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## Structure-Activity Relationships and Pharmacophore Model of a Non-Competitive Pyrazoline Containing Class of GluN2C/GluN2D Selective Antagonists

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### Abstract

Here we describe the synthesis and structure-activity relationship for a class of pyrazoline-containing dihydroquinolone negative allosteric modulators of the NMDA receptor that show strong subunit-selectivity for GluN2C- and GluN2D-containing receptors over GluN2A- and GluN2B-containing receptors. Several members of this class inhibit NMDA receptor responses in the nanomolar range, and are more than 50-fold selective over GluN1/GluN2A and GluN1/GluN2B NMDA receptors, as well as AMPA, kainate, GABA, glycine, nicotinic, serotonin, and purinergic receptors. Analysis of the purified enantiomers of one of the more potent and selective compounds shows that the *S*-enantiomer is both more potent and more selective than the *R*-enantiomer. The *S*-enantiomer had an IC<sub>50</sub> value of 0.17–0.22 μM at GluN2D- and GluN2C-containing receptors, respectively, and showed over 70-fold selectivity over other NMDA receptor subunits. The subunit-selectivity of this class of compounds should be useful in defining the role of GluN2C- and GluN2D-containing receptors in specific brain circuits in both physiological and patho-physiological conditions.

### Keywords

NMDA receptor; negative allosteric modulator; glutamate; Parkinson's disease; neuroprotection

### Introduction

Glutamatergic neurotransmission through ionotropic-glutamate receptors is the primary means of excitatory synaptic transmission in the mammalian central nervous system (CNS).

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**Supporting Information Available:** Supporting information including the experimental detail for the synthesis of all intermediates, solubility determination methods and data analysis, table S1 with A-ring modifications, table S2 with hetero-aromatic C-ring substitutions, table S3 with C-ring modifications, table S4 with off-target data from NIMH-PDSP, figure S1 showing mutant receptor responses, figure S2 showing plasma stability, compound solubility and Scheme S1 showing the synthesis of the mono-fluoro butyrate is available free of charge via the Internet at <http://pubs.acs.org>.

The receptor family comprises the  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), N-methyl-D-aspartate (NMDA) and kainate receptors.<sup>1,2</sup> NMDA receptors are widely expressed in the CNS and are thought to be involved in a range of important physiological processes including axonal guidance, synaptic plasticity, and memory formation.<sup>2-5</sup> NMDA receptors are also thought to play an important role in pathophysiological conditions including Parkinson's disease, schizophrenia, depression, and ischemia.<sup>2,6-8</sup>

NMDA receptors mediate the slow component of excitatory synaptic transmission and require the binding of both glutamate and glycine for channel activation. Glycine binds to the GluN1 subunits, which have eight splice variants encoded by a single gene.<sup>9-11</sup> The GluN2 subunits (GluN2A-D) bind glutamate, and are encoded by four distinct genes.<sup>12</sup> The GluN2 subunits control many of the functional and pharmacological properties of the receptor, including agonist EC<sub>50</sub>, single channel open time and open probability, as well as deactivation-time course following removal of glutamate.<sup>2,13-17</sup> NMDA receptor deactivation-time course determines the time course for the slow, Ca<sup>2+</sup>-permeable component of synaptic transmission.<sup>18</sup> Typically, NMDA receptors are blocked by extracellular Mg<sup>2+</sup> at resting membrane potentials, and the requirements of the glutamate release and depolarization-induced relief of Mg<sup>2+</sup> block have led to the idea that the NMDA receptors act as coincidence detectors in the brain.<sup>19,20</sup> The Mg<sup>2+</sup> IC<sub>50</sub> and the kinetics of block and unblock also vary according to the GluN2 subunit.<sup>21</sup>

The GluN1 subunits are expressed throughout the CNS, but GluN2 subunit composition and expression vary both during development and anatomically.<sup>8,22-28</sup> The spatially-restricted expression patterns, together with distinct functional and pharmacological differences imparted by the GluN2 subunits, make NMDA receptor subunit-selective modulators of therapeutic interest for several neurological disorders, including stroke, schizophrenia, treatment-resistant depression and Parkinson's disease.<sup>7,8,25,29</sup> Subunit-selectivity will restrict modulator actions to brain regions that express the subunit of interest, potentially limiting side effects that occur as a result of global NMDA receptor blockade.

We previously have described the discovery, preliminary SAR of 25 compounds and pharmacological mechanism of a representative member of the dihydroquinolone-pyrazoline (DQP) class of GluN2C/D subunit-selective antagonists.<sup>30</sup> In our previous study, the most potent analog, **DQP-1105**, had an IC<sub>50</sub> of 2.7  $\mu$ M at GluN2D-containing NMDA receptors and was 41-fold selective over GluN2B-containing receptors (Figure 1A).<sup>30</sup> In this report we provide an extensive exploration of the SAR, confirmation of mechanism of action, off-target analysis, an analysis of the stereoselectivity for a representative member of the class and data regarding metabolic stability and potential for blood-brain barrier (BBB) penetration. These efforts have yielded potent and selective analogues as well as insights into the pharmacophore for these pyrazoline-containing compounds.

## RESULTS

### Chemistry

The structure-activity relationship around the quinolone-pyrazoline core was probed by testing the potency and selectivity of analogues which contained a variety of aromatic substitutions in combination with perturbations of the acyl chain moiety (Figure 1B). A representative synthesis of these analogs is shown in Scheme 1. Briefly, anthranilic acids were reacted with triphosgene under standard conditions to yield the isatoic anhydride derivatives **80**. These compounds were then converted, via the Weinreb amide **81** and a subsequent lithium halogen exchange reaction with aryl bromides **82**, to the appropriate benzophenones, **83**.<sup>31</sup> The substituted quinolone core was accessed by condensation of **83** with ethyl acetoacetate using microwave irradiation yielding compounds **84**. The resultant methyl ketone underwent base-catalyzed condensation with an appropriate aryl aldehyde, **85**, yielding the  $\alpha,\beta$ -unsaturated ketone compounds **86**. These intermediates could be treated with hydrazine monohydrate in ethanol, utilizing microwave irradiation, to yield the pyrazoline-containing compounds typified by **87**. The pyrazoline amine was then functionalized with succinic anhydride (**88**), glutaric anhydride (**89**), or maleic anhydride (**90**) to yield the fully saturated or *cis*-double bond acyl chain derivatives (Scheme 1).

Standard esterification conditions from compound **4** yielded the saturated mono-methyl ester analog, **56** (Scheme 2). Reduction of the acid in compound **26** with  $\text{BH}_3\text{-DMS}$  led to the primary alcohol containing compound, **64**; a coupling reaction with compound **26** and  $\text{NH}_3$  gave the primary amide compound, **65** (Scheme 2). The unsaturated fumaric esters could be accessed under standard amide coupling conditions using (*E*)-4-methoxy-4-oxobut-2-enoic acid and compound **55f**, yielding compound **54g**, or with acylation of **22f** using (*E*)-methyl 4-chloro-4-oxobut-2-enoate, yielding compound **60** (Scheme 2). Additionally, the acyl chain was replaced with the alkyl chain by reacting the pyrazoline derivative **26f** and methyl 4-oxobutanoate under reductive amination conditions to give compound **66** (Scheme 2). Mono-fluoro butyrate **68c** (Scheme S1) was synthesized in three steps and also coupled to compound **26f** yielding **68** as an isostere of the hydroxyl-containing compound **64** (Scheme 2).<sup>32, 33</sup> The ester containing compounds, **54g**, **60** and **66**, could be saponified under basic conditions yielding the target scaffolds (**54**, **58** and **67**, Scheme 2). All compounds were assayed for activity using two-electrode voltage clamp recordings from *Xenopus laevis* oocytes recombinantly expressing the desired NMDA receptor subtypes (see *Methods*).

### A-Ring substituent optimization

We first evaluated the effect of substituents on the A-phenyl ring (Figure 1B) by holding the chlorine substitution on the quinolone core constant and evaluating the substitutions shown in Tables 1 and S1. At the A-ring, 4-substituted phenyl derivatives resulted in the best potency and selectivity. This observation led to the identification of **5** (Table 1), with a nitro group at R<sub>7</sub>, which was the most potent *para*-substituted analogue at GluN2D-containing receptors compared to the un-substituted A-ring, compound **1** (1.1  $\mu\text{M}$  vs. 88  $\mu\text{M}$ , respectively, Table 1). Realizing the substantial liabilities associated with the nitro group, we explored bio-isosteres of the substitution; replacing this group with a carboxylic acid **6** (Table 1) which showed no activity. By contrast, the methyl ester **7** (Table 1) remained

active but had decreased potency at GluN2D-containing receptors (32  $\mu\text{M}$  vs. 1.1  $\mu\text{M}$ ). Interestingly,  $\text{sp}^3$  hybridization is tolerated, but not preferred, as can be seen with the trifluoromethyl-containing compound **8** ( $\text{IC}_{50}$  4.1  $\mu\text{M}$ , GluN2D, Table 1). Although substitution at any of the three available positions on the A-ring is tolerated, substitution at either the  $\text{R}_5$  or  $\text{R}_6$  position showed no improvement in potency or selectivity with any of the analogs tested (**12–20, 71**; Table S1). Similarly, analogs with the A-ring replaced with furan, thiophene and pyridine substituents were evaluated and were inactive (Table S2).

A manual Hansch analysis, similar to that of the Topliss approach, was employed to better understand the physicochemical properties governing potency.<sup>34</sup> Analysis of the steric,  $\sigma$  and  $\pi$  substituent effects for the A-ring suggests that only the *para*- $\sigma$  contribution is directly associated with the  $\text{IC}_{50}$  values ( $r^2=0.75$ ,  $p < 0.05$  Pearson two-tailed correlation analysis) at the GluN2C- and GluN2D-containing NMDA receptors (**Compounds 2–5** and **8–10**, Table 1 and Figure 2A).<sup>34, 35</sup>

### B-Ring substituent optimization

We next modified the B-ring substituents with the aim of understanding the substituent effects at the *meta*- and *para*-positions (Figure 1B).<sup>34</sup> The *para*-bromo substitutions at the A- and B-rings in compound **21** showed enhanced potency at GluN2C- and GluN2D-containing receptors, with  $\text{IC}_{50}$  values of 0.71  $\mu\text{M}$  and 0.39  $\mu\text{M}$  respectively (Table 2). Interestingly, this compound also showed less selectivity for GluN2D- over GluN2A-containing receptors (33-fold) as compared to GluN2B-containing receptors (59-fold), suggesting a more favorable interaction with GluN2A-containing receptors had been formed (Table 2).

Co-varying A-ring *para*-substituents with the *para*-bromo B-ring substitution allowed us to determine that the *para*-chloro A-ring substitution was optimal for potency (**22–24**, Table 2). A similar trend was observed using the *para*-chloro-substitution on the B-ring while co-varying the A-ring substituents (**25–28**, Table 2). Therefore, this *para*-chloro-substitution on the A-ring was used for further SAR elaboration. We varied substituents at the *meta*- and *para*- position of the B-ring which identified numerous analogs that were highly potent and selective (Table 3). Notably, the *para*-fluoro containing compound **29** and the *meta*-fluoro containing compound **34** were both potent congeners that showed over 90-fold selectivity for GluN2D- over GluN2B-containing receptors; compound **34** also showed 67-fold selectivity over GluN2A-containing receptors (Table 3).

The Hansch evaluation of the *para*- $\sigma$  substituent effects at the B-ring show a seemingly parabolic relationship when compared to potency for only GluN2A-, GluN2C- and GluN2D-containing receptors (**Compounds 26** and **29–33**, Table 3, Figure 2B and C), with an optimal  $\sigma$  value corresponding to the bromo- and chloro- substitutions at all three receptors. At the *meta*-position of the B-ring, the correlation between the potency and the hydrophobic  $\pi$  value for substitutions at GluN2A-containing receptors also appears parabolic (**Compounds 34–39**, Table 3, Figure 2D). The decrease in potency at GluN2A-containing receptors observed with compound **39**, which was *meta*-substituted with the  $\text{CF}_3$  group (Table 3 and Figure 2D), could be a result of steric clashes with the receptor, or, as was

observed with the *para*- $\sigma$  substituents, could suggest that the optimal hydrophobicity at the GluN2A-containing receptors is attained with the *meta*-chloro substitution.

From this analysis of *meta*- and *para*-substitutions, we hypothesized that combining an optimal *para*-substitution for potency at GluN2D-containing receptors with a *meta*-substitution that was less active at GluN2A-containing receptors might improve selectivity. Both Cl- and F- substitutions gave optimal potency and selectivity when mono-substituted on the B-ring, leading us to co-vary these groups (Table 4). We synthesized compound **40**, which has a *meta*-fluoro and *para*-chloro substitution pattern on the B-ring. This compound maintained potency but did not increase selectivity (Table 4). Several other compounds that were di-substituted on the B-ring exhibited submicromolar potency at GluN2D-containing receptors, but all showed modest selectivity over GluN2A-containing receptors (**41–45**, Table 4).

### C-Ring substitutions with optimized A- and B-ring substituents

We next made a series of substitutions to the C-ring on the quinolone core (Table S3). Beginning with a methyl group at R<sub>1</sub> (Figure 1B) in combination with either the *para*-chloro- or the *meta*-fluoro-substitution on the B-ring, we synthesized compounds **46** and **47**, which decreased the potency as compared to the more favorable compounds with only B-ring and A-ring substitutions (Table S3). Interestingly, the modifications showed variability with regards to the relative selectivity for GluN2A- over GluN2B-containing receptors, suggesting that there remains room in this portion of the binding pocket for potential optimization of selectivity (**Compounds 48–53**, Table S3).

### Acyl-chain perturbations

We subsequently evaluated a series of perturbations to the acyl chain of the pyrazoline nitrogen (Table 5). Restricting the conformation to a *cis*-configuration with the maleate derivative maintained similar potency to the parent compound in each instance tested (**55** and **59** vs. **4** and **22** respectively, Table 5). The *trans*-fumaric derivative **58** was the most potent compound identified, but was no more selective over GluN2A- or GluN2B-containing receptors than the saturated derivative **22** (Tables 5 and 2). The succinic ester containing compound, **56**, was inactive, as was the fumaric ester, **60** (Table 5). We also evaluated glutaric-containing derivatives such as compound **61** (Table 5), which showed similar potencies to that of the succinic derivative **22** (Tables 5 and 2) at all receptors tested, suggesting that the length of the acyl chain is not crucial for activity.

