$ = 1.4 Hz), 130.11 (d, $J_{CF}$ = 10.7 Hz), 129.74, 129.06, 127.91, 127.48, 127.26, 124.92, 124.80, 120.65 (d, $J_{CF}$ = 7.5 Hz), 120.07 (dd, $J_{CF}$ = 25.5/10.7 Hz), 113.26, 112.40 (dd, $J_{CF}$ = 24.7/8.3 Hz), 61.39, 61.05 (d, $J_{CF}$ = 16.9 Hz), 14.50; *m/z* MS (TOF ES$^+$) $C_{25}H_{20}F_2NO_3$ $[MH]^+$ calcd 420.1; found 420.2; LC-MS $t_R$: 6.09.

**1-([1,1'-Biphenyl]-2-ylmethyl)-5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (16a).** Ethyl 1-([1,1'-biphenyl]-2-ylmethyl)-5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **(15a)** (100 mg, 0.24 mmol) was dispersed in 1,4-dioxane/2 M $HCl_{(aq)}$ (1:1, 2 mL) and heated at 80 °C for 5 h. TLC analysis (EtOAc) indicated complete hydrolysis had taken place. The mixture was cooled to rt, before diluting with water (20 mL) and extracting with DCM (3 × 20 mL). Concentration of the combined organic layers gave 90 mg of pale yellow solid (96%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 14.32 (s, 1H), 8.48 (s, 1H), 7.50–7.27 (m, 7H), 7.23–7.16 (m, 2H), 7.09 (ddd, $J$ = 10.2/9.1/3.4 Hz, 1H), 6.94 (d, $J$ = 7.7 Hz, 1H), 5.58 (d, $J$ = 3.0 Hz, 2H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.71, 165.83, 158.24 (dd, $J_{CF}$ = 265.1/2.7 Hz), 151.52, 147.94 (dd, $J_{CF}$ = 248.7/4.5 Hz), 141.25, 139.69, 132.10 (d, $J_{CF}$ = 2.1 Hz), 131.07, 131.03, 129.02, 128.87, 128.59, 128.33, 128.13, 126.25 (d, $J_{CF}$ = 1.6 Hz), 121.35 (dd, $J_{CF}$ = 25.6/10.8 Hz), 118.33 (d, $J_{CF}$ = 9.2 Hz), 113.19 (dd, $J_{CF}$ = 23.98.0 Hz), 110.22, 60.87 (d, $J_{CF}$ = 16.9 Hz); *m/z* MS (TOF ES$^+$) $C_{23}H_{16}F_2NO_3$ $[MH]^+$ calcd 392.1; found 392.1; LC-MS $t_R$: 6.00.

**1-([1,1'-Biphenyl]-3-ylmethyl)-5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (16b).** Ethyl 1-([1,1'-biphenyl]-3-ylmethyl)-5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **(15b)** (101 mg, 0.24 mmol) was dispersed in 1,4-dioxane/0.2 M $HCl_{(aq)}$ (1:1, 2 mL) and heated at 60 °C for 3 h. TLC analysis (EtOAc/DCM 1:1) indicated no progress. Heating was continued at 80

°C for a further 1.25 h, before adding in 2M $HCl_{(aq)}$ (0.15 mL). Heating was continued at 80 °C for a further 1.75 h, followed by rt stirring overnight. After a final 6 h of heating under reflux, the mixture was cooled, diluted with water, and the resulting precipitate collected by filtration (vacuum), with washings of water and PE. After drying, 85 mg of pale yellow solid was obtained (91%). $^1$H NMR (400 MHz, DMSO) δ 14.78 (s, 1H), 9.18 (s, 1H), 7.78 (ddd, $J$ = 13.8/9.1/4.4 Hz, 1H), 7.68–7.56 (m, 3H), 7.54 (s, 1H), 7.50–7.31 (m, 5H), 7.09 (d, $J$ = 7.7 Hz, 1H), 5.94 (d, $J$ = 3.9 Hz, 2H); $^{13}$C NMR (101 MHz, DMSO) δ 177.06, 165.24, 157.16 (dd, $J_{CF}$ = 261.6/2.3 Hz), 152.57, 147.26 (dd, $J_{CF}$ = 232.5/7.8 Hz), 140.62, 139.60, 137.16 (d, $J_{CF}$ = 2.4 Hz), 130.32 (dd, $J_{CF}$ = 12.0/2.8 Hz), 129.46, 128.95, 127.67, 126.73, 126.18, 124.61, 124.33, 121.88 (dd, $J_{CF}$ = 25.7/10.3 Hz), 117.55 (d, $J_{CF}$ = 10.0 Hz), 113.26 (dd, $J_{CF}$ = 23.2/9.0 Hz), 109.40, 60.70 (d, $J_{CF}$ = 15.3 Hz); *m/z* MS (TOF ES$^+$) $C_{23}H_{16}F_2NO_3$ $[MH]^+$ calcd 392.1; found 392.1; LC-MS $t_R$: 6.05.

***tert*-Butyl prop-2-yn-1-ylcarbamate (18).** Propargylamine **(17)** (5.126 g, 93.06 mmol) was dissolved in water (100 mL) with stirring. To this was added $Boc_2O$ (21.325 g, 97.71 mmol, 1.05 eq), and the mixture was stirred at rt overnight. TLC analysis (MeOH/DCM 1:9) indicated complete conversion. The mixture was extracted with DCM (3 × 30 mL), and the combined organic extracts washed with water (50 mL). Concentration of the organic layers gave 13.902 g of pale yellow oil, which rapidly crystallised to off-white crystals (96%). $^1$H NMR (400 MHz, $CDCl_3$) δ 4.70 (s, 1H), 3.92 (d, $J$ = 3.0 Hz, 2H), 2.21 (t, $J$ = 2.5 Hz, 1H), 1.45 (s, 9H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 146.90, 85.32, 80.21, 71.38, 28.48, 27.57.

***tert*-Butyl (3-(4-(hydroxymethyl)phenyl)prop-2-yn-1-yl)carbamate (19).**[39] 4-Iodobenzyl alcohol (2.02 g, 8.55 mmol), *tert*-butyl prop-2-yn-1-ylcarbamate **(18)** (1.724 g, 11.11 mmol, 1.3 eq) and TEA (3.573 mL, 25.64 mmol, 3 eq) were placed in a flask in dry THF (20 mL), and degassed by sonication (10 min). CuI (82 mg, 0.43 mmol, 0.05 eq) and $PdCl_2(PPh_3)_2$ (302 mg, 0.43 mmol, 0.05 eq) were added, and the mixture stirred at rt overnight. TLC analysis (EtOAc/PE 3:7) indicated complete depletion of the alkyne. The mixture was diluted with EtOAc (100 mL), before filtering through a bed of celite (vacuum). The filtrate was washed with water (2 x 50 mL) and brine (50

mL), before concentration and further purification by FCC (eluent EtOAc/PE 0:100 to 30:70) to give 933 mg of light brown solid (42%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, $J$ = 8.2 Hz, 2H), 7.30 (d, $J$ = 8.4 Hz, 2H), 4.77 (s, 1H), 4.69 (d, $J$ = 5.5 Hz, 2H), 4.15 (d, $J$ = 4.7 Hz, 2H), 1.76 (t, $J$ = 5.8 Hz, 1H), 1.47 (s, 9H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 150.57, 141.26, 132.02, 126.89, 122.08, 85.55, 83.07, 71.61, 65.05, 31.35, 28.52; *m/z* MS (TOF ES$^+$) $C_{11}H_{12}NO_3$ [M-$^t$Bu+2H]$^+$ calcd 206.1; found 206.2; LC-MS $t_R$: 5.54.

***tert*-Butyl (3-(4-(bromomethyl)phenyl)prop-2-yn-1-yl)carbamate (20).** *tert*-Butyl (3-(4-(hydroxymethyl)phenyl)prop-2-yn-1-yl)carbamate **(19)** (880 mg, 3.37 mmol) and $CBr_4$ (1.227 g, 3.70 mmol, 1.1 eq) were dissolved in DCM (20 mL), before cooling over an ice bath. $PPh_3$ (970 mg, 3.70 mmol, 1.1 eq) was added, and the mixture allowed to warm to rt with stirring. After 1.5 h of stirring at rt, TLC analysis (EtOAc/PE 3:7) indicated good progression. $PPh_3$ (0.5 eq) was added, and stirring continued overnight. The mixture was concentrated under reduced pressure, and the crude residue further purified by FCC (eluent EtOAc/PE 0:100 to 30:70) to give 595 mg of shiny off-white solid (55%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.40–7.34 (m, 2H), 7.34–7.27 (m, 2H), 4.75 (s, 1H), 4.46 (s, 2H), 4.14 (d, $J$ = 4.7 Hz, 2H), 1.46 (s, 9H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 155.40, 137.97, 132.16, 129.08, 122.98, 86.40, 82.68, 79.62, 32.96, 31.31, 28.46; *m/z* MS (TOF ES$^+$) $C_{11}H_{11}BrNO_2$ [M-$^t$Bu+2H]$^+$ calcd 268.0; found 268.0; LC-MS $t_R$: 6.29.

**Ethyl 1-(4-(3-((tert-butoxycarbonyl)amino)prop-1-yn-1-yl)benzyl)-5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (21).** Ethyl 5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **(2a)** (199 mg, 0.79 mmol), was alkylated with *tert*-butyl (3-(4-(bromomethyl)phenyl)prop-2-yn-1-yl)carbamate **(20)**, according to general procedure C. The resulting crude product was further purified by FCC (eluent EtOAc/PE 5:95 to 100:0) to give 392 mg (67%) of yellow solid $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.38 (d, $J$ = 8.3 Hz, 2H), 7.22 (ddd, $J$ = 13.5/9.0/4.3 Hz, 1H), 7.05 (d, $J$ = 8.1 Hz, 2H), 6.96 (ddd, $J$ = 10.3/9.1/3.4 Hz, 1H), 5.47 (d, $J$ = 2.8 Hz, 2H), 4.74 (s, 1H), 4.39 (q, $J$ = 7.1 Hz, 2H), 4.13 (d, $J$ = 4.5 Hz, 2H), 1.46 (s, 9H), 1.40 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.42, 165.20, 158.68 (d, $J_{CF}$ = 265.5 Hz),

155.40, 151.31, 147.68 (d, $J_{CF}$ = 243.2 Hz), 135.53 (d, $J_{CF}$ = 1.3 Hz), 132.55, 129.99, 126.07, 123.28, 120.66 (d, $J_{CF}$ = 7.8 Hz), 120.06 (dd, $J_{CF}$ = 25.5/10.5 Hz), 113.40, 112.49 (d, $J_{CF}$ = 33.3 Hz), 86.70, 82.33, 80.26, 61.46, 60.80 (d, $J_{CF}$ = 17.0 Hz), 31.30, 28.50, 14.51; *m/z* MS (TOF $ES^+$) $C_{27}H_{27}F_2N_2O_5$ $[MH]^+$ calcd 497.2; found 497.2; LC-MS $t_R$: 5.90.