The primary alcohol containing compound, **64**, retained similar activity as that of the parent compound **26** at GluN2C- and GluN2D-containing receptors (IC<sub>50</sub> of 1.7 and 0.69  $\mu$ M, respectively) while improving selectivity over GluN2A-containing receptors to 90-fold (Table 5). The primary amide derivative of the succinate acyl chain in compound **65** retained activity but showed decreased potency and selectivity compared to the alcohol and acid moieties (Table 5). Replacing the amide linkage to the pyrazoline with the alkyl derivative in compound **67** slightly diminished potency at GluN2D-containing receptors over the parent compound **26**, but maintained selectivity over the other receptor subtypes (Table 5 and 2). The mono-fluoro isostere of the hydroxyl-group in compound **64** was tested with

compound **68** (Table 5). While this compound retained the ability to accept a hydrogen bond, it was inactive.

### Stereochemical preference of a representative analog

Finally, we evaluated the selectivity and potency of purified enantiomers for a representative member of this class of compounds. The enantiomers of the racemic final product **26** were separable via reverse phase chiral chromatography using an OD-RH column (Figure 3, see *Methods*). Absolute stereochemistry of the second peak to elute during the enantiomeric resolution was assigned using X-ray crystallography as the *R*-enantiomer (**70**) (Figure 3, see *Methods*). Evaluation of the purified enantiomers showed that the *S*-enantiomer, **69**, is 11-fold more potent at GluN2D-containing receptors ( $IC_{50}$  0.17  $\mu$ M) than the *R*-enantiomer, **70** ( $IC_{50}$  1.9  $\mu$ M; Figure 4 and Table 6). In addition, **69** shows enhanced selectivity for GluN2C- and GluN2D- over GluN2A- and GluN2B-containing receptors as compared to the racemic **26** and the *R*-enantiomer, compound **70**, due to the enhanced potency at the GluN2D-containing receptors (Figure 4 and Table 6).

### Mechanism and site of action

A previous study of a representative member suggested that this class of compounds inhibits NMDA receptor function in a voltage-independent and non-competitive manner.<sup>30</sup> We confirmed this mechanism for compound **58**. Inhibition of GluN1/GluN2D responses by 1  $\mu$ M **58** was not surmounted by increasing both glutamate and glycine from 30  $\mu$ M to 3 mM ( $4.1 \pm 0.58\%$  of control in 30  $\mu$ M,  $3.1 \pm 0.50\%$  of control in 3 mM;  $n=6$ , unpaired t-test), implying that the compound is non-competitive at the glutamate and glycine binding sites. Moreover, inhibition produced by 1  $\mu$ M **58** was not significantly different at  $-40$  or  $+30$  mV ( $4.9 \pm 1.3\%$  and  $4.8 \pm 1.2\%$  of control, respectively,  $n=8$ , Student's t-test,  $p=0.93$ ), suggesting that the receptor blockade by this more potent compound is also voltage-independent. We also examined whether these more potent analogues interacted with the binding site first identified for both the previously described **DQP-1105** (Figure 1A) and the quinazoline-4-one (QNZ), **QNZ-46**.<sup>30, 36</sup> Mutations to the wild type GluN2D receptor (Q801Y, L705F and A752V), previously shown to decrease sensitivity of the GluN2D receptor to blockade by either **DQP-1105** and/or **QNZ-46** in the membrane proximal region of the bi-lobed ligand binding domain encoded by the S2 region of the polypeptide chain were evaluated.<sup>30, 36</sup> A test of the effectiveness of racemic **26** revealed that these mutants each significantly reduced the degree of inhibition, consistent with it acting at a similar site as **DQP-1105** and **QNZ-46** (Figure S1).

### Evaluation of off-target effects

We next evaluated the off-target actions for the racemic compounds **26** and **58** in a series of two-electrode voltage-clamp recordings using recombinant ligand-gated ion channels expressed in *Xenopus* oocytes (Table 7, see *Methods*). Compounds **26** and **58** were tested at 3  $\mu$ M on the AMPA receptors (GluA1–4), kainate receptors (GluK1–2 and GluK2/GluK5), the serotonin receptor (5HT<sub>3A</sub>), the gamma-amino butyric acid receptors (GABA<sub>A</sub> and GABA<sub>C</sub>), the glycine receptor (glycine  $\alpha$ 1), nicotinic acetylcholine receptors comprised of  $\alpha_1\beta_1\delta\gamma$ ,  $\alpha_3\beta_4$ ,  $\alpha_4\beta_2$ ,  $\alpha_7$ , or  $\alpha_9/\alpha_{10}$ , and purinergic P2<sub>X2</sub> receptors. Of the ion channel classes



evaluated, compounds **26** and **58** altered agonist-induced currents by less than 10%, with the exception of the nicotinic acetylcholine receptors, which exhibited 13–28% inhibition (Table 7).

We also tested the actions of racemic compounds **26** and **58** at 5  $\mu\text{M}$  on 42 different ion channels, G-protein coupled receptors, and transporters via the National Institutes of Mental Health (NIMH) psychoactive drug screening program (PDSP; Supplemental Table S4). The primary binding assay demonstrated that compounds **26** and **58** had a minimal effect on the receptors and transporters, with initial screens showing inhibition of three receptors by **26** (5HT<sub>6</sub>, H<sub>2</sub>, kappa-opioid) and four receptors by **58** (5-HT<sub>1E</sub>, 5-HT<sub>6</sub>, kappa-opioid, mu-opioid). For both compounds, the  $K_i$  values at these receptors were greater than 10  $\mu\text{M}$  on all receptors. The data collected from both the two-electrode voltage-clamp experiments and the PDSP demonstrate the utility of this class of compounds as selective inhibitors of the GluN2C- and GluN2D-containing NMDA receptors.

### Plasma stability, aqueous solubility, BBB penetration and human liver microsomal stability

Three of the more potent analogs, **58**, **26** and **64** were evaluated for plasma stability. The compounds showed minimal degradation in human, rat or mouse plasma over a two-hour time-course (Figure S2). The aqueous solubility of compound **26** was evaluated in oocyte recording buffer using nephelometry and assessed to be soluble at > 80  $\mu\text{M}$  (see *Methods* and Supplemental pg. S8).

The topological polar surface area (TPSA) of the carboxylic acid compounds was calculated to be outside the optimal range (< 90  $\text{\AA}^2$ ) for blood-brain barrier (BBB) penetration.<sup>37</sup> However, reduction of the acid to the alcohol moves the properties of this class closer to a typical range for CNS penetration (**64**; 102.0  $\text{\AA}^2$ , QikProp).<sup>37</sup> In order to assess the potential for BBB penetration, compounds **26** and **64** were selected for evaluation in the MDR1-MDCK permeability assay which has been demonstrated to accurately predict BBB penetration due to the overexpression of p-glycoprotein (P-gp) and high transepithelial electrical resistance of the cell line (Table 8).<sup>38</sup> As was anticipated with the carboxylic acid containing **26**, the potential for BBB penetration was low (Table 8). The results for the hydroxyl-containing compound **64** also suggested low BBB potential; however, the permeability coefficient ( $P_{\text{app}}$  A–B) was much closer to the recommended  $3.0 \times 10^{-6}$  cm/s (**64**, ( $P_{\text{app}}$  A–B;  $2.46 \times 10^{-6}$  cm/s)) than that of the carboxylic acid-containing compound (**26**, ( $P_{\text{app}}$  A–B;  $0.47 \times 10^{-6}$  cm/s)), suggesting that efflux may be problematic with this congener.<sup>38</sup> In order to evaluate an analog with lower TPSA, the mono-fluoro-containing compound, **68**, (Table 5, TPSA 79.08  $\text{\AA}^2$ ; QikProp) was assessed in the MDR1-MDCK assay and was classified as being highly brain penetrable ( $P_{\text{app}}$  A–B;  $3.88 \times 10^{-6}$  cm/s;  $P_{\text{app}}$  B–A;  $9.52 \times 10^{-6}$  cm/s; Table 8).<sup>38</sup>

The same compounds were also evaluated for metabolic stability using human liver microsomes. While the carboxylic acid containing **26** showed minimal degradation over the 60 minute assay, the hydroxyl-containing derivative **64** had a half-life of 13 minutes (Table 9). The half-life of the mono-fluoro compound, **68**, was determined to be 35 minutes in the

human liver microsomal assay (Table 9). These data suggest that the acyl-chain is a candidate for further optimization of desirable pharmacokinetic properties.

## Discussion and Conclusion

This study describes the development of potent, selective, and soluble negative allosteric modulators for GluN2C- and GluN2D-containing NMDA receptors that act on the membrane proximal lobe of the GluN2 glutamate binding domain. We describe here several compounds with  $IC_{50}$  values in the 100–500 nanomolar range that show 50–200 fold selectivity over GluN2A- and GluN2B-containing receptors. We have taken a classical approach to the SAR, allowing proposal of features for a hypothetical pharmacophore. The A-ring substituents that were explored directly correlate potency with the *para*- $\sigma$  substituent coefficients at only GluN2C- and GluN2D-containing receptors, when  $R_1$  was substituted with chloro (Figure 2A and 5A). While we maintained selectivity with many of the analogs, our results suggest there is a conserved portion of the binding pocket among GluN2A-, GluN2C- and GluN2D-containing receptors with respect to the *para*-position of the B-ring; the electronic effects relative to the  $\sigma$  substituent coefficient show similar trends when plotted as a function of potency (Figure 2B, C and 5A). We visualized the electron density of these analogs by carrying out Hartree-Fock calculations using the 6-31G\* basis set. While several of the analogs substituted at the *para*-position of the B-ring could theoretically accept a hydrogen bond, only the bromo- and chloro-containing compounds (**26** and **21**) exhibit an accessible deficiency of electron density at the terminal position of the substitutions. We hypothesize that the apparent sigma hole found at the bromo and chloro atoms could enhance potency by forming a halogen bond with an oxygen electron donor at the receptors (Figure 5B).<sup>39, 40</sup> Furthermore, the SAR has revealed that rigidifying the acyl-chain in the trans-conformation can enhance potency at the GluN2D-containing receptors, while the length of the chain was found not to be crucial for activity (Figure 5A). The finding that the hydroxyl-containing compound **64** (Table 5) retains both potency and selectivity suggests that the charge on the carboxylic acid is not crucial for either property. While it would be ideal to interpret these data in the context of the receptor, no high quality crystallographic data exists for the region of the LBD where these compounds are thought to act, making docking studies challenging.

We expect a reduction in molecular weight in conjunction with further optimization of the topological polar surface area will be required to obtain optimal BBB penetration and pharmacokinetic properties. However, the improvements in potency and selectivity suggest this class of compounds should be useful as pharmacologic probes to evaluate the contributions of the GluN2C- and GluN2D-containing NMDA receptors in normal and patho-physiologic processes in isolated systems.

The GluN2C- and GluN2D-NMDA receptor subunits remain understudied, largely because of a lack of potent and selective pharmacological tools. However, these NMDA receptor subunits reside in a number of brain regions that are highly relevant for neurological disease. For example, expression of functional GluN2D in the subthalamic nuclei raises the possibility that GluN2D-selective inhibitors could attenuate neuronal firing rate and alter firing patterns in subthalamic neurons, which could be of utility in Parkinson's



disease.<sup>22, 24, 26, 28, 41–44</sup> In addition, expression of GluN2D-containing receptors in substantia nigra pars compacta neurons raises the possibility that GluN2D-selective antagonists might possess neuro-protective properties in Parkinson's disease by diminishing  $\text{Ca}^{2+}$  influx into the dopaminergic substantia nigra pars compacta neurons, which may lead to neuronal death.<sup>8, 45</sup> GluN2C is expressed widely in the cerebellum, and has also been suggested to have a role in both emotional learning and schizophrenia.<sup>46–48</sup> The compounds described here could therefore be tools with which to evaluate GluN2C- and GluN2D-containing receptor function in specific circuits implicated in these conditions.

## BIOLOGY EXPERIMENTAL

### Two-electrode voltage-clamp electrophysiology

The Emory University Institutional Animal Care and Use Committee approved all protocols involving the use of animals. *Xenopus laevis* oocytes were isolated and maintained as previously described.<sup>36</sup> The cDNAs for the desired NMDA receptor subunits (GenBank accession numbers U11418 and U08261; GluN1, D13211; GluN2A, U11419; GluN2B, M91563; GluN2C, L31611; GluN2D) were obtained from Drs. S. Heinemann (Salk Institute), S. Nakanishi (Kyoto University), and P. Seeburg (University of Heidelberg). Preparation of cRNA, injection of oocytes with RNA, and two-electrode voltage clamp recordings have been described elsewhere.<sup>30, 36, 49</sup> Briefly, oocytes were placed in a perfusion chamber with recording solution comprised of (in mM) 90 NaCl, 1 KCl, 0.5  $\text{BaCl}_2$ , 0.005 EDTA and 10 HEPES, pH adjusted to 7.4 with NaOH at 23 °C. The glass electrodes used had tip resistances of 0.5–2.0 M $\Omega$  and were filled with 0.3–3.0 M KCl. Compounds were made as 20 mM stock solutions in DMSO and diluted to final concentrations in recording solution (final DMSO was 0.1–0.05% vol/vol). The current recordings were performed using a Warner OC-725B or C amplifier at a holding potential of –40 mV.

Subunit selectivity was determined by recording from various ligand-gated ion channels expressed in *Xenopus laevis* oocytes as previously described.<sup>50</sup> The cDNA encoding GABA<sub>A</sub>, GABA<sub>C</sub> and glycine receptor cDNAs were provided by Dr. Weiss (University of Texas Health Science Center San Antonio), nicotinic acetylcholine and 5-HT<sub>3A</sub> serotonin receptor cDNAs were provided by Drs. Papke and Heinemann (University of Florida and Salk Institute), and purinergic receptor cDNA were provided by Dr. Hume (University of Michigan). The glutamate receptors GluA1–4, GluK1, and GluK2 were activated by 100  $\mu\text{M}$  glutamate. GluK1 and GluK2 expressing oocytes were incubated for 5 minutes in 1 mg/ml of concanavalin A prior to recording. The GluK2/5 receptor was activated with 100  $\mu\text{M}$  AMPA (2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid). The GABA<sub>A</sub> and GABA<sub>C</sub> receptors were activated by 20  $\mu\text{M}$  and 2  $\mu\text{M}$  *gamma*-aminobutyric acid, respectively. Acetylcholine was used activate the nicotinic acetylcholine  $\alpha_1\beta_1\delta\gamma$  (1  $\mu\text{M}$ ),  $\alpha_3\beta_4$  (10  $\mu\text{M}$ ),  $\alpha_2\beta_4$  (10  $\mu\text{M}$ ),  $\alpha_7$  (300  $\mu\text{M}$ ),  $\alpha_9\alpha_{10}$  (10  $\mu\text{M}$ ) receptors. The glycine  $\alpha_1$  and 5-HT<sub>3A</sub> currents were evoked by 50  $\mu\text{M}$  glycine receptor and 3  $\mu\text{M}$  serotonin, respectively. The human and rat P2X<sub>2</sub> purinergic receptors were activated with 9  $\mu\text{M}$  adenosine triphosphate.

The receptor binding profiles (Supplemental Table S2) and  $K_i$  determinations for compounds **26** and **58** was generously provided by the National Institute of Mental Health Psychoactive Drug Screening Program, Contract # HHSN-271-2008-025C (NIMH PDSP). The NIMH PDSP is directed by Bryan L. Roth MD, Ph.D. at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA. Data was collected using 5  $\mu\text{M}$  of compounds **26** and **58**.

### MDR1-MDCK Permeability

Cell monolayers were grown to confluence on collagen-coated, microporous, polycarbonate membranes in 12-well Costar Transwell<sup>®</sup> plates. The permeability assay buffer was Hanks Balanced Salt Solution containing 10 mM HEPES and 15 mM glucose at pH 7.4. The buffer in the receiver chamber also contained 1% bovine serum albumin. The dosing solution concentration in the assay buffer was 5  $\mu\text{M}$  for each compound tested. The cell monolayers were dosed on the apical side (A-to-B) or basolateral side (B-to-A) and incubated at 37 °C with 5% CO<sub>2</sub> in a humidified incubator. Samples were taken from the donor and receiver chambers at 120 minutes. Each determination was performed in duplicate. All samples were assayed by LC-MS/MS using electrospray ionization. The apparent permeability ( $P_{\text{app}}$ ) and percent recovery were calculated as follows.

$$P_{\text{app}} = (dC_r/dt) \times V_r / (A \times C_A) \quad (1)$$

$$\text{Percent recovery} = 100 \times ((V_r \times C_r^{\text{final}}) + (V_d \times C_d^{\text{final}})) / (V_d \times C_N) \quad (2)$$

where  $dC_r/dt$  is the slope of the cumulative concentration in the receiver compartment versus time in  $\mu\text{M s}^{-1}$ ;  $V_r$  is the volume in the receiver compartment in  $\text{cm}^3$ ;  $V_d$  is the volume in the donor compartment in  $\text{cm}^3$ ;  $A$  is the area of the insert ( $1.13 \text{ cm}^2$  for 12-well Transwell<sup>®</sup>);  $C_A$  is the average of the nominal dosing concentration and the measured 120 minute donor concentration in  $\mu\text{M}$ ;  $C_N$  is the nominal concentration of the dosing solution in  $\mu\text{M}$ ;  $C_r^{\text{final}}$  is the cumulative receiver concentration in  $\mu\text{M}$  at the end of the incubation period;  $C_d^{\text{final}}$  is the concentration of the donor in  $\mu\text{M}$  at the end of the incubation period. The MDR1-MDCK permeability assays were performed by Absorption Systems.

### Human liver microsomal stability

Human liver microsomes were obtained from XenoTech. The reaction mixture was prepared with 0.5 mg/mL human liver microsomes, 100 mM potassium phosphate (pH 7.4), 5 mM magnesium chloride, 1  $\mu\text{M}$  test compound. The reaction mixture was incubated in a shaking water bath at 37 °C for 3 minutes prior to the addition of NADPH (1 mM). Testosterone was run simultaneously in a separate vessel as a control. 100  $\mu\text{l}$  aliquots were taken at 0, 10, 20, 30 and 60 minutes for both test compound and testosterone. The aliquots were combined immediately with 400  $\mu\text{l}$  of ice cold 50/50 acetonitrile/deionized H<sub>2</sub>O containing 0.1% formic acid and internal standard to terminate the reaction. The samples were then mixed and centrifuged to precipitate microsomal proteins. All samples were assayed by LC-MS/MS using electrospray ionization and multiple reaction monitoring and the peak area responses to internal standard of the compounds at each time point was compared to the peak area

response at time 0 to determine the percent compound remaining. The human liver microsomal stability assays were performed by Absorption Systems.

### Data analysis

Potency of compounds was assessed by fitting the composite concentration-response curve obtained from the average of multiple recordings with the equation

$$Response = 100 / (1 + ([inhibitor\ concentration] / IC_{50})^N) \quad (3)$$

where  $IC_{50}$  is the concentration of compound that is required to inhibit the response half-maximally and  $N$  is the hill slope. Saturating conditions were assumed to give complete inhibition. Data for compounds that did not inhibit the steady-state current by at least 30% were not fit by the above equation and are designated as NE in the data tables.

## CHEMISTRY EXPERIMENTAL

Commercial vendors provided compounds **4**, **5**, **9–11**, **14**, **16**, **17**, **20**, **57** and **62**, that are not described below, which were 90% purity, as provided by the vendor, or determined independently as below. All reagents were obtained from commercial suppliers and used without further purification unless otherwise noted. Reaction progress was monitored by thin layer chromatography (TLC) on pre-coated glass plates (silica gel 60 F254, 0.25 mm). Proton, carbon and fluorine NMR spectra were recorded on INOVA-400 (400 MHz), VNMRS 400 (400 MHz), UNITY-600 (600 MHz), or INOVA-600 (600 MHz) instruments. Proton and carbon spectra were referenced to the residual solvent peak while fluorine spectra were referenced to trifluoroacetic acid residual peak. The Emory University Mass Spectrometry Center collected mass spectral data on either a VG 70-S Nier Johnson or JEOL instrument. Compound purity was assessed by reverse phase liquid chromatography using an Agilent Zorbax, 3.5  $\mu$ m, XD B-C18 column, 4.6  $\times$  50 mm (254 nm), or by elemental analyses, performed by Atlantic Microlab Inc. Purity for all compounds synthesized and tested was at or above 95% unless otherwise noted. Flash chromatography was performed on a Teledyne ISCO Combiflash Companion with pre-packaged disposable normal phase silica columns.

### Computational Analysis

Energy minimized conformations of the compounds being analyzed were generated using the OPLS\_2005 force field in MacroModel (MacroModel, version 9.9, Schrödinger, LLC, New York, NY, 2011). The TPSA approximations obtained from QikProp (QikProp, version 3.4, Schrödinger, LLC, New York, NY, 2011) using energy minimized conformations, as above. The Hartree-Fock calculations were carried out using the neutral compounds and the 6-31G\* basis set in Spartan '10 (Spartan '10, Wavefunction, Inc., Irvine, CA). The equilibrium geometry at the ground state in vacuum was calculated. For visualization purposes, the energy range in the electrostatic potential maps was limited from -100.00 to 280.00 kJ/mol.

## Separation and X-Ray Crystallography of Enantiomers

Separation of the final compounds used for biological testing from the racemic **26** was obtained using a ChiralPak OD-RH 30 mm X 250 mm, 5 $\mu$ m column using the following conditions: Flow rate 10 ml/min, injection volume 4–6 ml (2 mg/ml), 60% acetonitrile (0.1% Formic acid) : 40% H<sub>2</sub>O (0.1% Formic acid); **69** *R<sub>t</sub>*, 21.8; **70** *R<sub>t</sub>*, 25.1 minutes. The enantiomeric excess (e.e.) of the enantiomers, **69** and **70**, was determined using an Agilent 1200 HPLC pump on a ChiralPak OD-RH column (4.6 mm  $\times$  150 mm, 5 $\mu$ m) using the following conditions: flow rate 0.5 ml/min, injection volume 10  $\mu$ l, 60% acetonitrile (0.1% Formic acid) : 40% H<sub>2</sub>O (0.1% Formic acid); **69** (**S-26**,  $[\alpha]_D^{20}$  -34.0 (*c* = 0.32 mg/ml, chloroform), *R<sub>t</sub>*, 7.47 min, 100% e.e. **70** (**R-26**)  $[\alpha]_D^{20}$  + 36.0 (*c* = 0.245 mg/ml, MeOH), *R<sub>t</sub>*, 8.79 min, 98% e.e. Optical rotation data were collected using a Perkin-Elmer 314 instrument. The proton NMR spectrum was identical to that of racemic **26** for each enantiomer.

Single crystals of the second peak to elute from the separation of racemic **26** (**70**; retention time: 25.1 min.) were grown by slow evaporation of a solution of the compound in a mixture of methanol and water. Crystal data: C<sub>28</sub>H<sub>23</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>5</sub>, (*M* = 552.39): 1.124  $\times$  0.087  $\times$  0.056, orthorhombic, space group *P* 2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> (no.19), *a* = 8.0529(5) Å, *b* = 10.2097(5) Å, *c* = 31.2978(13) Å, *V* = 2573.2(2) Å<sup>3</sup>, *Z* = 4,  $\mu$ (MoK $\alpha$ ) = 0.315 mm<sup>-1</sup>, *D*<sub>calc</sub> = 1.490 g/mm<sup>3</sup>; temperature 173 K. Intensity data were collected on a Bruker APEX II CCD diffractometer with monochromated MoK $\alpha$  radiation ( $\lambda$  = 0.71073 Å) at 173 K, in the 2 $\theta$  range 2.6–53.4°. The user interface Olex2 was used for the crystallographic calculations and crystal structure visualization.<sup>51</sup> The structure was solved with Superflip by charge flipping and refined by least squares minimization using Shelxl.<sup>52,53</sup> A total of 15745 reflections were measured (2.602  $\leq$  2 $\theta$   $\leq$  53.41) while 5408 unique data (*R<sub>int</sub>* = 0.124) were used in the refinements. The final *R*<sub>1</sub> was 0.0590 (*I* > 2 $\sigma$ (*I*)) and the weighted *R* value *wR*<sub>2</sub> was 0.0874 (all data).

## General procedure for the synthesis of acylated quinolone-pyrazoline products (Procedure G)

In an appropriately sized microwaveable vessel, the pyrazol-3-yl-quinolin-2(1H)-one intermediate (1.00 equiv) was dissolved in anhydrous tetrahydrofuran (THF) (0.15 M) with 4 Å molecular sieves present. The appropriate anhydride (1.00 equiv) was added. The solution was microwaved (Biotage Initiator) with stirring for 20 minutes at 165 °C. The THF was removed under vacuum, and the organics were dissolved in dichloromethane (DCM), washed three times with acidified (pH 2, HCl) brine, dried over magnesium sulfate, filtered, concentrated under reduced pressure and subjected to flash column chromatography using a 0–10% MeOH:DCM gradient unless otherwise noted.

### 4-[3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobutanoic acid (**2**)

Compound **2** was prepared according to general procedure G using succinic anhydride (**88**) (0.062 g, 0.622 mmol) and **2f** (0.260 g, 0.622 mmol). After removal of the THF, the residue was dissolved in hot EtOAc and small portions of hexanes were added until a solid began to form. The solid was filtered and column chromatographed using 0–10% MeOH:DCM and

the title compound was obtained as a yellow solid. Yield 0.236 g, 73.2%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.42 (s, 1H), 12.15 (s, 1H), 7.68 – 7.38 (m, 6H), 7.27 (d, *J* = 7.4 Hz, 1H), 7.04 (td, *J* = 8.8, 2.4 Hz, 2H), 6.93 (d, *J* = 2.5 Hz, 1H), 6.85 – 6.76 (m, 2H), 5.35 – 5.30 (m, 1H), 3.80 – 3.67 (m, 1H), 2.83 – 2.74 (m, 1H), 2.48 – 2.39 (m, 2H), 2.34 – 2.25 (m, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 173.59, 168.66, 160.13, 152.45, 149.96, 138.39, 137.34, 134.56, 131.25, 129.47, 128.51, 127.55, 126.09, 124.64, 120.68, 117.65, 115.26, 115.05, 58.24, 45.23, 28.92, 28.59, 28.22. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –116.13 – –116.20 (m). HRMS (*m/z*): [M-H]<sup>–</sup> calcd for C<sub>28</sub>H<sub>20</sub>ClN<sub>3</sub>O<sub>4</sub>F, 516.11319; found, 516.11246. Anal. Calcd for C<sub>28</sub>H<sub>21</sub>ClN<sub>3</sub>O<sub>4</sub>F: C, 64.93; H, 4.09; N, 8.11. Found C, 61.01; H, 4.22; N, 7.15. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>t</sub> 0.95 min; > 95% purity; 75% ACN:H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>t</sub> 0.72 min; > 95% purity.

#### 4-[3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobutanoic acid (3)

Compound **3** was prepared according to general procedure G using **87** (0.046 g, 0.460 mmol) and **3f** (0.200 g, 0.460 mmol). The title compound was obtained by removing the THF, dissolving the crude mixture into hot EtOAc and adding small portions of hot hexanes until a yellow precipitate formed. The mixture was allowed to cool and filtered to give the title compound as a yellow solid. Yield 0.187 g, 76.0 %. <sup>1</sup>H NMR (400 MHz DMSO-*d*<sub>6</sub>) δ 12.42 (s, 1H), 12.15 (s, 1H), 7.64 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.61 – 7.38 (m, 5H), 7.27 (d, *J* = 7.9 Hz, 3H), 6.94 (d, *J* = 2.5 Hz, 1H), 6.79 (d, *J* = 8.2 Hz, 2H), 5.33 (dd, *J* = 12.1, 4.7 Hz, 1H), 3.75 (dd, *J* = 18.5, 12.0 Hz, 1H), 2.78 (dd, *J* = 18.4, 4.7 Hz, 1H), 2.49 – 2.23 (m, 4H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 173.55, 168.67, 160.09, 152.42, 149.96, 141.13, 137.32, 134.54, 131.59, 131.24, 129.47, 128.57, 128.46, 128.37, 127.38, 126.13, 126.06, 124.56, 120.66, 117.64, 58.29, 45.12, 28.57, 28.23. HRMS (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>28</sub>H<sub>22</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>4</sub>, 534.09819, found; 534.09774. Anal. Calcd for C<sub>28</sub>H<sub>21</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>4</sub>·0.40H<sub>2</sub>O: C, 62.09; H, 4.06; N: 7.76. Found C, 61.90; H, 4.13; N: 7.64.