**1-(4-(3-Aminoprop-1-yn-1-yl)benzyl)-5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride (22).** Ethyl 1-(4-(3-((tert-butoxycarbonyl)amino)prop-1-yn-1-yl)benzyl)-5,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **(21)** (230 mg, 0.46 mmol) was dispersed in a mixture of 1,4-dioxane/1 M $HCl_{(aq)}$ (1:1, 2 mL) and heated at 80 °C for 3.5 h, then at rt overnight (TLC analysis (MeOH/DCM 1:9) indicated complete *N*-Boc deprotection after 1.5 h). Heating was continued under reflux for a further 5 h, before cooling and concentration under reduced pressure, to give 205 mg of pale yellow solid (quantitative yield). $^1$H NMR (400 MHz, DMSO) δ 14.75 (s, 1H), 9.14 (s, 1H), 8.45 (s, 3H), 7.77 (ddd, *J* = 13.7, 9.1, 4.3 Hz, 1H), 7.56–7.31 (m, 3H), 7.21 (d, *J* = 8.3 Hz, 2H), 5.90 (d, *J* = 4.0 Hz, 2H), 3.97 (s, 2H); $^{13}$C NMR (101 MHz, DMSO) δ 177.04, 165.18, 158.46 (d, $J_{CF}$ = 261.7 Hz), 152.63, 147.97 (d, $J_{CF}$ = 262.4 Hz), 137.80, 131.87, 130.23, 126.15, 120.50, 119.70 (d, $J_{CF}$ = 23.1 Hz), 119.04, 113.35 (d, $J_{CF}$ = 13.3 Hz), 109.46, 85.09, 83.04, 60.31 (d, $J_{CF}$ = 4.2 Hz), 28.93; *m/z* MS (TOF $ES^+$) $C_{20}H_{15}F_2N_2O_3$ $[MH]^+$ calcd 369.1; found 369.1; LC-MS $t_R$: 4.36.

**Ethyl 3-(dimethylamino)-2-(2-fluorobenzoyl)acrylate (24).**[40] Ethyl-(2-fluorobenzyl)acetate **(23)** (1.04 g, 4.95 mmol) was dissolved in DMF (1 mL) and *N*,*N*-dimethylformamide, dimethyl acetal (627 mg, 5.26 mmol, 1 eq) added. The mixture was heated at 100 °C in the microwave reactor for 30 min, before pouring onto ice water (200 mL), and stirring for 5 min. The aqueous slurry was then extracted with $Et_2O$ (20 mL), before saturating the aqueous layer with NaCl and extracting again with $Et_2O$ (2 x 20 mL). The combined organic layers were washed with brine (30 mL) before concentration to give 1.185 g of yellow oil (90%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.59 (s, 1H), 7.45 – 7.31 (m, 1H), 7.16 (ddd, *J* = 7.6, 0.9 Hz, 1H), 7.01 (ddd, *J* = 10.3, 8.3,

0.8 Hz, 1H), 3.95 (q, *J* = 7.1 Hz, 2H), 3.28 (s, 3H), 2.90 (s, 3H), 0.88 (t, *J* = 7.1 Hz, 3H); *m/z* MS (TOF ES$^+$) $C_{14}H_{17}FNO_3$ $[MH]^+$ calcd 266.1; found 266.2.1; LC-MS $t_R$: 5.66.

**Ethyl 1-(4-methoxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (25a).**[40] Ethyl 3-(dimethylamino)-2-(2-fluorobenzoyl)acrylate **(24)** (100 mg, 0.38 mmol) and *p*-anisidine (47 mg, 0.38 mmol, 1 eq) were dissolved in dry DMF (2 mL) and heated at 140 °C in the microwave reactor for 1 h. TLC analysis (EtOAc/PE 1:1) indicated partial progression, so the mixture was heated at 140 °C (oil bath) over the weekend. LC-MS analysis after this time indicated only 1 major peak, the mixture was poured on to ice water, and the resulting precipitate filtered, washed with water, PE and dried, to give 87 mg of pale brown solid (71%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.55 (dd, *J* = 8.0, 1.4 Hz, 1H), 8.50 (s, 1H), 7.51 (ddd, *J* = 8.6, 7.1, 1.6 Hz, 1H), 7.45–7.37 (m, 1H), 7.37–7.28 (m, 2H), 7.16–7.04 (m, 2H), 6.99 (d, *J* = 8.4 Hz, 1H), 4.40 (q, *J* = 7.1 Hz, 2H), 3.92 (s, 3H), 1.40 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 174.59, 165.95, 160.61, 149.25, 141.14, 133.52, 132.40, 128.70, 128.62, 127.65, 125.33, 117.85, 115.55, 111.39, 61.08, 55.88, 14.59; *m/z* MS (TOF ES$^+$) $C_{19}H_{18}NO_4$ $[MH]^+$ calcd 324.1; found 324.2; LC-MS $t_R$: 5.27.

**Ethyl 1-([1,1'-biphenyl]-4-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (25b).** Ethyl 3-(dimethylamino)-2-(2-fluorobenzoyl)acrylate **(24)** (100 mg, 0.38 mmol) and 4-aminobiphenyl (64 mg, 0.38 mmol, 1 eq) were dissolved in dry DMF (2 mL) and heated at 140 °C (oil bath) over the weekend. LC-MS analysis after this time indicated only 1 major peak, the mixture was poured on to ice water, and the resulting precipitate filtered, washed with water, PE and dried, to give 85 mg of yellow solid (61%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.64–8.50 (m, 2H), 7.88–7.77 (m, 2H), 7.72–7.64 (m, 2H), 7.64–7.48 (m, 5H), 7.48–7.36 (m, 2H), 7.08 (dd, *J* = 13.6, 4.9 Hz, 1H), 4.41 (q, *J* = 7.1 Hz, 2H), 1.41 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 174.58, 165.83, 148.84, 143.27, 140.75, 139.83, 139.48, 132.50, 129.27, 129.16, 128.63, 128.46, 127.87, 127.74, 127.39, 125.47, 117.81, 111.63, 61.13, 14.60; *m/z* MS (TOF ES$^+$) $C_{24}H_{20}NO_3$ $[MH]^+$ calcd 370.1; found 370.2; LC-MS $t_R$: 5.77.

**1-(4-Methoxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (26a).** Ethyl 1-(4-methoxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **(25a)** (68 mg, 0.21 mmol) was hydrolysed according to general procedure D. On acidification of the aqueous layer during workup, a pink precipitate formed. This was collected by filtration (vacuum) and washed with water and PE, before drying to give 46 mg of pink solid (74%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 14.92 (s, 1H), 8.80 (s, 1H), 8.56 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.68 (ddd, *J* = 8.7, 7.1, 1.6 Hz, 1H), 7.57 (ddd, *J* = 8.1, 7.1, 1.0 Hz, 1H), 7.41–7.28 (m, 2H), 7.17 (d, *J* = 8.5 Hz, 1H), 7.15–7.05 (m, 2H), 3.93 (s, 4H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 178.92, 167.11, 161.03, 149.04, 141.64, 133.90, 132.77, 128.36, 126.90, 126.49, 126.07, 118.72, 115.71, 109.02, 55.94; *m/z* MS (TOF ES$^+$) $C_{17}H_{14}NO_4$ $[MH]^+$ calcd 296.1; found 296.2; LC-MS $t_R$: 5.25.

**1-([1,1'-Biphenyl]-4-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (26b).** Ethyl 1-([1,1'-biphenyl]-4-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **(25b)** (68 mg, 0.18 mmol) was hydrolysed according to general procedure D. On extracting the reaction mixture with $Et_2O$, the lithium salt of the product was found to suspend in the organic layer. This was collected by filtration (vacuum), before suspending in 2 M $HCl_{(aq)}$ (10 mL), and extracting the acidic suspension with DCM (2 x 10 mL). Concentration of the DCM layers gave 35 mg of pale yellow solid (57%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 14.88 (s, 1H), 8.86 (s, 1H), 8.59 (dt, *J* = 5.1, 2.5 Hz, 1H), 7.93–7.79 (m, 2H), 7.76–7.64 (m, 3H), 7.60 (ddd, *J* = 8.1, 7.1, 1.0 Hz, 1H), 7.57–7.39 (m, 5H), 7.27 (d, *J* = 8.1 Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 178.96, 166.99, 148.68, 143.94, 141.27, 139.21, 139.10, 134.01, 129.32, 129.31, 128.65, 127.54, 127.42, 127.01, 126.61, 126.11, 118.68, 109.23; *m/z* MS (TOF ES$^+$) $C_{22}H_{16}NO_3$ $[MH]^+$ calcd 342.1; found 342.2; LC-MS $t_R$: 5.72.