#### 4-[1-(3-Carboxypropanoyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-5-yl]benzoic acid (6)

Compound **6** was prepared according to general procedure G using **87** (0.045 g, 0.451 mmol) and **6f** (0.200 g, 0.451 mmol). The solvent was removed and the product was obtained by dissolving in hot EtOAc and adding hexanes until a solid began to precipitate. The solution was cooled and the product filtered to yield a white solid. Yield 0.040 g, 16.3 %. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.42 (bs, 3H), 7.79 (d, *J* = 8.0 Hz, 2H), 7.64 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.60 – 7.41 (m, 5H), 7.27 (d, *J* = 7.4 Hz, 1H), 6.96 – 6.82 (m, 3H), 5.39 (dd, *J* = 12.0, 4.8 Hz, 1H), 3.79 (dd, *J* = 18.4, 12.2 Hz, 1H), 2.79 (dd, *J* = 18.4, 4.7 Hz, 1H), 2.61 – 2.37 (m, 2H), 2.30 (t, *J* = 6.9 Hz, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 173.51, 172.19, 168.72, 167.04, 160.09, 152.41, 149.99, 146.92, 137.33, 134.56, 131.25, 129.68, 129.55, 129.51, 128.52, 128.48, 128.38, 126.12, 126.07, 125.54, 124.53, 120.68, 117.65, 58.71, 45.12, 28.53, 28.22. HRMS (*m/z*): [M+Na]<sup>+</sup> calcd for C<sub>29</sub>H<sub>22</sub>ClN<sub>3</sub>O<sub>6</sub>Na, 566.10893; found, 566.10923. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>t</sub> 0.71 min; > 95% purity; 75% ACN:H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>t</sub> 0.53 min; > 95% purity.

**4-{3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-[4-(methoxycarbonyl)phenyl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (7)**

Compound **7** was prepared according to general procedure G using **87** (0.055 g, 0.546 mmol) and **7f** (0.250 g, 0.546 mmol). The title compound was purified using flash chromatography (2–10% MeOH:DCM), followed by precipitation from hot EtOAc using hot hexanes. Yield 0.040 g, 13.1%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.38 (s, 1H), 12.06 (s, 1H), 7.84 – 7.72 (m, 2H), 7.64 – 7.35 (m, 6H), 7.22 (d, *J* = 7.4 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 3H), 5.36 (dd, *J* = 12.3, 4.6 Hz, 1H), 3.90 – 3.69 (m, 4H), 2.74 (dd, *J* = 18.5, 4.6 Hz, 1H), 2.51 – 2.33 (m, 2H), 2.30 – 2.21 (m, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 173.53, 168.77, 165.96, 160.10, 152.43, 150.02, 147.43, 137.34, 134.59, 131.29, 129.44, 128.41, 126.14, 125.76, 124.50, 120.67, 117.67, 58.68, 52.16, 45.09, 28.52, 28.22. HRMS (*m/z*): [M + Na]<sup>+</sup> calcd for C<sub>30</sub>H<sub>24</sub>ClN<sub>3</sub>O<sub>6</sub>Na, 580.12458; found; 580.12484. Anal. Calcd for C<sub>30</sub>H<sub>24</sub>ClN<sub>3</sub>O<sub>6</sub>·0.80H<sub>2</sub>O: C, 62.95; H, 4.51; N, 7.34. Found C, 63.04; H, 4.55; N, 7.36.

**4-{3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-[4-(trifluoromethyl)phenyl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (8)**

Compound **8** was prepared according to general procedure G using **87** (0.043 g, 0.427 mmol) and **8f** (0.200 g, 0.427 mmol). The title compound was obtained after flash chromatography (2–10% MeOH:DCM) followed by trituration from EtOAc as a yellow solid. Yield 0.030 g, 12.4 %. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.42 (s, 1H), 12.04 (s, 1H), 7.65 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.61 – 7.40 (m, 7H), 7.26 (d, *J* = 7.4 Hz, 1H), 6.99 (d, *J* = 8.0 Hz, 2H), 6.93 (d, *J* = 2.3 Hz, 1H), 5.43 (dd, *J* = 12.2, 4.7 Hz, 1H), 3.79 (dd, *J* = 18.5, 12.2 Hz, 1H), 2.80 (dd, *J* = 18.6, 4.7 Hz, 1H), 2.60 – 2.40 (m, 2H), 2.29 (t, *J* = 6.8 Hz, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 174.14, 169.46, 160.72, 153.09, 150.68, 147.32, 138.01, 135.23, 131.92, 130.09, 129.22, 129.12, 129.05, 126.88, 126.79, 126.72, 126.05, 125.13, 121.31, 118.31, 59.19, 45.74, 29.19, 28.89. HRMS (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>29</sub>H<sub>22</sub>ClN<sub>3</sub>O<sub>4</sub>F<sub>3</sub>, 568.12455; found, 568.12554. C<sub>29</sub>H<sub>21</sub>ClN<sub>3</sub>O<sub>4</sub>F<sub>3</sub>·0.07EtOAc; HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>f</sub> 1.1 min; > 95% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>f</sub> 0.86 min; > 95% purity.

**4-[3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobutanoic acid (9)**

Compound **9** was prepared according to general procedure G using **87** (0.058 g, 0.582 mmol) and **9f** (0.250 g, 0.582 mmol). After removal of the THF, the title compound was obtained by dissolving the crude mixture into hot EtOAc and adding small portions of hot hexanes until a yellow precipitate formed, the mixture was allowed to cool and filtered to give the title compound as a yellow solid. Yield 0.132 g, 42.8%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.40 (s, 1H), 12.16 (s, 1H), 7.64 (dd, *J* = 8.9, 2.4 Hz, 1H), 7.60 - 7.49 (m, 3H), 7.45 (d, *J* = 8.7 Hz, 1H), 7.43 – 7.38 (m, 1H), 7.29 (d, *J* = 7.0 Hz, 1H), 6.93 (d, *J* = 2.5 Hz, 1H), 6.82-6.66 (m, 4H), 5.24 (dd, *J* = 12.1, 4.6 Hz, 1H), 3.89 - 3.62 (m, 4H), 2.78 (dd, *J* = 18.3, 4.6 Hz, 1H), 2.45 (t, *J* = 7.0 Hz, 2H), 2.27 (t, *J* = 6.9 Hz, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 173.58, 168.49, 160.14, 158.22, 152.39, 149.86, 137.30, 134.58, 134.31, 131.22, 129.42, 128.57, 128.43, 128.35, 126.77, 126.09, 126.02, 124.74, 120.71, 117.63, 113.71, 58.47, 55.10, 45.27, 28.63, 28.24. HRMS (*m/z*): [M+Na]<sup>+</sup> calcd for



$C_{29}H_{24}ClN_3O_5Na$ , 552.12967; found, 552.13018. Anal. Calcd for  $C_{29}H_{24}ClN_3O_5 \cdot 1.00H_2O$ : C, 63.56; H, 4.78; N, 7.67. Found C, 63.68; H, 4.57; N, 7.59.

#### 4-[3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(3-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobutanoic acid (**12**)

Compound **12** was prepared according to general procedure G using **87** (0.120 g, 1.20 mmol) and **12f** (0.500 g, 1.20 mmol). After removal of the THF, the title compound was obtained by precipitation from EtOAc using hexanes, as a yellow solid. Yield 0.380 g, 62%.  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.42 (s, 1H), 12.15 (s, 1H), 7.65 (dd,  $J = 8.7, 2.6$  Hz, 1H), 7.53 (m, 2H), 7.44 (m, 3H), 7.26 (d,  $J = 7.0$  Hz, 2H), 7.03 (t,  $J = 8.7$  Hz, 1H), 6.93 (d,  $J = 2.3$  Hz, 1H), 6.64 (dd,  $J = 12.3, 8.8$  Hz, 2H), 5.35 (dd,  $J = 12.3, 4.4$  Hz, 1H), 3.75 (dd,  $J = 18.6, 12.1$  Hz, 1H), 2.81 (dd,  $J = 17.7, 4.7$  Hz, 1H), 2.60 – 2.23 (m, 4H).  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  173.57, 168.75, 163.36, 160.94, 160.12, 152.47, 149.98, 144.95, 144.88, 137.33, 134.56, 131.25, 130.52, 130.45, 129.40, 128.56, 128.42, 128.38, 128.29, 126.14, 126.07, 124.53, 121.50, 120.65, 117.65, 114.00, 113.79, 112.34, 112.13, 58.41, 45.14, 28.53, 28.20. HRMS ( $m/z$ ):  $[M-H]^-$  calcd for  $C_{28}H_{20}ClN_3O_4F$ ; 516.11319; found, 516.11239. Anal. Calcd for  $C_{28}H_{21}ClN_3O_4F \cdot 0.40H_2O$ : C, 64.93; H, 4.09; N, 8.11. Found C, 64.11; H, 4.03; N, 7.94. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  0.97 min; > 95% purity; 75% ACN:H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  0.73 min; > 95% purity.

#### 4-[3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobutanoic acid (**13**)

Compound **13** was prepared according to general procedure G using **87** (0.046 g, 0.460 mmol) and **13f** (0.200 g, 0.460 mmol). After removal of the THF, the title compound was obtained by precipitating from EtOAc using hexanes, as a yellow solid. Yield 0.156 g, 63.4%.  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.39 (s, 1H), 12.04 (s, 1H), 7.64 – 7.57 (m, 1H), 7.56 – 7.34 (m, 5H), 7.27 – 7.17 (m, 3H), 6.97 – 6.87 (m, 2H), 6.68 (d,  $J = 6.8$  Hz, 1H), 5.29 (dd,  $J = 12.2, 4.8$  Hz, 1H), 3.71 (dd,  $J = 18.5, 12.0$  Hz, 1H), 2.84 – 2.73 (m, 1H), 2.50 – 2.32 (m, 2H), 2.25 (t,  $J = 6.7$  Hz, 2H).  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  173.55, 168.78, 152.53, 150.00, 144.54, 137.35, 134.62, 133.05, 130.47, 129.37, 128.60, 128.45, 127.14, 126.15, 125.51, 124.51, 124.11, 120.64, 58.40, 45.10, 28.53, 28.18. HRMS ( $m/z$ ):  $[M+Na]^+$  calcd for  $C_{28}H_{21}Cl_2N_3O_4Na$ ; 556.08013; found, 556.07988. Anal. Calcd for  $C_{28}H_{21}Cl_2N_3O_4 \cdot 0.20H_2O$ : C, 61.68; H, 4.10; N, 7.71. Found C, 61.74; H, 4.27; N, 7.32.

#### 4-[3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobutanoic acid (**15**)

Compound **15** was prepared according to general procedure G using **87** (0.029 g, 0.29 mmol) and **15f** (0.13 g, 0.29 mmol). The title compound was purified using flash chromatography (2–10% MeOH:DCM) and isolated as a yellow solid. Yield 0.090 g, 58.7%.  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.41 (s, 1H), 12.08 (s, 1H), 7.64 (dt,  $J = 8.8, 2.1$  Hz, 1H), 7.58 – 7.42 (m, 4H), 7.39 (d,  $J = 7.3$  Hz, 1H), 7.27 (d,  $J = 7.3$  Hz, 1H), 7.13 (t,  $J = 7.9$  Hz, 1H), 6.93 (d,  $J = 2.2$  Hz, 1H), 6.77 (dd,  $J = 8.2, 2.4$  Hz, 1H), 6.59 (s, 1H), 6.32 (d,  $J = 7.7$  Hz, 1H), 5.27 (dd,  $J = 12.0, 4.7$  Hz, 1H), 3.77 – 3.63 (m, 4H), 2.85 (dd,  $J = 18.4, 4.7$  Hz, 1H), 2.48 – 2.36 (m, 2H), 2.28 (t,  $J = 6.8$  Hz, 2H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$

173.54, 168.64, 160.16, 159.25, 152.47, 149.91, 143.75, 137.32, 134.64, 131.21, 129.59, 129.15, 128.59, 128.48, 128.37, 128.30, 126.09, 124.56, 120.68, 117.63, 117.39, 112.37, 111.35, 58.88, 54.98, 45.23, 28.56, 28.22. HRMS ( $m/z$ ):  $[M-H]^-$  calcd for  $C_{29}H_{23}ClN_3O_5$ , 528.13317; found, 528.13351. Anal. Calcd for  $C_{29}H_{23}ClN_3O_5 \cdot 0.70H_2O$ : C, 64.32; H, 4.54; N, 7.76. Found C, 64.38; H, 4.67; N: 7.67.

#### 4-[3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobutanoic acid (**18**)

Compound **18** was prepared according to general procedure G using **87** (0.046 g, 0.460 mmol) and **18f** (0.200 g, 0.460 mmol). After removal of the THF, the title compound was obtained by precipitation from EtOAc using hexanes, as a yellow solid. Yield 0.136 g, 55.3%.  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.40 (s, 1H), 12.13 (s, 1H), 7.63 (dd,  $J = 8.6, 1.6$  Hz, 1H), 7.56-7.49 (m, 2H), 7.45-7.41 (m, 4H), 7.26 (t,  $J = 7.6$  Hz, 1H), 7.19-7.15 (m, 2H), 6.93 (s, 1H), 6.41 (d,  $J = 7.4$  Hz, 1H), 3.87 (dd,  $J = 18.4, 12.4$  Hz, 1H), 2.75 (dd,  $J = 18.2, 4.7$  Hz, 1H), 2.60-2.54 (m, 1H), 2.46-2.32 (m, 2H), 2.39-2.30 (m, 2H).  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  173.55, 168.78, 160.02, 152.56, 150.10, 138.66, 134.68, 131.27, 130.52, 129.36, 128.78, 128.37, 128.58, 126.13, 125.90, 124.33, 120.66, 117.64, 56.40, 44.20, 28.43, 28.22. HRMS ( $m/z$ ):  $[M+Na]^+$  calcd for  $C_{28}H_{21}Cl_2N_3O_4Na$ , 556.08013; found 556.08038. Anal. Calcd for  $C_{28}H_{21}Cl_2N_3O_4 \cdot 0.30H_2O$ : C, 62.30; H, 4.03; N, 7.78. Found C, 62.27; H, 4.32; N, 7.49.

#### 4-[5-(2-Bromophenyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobutanoic acid (**19**)

Compound **19** was prepared according to general procedure G using **87** (0.063 g, 0.627 mmol) and **19f** (0.300 g, 0.627 mmol). After removal of the THF, the title compound was obtained by precipitation from EtOAc and hexanes, as a yellow solid. Yield 0.140 g, 38.6%.  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.41 (s, 1H), 12.05 (s, 1H), 7.64 (dd,  $J = 8.9, 2.5$  Hz, 1H), 7.59 (d,  $J = 7.5$  Hz, 1H), 7.53 (p,  $J = 7.6$  Hz, 2H), 7.42 (dd,  $J = 11.1, 7.9$  Hz, 3H), 7.26 – 7.15 (m, 3H), 6.93 (d,  $J = 2.3$  Hz, 1H), 6.39 – 6.32 (m, 1H), 5.48 (dd,  $J = 12.1, 4.6$  Hz, 1H), 3.87 (dd,  $J = 18.3, 12.0$  Hz, 1H), 2.73 (dd,  $J = 18.4, 4.6$  Hz, 1H), 2.62 – 2.51 (m, 1H), 2.49 – 2.28 (m, 3H).  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  173.53, 168.76, 160.06, 152.42, 150.11, 140.19, 137.33, 132.58, 131.25, 129.08, 128.41, 126.12, 124.30, 120.64, 117.63, 58.69, 44.33, 28.41, 28.21. HRMS ( $m/z$ ):  $[M+Na]^+$  calcd for  $C_{28}H_{21}ClBrN_3O_4Na$ ; 600.02962, found 600.02945. Anal. Calcd for  $C_{28}H_{21}ClBrN_3O_4$ : C, 58.10; H, 3.66; N, 7.26. Found C, 54.48; H, 3.54; N, 6.55. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  1.17 min; > 95% purity; 75% ACN:H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  1.17 min; > 95% purity.

#### 4-[5-(4-Bromophenyl)-3-[4-(4-bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobutanoic acid (**21**)

Compound **21** was prepared according to general procedure G using **87** (0.057 g, 0.573 mmol) and **21f** (0.300 g, 0.573 mmol). There was a yellow solid present in the reaction vessel which was filtered, dried and determined to be the title compound. Yield 0.320 g, 90%.  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.30 (s, 1H), 12.18 (s, 1H), 7.75 (d,  $J = 8.2$  Hz, 1H), 7.66 (d,  $J = 8.2$  Hz, 1H), 7.58 (t,  $J = 7.8$  Hz, 1H), 7.48 – 7.39 (m, 3H), 7.36 (d,  $J = 8.1$

Hz, 1H), 7.22 (d,  $J = 8.1$  Hz, 1H), 7.15 (t,  $J = 7.7$  Hz, 1H), 7.03 (d,  $J = 8.2$  Hz, 1H), 6.74 (d,  $J = 7.9$  Hz, 2H), 5.33 (dd,  $J = 11.8, 4.4$  Hz, 1H), 3.74 (dd,  $J = 18.7, 12.1$  Hz, 1H), 2.76 (dd,  $J = 18.5, 4.4$  Hz, 1H), 2.65 – 2.39 (m, 2H), 2.39 – 2.30 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  173.52, 168.65, 160.14, 152.69, 149.89, 141.52, 138.55, 134.40, 131.73, 131.45, 131.24, 130.82, 127.63, 127.28, 123.41, 122.41, 121.82, 120.09, 119.07, 115.59, 58.28, 45.14, 28.49, 28.23. HRMS ( $m/z$ ):  $[\text{M}-\text{H}]^-$  calcd for  $\text{C}_{28}\text{H}_{20}\text{Br}_2\text{N}_3\text{O}_4$ , 619.98260; found, 619.98231. Anal. Calcd for  $\text{C}_{28}\text{H}_{21}\text{Br}_2\text{N}_3\text{O}_4$ : C, 53.96; H, 3.40; N, 6.74. Found C, 52.23; H, 3.67; N, 5.55. HPLC 85% MeOH:H<sub>2</sub>O 0.1% Formic Acid)  $R_t$  1.2 min; > 95% purity; 75% ACN:H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  0.84 min; > 94% purity.

#### 4-{3-[4-(4-Bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (22)

Compound **22** was prepared according to general procedure G using **87** (0.105 g, 1.04 mmol) and **22f** (0.500 g, 1.044 mmol). After removal of the THF, the residue was partitioned between EtOAc and acidified brine. The organics were washed three times, dried over magnesium sulfate and concentrated under vacuum. The title compound was obtained after column chromatography using 10% MeOH:DCM. Yield 0.240 g, 39.7%.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.29 (s, 1H), 12.05 (s, 1H), 7.74 (dd,  $J = 8.1, 2.2$  Hz, 1H), 7.65 (dd,  $J = 8.1, 2.2$  Hz, 1H), 7.58 (t,  $J = 7.6$  Hz, 1H), 7.42 (d,  $J = 8.3$  Hz, 1H), 7.36 (dd,  $J = 8.1, 2.3$  Hz, 1H), 7.34 - 7.25 (m, 2H), 7.22 (dd,  $J = 8.2, 2.3$  Hz, 1H), 7.15 (t,  $J = 7.6$  Hz, 1H), 7.03 (d,  $J = 8.1$  Hz, 1H), 6.85 - 6.75 (m, 2H), 5.35 (dd,  $J = 11.9, 4.4$  Hz, 1H), 3.73 (dd,  $J = 18.4, 12.1$  Hz, 1H), 2.76 (dd,  $J = 18.6, 4.4$  Hz, 1H), 2.65 - 2.42 (m, 2H), 2.34 (t,  $J = 6.7$  Hz, 2H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  173.54, 168.69, 160.17, 152.70, 149.90, 141.10, 138.58, 134.42, 131.61, 131.45, 131.27, 130.84, 128.34, 127.30, 123.44, 122.42, 119.08, 115.60, 58.23, 45.16, 28.48, 28.23. HRMS ( $m/z$ ):  $[\text{M}-\text{H}]^-$  calcd for  $\text{C}_{28}\text{H}_{20}\text{BrClN}_3\text{O}_4$ , 576.03312; found, 576.03267. Anal. Calcd for  $\text{C}_{28}\text{H}_{21}\text{BrClN}_3\text{O}_4 \cdot 0.50\text{H}_2\text{O}$ : C, 57.21; H, 3.77; N, 7.15. Found C, 57.02; H, 3.72; N, 7.05.

#### 4-{3-[4-(4-Bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (23)

Compound **23** was prepared according to general procedure G using **87** (0.054 g, 0.541 mmol) and **23f** (0.250 g, 0.541 mmol). The title compound, a yellow solid, was obtained after purifying using flash chromatography (0–10% MeOH:DCM). Yield 0.100 g, 32.9%.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.29 (s, 1H), 12.11 (s, 1H), 7.74 (d,  $J = 8.0$  Hz, 1H), 7.66 (d,  $J = 8.2$  Hz, 1H), 7.58 (t,  $J = 7.7$  Hz, 1H), 7.43 (d,  $J = 8.3$  Hz, 1H), 7.36 (d,  $J = 8.0$  Hz, 1H), 7.23 (d,  $J = 8.2$  Hz, 1H), 7.15 (t,  $J = 7.6$  Hz, 1H), 7.05 (t,  $J = 8.9$  Hz, 3H), 6.83 (dd,  $J = 8.4, 5.2$  Hz, 2H), 5.35 (dd,  $J = 12.0, 4.4$  Hz, 1H), 3.72 (dd,  $J = 18.5, 12.0$  Hz, 1H), 2.78 (dd,  $J = 18.4, 4.4$  Hz, 1H), 2.65 - 2.43 (m, 2H), 2.34 (t,  $J = 6.7$  Hz, 2H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  173.56, 168.64, 160.16, 152.71, 149.87, 138.56, 138.36, 134.42, 131.69, 131.46, 131.24, 130.86, 127.46, 127.38, 127.29, 123.47, 122.43, 121.80, 119.08, 115.59, 115.18, 114.96, 58.15, 45.26, 28.50, 28.23.  $^{19}\text{F}$  NMR (376 MHz, DMSO- $d_6$ )  $\delta$  -116.06 – -116.19 (m). HRMS ( $m/z$ ):  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{28}\text{H}_{22}\text{BrFN}_3\text{O}_4$ , 562.07722, found; 562.07669. Anal. Calcd for  $\text{C}_{28}\text{H}_{21}\text{BrFN}_3\text{O}_4$ : C, 59.80; H, 3.76; N, 7.47. Found C, 50.97; H,

3.55; N, 6.00. HPLC 75–95% MeOH:H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>t</sub> 0.89 min; > 95% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>t</sub> 0.67 min; > 95% purity.

#### 4-{3-[4-(4-Bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (24)

Compound **24** was prepared according to general procedure G using **87** (0.023 g, 0.23 mmol) and **24f** (0.100 g, 0.23 mmol). The title compound was obtained after purifying using flash chromatography (0–10% MeOH:DCM) as an off-white solid. Yield 0.110 g, 86%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.29 (s, 1H), 12.20 (s, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.67 – 7.54 (m, 2H), 7.47 – 7.34 (m, 2H), 7.23 (d, *J* = 5.4 Hz, 4H), 7.15 (t, *J* = 7.6 Hz, 1H), 7.03 (d, *J* = 8.3 Hz, 1H), 6.77 (d, *J* = 6.7 Hz, 2H), 5.32 (dd, *J* = 11.8, 4.7 Hz, 1H), 3.75 (dd, *J* = 18.2, 11.9 Hz, 1H), 2.77 (dd, *J* = 18.3, 4.5 Hz, 1H), 2.62 – 2.41 (m, 2H), 2.33 (t, *J* = 6.7 Hz, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 173.54, 168.57, 160.17, 152.68, 149.84, 142.25, 138.54, 134.42, 131.74, 131.40, 131.25, 131.16, 130.84, 128.36, 127.28, 127.03, 125.33, 123.55, 122.39, 121.77, 119.11, 115.57, 58.87, 45.39, 28.54, 28.24. HRMS (*m/z*): [M-H]<sup>-</sup> calcd for C<sub>28</sub>H<sub>21</sub>BrN<sub>3</sub>O<sub>4</sub>, 542.07209; found, 542.07235. Anal. Calcd for C<sub>28</sub>H<sub>22</sub>BrN<sub>3</sub>O<sub>4</sub>: C, 61.89; H, 3.89; N, 7.73. Found C, 56.02; H, 4.02; N, 6.78. HPLC 95% MeOH:H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>t</sub> 0.91 min; > 95% purity; 65% ACN: H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>t</sub> 0.70 min; > 95% purity.

#### 4-{5-(4-Bromophenyl)-3-[4-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (25)

Compound **25** was prepared according to general procedure G using **87** (0.031 g, 0.31 mmol) and **25f** (0.150 g, 0.31 mmol). The title compound was obtained after purifying using flash chromatography (0–10% MeOH:DCM) as an off-white solid. Yield 0.048 g, 27%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.30 (s, 1H), 7.64 – 7.48 (m, 2H), 7.42 (t, *J* = 7.9 Hz, 5H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.15 (t, *J* = 7.6 Hz, 1H), 7.02 (d, *J* = 8.1 Hz, 1H), 6.74 (d, *J* = 8.0 Hz, 2H), 5.33 (dd, *J* = 11.9, 4.4 Hz, 1H), 3.72 (dd, *J* = 18.5, 12.0 Hz, 1H), 2.79 – 2.69 (m, 1H), 2.53 – 2.36 (m, 2H), 2.32 (t, *J* = 6.9 Hz, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 174.14, 169.65, 160.19, 152.36, 149.87, 141.71, 138.60, 133.96, 133.25, 131.59, 131.44, 131.23, 130.56, 128.37, 127.64, 127.26, 123.65, 122.43, 120.06, 119.18, 115.66, 58.23, 45.12, 30.27, 29.69. HRMS (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>28</sub>H<sub>22</sub>BrClN<sub>3</sub>O<sub>4</sub>, 578.04767; found, 578.04719. Anal. Calcd for C<sub>28</sub>H<sub>21</sub>BrClN<sub>3</sub>O<sub>4</sub>·1.20H<sub>2</sub>O: C, 56.01; H, 3.93; N, 7.00. Found C, 56.02; H, 4.02; N, 6.78.

#### 4-{5-(4-Chlorophenyl)-3-[4-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (26)

Compound **26** was prepared according to general procedure G using **87** (0.046 g, 0.460 mmol) and **26f** (0.200 g, 0.460 mmol). The title compound was obtained after removing the THF under vacuum, precipitating from EtOAc with hexanes and further purification using flash chromatography (2–10% MeOH:DCM) as a yellow solid. Some compound was lost due to spillage. Yield 0.054 g, 22%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.29 (s, 1H), 12.06 (s, 1H), 7.65 – 7.55 (m, 2H), 7.52 (dd, *J* = 8.2, 2.3 Hz, 1H), 7.48 – 7.38 (m, 2H), 7.34 – 7.24 (m, 3H), 7.15 (t, *J* = 7.6 Hz, 1H), 7.03 (d, *J* = 8.1 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 2H), 5.35

(dd,  $J = 11.9, 4.5$  Hz, 1H), 3.73 (dd,  $J = 18.5, 12.0$  Hz, 1H), 2.77 (dd,  $J = 18.4, 4.4$  Hz, 1H), 2.65 – 2.43 (m, 2H), 2.34 (t,  $J = 6.7$  Hz, 2H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  173.54, 168.67, 160.17, 152.72, 149.89, 141.09, 138.57, 134.04, 133.22, 131.59, 131.46, 130.55, 128.32, 127.31, 122.42, 119.15, 115.59, 58.21, 45.16, 28.47, 28.20. HRMS ( $m/z$ ):  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{28}\text{H}_{22}\text{Cl}_2\text{N}_3\text{O}_4$ , 534.09819; found, 534.09787. Anal. Calcd for  $\text{C}_{28}\text{H}_{21}\text{Cl}_2\text{N}_3\text{O}_4$ : C, 62.93; H, 3.96; N, 7.86. Found C, 62.38; H, 4.03; N, 7.73. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid)  $R_f$  0.95 min; > 95% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid)  $R_f$  0.61 min; > 95% purity.