**Ethyl 1-((1-(*tert*-butoxycarbonyl)piperidin-4-yl)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (27)** Ethyl 3-(dimethylamino)-2-(2-fluorobenzoyl)acrylate **(24)** (500 mg, 1.88 mmol), *N*-Boc-(4-aminomethyl)piperidine (404 mg, 1.88 mmol, 1 eq) and $Cs_2CO_3$ (735 mg, 2.26 mmol, 1.2 eq) were dissolved in dry DMF (10 mL) under an atmosphere of nitrogen gas. The mixture was heated at 100 °C (oil bath) for 6.5 h, after which time TLC analysis (EtOAc/PE 6:4) indicated

disappearance of starting material. The mixture was cooled to rt, before diluting with ice water and stirring for 30 min. The resulting precipitate was collected by filtration (vacuum) to give 619 mg of off-white solid (79%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.56 (dd, *J* = 8.1, 1.5 Hz, 1H), 8.40 (s, 1H), 7.70 (ddd, *J* = 8.6, 7.1, 1.6 Hz, 1H), 7.46 (td, *J* = 7.6, 0.8 Hz, 1H), 7.41 (d, *J* = 8.5 Hz, 1H), 4.41 (q, *J* = 7.1 Hz, 2H), 4.34 – 3.84 (m, 4H), 2.63 (t, *J* = 12.2 Hz, 2H), 2.09 (ddt, *J* = 15.4, 7.6, 3.8 Hz, 1H), 1.60 (d, *J* = 17.3 Hz, 2H), 1.46 (s, 9H), 1.42 (t, *J* = 7.1 Hz, 3H), 1.37–1.10 (m, 2H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 174.32, 166.09, 154.70, 149.49, 139.07, 132.76, 129.51, 128.49, 125.34, 115.67, 110.94, 79.99, 61.17, 59.33, 43.40, 35.69, 29.83, 28.56, 14.60; *m/z* MS (TOF ES$^+$) $C_{23}H_{31}N_2O_5$ $[MH]^+$ calcd 415.2; found 415.3; LC-MS $t_R$: 5.42.

**1-((1-(*tert*-Butoxycarbonyl)piperidin-4-yl)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (28).** Ethyl 1-((1-(*tert*-butoxycarbonyl)piperidin-4-yl)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **(27)** (250 mg, 0.60 mmol) was dispersed in THF/water (1:1, 6 mL) and the flask atmosphere purged with nitrogen gas. $LiOH.H_2O$ (101 mg, 2.41 mmol, 4 eq) was added, and the mixture stirred at rt overnight. TLC analysis (MeOH/DCM 5:95) indicated conversion was complete. The mixture was diluted with water (20 mL) and washed with $Et_2O$ (30 mL), before careful acidification (~pH 4-5) by dropwise addition of 2 M $HCl_{(aq)}$, causing a precipitate to form. This was collected by filtration (vacuum) to give 202 mg of off-white solid (87%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 14.85 (s, 1H), 8.69 (s, 1H), 8.58 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.87 (ddd, *J* = 8.7, 7.2, 1.6 Hz, 1H), 7.68–7.54 (m, 2H), 4.19 (s, 4H), 2.63 (t, *J* = 12.0 Hz, 2H), 2.11 (dtd, *J* = 15.4, 7.7, 3.9 Hz, 1H), 1.83–1.52 (m, *J* = 13.5 Hz, 2H), 1.45 (d, *J* = 7.0 Hz, 9H), 1.32 (dt, *J* = 23.1, 13.5 Hz, 2H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 178.62, 167.03, 154.66, 148.79, 139.62, 134.26, 127.79, 126.85, 126.48, 116.46, 108.82, 80.13, 59.94, 43.06, 36.02, 29.82, 28.54; *m/z* MS (TOF ES$^+$) $C_{21}H_{27}N_2O_5$ $[MH]^+$ calcd 387.2; found 387.2; LC-MS $t_R$: 5.45.

**4-Oxo-1-(piperidin-4-ylmethyl)-1,4-dihydroquinoline-3-carboxylic acid hydrochloride (29).** 1-((1-(*tert*-Butoxycarbonyl)piperidin-4-yl)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(28)** (155 mg, 0.40 mmol) was dissolved in DCM (2 mL) with stirring at rt, before addition of 4M

HCl in 1,4-dioxane (2 mL). The mixture was stirred at rt for 1 h, before addition of PE, followed by collection of the resulting precipitate by filtration (vacuum). The collected solid was further washed with PE, before drying to give 149 mg of off-white solid (quantitative yield). $^{1}$H NMR (400 MHz, DMSO) δ 9.18–8.95 (m, *J* = 16.9 Hz, 2H), 8.89–8.62 (m, *J* = 10.1 Hz, 1H), 8.41 (dd, *J* = 8.1, 1.5 Hz, 1H), 8.11 (d, *J* = 8.7 Hz, 1H), 7.99 (ddd, *J* = 8.7, 7.1, 1.6 Hz, 1H), 7.69 (dd, *J* = 11.2, 3.9 Hz, 1H), 4.56 (d, *J* = 7.4 Hz, 2H), 3.23 (d, *J* = 12.5 Hz, 2H), 2.75 (q, *J* = 12.3 Hz, 2H), 2.16 (ddd, *J* = 11.2, 7.5, 3.6 Hz, 1H), 1.71 (d, *J* = 12.8 Hz, 2H), 1.60–1.38 (m, 2H); $^{13}$C NMR (101 MHz, DMSO) δ 177.83, 166.08, 149.81, 139.52, 134.30, 126.43, 125.97, 125.61, 118.45, 107.40, 57.13, 42.36, 32.69, 25.50; *m/z* MS (TOF ES$^{+}$) $C_{16}H_{19}N_2O_3$ $[MH]^{+}$ calcd 287.1; found 287.2; LC-MS $t_R$: 3.70.

**Ethyl 4-oxo-1-(piperidin-4-ylmethyl)-1,4-dihydroquinoline-3-carboxylate hydrochloride (30).** Ethyl 1-((1-(*tert*-butoxycarbonyl)piperidin-4-yl)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **(27)** (342 mg, 0.83 mmol) was dissolved in DCM (2 mL) and stirred at rt before addition of 4M HCl in 1,4-dioxane (2 mL). The mixture was stirred at rt for 1 h before addition of PE and attempted collection of the resulting precipitate by filtration (vacuum). However, the product was found to be hygroscopic, so the solid was redissolved in MeOH/DCM and combined with the filtrate, before concentration under reduced pressure, to give 325 mg of off-white solid (quantitative yield). $^{1}$H NMR (400 MHz, DMSO) δ 9.11 (d, *J* = 10.1 Hz, 1H), 8.90 (d, *J* = 9.9 Hz, 1H), 8.68 (s, 1H), 8.26 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.86 (d, *J* = 8.5 Hz, 1H), 7.83–7.74 (m, 1H), 7.49 (t, *J* = 7.5 Hz, 1H), 4.35 (d, *J* = 7.3 Hz, 2H), 4.23 (q, *J* = 7.1 Hz, 2H), 3.23 (d, *J* = 12.3 Hz, 2H), 2.86 – 2.64 (m, 2H), 2.13 (dd, *J* = 9.1, 5.8 Hz, 1H), 1.70 (d, *J* = 12.8 Hz, 2H), 1.50 (td, *J* = 15.6, 3.6 Hz, 2H), 1.29 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, DMSO) δ 172.79, 164.78, 149.74, 139.00, 132.72, 128.35, 126.46, 124.96, 117.54, 109.70, 59.82, 56.47, 42.38, 32.53, 25.58, 14.36; *m/z* MS (TOF ES$^{+}$) $C_{18}H_{23}N_2O_3$ $[MH]^{+}$ calcd 315.2; found 315.2; LC-MS $t_R$: 3.67.

**1-((1-Benzylpiperidin-4-yl)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (31).** Ethyl 4-oxo-1-(piperidin-4-ylmethyl)-1,4-dihydroquinoline-3-carboxylate hydrochloride **(30)** (80 mg, 0.23 mmol), $K_2CO_3$ (79 mg, 0.57 mmol, 2.5 eq), BnBr (47 mg, 0.28 mmol, 1.2 eq) were dispersed

in MeCN (2 mL) and stirred at rt overnight. MeCN was removed under reduced pressure, and the residue dispersed in water, causing a precipitate to form. This was collected by filtration (vacuum), and washed with PE to give 60 mg of off-white solid. This was purified by FCC (MeOH/DCM 0:100 to 10:90) to give 48 mg of glassy solid. This was taken up in water/THF (1:1, 4 mL), and the vessel purged with nitrogen gas, before addition of $LiOH.H_2O$ (39 mg, 0.92 mmol, 4 eq). The mixture was stirred at rt overnight, before diluting with water (20 mL) and washing with $Et_2O$ (20 mL). The aqueous layer was then neutralised with careful addition of 2M $HCl_{(aq)}$, before extraction with EtOAc (3 x 20 mL). Finally, concentration of the EtOAc layers gave 35 mg of off-white solid (40%). $^1$H NMR (400 MHz, DMSO) δ 15.23 (s, 1H), 9.01 (s, 1H), 8.40 (dd, *J* = 8.1, 1.5 Hz, 1H), 8.10 (d, *J* = 8.7 Hz, 1H), 7.97 (ddd, *J* = 8.7, 7.0, 1.6 Hz, 1H), 7.67 (t, *J* = 7.2 Hz, 1H), 7.39–7.15 (m, 5H), 4.50 (d, *J* = 7.4 Hz, 2H), 3.41 (s, 2H), 2.78 (d, *J* = 11.4 Hz, 2H), 1.95–1.62 (m, 3H), 1.56 – 1.27 (m, 4H); $^{13}$C NMR (101 MHz, DMSO) δ 177.73, 166.07, 149.64, 139.54, 137.41, 134.27, 128.62, 128.10, 126.79, 126.36, 125.91, 125.51, 118.45, 107.26, 62.13, 58.12, 52.44, 34.95, 28.70; *m/z* MS (TOF ES$^+$) $C_{23}H_{25}N_2O_3$ $[MH]^+$ calcd 377.2; found 377.2; LC-MS $t_R$: 4.16.