#### 4-{3-[4-(4-Chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (27)

Compound **27** was prepared according to general procedure G using **87** (0.029 g, 0.287 mmol) and **27f** (0.120 g, 0.287 mmol). The title compound was obtained after removal of the residual solvent, dissolving the crude material in DCM and washing 3X with acidified brine. The organics were collected, dried over magnesium sulfate and concentrated to yield the title compound as a brown solid. Yield 0.085 g, 57%.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.30 (s, 1H), 12.08 (s, 1H), 7.65 – 7.49 (m, 3H), 7.47 – 7.34 (m, 2H), 7.34 – 7.26 (m, 1H), 7.15 (t,  $J = 7.7$  Hz, 1H), 7.10 – 7.00 (m, 3H), 6.88 – 6.80 (m, 2H), 5.35 (dd,  $J = 11.7, 4.3$  Hz, 1H), 3.72 (dd,  $J = 18.1, 12.0$  Hz, 1H), 2.78 (dd,  $J = 18.5, 4.3$  Hz, 1H), 2.64 – 2.42 (m, 2H), 2.33 (t,  $J = 6.6$  Hz, 2H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  174.19, 169.29, 160.82, 153.38, 150.51, 139.22, 138.99, 134.70, 133.85, 132.08, 131.24, 128.97, 128.11, 128.05, 127.94, 124.20, 123.06, 119.81, 116.25, 115.76, 115.62, 58.80, 45.91, 29.16, 28.87. HRMS ( $m/z$ ):  $[\text{M}-\text{H}]^-$  calcd for  $\text{C}_{28}\text{H}_{20}\text{ClFN}_3\text{O}_4$ , 516.11319; found, 516.11362. Anal. Calcd for  $\text{C}_{28}\text{H}_{21}\text{ClFN}_3\text{O}_4 \cdot 0.70\text{DCM}$ : C, 59.70; H, 3.91; N, 7.28. Found C, 59.54; H, 4.15; N, 7.20.

#### 4-{3-[4-(4-Chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (28)

Compound **28** was prepared according to general procedure G using **87** (0.055 g, 0.550 mmol) and **28f** (0.220 g, 0.550 mmol). The title compound was obtained by filtering from DCM after removal of the THF *in vacuo*. Yield 0.204 g, 74%.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.29 (s, 1H), 12.13 (s, 1H), 7.66 – 7.54 (m, 2H), 7.54 – 7.39 (m, 3H), 7.32 – 7.11 (m, 5H), 7.03 (d,  $J = 8.1$  Hz, 1H), 6.77 (dd,  $J = 6.5, 3.0$  Hz, 2H), 5.32 (dd,  $J = 11.9, 4.3$  Hz, 1H), 3.75 (dd,  $J = 18.4, 12.1$  Hz, 1H), 2.77 (dd,  $J = 18.3, 4.3$  Hz, 1H), 2.64 – 2.39 (m, 2H), 2.33 (t,  $J = 6.6$  Hz, 2H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  173.55, 168.57, 160.17, 152.70, 149.84, 142.23, 138.54, 134.03, 133.17, 131.48, 131.40, 130.57, 128.33, 128.25, 127.28, 127.03, 125.33, 123.62, 122.39, 119.18, 115.57, 58.86, 45.39, 28.53, 28.23. HRMS ( $m/z$ ):  $[\text{M}-\text{H}]^-$  calcd for  $\text{C}_{28}\text{H}_{21}\text{ClN}_3\text{O}_4$ , 498.12261; found, 498.12276. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid)  $R_f$  0.87 min; > 95% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid)  $R_f$  0.69 min; > 95% purity.

#### 4-{5-(4-Chlorophenyl)-3-[4-(4-fluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (29)

Compound **29** was prepared according to general procedure G using **87** (0.072 g, 0.718 mmol) and **29f** (0.300 g, 0.718 mmol). The title compound was obtained by filtering from

DCM after removal of the THF *in vacuo*. Yield 0.210 g, 57%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.27 (s, 1H), 12.09 (s, 1H), 7.58 (t, *J* = 7.7 Hz, 1H), 7.43 (d, *J* = 7.6 Hz, 2H), 7.40 - 7.25 (m, 5H), 7.15 (t, *J* = 7.7 Hz, 1H), 7.04 (d, *J* = 8.2 Hz, 1H), 6.86 (d, *J* = 8.2 Hz, 2H), 5.34 (dd, *J* = 11.9, 4.6 Hz, 1H), 3.74 (dd, *J* = 18.5, 11.9 Hz, 1H), 2.79 (dd, *J* = 18.4, 4.6 Hz, 1H), 2.64 - 2.42 (m, 2H), 2.33 (t, *J* = 6.8 Hz, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 173.54, 168.67, 160.21, 152.82, 150.17, 141.11, 138.53, 131.57, 131.44, 130.80, 128.30, 127.36, 123.62, 122.38, 119.40, 115.57, 115.31, 115.10, 58.21, 45.16, 28.50, 28.20. HRMS (*m/z*): [M+Na]<sup>+</sup> calcd for C<sub>28</sub>H<sub>21</sub>ClFN<sub>3</sub>O<sub>4</sub>Na, 540.10968; found, 540.10938. Anal. Calcd for C<sub>28</sub>H<sub>21</sub>ClFN<sub>3</sub>O<sub>4</sub>·0.90H<sub>2</sub>O: C, 62.96; H, 4.30; N, 7.86. Found C, 62.80; H, 4.06; N, 7.80.

#### 4-[5-(4-Chlorophenyl)-3-(2-oxo-4-p-tolyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobutanoic acid (30)

Compound **30** was prepared according to general procedure G using **87** (0.060 g, 0.604 mmol) and **30f** (0.250 g, 0.604 mmol). The title compound was obtained as a yellow solid after purifying using flash chromatography (2–10% MeOH:DCM) followed by precipitation from EtOAc using hexanes. Yield 0.260 g, 84%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.24 (s, 1H), 12.08 (s, 1H), 7.57 (t, *J* = 7.5 Hz, 1H), 7.42 (d, *J* = 8.2 Hz, 1H), 7.36 – 7.21 (m, 5H), 7.18 – 7.04 (m, 3H), 6.84 (dd, *J* = 8.5, 2.3 Hz, 2H), 5.32 (dd, *J* = 11.9, 4.4 Hz, 1H), 3.68 (dd, *J* = 18.4, 12.0 Hz, 1H), 2.74 (dd, *J* = 18.4, 4.5 Hz, 1H), 2.63 – 2.39 (m, 5H), 2.31 (t, *J* = 6.7 Hz, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 173.55, 168.65, 160.25, 152.86, 151.26, 141.13, 138.55, 137.66, 132.18, 131.51, 131.28, 129.24, 128.79, 128.69, 128.54, 128.26, 127.45, 123.25, 122.23, 119.43, 115.52, 58.17, 45.20, 28.79, 28.60, 28.24, 20.90. HRMS (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>29</sub>H<sub>25</sub>ClN<sub>3</sub>O<sub>4</sub>, 514.15281; found, 514.15260. Anal. Calcd for C<sub>29</sub>H<sub>24</sub>ClN<sub>3</sub>O<sub>4</sub>·0.80H<sub>2</sub>O: C, 65.92; H, 4.88; N, 7.95. Found C, 65.86; H, 4.84; N, 7.75.

#### 4-{5-(4-Chlorophenyl)-3-[4-(4-methoxyphenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (31)

Compound **31** was prepared according to general procedure G using **87** (0.047 g, 0.465 mmol) and **31f** (0.200 g, 0.465 mmol). The title compound was obtained as a white solid by filtering from DCM after removal of the THF *in vacuo*. Yield 0.165 g, 67%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 14.05 (s, 1H), 12.24 (s, 1H), 7.57 (t, *J* = 7.7 Hz, 1H), 7.42 (d, *J* = 8.3 Hz, 2H), 7.38 – 7.06 (m, 6H), 7.01 (d, *J* = 8.5 Hz, 1H), 6.83 (d, *J* = 7.8 Hz, 3H), 5.33 (dd, *J* = 11.8, 4.4 Hz, 1H), 3.85 (s, 3H), 3.67 (dd, *J* = 18.4, 12.1 Hz, 1H), 2.78-2.53 (m, 2H), 2.41 – 2.22 (m, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 175.22, 173.68, 168.75, 160.42, 160.28, 159.20, 152.91, 151.06, 141.19, 138.54, 131.50, 131.26, 131.01, 130.92, 130.00, 128.39, 128.24, 127.68, 127.44, 127.00, 123.52, 122.23, 119.66, 115.54, 113.68, 113.56, 58.16, 55.17, 45.24, 32.60, 28.76, 28.44. HRMS (*m/z*): [M+K]<sup>+</sup> calcd for C<sub>29</sub>H<sub>24</sub>ClN<sub>3</sub>O<sub>5</sub>, 568.10361; found, 568.10341. Anal. Calcd for C<sub>29</sub>H<sub>24</sub>ClN<sub>3</sub>O<sub>5</sub>: C, 65.72; H, 4.56; N, 7.92. Found C, 57.27; H, 4.20; N, 6.50. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>t</sub> 0.89 min; > 95% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>t</sub> 0.67 min; > 95% purity.



**4-{5-(4-Chlorophenyl)-3-[4-(4-cyanophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (32)**

Compound **32** was prepared according to general procedure G using **87** (0.059 g, 0.588 mmol) and **32f** (0.150 g, 0.588 mmol). The title compound was obtained as a yellow solid after precipitation from EtOAc using hexanes followed by purification using flash chromatography with 10% MeOH:DCM as a yellow solid. Yield 0.150 g, 48.6%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.33 (s, 1H), 12.09 (s, 1H), 7.97 (ddd, *J* = 11.8, 7.7, 1.7 Hz, 2H), 7.64 – 7.56 (m, 2H), 7.50 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 1H), 7.35 – 7.27 (m, 2H), 7.14 (t, *J* = 7.7 Hz, 1H), 6.95 (dd, *J* = 8.2, 1.2 Hz, 1H), 6.89 – 6.84 (m, 2H), 5.35 (dd, *J* = 12.0, 4.4 Hz, 1H), 3.79 (dd, *J* = 18.5, 12.0 Hz, 1H), 2.89 (dd, *J* = 18.5, 4.5 Hz, 1H), 2.57 – 2.23 (m, 4H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 173.45, 168.70, 160.12, 152.50, 149.37, 140.99, 140.55, 138.61, 132.20, 131.62, 130.59, 130.50, 129.79, 128.37, 128.25, 127.32, 127.23, 123.18, 122.52, 118.75, 118.63, 115.65, 111.18, 58.30, 45.00, 28.77, 28.22. HRMS (*m/z*): [M-H]<sup>-</sup> calcd for C<sub>29</sub>H<sub>20</sub>ClN<sub>4</sub>O<sub>4</sub>, 523.11786; found, 523.11828. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>t</sub> 0.67 min; 87% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>t</sub> 0.89 min; 85% purity.

**4-[5-(4-Chlorophenyl)-3-{2-oxo-4-(4-(trifluoromethyl)phenyl)-1,2-dihydroquinolin-3-yl}-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobutanoic acid (33)**

Compound **33** was prepared according to general procedure G using **87** (0.039 g, 0.385 mmol) and **33f** (0.180 g, 0.220 mmol). The title compound was obtained after flash column chromatography using 0–10% MeOH:DCM and precipitation from EtOAc using hexanes as a yellow solid. Yield 0.125 g, 57.2%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.34 (s, 1H), 12.07 (s, 1H), 7.90 (d, *J* = 8.1 Hz, 1H), 7.85 (d, *J* = 8.1 Hz, 1H), 7.65 (d, *J* = 8.1 Hz, 1H), 7.65 (d, *J* = 8.1 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.53 (d, *J* = 8.1 Hz, 1H), 7.45 (d, *J* = 8.2 Hz, 1H), 7.26 (d, *J* = 8.2 Hz, 2H), 7.15 (t, *J* = 7.7 Hz, 1H), 6.98 (d, *J* = 8.1 Hz, 1H), 6.86 (d, *J* = 8.2 Hz, 2H), 5.36 (dd, *J* = 12.0, 4.6 Hz, 1H), 3.80 (dd, *J* = 18.4, 12.0 Hz, 1H), 2.91 (dd, *J* = 18.5, 4.8 Hz, 1H), 2.52 - 2.21 (m, 4H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 173.42, 168.68, 160.19, 152.58, 149.61, 141.06, 139.85, 138.62, 131.62, 130.35, 129.69, 128.31, 127.27, 125.20, 123.28, 122.54, 118.95, 115.63, 58.23, 45.04, 28.32, 28.05. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ -61.595 (s). HRMS (*m/z*): [M-H]<sup>-</sup> calcd for C<sub>29</sub>H<sub>20</sub>ClF<sub>3</sub>N<sub>3</sub>O<sub>4</sub>, 566.10999; found, 566.11036. Anal. Calcd for C<sub>29</sub>H<sub>21</sub>ClF<sub>3</sub>N<sub>3</sub>O<sub>4</sub>·0.40H<sub>2</sub>O: C, 60.56; H, 3.82; N, 7.31. Found C, 60.57; H, 4.00; N, 7.23.

**4-{5-(4-Chlorophenyl)-3-[4-(3-fluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (34)**

Compound **34** was prepared according to general procedure G using **87** (0.060 g, 0.598 mmol) and **34f** (0.250 g, 0.367 mmol). The title compound was obtained after precipitating from EtOAc using hexanes followed by flash column chromatography using 10% MeOH:DCM, as a yellow solid. Yield 0.190 g, 61.3%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.24 (s, 1H), 12.08 (s, 1H), 7.57 (t, *J* = 7.5 Hz, 1H), 7.42 (d, *J* = 8.2 Hz, 1H), 7.36 - 7.21 (m, 5H), 7.18 - 7.04 (m, 3H), 6.84 (d, *J* = 8.2 Hz, 2H), 5.32 (dd, *J* = 12.0, 4.5 Hz, 1H), 3.68 (dd, *J* = 18.5, 12.0 Hz, 1H), 2.74 (dd, *J* = 18.5, 4.5 Hz, 1H), 2.52 - 2.39 (m, 4H). <sup>13</sup>C NMR (100 MHz DMSO-*d*<sub>6</sub>) δ 173.48, 168.60, 160.19, 149.62, 141.15, 138.53, 131.53, 131.45,

130.35, 128.37, 127.33, 127.16, 122.44, 119.07, 115.57, 58.27, 45.11, 28.79, 28.47, 28.18.  $^{19}\text{F}$  NMR (376 MHz, DMSO- $d_6$ )  $\delta$  -113.29 – -113.63 (m). HRMS ( $m/z$ ):  $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{28}\text{H}_{21}\text{ClFN}_3\text{O}_4\text{Na}$ , 540.10968; found, 540.11014. Anal. Calcd for  $\text{C}_{28}\text{H}_{21}\text{ClFN}_3\text{O}_4 \cdot 0.90\text{H}_2\text{O}$ : C, 62.96; H, 4.30; N, 7.87. Found C, 63.04; H, 4.38; N, 7.55.