**4-Oxo-1-((1-(phenylcarbamoyl)piperidin-4-yl)methyl)-1,4-dihydroquinoline-3-carboxylic acid (32).** Ethyl 4-oxo-1-(piperidin-4-ylmethyl)-1,4-dihydroquinoline-3-carboxylate hydrochloride **(30)** (80 mg, 0.23 mmol) was dispersed in DCM (2 mL), with addition of DIPEA (0.060 mL, 0.35 mmol, 1.5 eq) and stirred at rt, before addition of phenyl isocyanate (0.027 mL, 0.25 mmol, 1.1 eq). The mixture was stirred at rt overnight. Further phenyl isocyanate (0.2 eq) with stirring at rt for a further 3 h, before diluting the reaction mixture with EtOAc (30 mL) and washing with 2 M $NaOH_{(aq)}$ (20 mL), 2M $HCl_{(aq)}$ (20 mL) and brine (20 mL). The organic layer was concentrated and further purified by FCC (eluent MeOH/DCM 0:100 to 10:90) to give 88 mg of yellow solid. This was taken up in water/THF (1:1, 4 mL), and the vessel purged with nitrogen gas, before addition of $LiOH.H_2O$ (39 mg, 0.92 mmol, 4 eq). The mixture was stirred at rt overnight, before diluting with water (20 mL) and washing with $Et_2O$ (20 mL). The aqueous layer was then acidified (~pH 4) with 2M $HCl_{(aq)}$, causing a precipitate to form. This was collected by filtration (vacuum) to give 20 mg

of off-white solid (22%). $^{1}$H NMR (400 MHz, DMSO) δ 15.24 (s, 1H), 9.05 (s, 1H), 8.46 (s, 1H), 8.42 (dd, *J* = 8.1, 1.4 Hz, 1H), 8.14 (d, *J* = 8.7 Hz, 1H), 7.99 (ddd, *J* = 8.6, 7.1, 1.4 Hz, 1H), 7.69 (dd, *J* = 7.5 Hz, 1H), 7.44 (d, *J* = 7.6 Hz, 2H), 7.21 (dd, *J* = 7.9 Hz, 2H), 6.91 (dd, *J* = 7.3 Hz, 1H), 4.53 (d, *J* = 7.5 Hz, 2H), 4.13 (d, *J* = 13.4 Hz, 2H), 2.68 (t, *J* = 12.1 Hz, 2H), 2.24–1.97 (m, 1H), 1.54 (d, *J* = 10.9 Hz, 2H), 1.28 (ddd, *J* = 19.5, 9.8, 6.2 Hz, 2H); $^{13}$C NMR (101 MHz, DMSO) δ 177.78, 166.09, 154.73, 149.68, 140.68, 139.58, 134.28, 128.23, 126.38, 125.94, 125.56, 121.54, 119.50, 118.47, 107.32, 57.81, 43.47, 35.12, 28.63; *m/z* MS (TOF ES$^{+}$) $C_{23}H_{24}N_3O_4$ $[MH]^{+}$ calcd 406.2; found 406.2; LC-MS $t_R$: 5.13.

**Pharmacology.**

*Radioligand binding assays.* FlpInCHO cells stably expressing the human $M_1$ muscarinic acetylcholine receptor (FlpInCHO-h$M_1$ mAChR) were generated as described previously[41] and maintained in DMEM containing 5% FBS under humidified conditions at 37°C and 5% $CO_2$. Radioligand binding experiments were performed on whole cells, seeded at 20,000 cells per well in complete DMEM into 96-well ISOPLATE TC plates. Next, cells were allowed to grow for 8 hours at 37 °C, then serum starved overnight. Following a wash with phosphate-buffered saline (100 µl) the cells were then resuspended in binding buffer (10 mM HEPES, 100 mM NaCl, 10 mM $MgCl_2$, pH 7.4). Assays were performed in a total volume of 200 µl with a 1/10 dilution of drug, for a duration of 4 hours at 4 °C. Assays were terminated by buffer removal followed by rapid washing, twice, with ice-cold 0.9% NaCl (100 µl). Plates were allowed to dry inverted for 30 min; OptiPhase Supermix scintillation cocktail (100 µl) was added, plates were sealed (TopSeal$^{TM}$), and radioactivity was measured in a MicroBeta$^2$ LumiJET microplate counter. All inhibition binding experiments were performed with 0.1 nM [$^3$H]-NMS ($K_D$ concentration) in presence of increasing concentrations of ACh with or without increasing concentrations of BQCA analogues.

*Intracellular calcium mobilisation assays.* On the day prior to the experiment, FlpInCHO-h$M_1$ mAChR cells were seeded at 20,000 cells per well (100 µL final volume per well) into clear 96-well

plates in DMEM containing 5% FBS and incubated 8 hours under humidified conditions at 37°C and 5% $CO_2$ prior serum starving the cells overnight. On the day of the experiment, the assay plates were washed once with 100 μL per well of phosphate buffered saline. The cells were then incubated with $Ca^{2+}$ assay buffer (150 mM NaCl, 2.6 mM KCl, 1.18 mM $MgCl_2 \cdot 6H_2O$, 10 mM D-glucose, 10 mM HEPES, 2.2 mM $CaCl_2 \cdot 2H_2O$, 0.5% (w/v) bovine serum albumin (BSA), and 4 mM Probenecid, pH = 7.4) containing 1 μM Fluo-4-AM for 1 hour under humidified conditions at 37°C and 0% $CO_2$. Each assay plate was then loaded into a FLEXstation™ (Molecular Devices Inc., Sunnyvale, CA) with its stock compound plate. The FLEXstation measured fluorescence over a 100 second time period using 485 nm excitation and 520 nm emission wavelengths and performed the addition of drugs (1/10 dilution) at the 20 second time point. For all interaction studies, ACh and BQCA (or its analogue) were co-added (simultaneously) at 20 seconds. For each, the peak of maximum florescence between the time 20 to 100 seconds was chosen and corrected to the baseline (fluorescence from time 0 to 19 seconds), then normalised to 100 μM ATP as internal control.

*Data analysis.* Competition binding curves between [$^3$H]NMS and ACh in the absence or presence of BQCA or its analogues, were fitted to the allosteric ternary complex model as described previously[7]. Competition-binding curves between [$^3$H]NMS and ACh in the absence or presence of BQCA or analogues were fitted to the following allosteric ternary complex model (equation 1):

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

where Y is percentage (vehicle control) binding, [A], [B], and [I] are the concentrations of [$^3$H]NMS, BQCA (or analogues) and ACh respectively, $K_A$ and $K_B$ are the equilibrium dissociation constants of [$^3$H]NMS and BQCA, respectively, $K_B$ is the equilibrium dissociation constant of CCh and α′ and α are the cooperativities (or analogues) between BQCA and [$^3$H]NMS or CCh respectively. Values of α (or α′) > 1 denote positive cooperativity; values < 1 (but > 0) denote negative cooperativity, and values =1 denote neutral cooperativity.

Functional data were analysed using an operational model of allosterism and agonism according to equation 2: [10,42]

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

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, $\tau_A$ and $\tau_B$ are operational measures of orthosteric and allosteric ligand efficacy (which incorporate both signal efficiency and receptor density), respectively, α is the binding cooperativity parameter between the orthosteric and allosteric ligand, and β denotes the magnitude of the allosteric effect of the modulator on the efficacy of the orthosteric agonist. In all instances, the equilibrium dissociation constant of each agonist and BQCA (or analogues) was fixed to that determined from the binding assays.

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### Notes

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The authors declare no competing financial interest.

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## ABBREVIATIONS USED

BQCA, benzyl quinolone carboxylic acid (1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid); CHO, Chinese hamster ovary; DIPEA, *N,N*-diisopropylethylamine; FCC, flash-column chromatography; PE, petroleum-ether 40-60; PLC, preparative layer chromatography; TEA, triethylamine.

## REFERENCES

(1) Medeiros, R. R.; Kitazawa, M. M.; Caccamo, A. A.; Baglietto-Vargas, D. D.; Estrada-Hernandez, T. T.; Cribbs, D. H. D.; Fisher, A. A.; LaFerla, F. M. F. Loss of Muscarinic $M_1$ Receptor Exacerbates Alzheimer's Disease-Like Pathology and Cognitive Decline. *Am. J. Pathol.* **2011**, *179*, 980–991.

(2) Langmead, C. J.; Watson, J.; Reavill, C. Muscarinic acetylcholine receptors as CNS drug targets. *Pharmacol. Therapeut.* **2008**, *117*, 232–243.

(3) Holden, M.; Kelly, C. Use of cholinesterase inhibitors in dementia. *Adv. Psychiat. Treat.* **2002**, *8*, 89–96.

(4) Wess, J.; Eglen, R. M.; Gautam, D. Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. *Nat. Rev. Drug Discov.* **2007**, *6*, 721–733.

(5) Decker, M.; Holzgrabe, U. $M_1$ Muscarinic Acetylcholine Receptor Allosteric Modulators as Potential Therapeutic Opportunities for Treating Alzheimer's Disease. *Med. Chem. Commun.* **2012**, *3*, 752.

(6) Ma, L.; Seager, M. A.; Seager, M. A.; Wittmann, M.; Jacobson, M.; Bickel, D.; Burno, M.; Jones, K.; Graufelds, V. K.; Xu, G.; Pearson, M.; McCampbell, A.; Gaspar, R.; Shughrue, P.; Danziger, A.; Regan, C.; Flick, R.; Pascarella, D.; Garson, S.; Doran, S.; Kreatsoulas, C.; Veng, L.; Lindsley, C. W.; Shipe, W. D.; Kuduk, S. D.; Sur, C.; Kinney, G.; Seabrook, G. R.; Ray, W. J. Selective activation of the $M_1$ muscarinic acetylcholine receptor achieved by allosteric potentiation. *P. Natl. Acad. Sci. USA* **2009**, *106*, 15950–15955.

(7) Canals, M.; Lane, J. R.; Wen, A.; Scammells, P. J.; Sexton, P. M.; Christopoulos, A. A Monod-Wyman-Changeux Mechanism Can Explain G Protein-coupled Receptor (GPCR) Allosteric Modulation. *J. Biol. Chem.* **2012**, *287*, 650–659.

(8) Shirey, J. K.; Brady, A. E.; Jones, P. J.; Davis, A. A.; Bridges, T. M.; Kennedy, J. P.; Jadhav, S. B.; Menon, U. N.; Xiang, Z.; Watson, M. L.; Christian, E. P.; Doherty, J. J.; Quirk, M. C.; Snyder, D. H.; Lah, J. J.; Levey, A. I.; Nicolle, M. M.; Lindsley, C. W.; Conn, P. J. A Selective Allosteric Potentiator of the $M_1$ Muscarinic Acetylcholine Receptor Increases Activity of Medial Prefrontal Cortical Neurons and Restores Impairments in Reversal Learning. *J. Neurosci.* **2009**, *29*, 14271–14286.

(9) Yang, F. V.; Shipe, W. D.; Bunda, J. L.; Nolt, M. B.; Wisnoski, D. D.; Zhao, Z.; Barrow, J. C.; Ray, W. J.; Ma, L.; Wittmann, M.; Seager, M. A.; Koeplinger, K. A.; Hartman, G. D.; Lindsley, C. W. Parallel synthesis of N-biaryl quinolone carboxylic acids as selective $M_1$ positive allosteric modulators. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 531–536.

(10) Leach, K.; Sexton, P. M.; Christopoulos, A. Allosteric GPCR modulators: taking advantage of permissive receptor pharmacology. *Trends Pharmacol. Sci.* **2007**, *28*, 382–389.

(11) Conn, P. J.; Kuduk, S. D.; Doller, D. Drug Design Strategies for GPCR Allosteric Modulators. *Annu. Rep. Med. Chem.* **2012**, 47, 441–457.

(12) Gould, R. G.; Jacobs, W. A. The synthesis of certain substituted quinolines and 5, 6-benzoquinolines. *J. Am. Chem. Soc.* **1939**, *61*, 2890–2895.

(13) Cao, X.; You, Q. D.; Li, Z.-Y.; Yang, Y.; Wang, X. J. Microwave-Assisted Simple Synthesis of Substituted 4-Quinolone Derivatives. *Synth. Commun.* **2009**, *39*, 4375–4383.

(14) Eaton, P. E.; Carlson, G. R.; Lee, J. T. Phosphorus pentoxide-methanesulfonic acid. Convenient alternative to polyphosphoric acid. *J. Org. Chem.* **2001**, *38*, 4071–4073.

(15) Zewge, D.; Chen, C.-Y.; Deer, C.; Dormer, P. G.; Hughes, D. L. A Mild and Efficient Synthesis of 4-Quinolones and Quinolone Heterocycles. *J. Org. Chem.* **2007**, *72*, 4276–4279.

(16) Birch, M.; Sibley, G. E. M.; Law, D.; Oliver, J. D. Antifungal Combination Therapy. *WIPO* **2009**, WO 2009/144473.

(17) Maffioli, S. I.; Marzorati, E.; Marazzi, A. Mild and Reversible Dehydration of Primary Amides with PdCl 2in Aqueous Acetonitrile. *Org. Lett.* **2005**, *7*, 5237–5239.

(18) Koguro, K.; Oga, T.; Mitsui, S.; Orita, R. Novel Synthesis of 5□Substituted Tetrazoles from Nitriles. *Synthesis* **1998**, *29*, 910–914.

(19) Škugor, M. M.; Štimac, V.; Palej, I.; Lugarić, Đ.; Paljetak, H. Č.; Filić, D.; Modrić, M.; Đilović, I.; Gembarovski, D.; Mutak, S.; Haber, V. E.; Holmes, D. J.; Ivezić-Schoenfeld, Z.; Alihodžić, S. Synthesis and Biological Activity of 4"-O-Acyl Derivatives of 14- and 15-Membered Macrolides Linked to ω-Quinolone-Carboxylic Unit. *Bioorg. Med. Chem.* **2010**, *18*, 6547–6558.

(20) Johnson, M. R. Soluble Amide & Ester Pyrazinoylguanidine Sodium Channel Blockers, *US Patent Office* **2009**, US 2009/0324724.

(21) Guicherit, O. M.; Boyd, E. A.; Brunton, S. A.; Price, S.; Stibbard, J. H. A.; Mackinnon, C. H. Mediators of Hedgehog Signaling Pathways, Compositions and Uses Related Thereto *WIPO* **2006**, WO 2006/050506.

(22) Kuduk, S. D.; Chang, R. K.; Di Marco, C. N.; Ray, W. J.; Ma, L.; Wittmann, M.; Seager, M. A.; Koeplinger, K. A.; Thompson, C. D.; Hartman, G. D.; Bilodeau, M. T. Quinolizidinone carboxylic acid selective $M_1$ allosteric modulators: SAR in the piperidine series. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1710–1715.

(23) Kuduk, S. D.; Chang, R. K.; Di Marco, C. N.; Ray, W. J.; Ma, L.; Wittmann, M.; Seager, M. A.; Koeplinger, K. A.; Thompson, C. D.; Hartman, G. D.; Bilodeau, M. T. Quinolizidinone Carboxylic Acids as CNS Penetrant, Selective $M_1$ Allosteric Muscarinic Receptor Modulators. *ACS Med. Chem. Lett.* **2010**, *1*, 263–267.

(24) Kuduk, S. D.; Chang, R. K.; Di Marco, C. N.; Pitts, D. R.; Greshock, T. J.; Ma, L.; Wittmann, M.; Seager, M. A.; Koeplinger, K. A.; Thompson, C. D.; Hartman, G. D.; Bilodeau, M. T.; Ray, W. J. Discovery of a Selective Allosteric $M_1$ Receptor Modulator with Suitable Development Properties Based on a Quinolizidinone Carboxylic Acid Scaffold. *J. Med. Chem.* **2011**, *54*, 4773–4780.

(25) Wise, W. M.; Brandt, W. W. An Investigation of Some Hydroxamic Acids. *J. Am. Chem. Soc.* **1955**, *77*, 1058–1059.

(26) Meanwell, N. A. Synopsis of Some Recent Tactical Application of Bioisosteres in Drug Design. *J. Med. Chem.* **2011**, *54*, 2529–2591.

(27) Bell, R. P.; Higginson, W.; Bell, R. P.; Higginson, W. The catalyzed dehydration of acetaldehyde hydrate, and the effect of structure on the velocity of protolytic reactions. *P. Roy. Soc. A-Math. Phy.* **1949**, *197*, 141–159.

(28) Barbosa, J.; Barrón, D.; Jiménez-Lozano, E.; Sanz-Nebot, V. Comparison Between Capillary Electrophoresis, Liquid Chromatography, Potentiometric and Spectrophotometric Techniques for Evaluation of p*K*a Values of Zwitterionic Drugs in Acetonitrile–Water Mixtures. *Anal. Chim. Acta* **2001**, *437*, 309–321.

(29) Reid, P. R.; Bridges, T. M.; Sheffler, D. J.; Cho, H. P.; Lewis, L. M.; Days, E.; Daniels, J. S.; Jones, C. K.; Niswender, C. M.; Weaver, C. D.; Conn, P. J.; Lindsley, C. W.; Wood, M. R. Discovery and optimization of a novel, selective and brain penetrant $M_1$ positive allosteric modulator (PAM): The development of ML169, an MLPCN probe. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2697–2701.

(30) Kuduk, S. D.; Di Marco, C. N.; Cofre, V.; Pitts, D. R.; Ray, W. J.; Ma, L.; Wittmann, M.; Seager, M. A.; Koeplinger, K. A.; Thompson, C. D.; Hartman, G. D.; Bilodeau, M. T. Pyridine containing $M_1$ positive allosteric modulators with reduced plasma protein binding. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 657–661.

(31) Kuduk, S. D.; DiPardo, R. M.; Beshore, D. C.; Ray, W. J.; Ma, L.; Wittmann, M.; Seager, M. A.; Koeplinger, K. A.; Thompson, C. D.; Hartman, G. D.; Bilodeau, M. T. Hydroxy cycloalkyl fused pyridone carboxylic acid $M_1$ positive allosteric modulators. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2538–2541.

(32) Kuduk, S. D.; Di Marco, C. N.; Cofre, V.; Pitts, D. R.; Ray, W. J.; Ma, L.; Wittmann, M.; Veng, L.; Seager, M. A.; Koeplinger, K. A.; Thompson, C. D.; Hartman, G. D.; Bilodeau, M. T. N-Heterocyclic derived $M_1$ positive allosteric modulators. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1334–1337.

(33) Kuduk, S. D.; Di Marco, C. N.; Cofre, V.; Ray, W. J.; Ma, L.; Wittmann, M.; Seager, M. A.; Koeplinger, K. A.; Thompson, C. D.; Hartman, G. D.; Bilodeau, M. T. Fused heterocyclic $M_1$ positive allosteric modulators. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2769–2772.

(34) Kuduk, S. D.; Chang, R. K.; Greshock, T. J.; Ray, W. J.; Ma, L.; Wittmann, M.; Seager, M. A.; Koeplinger, K. A.; Thompson, C. D.; Hartman, G. D.; Bilodeau, M. T. Identification of Amides as Carboxylic Acid Surrogates for Quinolizidinone-Based $M_1$ Positive Allosteric Modulators. *ACS Med. Chem. Lett.* **2012**, *3*, 1070–1074.

(35) Podányi, B.; Keresztúri, G.; Vasvári-Debreczy, L.; Hermecz, I.; Tóth, G. An NMR Study of Halogenated 1,4-Dihydro-1-ethyl-4-oxoquinoline-3-carboxylates. *Magn. Reson. Chem.* **1996**, *34*, 972–978.

(36) Lauer, W. M.; Arnold, R. T.; Tiffany, B.; Tinker, J. The Synthesis of Some Chloromethoxyquinolines1. *J. Am. Chem. Soc.* **2001**, *68*, 1268–1269.

(37) Zhang, Y.; Guiguemde, W. A.; Sigal, M.; Zhu, F.; Connelly, M. C.; Nwaka, S.; Guy, R. K. Synthesis and structure–activity relationships of antimalarial 4-oxo-3-carboxyl quinolones. *Bioorg. Med. Chem.* **2010**, *18*, 2756–2766.

(38) Lindsley, C. W.; Shipe, W. D.; Yang, F. V.; Bunda, J. L. Benzyl-Substituted Quinolone $M_1$ Receptor Positive Allosteric Modulators. *WIPO* **2008**, WO 2008/002621.