#### 4-{5-(4-Chlorophenyl)-3-[4-(3-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (35)

Compound **35** was prepared according to general procedure G using **87** (0.069 g, 0.691 mmol) and **35f** (0.300 g, 0.691 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 2–10% MeOH:DCM. Yield 0.170 g, 46.1%.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.25 (s, 1H), 12.04 (s, 1H), 7.58 – 7.51 (m, 2H), 7.51 – 7.46 (m, 1H), 7.39 (d,  $J$  = 8.2 Hz, 1H), 7.36 – 7.32 (m, 1H), 7.25 (ddt,  $J$  = 7.1, 4.9, 2.5 Hz, 2H), 7.19 (d,  $J$  = 7.5 Hz, 1H), 7.12 (t,  $J$  = 7.7 Hz, 1H), 7.01 – 6.94 (m, 1H), 6.81 – 6.73 (m, 2H), 5.34 – 5.28 (m, 1H), 3.83 – 3.74 (m, 1H), 2.84 – 2.70 (m, 1H), 2.52 – 2.34 (m, 2H), 2.31 – 2.24 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  173.50, 168.64, 160.20, 152.66, 149.46, 141.16, 138.53, 137.44, 137.28, 133.18, 131.56, 131.47, 130.15, 130.00, 129.59, 128.49, 128.40, 127.29, 127.16, 123.43, 122.48, 119.17, 115.58, 58.30, 45.10, 28.78, 28.49, 28.19. HRMS ( $m/z$ ):  $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{28}\text{H}_{21}\text{Cl}_2\text{N}_3\text{O}_4\text{Na}$ , 556.08013; found, 556.07990. Anal. Calcd for  $\text{C}_{28}\text{H}_{21}\text{Cl}_2\text{N}_3\text{O}_4 \cdot 0.70\text{H}_2\text{O}$ : C, 61.48; H, 4.13; N, 7.68. Found C, 61.51; H, 4.11; N, 7.48.

#### 4-{5-(4-Chlorophenyl)-3-[2-oxo-4-(*m*-tolyl)-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (36)

Compound **36** was prepared according to general procedure G using **87** (0.060 g, 0.604 mmol) and **36f** (0.250 g, 0.604 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0–10% MeOH:DCM followed by precipitation from hot EtOAc and hexanes. Yield 0.240 g, 77.0%.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.21 (s, 1H), 12.05 (s, 1H), 7.53 (t,  $J$  = 7.6 Hz, 1H), 7.42 – 7.27 (m, 3H), 7.27 – 7.17 (m, 2H), 7.16 – 7.06 (m, 2H), 7.00 (q,  $J$  = 10.2, 8.9 Hz, 2H), 6.80 – 6.67 (m, 2H), 5.33 – 5.23 (m, 1H), 3.80 – 3.62 (m, 1H), 2.76 (dd,  $J$  = 18.4, 4.6 Hz, 0.5H), 2.64 (dd,  $J$  = 18.5, 4.6 Hz, 0.5H), 2.57 – 2.34 (m, 5H), 2.31 – 2.18 (m, 2H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  173.61, 173.54, 168.63, 168.57, 160.29, 152.88, 152.70, 151.33, 151.24, 141.25, 141.20, 138.52, 137.49, 137.43, 135.17, 135.03, 131.53, 131.30, 130.25, 128.84, 128.36, 128.32, 128.13, 127.50, 127.41, 127.34, 127.26, 126.39, 125.46, 123.16, 122.28, 119.42, 115.55, 115.51, 58.28, 58.23, 45.25, 28.90, 28.79, 28.67, 28.57, 28.35, 28.24, 28.14, 21.09, 21.02, 20.97, 20.91. HRMS ( $m/z$ ):  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{29}\text{H}_{25}\text{ClN}_3\text{O}_4$ , 514.15281; found, 514.15263. Anal. Calcd for  $\text{C}_{29}\text{H}_{24}\text{ClN}_3\text{O}_4 \cdot 1.50\text{H}_2\text{O} \cdot 0.06\text{EtOAc}$ : C, 64.29; H, 5.07; N, 7.69. Found C, 64.65; H, 4.90; N, 7.29.

#### 4-{5-(4-Chlorophenyl)-3-[4-(3-methoxyphenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (37)

Compound **37** was prepared according to general procedure G using **87** (0.070 g, 0.698 mmol) and **37f** (0.300 g, 0.698 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0–10% MeOH:DCM. Yield 0.090 g, 24.3%.  $^1\text{H}$

NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 12.23 (s, 1H), 12.03 (s, 1H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.48 – 7.35 (m, 2H), 7.32 – 7.21 (m, 2H), 7.18 – 7.12 (m, 1H), 7.09 (t, *J* = 8.3 Hz, 2H), 6.95 (d, *J* = 9.9 Hz, 1H), 6.85 – 6.72 (m, 3H), 5.37 – 5.30 (m, 1H), 3.84 – 3.64 (m, 4H), 2.83 – 2.72 (m, 1H), 2.60 – 2.51 (m, 2H), 2.31 (t, *J* = 7.1 Hz, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 173.52, 168.60, 160.26, 159.03, 152.90, 150.95, 141.24, 141.19, 138.52, 138.50, 136.50, 136.46, 131.56, 131.48, 131.33, 131.31, 129.43, 129.38, 128.35, 128.21, 128.14, 127.46, 127.43, 127.19, 123.24, 123.14, 122.32, 121.65, 120.70, 119.31, 119.27, 115.51, 115.13, 114.02, 113.88, 113.58, 58.25, 55.26, 54.99, 45.30, 45.19, 28.59, 28.22. HRMS (*m/z*): [M + H]<sup>+</sup> calcd for C<sub>29</sub>H<sub>25</sub>ClN<sub>3</sub>O<sub>5</sub>, 530.14773; found, 530.14715. Anal. Calcd for C<sub>29</sub>H<sub>24</sub>ClN<sub>3</sub>O<sub>5</sub>: C, 65.72; H, 4.56; N, 7.93. Found C, 65.52; H, 4.72; N, 7.97.

#### 4-{5-(4-Chlorophenyl)-3-[4-(3-cyanophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (38)

Compound **38** was prepared according to general procedure G using **87** (0.021 g, 0.212 mmol) and **38f** (0.090 g, 0.212 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0–10% MeOH:DCM. Yield 0.034 g, 31.0%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.35 (s, 1H), 12.10 (s, 1H), 8.04 – 7.91 (m, 1H), 7.84 – 7.67 (m, 2H), 7.65 – 7.55 (m, 2H), 7.43 (d, *J* = 8.2 Hz, 1H), 7.31 (d, *J* = 8.3 Hz, 2H), 7.16 (t, *J* = 7.7 Hz, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 6.82 (m, 2H), 5.40 – 5.32 (m, 1H), 3.96 – 3.81 (m, 1H), 2.87 (dt, *J* = 18.3, 4.1 Hz, 1H), 2.42 (d, *J* = 7.6 Hz, 2H), 2.35 – 2.26 (m, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 173.58, 173.42, 168.61, 160.13, 152.58, 148.90, 141.02, 138.54, 136.80, 136.70, 134.66, 133.48, 133.05, 132.18, 132.09, 131.73, 131.57, 129.51, 129.32, 128.47, 128.41, 127.34, 127.25, 127.04, 123.64, 123.53, 122.54, 119.03, 118.52, 115.59, 111.60, 111.40, 58.27, 45.03, 28.75, 28.32, 28.10. HRMS (*m/z*): [M-H]<sup>-</sup> calcd for C<sub>29</sub>H<sub>20</sub>ClN<sub>4</sub>O<sub>4</sub>, 523.11786; found, 523.11778. Anal. Calcd for C<sub>29</sub>H<sub>21</sub>ClN<sub>4</sub>O<sub>4</sub>: C, 66.35; H, 4.03; N, 10.67. Found C, 63.97; H, 4.59; N, 9.39. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>f</sub> 0.68 min; > 95% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>f</sub> 0.59 min; > 95% purity.

#### 4-[5-(4-Chlorophenyl)-3-{2-oxo-4-[3-(trifluoromethyl)phenyl]-1,2-dihydroquinolin-3-yl}-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobutanoic acid (39)

Compound **39** was prepared according to general procedure G using **87** (0.054 g, 0.53 mmol) and **39f** (0.250 g, 0.53 mmol). The title compound was obtained as a yellow solid using a 0–8% MeOH:DCM. Yield 0.073 g, 24%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.34 (s, 1H), 12.06 (s, 1H), 7.96–7.84 (m, 1H), 7.82 – 7.66 (m, 2H), 7.59 (t, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.3 Hz, 1H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.23 (d, *J* = 8.1 Hz, 1H), 7.20 – 7.13 (m, 1H), 6.98 (d, *J* = 8.2 Hz, 0.5H), 6.92 (d, *J* = 8.2 Hz, 0.5H), 6.84 (d, *J* = 8.1 Hz, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 5.34 (dt, *J* = 12.2, 4.3 Hz, 1H), 3.95 – 3.70 (m, 1H), 3.00 – 2.74 (m, 1H), 2.54 – 2.23 (m, 4H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 173.40, 173.34, 168.62, 168.52, 160.20, 160.17, 152.67, 152.61, 149.38, 141.10, 141.03, 138.57, 136.57, 136.47, 133.59, 132.57, 131.60, 131.51, 129.50, 129.33, 129.15, 128.92, 128.49, 128.38, 128.30, 127.67, 127.25, 127.17, 127.13, 126.47, 125.07, 123.62, 123.45, 122.54, 122.36, 119.26, 119.11, 115.61, 58.26, 58.21, 45.12, 45.01, 28.37, 28.29, 28.19, 28.08. HRMS (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>29</sub>H<sub>22</sub>ClF<sub>3</sub>N<sub>3</sub>O<sub>4</sub>, 568.12455; found, 568.12417. Anal. Calcd for C<sub>29</sub>H<sub>21</sub>ClF<sub>3</sub>N<sub>3</sub>O<sub>4</sub>·0.20H<sub>2</sub>O: C, 60.94; H, 3.77; N, 7.35. Found C, 60.79; H, 3.96; N, 7.33.

**4-{3-[4-(4-Chloro-3-fluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (40)**

Compound **40** was prepared according to general procedure G using **87** (0.040 g, 0.398 mmol) and **40f** (0.180 g, 0.398 mmol). The title compound was obtained after flash column chromatography using 0–10% MeOH:DCM as a yellow solid followed by precipitation from EtOAc and hexanes. Yield 0.096 g, 43.7%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.33 (s, 1H), 12.12 (s, 1H), 7.79 – 7.64 (m, 1H), 7.62 – 7.51 (m, 2H), 7.46 – 7.38 (m, 2H), 7.33 – 7.23 (m, 2H), 7.21 – 7.00 (m, 2H), 6.93 – 6.78 (m, 2H), 5.39 (dt, *J* = 12.1, 4.4 Hz, 1H), 3.85 – 3.72 (m, 1H), 2.91 – 2.79 (m, 1H), 2.66 – 2.39 (m, 2H), 2.34 (td, *J* = 7.0, 2.2 Hz, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 173.61, 173.47, 173.44, 168.68, 168.66, 168.09, 168.05, 160.11, 157.84, 156.19, 152.77, 152.70, 152.63, 148.67, 141.03, 140.99, 138.55, 136.45, 136.36, 136.30, 131.63, 131.57, 130.63, 130.53, 128.34, 128.28, 127.32, 127.21, 127.12, 127.00, 126.21, 123.57, 123.45, 122.54, 119.69, 119.58, 118.92, 117.28, 115.58, 58.28, 58.17, 45.15, 28.77, 28.39, 28.17. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ -116.32 – -116.51 (m). HRMS (*m/z*): [M+K]<sup>+</sup> calcd for C<sub>28</sub>H<sub>20</sub>Cl<sub>2</sub>FN<sub>3</sub>O<sub>4</sub>K, 590.04465; found, 590.04631. Anal. Calcd for C<sub>28</sub>H<sub>20</sub>Cl<sub>2</sub>FN<sub>3</sub>O<sub>4</sub> · 0.80H<sub>2</sub>O · 0.10EtOAc: C, 59.26; H, 3.92; N, 7.30. Found C, 59.16; H, 4.21; N, 7.14.

**4-{3-[4-(3-Chloro-4-fluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (41)**

Compound **41** was prepared according to general procedure G using **87** (0.040 g, 0.398 mmol) and **41f** (0.180 g, 0.398 mmol). The title compound was obtained after purifying using flash column chromatography using 0–10% MeOH:DCM as a yellow solid. Yield 0.120 g, 54.6%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.32 (s, 1H), 12.12 (s, 1H), 7.73 – 7.50 (m, 3H), 7.44 (dd, *J* = 8.2, 1.7 Hz, 2H), 7.29 (td, *J* = 8.8, 2.0 Hz, 2H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.09 – 7.01 (m, 1H), 6.88 (dt, *J* = 8.6, 2.4 Hz, 2H), 5.38 (dd, *J* = 10.4, 4.6 Hz, 1H), 3.90 – 3.76 (m, 1H), 2.93 – 2.77 (m, 1H), 2.66 – 2.40 (m, 2H), 2.40 – 2.28 (m, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 173.63, 173.48, 173.44, 168.68, 160.15, 155.93, 152.68, 152.60, 148.76, 148.72, 141.01, 138.50, 133.00, 132.90, 131.93, 131.62, 131.57, 131.50, 130.63, 130.51, 129.53, 128.39, 128.34, 127.38, 127.23, 127.11, 123.74, 123.66, 122.50, 119.77, 119.46, 119.24, 116.87, 116.66, 115.56, 58.25, 45.10, 28.78, 28.43, 28.38, 28.15. HRMS (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>28</sub>H<sub>21</sub>Cl<sub>2</sub>FN<sub>3</sub>O<sub>4</sub>, 552.08877; found, 552.09030. Anal. Calcd for C<sub>28</sub>H<sub>20</sub>Cl<sub>2</sub>FN<sub>3</sub>O<sub>4</sub>: C, 60.88; H, 3.64; N, 7.61. Found C, 58.29; H, 4.04; N, 6.66. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>t</sub> 0.93 min; > 95% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>t</sub> 0.55 min; > 95% purity.