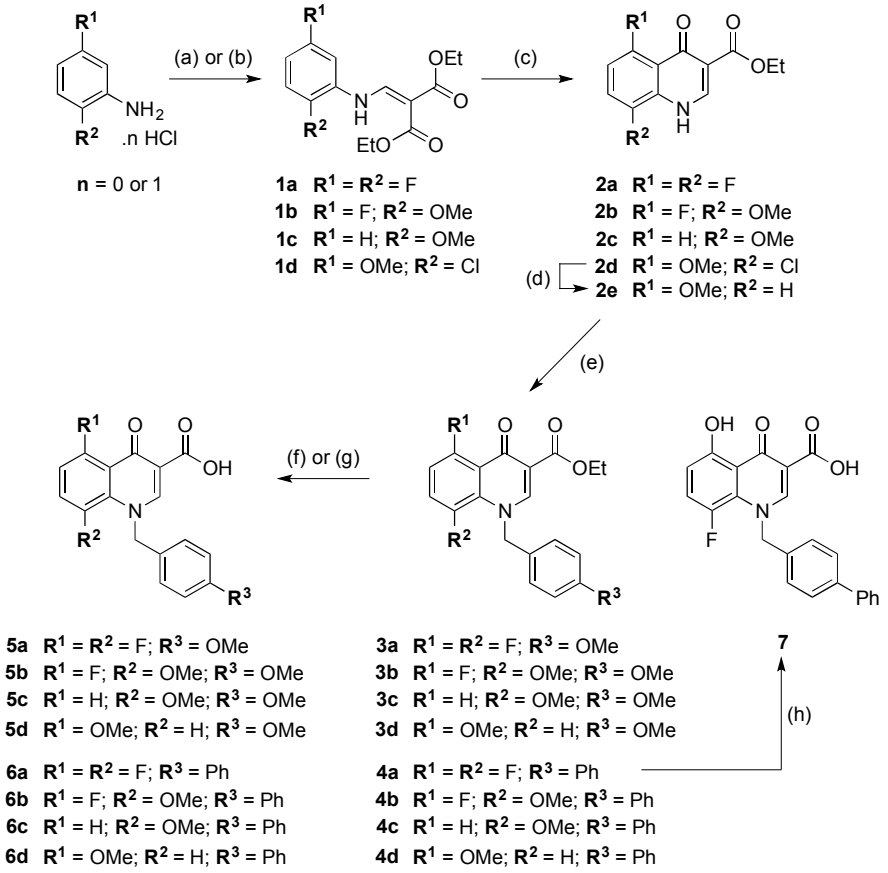
(39) Casara, P.; Jund, K.; Bey, P. General synthetic access to α-allenyl amines and α-allenyl-α-aminoacids as potential enzyme activated irreversible inhibitors of PLP dependent enzymes. *Tetrahedron Lett.* **2001**, *25*, 1891–1894.

(40) Bunce, R. A.; Lee, E. J.; Grant, M. T. Ethyl 1,4-dihydro-4-oxo-3-quinolinecarboxylates by a tandem addition-elimination-SNAr reaction. *J. Heterocyclic Chem.* **2011**, *48*, 620–625.

(41) Avlani, V. A.; Gregory, K. J.; Morton, C. J.; Parker, M. W.; Sexton, P. M.; Christopoulos, A. Critical Role for the Second Extracellular Loop in the Binding of Both Orthosteric and Allosteric G Protein-coupled Receptor Ligands. *J. Biol. Chem.* **2007**, *282*, 25677–25686.

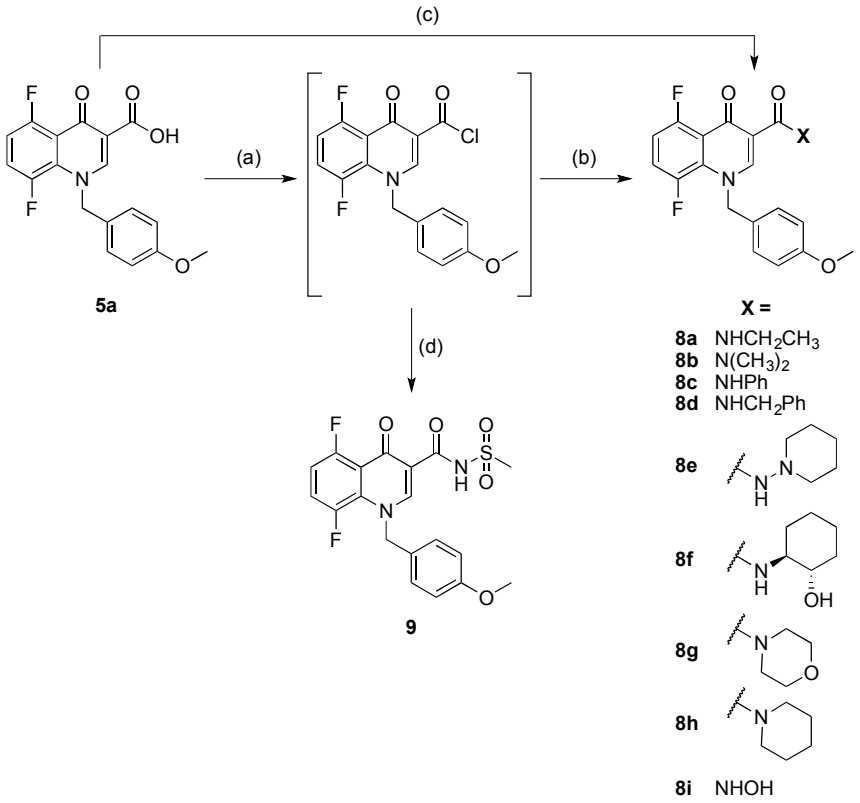
(42) Aurelio, L.; Valant, C.; Flynn, B. L.; Sexton, P. M.; Christopoulos, A.; Scammells, P. J. Allosteric Modulators of the Adenosine A 1Receptor: Synthesis and Pharmacological Evaluation of 4-Substituted 2-Amino-3-benzoylthiophenes. *J. Med. Chem.* **2009**, *52*, 4543–4547.

**Scheme 1: Synthesis of BQCA analogues with varied 5- and 8- substituents**[a]



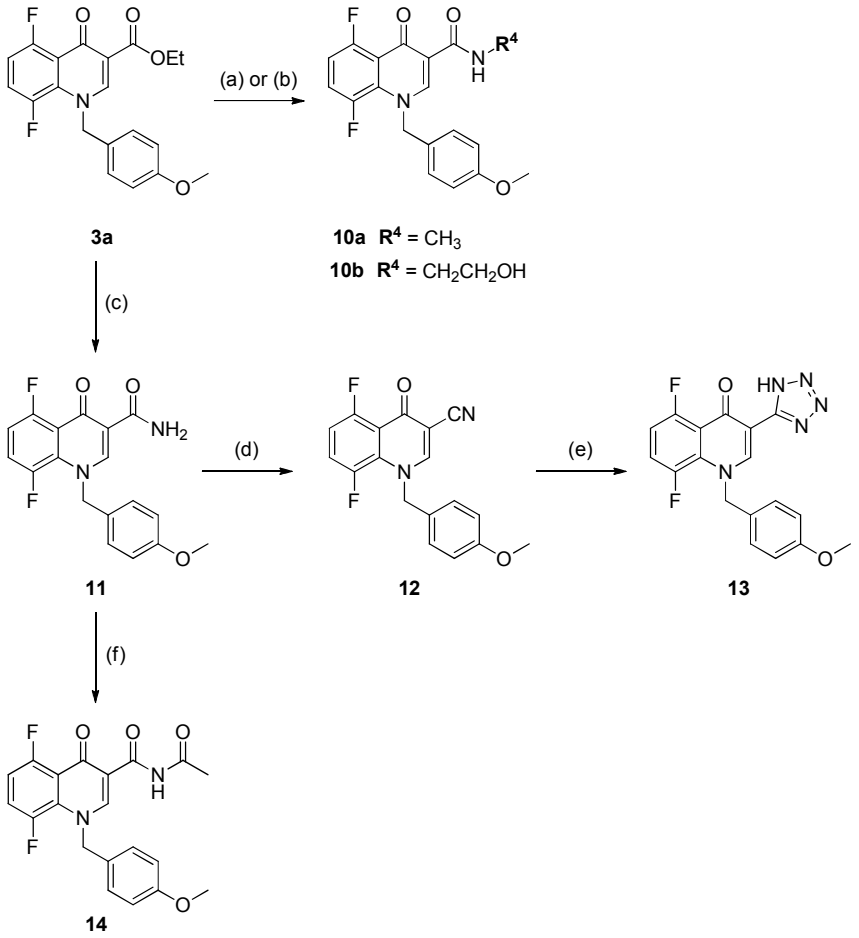

[a]Reagents and conditions: (a) diethylethoxymethylene malonate, 100-120 °C, 71-86%; (b) diethylethoxymethylene malonate, $Et_3N$, 100 °C, 79%; (c) Eaton's reagent, 90-100 °C, 35-84%; (d) $H_2$, 10% Pd/C, EtOH, 100%; (e) alkyl halide, KI, $K_2CO_3$, DMF, rt, 32-86%; (f) 1,4-dioxane/$HCl_{(aq)}$, reflux, 62-90%; (g) $LiOH.H_2O$, THF, water, rt, 49-98%; (h) i. 1 M $NaOH_{(aq)}$, 1,4-dioxane, 80 °C; ii. $LiOH.H_2O$, reflux; iii. microwave 160 °C, 1 hour; 53%.

**Scheme 2: Synthesis of amide/*N*-acyl-sulfonamide derivatives of 5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid**[a]



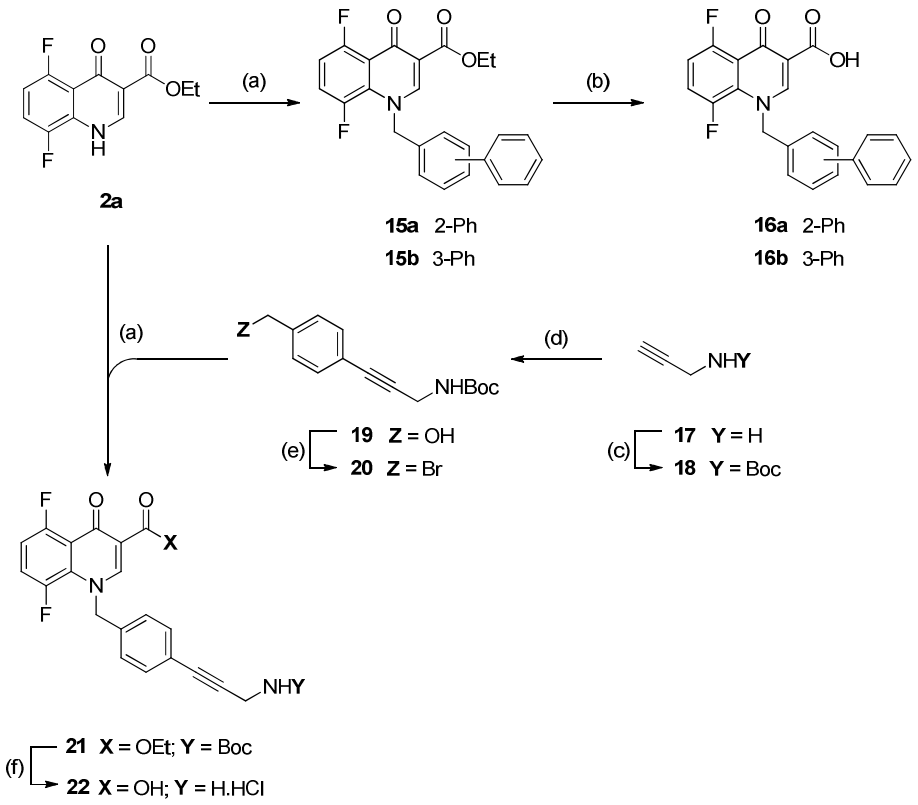

[a]Reagents and conditions: (a) i. DCM, 0 °C; ii. Oxalyl chloride, DMF; (b) amine or amine hydrochloride + TEA, DCM, 18-83%; (c) i. HCTU, amine, DMF; ii. DIPEA, DCM, 62-100%; (d) i. methanesulfonamide, DCM, 0 °C; ii. TEA, 0 °C to rt; 39%.

**Scheme 3 Synthesis of amide and acid isostere analogues of 5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid**[a]



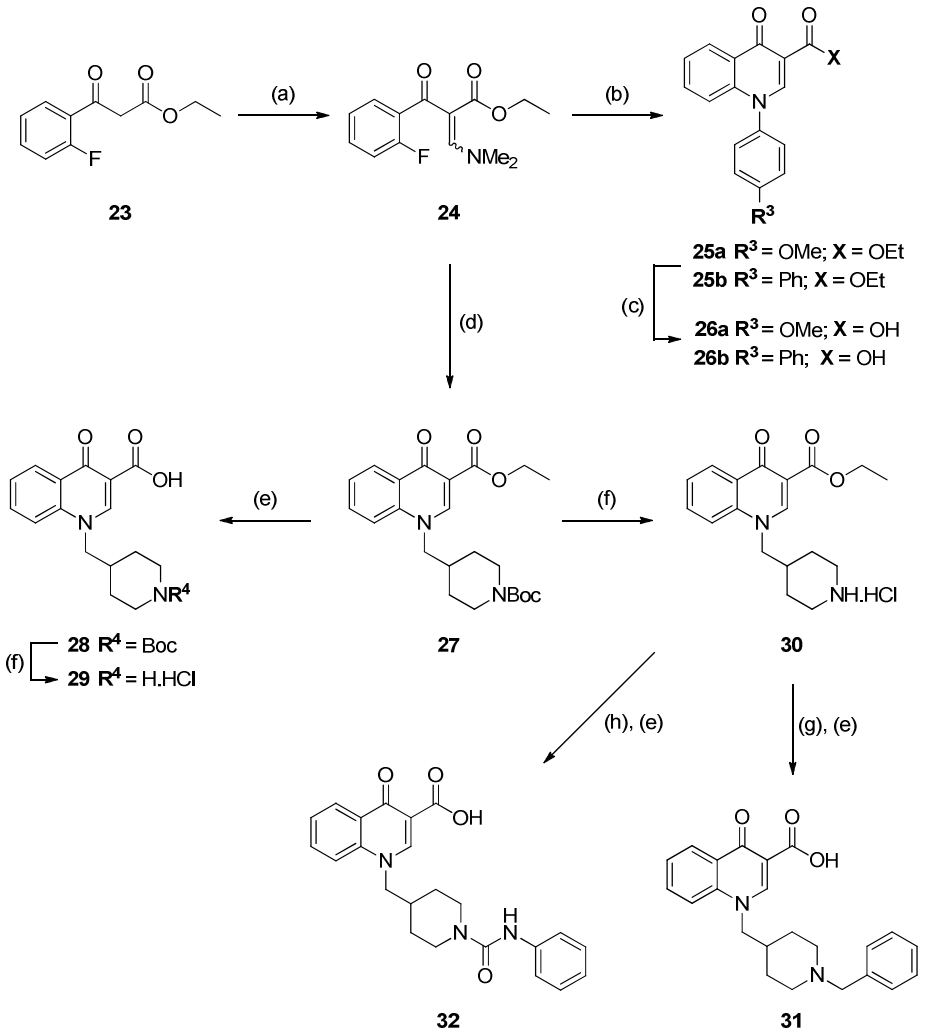

[a]Reagents and conditions: (a) 40% $MeNH_{2\,(aq)}$, EtOH, rt, 83%; (b) Ethanolamine, EtOH, rt, 88%; (c) 28-30% $NH_{3\,(aq)}$, EtOH, rt, 88%; (d) i. $PdCl_2$, MeCN, water, MeOH, THF, rt; ii. 50 °C, 35%; (e) $NaN_3$, TEA.HCl, toluene, reflux, 38%; (f) $Ac_2O$, pyridine, 100 °C, 64%.

**Scheme 4 Synthesis of 5,8-difluoro-1-(substituted-benzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid analogues**[a]




[a]Reagents and conditions: (a) alkyl halide, KI, $K_2CO_3$, DMF, rt, 67-75%; (b) 2 M HCl $_{(aq)}$, 1,4-dioxane, 80 °C to reflux, 91-96%; (c) $Boc_2O$, water, rt, 96%; (d) 4-iodobenzyl alcohol, TEA, CuI, $PdCl_2(PPh_3)_2$, THF, 42%; (e) $PPh_3$, $CBr_4$, DCM, 0 °C to rt, 55%; (f) 1 M HCl $_{(aq)}$, 1,4-dioxane, 80 °C to reflux, 100%.

**Scheme 5 Synthesis of 4-oxo-1,4-dihydroquinoline-3-carboxylic acid bearing *N*-aryl or *N*-(substituted piperidine-4-methyl) groups[a]**




[a]Reagents and conditions: (a) *N,N*-dimethylformamide, dimethyl acetal, DMF, microwave 100 °C, 30 min, 90%; (b) substituted aniline, dry DMF, 140 °C, 61-71%; (c) $LiOH.H_2O$, THF, water, rt, 57-74%; (d) *N*-Boc-(4-aminomethyl)piperidine, dry DMF, 100 °C, 79%; (e) $LiOH.H_2O$, THF, water, rt, 22-87%; (f) 4 M HCl/1,4-dioxane, DCM, 100%; (g) BnBr, $K_2CO_3$, MeCN, rt; (h) phenyl isocyanate, DIPEA, DCM, rt.

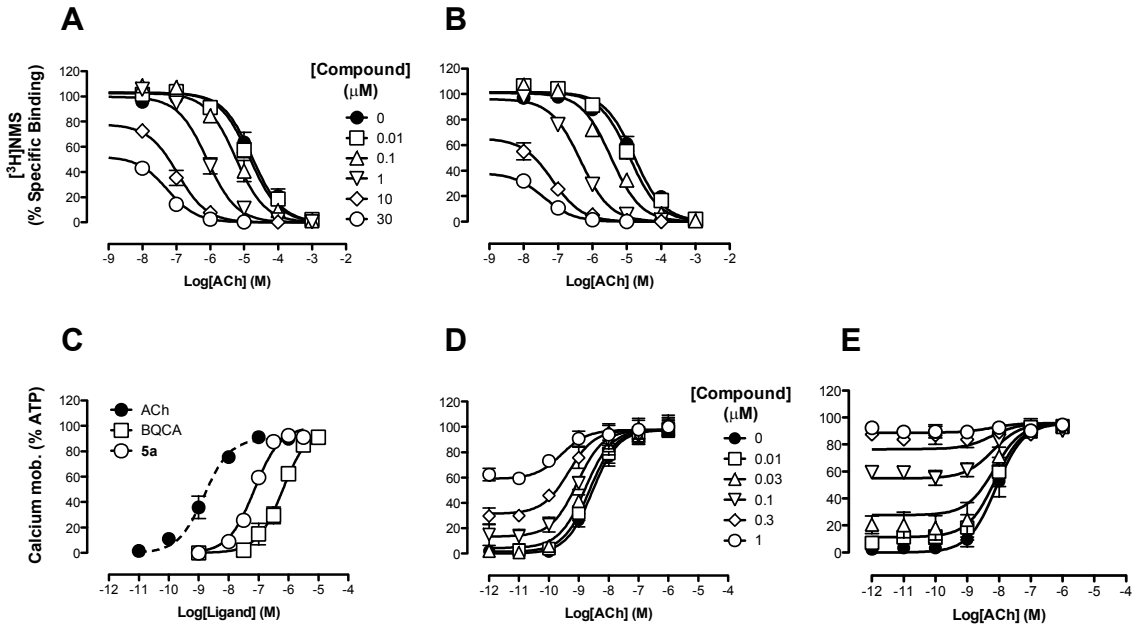
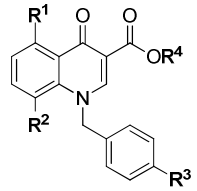


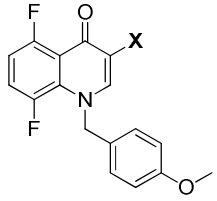

**Figure 1. Pharmacological characterization of BQCA and 5a in radioligand binding and calcium mobilization assays.** (A and B) Radioligand binding experiments using $M_1$-expressing CHO cells in presence of a $K_D$ concentration of the radiolabelled antagonist [$^3$H]-NMS (0.1 nM), increasing concentrations of ACh with or without increasing concentrations of either BQCA (A) or **5a** (B). (C, D and E) Calcium mobilization experiments. (C) Increasing concentration of ACh, BQCA and **5a** alone. (D and E) Increasing concentrations of ACh with or without increasing concentrations of either BQCA (D) or **5a** (E). Values represent the mean ± S.E.M. from at least three to four experiments performed in duplicate.

**Table 1: Binding and functional parameters for analogues of BQCA with varying 5- and 8- substituents, bearing an *N*-(4-methoxybenzyl) or *N*-(4-phenylbenzyl) pendant[a]**




| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | p$K_B$ | Log$\alpha$ ($\alpha$) | Log$\tau_B$ ($\tau_B$) | Log$\alpha\beta$ ($\alpha\beta$) |
|---|---|---|---|---|---|---|---|---|
| **BQCA** | H | H | OMe | H | 4.72 ± 0.07 | 2.60 ± 0.11 (400) | 1.45 ± 0.09 (28) | 2.72 ± 0.13 (525) |
| **3a** | F | F | OMe | Et | 5.12 ± 0.15 | 0.18 ± 0.09 (1.5) | | |
| **3b** | F | OMe | OMe | Et | 4.82 ± 0.28 | −0.10 ± 0.27 (1) | | |
| **3c** | H | OMe | OMe | Et | 4.51 ± 0.04 | −0.99 ± 1.04 (0.1) | | |
| **3d** | OMe | H | OMe | Et | 4.22 ± 0.06 | 1.19 ± 0.15 (16) | | |
| **4a** | F | F | Ph | Et | 3.75 ± 0.10 | 0.63 ± 0.24 (4) | | |
| **4b** | F | OMe | Ph | Et | 5.32 ± 0.11 | 0.59 ± 0.12 (4) | | |
| **4c** | H | OMe | Ph | Et | 4.08 ± 0.07 | 0.69 ± 0.20 (5) | | |
| **4d** | OMe | H | Ph | Et | 5.16 ± 0.15 | 0.70 ± 0.14 (5) | | |
| **5a** | F | F | OMe | H | 4.96 ± 0.13 | 2.62 ± 0.10(420) | 2.18 ± 0.06 (150) | 2.31 ± 0.28 (205) |
| **5b** | F | OMe | OMe | H | 4.86 ± 0.03 | 1.31 ± 0.09 (20) | | |
| **5c** | H | OMe | OMe | H | 4.75 ± 0.05 | 1.68 ± 0.10 (48) | | |
| **5d** | OMe | H | OMe | H | 4.62 ± 0.12 | 2.20 ± 0.14 (160) | 1.75 ± 0.17 (55) | |
| **6a** | F | F | Ph | H | 5.36 ± 0.10 | 3.16 ± 0.13 (1450) | 2.37 ± 0.20 (240) | 2.87 ± 0.24 (740) |
| **6b** | F | OMe | Ph | H | 5.84 ± 0.04 | 0.87 ± 0.12 (7) | | |
| **6c** | H | OMe | Ph | H | 5.71 ± 0.05 | 0.98 ± 0.11 (10) | | |
| **6d** | OMe | H | Ph | H | 5.67 ± 0.10 | 1.70 ± 0.10 (50) | 1.92 ± 0.10 (85) | |
| **7** | OH | F | Ph | H | 5.55 ± 0.17 | 2.41 ± 0.26 (257) | | |

**Table 2: Binding and functional parameters for analogues of 5,8-difluoro-1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid bearing amide or acid isostere groups** [a]



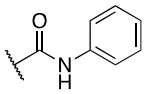
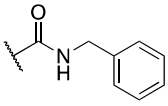
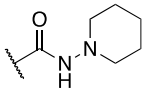
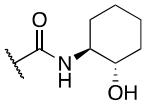
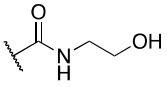
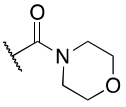
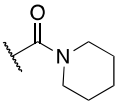
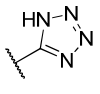
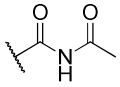
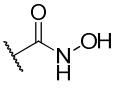
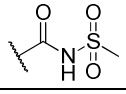
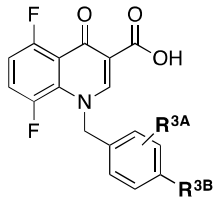
| | X | p$K_B$ | Log$\alpha$ ($\alpha$) | Log$\tau_B$ ($\tau_B$) | Log$\alpha\beta$ ($\alpha\beta$) |
|---|---|---|---|---|---|
| **11** | $CONH_2$ | 5.17 ± 0.12 | 1.51 ± 0.12 (32) | | |
| **10a** | $CONHCH_3$ | 4.92 ± 0.12 | 0.28 ± 0.10 (2) | | |
| **8a** | CONHEt | 5.78 ± 0.18 | 0.17 ± 0.10 (1.5) | | |
| **8b** | $CON(CH_3)_2$ | 4.48 ± 0.19 | 0.85 ± 0.15 (7) | | |
| **8c** |  | 4.80 ± 0.37 | −0.01 ± 0.16 (1.0) | | |
| **8d** |  | 4.63 ± 0.21 | 1.75 ± 0.20 (56) | 1.25 ± 0.20 (18) | |
| **8e** |  | 5.07 ± 0.15 | 1.45 ± 0.16 (28) | | |
| **8f** |  | 4.99 ± 0.07 | 2.22 ± 0.10 (170) | 2.36 ± 0.06 (230) | 2.92 ± 0.06 (840) |
| **10b** |  | 4.65 ± 0.17 | 0.96 ± 0.12 (9) | | |
| **8g** |  | 4.97 ± 0.20 | 0.29 ± 0.13 (2) | | |
| **8h** |  | 4.71 ± 0.24 | 1.26 ± 0.21 (18) | | |
| **12** | CN | 4.66 ± 0.20 | 0.74 ± 0.17 (6) | | |
| **13** |  | 4.97 ± 0.18 | 1.89 ± 0.17 (78) | 0.99 ± 0.07 (10) | |
| **14** |  | 4.35 ± 0.07 | 1.04 ± 0.57 (11) | | |
| **8i** |  | 4.80 ± 0.20 | 1.52 ± 0.17 (33) | | |
| **9** |  | 4.63 ± 0.06 | 1.47 ± 0.19 (30) | | |

**Table 3: Binding and functional parameters for 5,8-difluoro-1-(substituted-benzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid analogues**[a]



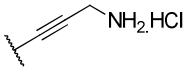
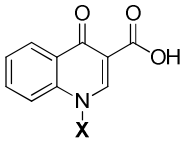
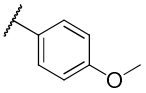
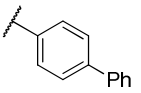
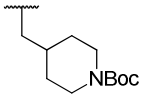
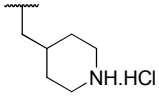
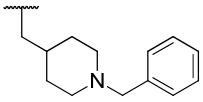
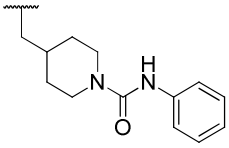
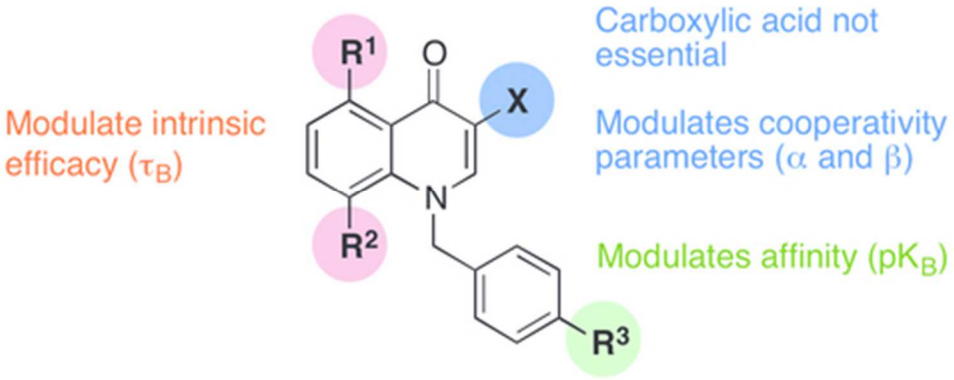

| | $R^{3A}$ | $R^{3B}$ | $pK_B$ | Logα (α) | Log$\tau_B$ ($\tau_B$) | Logαβ (αβ) |
|---|---|---|---|---|---|---|
| **16a** | 2-Ph | H | 5.00 ± 0.04 | 1.87 ± 0.08 (74) | 1.10 ± 0.23 (13) | |
| **16b** | 3-Ph | H | 5.55 ± 0.16 | 1.64 ± 0.15 (44) | | |
| **22** | - |  | 4.96 ± 0.03 | 2.29 ± 0.07 (195) | 1.82 ± 0.14 (65) | 2.35 ± 0.09 (225) |

**Table 4: Binding and functional parameters for 4-oxo-1,4-dihydroquinoline-3-carboxylic acid derivatives bearing *N*-aryl or N-(substituted piperidine-4-methyl) groups**[a]



| | X | $pK_B$ | $Log\alpha_{ACh}$ |
|---|---|---|---|
| **26a** |  | 4.65 ± 0.21 | 0.52 ± 0.15 (3) |
| **26b** |  | 4.73 ± 0.26 | −0.07 ± 0.10 (1) |
| **28** |  | 4.70 ± 0.10 | 0.45 ± 0.09 (3) |
| **29** |  | 4.82 ± 0.12 | 0.17 ± 0.10 (1.5) |
| **31** |  | 5.13 ± 0.05 | −0.02 ± 0.22 (1) |
| **32** |  | 4.66 ± 0.08 | 0.99 ± 0.27 (10) |




Graphical Abstract
40x16mm (300 x 300 DPI)