**4-{5-(4-Chlorophenyl)-3-[4-(3,4-dichlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (42)**

Compound **42** was prepared according to general procedure G using **87** (0.043 g, 0.427 mmol) and **42f** (0.200 g, 0.427 mmol). The title compound was obtained after purifying flash column chromatography using 0–10% MeOH:DCM as a yellow solid. Yield 0.102 g, 42.0%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.31 (s, 1H), 12.10 (s, 1H), 7.83 – 7.70 (m, 1H), 7.63 – 7.54 (m, 2H), 7.46 – 7.37 (m, 2H), 7.32 – 7.22 (m, 2H), 7.16 (t, *J* = 7.7 Hz, 1H), 7.04 (t, *J* = 7.0 Hz, 1H), 6.87 – 6.80 (m, 2H), 5.37 (dd, *J* = 12.0, 4.3 Hz, 1H), 3.88 – 3.74 (m,

1H), 2.90 – 2.74 (m, 1H), 2.53 – 2.39 (m, 2H), 2.38 – 2.29 (m, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 174.04, 169.33, 160.76, 153.24, 149.13, 141.66, 139.20, 136.68, 132.40, 132.27, 132.19, 131.96, 131.08, 130.76, 129.73, 129.05, 128.98, 127.99, 127.83, 127.74, 123.18, 119.69, 116.23, 58.93, 45.75, 29.62, 29.11, 29.07, 28.86. HRMS (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>28</sub>H<sub>21</sub>Cl<sub>3</sub>N<sub>3</sub>O<sub>4</sub>, 568.05922; found, 568.06168. Anal. Calcd for C<sub>28</sub>H<sub>20</sub>Cl<sub>3</sub>N<sub>3</sub>O<sub>4</sub> · 0.80H<sub>2</sub>O: C, 57.66; H, 3.73; N, 7.20. Found C, 57.70; H, 3.65; N: 6.94.

#### 4-{5-(4-Chlorophenyl)-3-[4-(3,4-difluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (43)

Compound **43** was prepared according to general procedure G using **87** (0.0427 g, 0.427 mmol) and **43f** (0.200 g, 0.427 mmol). The title compound was obtained after flash column chromatography using 0–10% MeOH:DCM as a yellow solid. Yield 0.110 g, 68.8%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.31 (s, 1H), 12.13 (s, 1H), 7.63 – 7.52 (m, 3H), 7.46 – 7.39 (m, 1H), 7.35 – 7.28 (m, 1H), 7.29 – 7.23 (m, 2H), 7.20 – 7.10 (m, 1H), 7.06 (d, *J* = 8.2 Hz, 1H), 6.95 – 6.84 (m, 2H), 5.43 – 5.33 (m, 1H), 3.88 – 3.73 (m, 1H), 2.86 (dd, *J* = 18.5, 4.4 Hz, 1H), 2.66 – 2.29 (m, 4H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 174.08, 169.36, 160.79, 153.45, 149.53, 141.69, 139.20, 139.20, 132.28, 132.14, 128.99, 128.91, 127.98, 127.80, 124.26, 123.12, 119.83, 116.22, 58.92, 45.79, 29.07, 28.84. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –5.88 – –6.23 (m), –6.44 – –6.75 (m). HRMS (*m/z*): [M-H]<sup>–</sup> calcd for C<sub>28</sub>H<sub>19</sub>ClF<sub>2</sub>N<sub>3</sub>O<sub>4</sub>, 534.10376; found, 534.10358. Anal. Calcd for C<sub>28</sub>H<sub>20</sub>ClF<sub>2</sub>N<sub>3</sub>O<sub>4</sub> · 0.40H<sub>2</sub>O: C, 61.92; H, 3.86; N, 7.74. found C, 61.76; H, 4.15; N, 7.52.

#### 4-{5-(4-Chlorophenyl)-3-[4-(3,5-difluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (44)

Compound **44** was prepared according to general procedure G using **87** (0.041 g, 0.413 mmol) and **44f** (0.180 g, 0.413 mmol). The title compound as a yellow solid was obtained after purifying using flash column chromatography with 0–10% MeOH:DCM. Yield 0.133 g, 56.5%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.33 (s, 1H), 12.07 (s, 1H), 7.59 (t, *J* = 7.7 Hz, 1H), 7.47 – 7.33 (m, 2H), 7.29 (d, *J* = 8.2 Hz, 2H), 7.23 (d, *J* = 8.5 Hz, 1H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 2H), 6.89 (d, *J* = 8.1 Hz, 2H), 5.40 (dd, *J* = 12.0, 4.6 Hz, 1H), 3.86 (dd, *J* = 18.4, 12.0 Hz, 1H), 2.90 (dd, *J* = 18.5, 4.6 Hz, 1H), 2.59 – 2.41 (m, 2H), 2.33 (t, *J* = 6.8 Hz, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 173.65, 173.46, 168.64, 163.55, 163.43, 161.11, 160.95, 160.11, 152.68, 148.60, 141.09, 139.05, 138.95, 138.52, 131.58, 128.55, 128.40, 127.26, 127.13, 123.41, 122.57, 118.79, 115.58, 113.31, 113.12, 112.32, 112.12, 103.85, 58.30, 45.02, 28.80, 28.37, 28.15. <sup>19</sup>F (376 MHz, DMSO-*d*<sub>6</sub>) δ –109.98 – –110.05 (m). HRMS (*m/z*): [M-H]<sup>–</sup> calcd for C<sub>28</sub>H<sub>19</sub>ClF<sub>2</sub>N<sub>3</sub>O<sub>4</sub>, 534.10376; found, 534.10402. Anal. Calcd for C<sub>28</sub>H<sub>20</sub>ClF<sub>2</sub>N<sub>3</sub>O<sub>4</sub> · 0.50H<sub>2</sub>O: C, 61.71, H: 3.88, N: 7.71; C: 61.52, H: 4.05, N: 7.42.

#### 4-{5-(4-Chlorophenyl)-3-[4-(3,5-dichloro(phenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobutanoic acid (45)

Compound **45** was prepared according to general procedure G using **87** (0.038 g, 0.384 mmol) and **45f** (0.180 g, 0.384 mmol). The title compound was obtained as a yellow solid after purifying using flash column chromatography with 0–10% MeOH:DCM. Yield 0.080

g, 36.6%.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.33 (s, 1H), 12.10 (s, 1H), 7.84 - 7.71 (m, 1H), 7.64 - 7.56 (m, 2H), 7.51 - 7.39 (m, 2H), 7.29 (t,  $J = 7.3$  Hz, 2H), 7.17 (t,  $J = 7.6$  Hz, 1H), 7.06 (t,  $J = 6.9$  Hz, 1H), 6.84 (d,  $J = 8.1$  Hz, 2H), 5.39 (dd,  $J = 12.0, 4.3$  Hz, 1H), 3.89 - 3.75 (m, 1H), 2.85 - 2.67 (m, 1H), 2.64 - 2.30 (m, 4H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  173.46, 168.66, 160.11, 152.60, 152.53, 148.48, 141.01, 138.52, 136.01, 131.77, 131.59, 131.31, 131.11, 130.39, 130.13, 129.07, 128.40, 128.33, 127.35, 127.17, 127.06, 122.55, 119.02, 115.57, 58.24, 45.09, 28.43, 28.38, 28.17. HRMS calcd for  $\text{C}_{28}\text{H}_{19}\text{Cl}_3\text{N}_3\text{O}_4$  [M-H] $^-$ ; 566.04466, found; 566.04483. Anal.  $\text{C}_{28}\text{H}_{20}\text{Cl}_3\text{N}_3\text{O}_4 \cdot 0.30\text{H}_2\text{O}$ ; C, 58.57; H, 3.62; N, 7.32. Found C, 58.58; H, 3.75; N, 7.23.

#### 4-{5-(4-Chlorophenyl)-3-[4-(4-chlorophenyl)-6-methyl-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (46)

Compound **46** was prepared according to general procedure G using **87** (0.039 g, 0.39 mmol) and **46f** (0.175 g, 0.39 mmol). The title compound was obtained after flash column chromatography using 0–8% MeOH:DCM as a yellow solid. Yield 0.114 g, 53.3%.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.18 (s, 1H), 11.79 (s, 1H), 7.56 (dt,  $J = 8.2, 2.3$  Hz, 1H), 7.48 (dt,  $J = 8.2, 2.3$  Hz, 1H), 7.41 - 7.34 (m, 2H), 7.30 (dd,  $J = 8.3, 2.2$  Hz, 1H), 7.27 - 7.20 (m, 3H), 6.80 - 6.72 (m, 3H), 5.35 - 5.25 (m, 1H), 3.75 - 3.59 (m, 1H), 2.76 - 2.65 (m, 1H), 2.61 - 2.33 (m, 2H), 2.26 - 2.32 (m, 2H), 2.19 (s, 3H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  173.54, 168.64, 160.00, 152.85, 149.66, 141.10, 136.63, 134.07, 133.16, 132.79, 131.56, 131.48, 131.40, 130.53, 128.37, 128.30, 127.30, 126.53, 123.47, 119.05, 115.59, 58.18, 45.18, 28.47, 28.19, 20.58. HRMS ( $m/z$ ): [M-H] $^-$  calcd for  $\text{C}_{29}\text{H}_{22}\text{Cl}_2\text{N}_3\text{O}_4$ , 546.09929; found, 546.09872. Anal. Calcd for  $\text{C}_{29}\text{H}_{23}\text{Cl}_2\text{N}_3\text{O}_4 \cdot 0.50\text{H}_2\text{O}$ : C, 62.49; H, 4.34; N, 7.54. Found C, 62.44; H, 4.55; N 7.39.

#### 4-{5-(4-Chlorophenyl)-3-[4-(3-fluorophenyl)-6-methyl-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (47)

Compound **47** was prepared according to general procedure G using **87** (0.037 g, 0.37 mmol) and **47f** (0.160 g, 0.37 mmol). The title compound was obtained after flash column chromatography using 0–10% MeOH:DCM as a brown solid. Yield 0.094 g, 47.7%.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.24 (s, 1H), 12.12 (s, 1H), 7.64 - 7.47 (m, 1H), 7.42 (d,  $J = 8.5$  Hz, 1H), 7.40 - 7.32 (m, 2H), 7.32 - 7.22 (m, 3H), 7.11 (dd,  $J = 23.8, 8.5$  Hz, 1H), 6.86 - 6.77 (m, 3H), 5.35 (dt,  $J = 12.5, 3.4$  Hz, 1H), 3.80 (dd,  $J = 18.6, 12.1$  Hz, 1H), 2.81 (dt,  $J = 18.5, 5.2$  Hz, 1H), 2.53 - 2.39 (m, 2H), 2.31 (t,  $J = 6.8$  Hz, 2H), 2.23 (s, 3H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  173.46, 168.57, 162.67, 161.11, 160.03, 152.88, 152.81, 149.39, 141.15, 137.59, 136.61, 132.77, 131.58, 131.52, 131.38, 130.25, 128.36, 128.20, 127.33, 127.15, 126.57, 126.52, 125.83, 124.79, 123.38, 123.30, 118.98, 116.66, 116.52, 115.57, 115.19, 115.05, 58.25, 45.16, 28.98, 28.47, 28.19, 20.61.  $^{19}\text{F}$  NMR (376 MHz, DMSO- $d_6$ )  $\delta$  -113.28 - -113.50 (m). HRMS ( $m/z$ ): [M-H] $^-$  calcd for  $\text{C}_{29}\text{H}_{22}\text{ClFN}_3\text{O}_4$ , 530.12884; found, 530.12883. Anal. Calcd for  $\text{C}_{29}\text{H}_{23}\text{ClFN}_3\text{O}_4 \cdot 1.20\text{H}_2\text{O}$ : C, 62.92; H, 4.62; N, 7.59. Found C, 62.82; H, 4.37; N, 7.34.



**4-{3-[6-Chloro-4-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (48)**

Compound **48** was prepared according to general procedure G using **87** (0.038 g, 0.38 mmol) and **48f** (0.180 g, 0.38 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0–8% MeOH:DCM. Yield 0.033 g, 15.1%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.44 (s, 1H), 12.08 (s, 1H), 7.69 – 7.58 (m, 2H), 7.54 (dd, *J* = 8.2, 2.3 Hz, 1H), 7.44 (dd, *J* = 8.5, 2.7 Hz, 2H), 7.35 – 7.23 (m, 3H), 6.93 (d, *J* = 2.3 Hz, 1H), 6.85 – 6.76 (m, 2H), 5.35 (dd, *J* = 12.0, 4.4 Hz, 1H), 3.72 (dd, *J* = 18.5, 12.0 Hz, 1H), 2.76 (dd, *J* = 18.5, 4.4 Hz, 1H), 2.64 – 2.38 (m, 2H), 2.33 (t, *J* = 6.7 Hz, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 173.50, 168.71, 159.96, 152.33, 148.76, 141.00, 137.33, 133.52, 133.36, 131.61, 131.48, 131.38, 130.52, 128.52, 128.32, 127.27, 126.25, 125.91, 124.80, 120.43, 117.67, 58.25, 45.08, 28.47, 28.19. HRMS (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>28</sub>H<sub>21</sub>Cl<sub>3</sub>N<sub>3</sub>O<sub>4</sub>, 568.05922; found, 568.05881. Anal. Calcd for C<sub>28</sub>H<sub>20</sub>Cl<sub>3</sub>N<sub>3</sub>O<sub>4</sub>·0.40H<sub>2</sub>O: C, 58.38; H, 3.64; N, 7.29. Found C, 58.29; H, 3.73; N, 7.24.

**4-{3-[6-Chloro-4-(3-fluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (49)**

Compound **49** was prepared according to general procedure G using **87** (0.035 g, 0.35 mmol) and **49f** (0.160 g, 0.35 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0–8% MeOH:DCM. Yield 0.039 g, 20.0%. <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 12.45 (s, 1H), 12.05 (s, 1H), 7.65 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.63 – 7.53 (m, 1H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.42 – 7.32 (m, 2H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.29 – 7.25 (m, 1H), 7.19 (dd, *J* = 9.2, 2.1 Hz, 1H), 7.12 (d, *J* = 7.5 Hz, 1H), 6.94 (d, *J* = 2.4 Hz, 1H), 6.83 (dd, *J* = 11.9, 8.3 Hz, 2H), 5.36 (dt, *J* = 12.2, 4.6 Hz, 1H), 2.88 – 2.80 (m, 1H), 2.51 – 2.44 (m, 2H), 2.31 (t, *J* = 6.9 Hz, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 174.13, 169.33, 163.45, 161.82, 160.66, 153.02, 152.95, 149.14, 141.73, 137.95, 137.52, 132.03, 131.21, 129.05, 127.96, 127.78, 126.91, 126.57, 125.39, 125.30, 121.01, 118.31, 116.19, 58.96, 45.71, 29.12, 28.85. HRMS (*m/z*): [M-H]<sup>-</sup> calcd for C<sub>28</sub>H<sub>19</sub>Cl<sub>2</sub>FN<sub>3</sub>O<sub>4</sub>, 550.07421; found, 550.07485. Anal. Calcd for C<sub>28</sub>H<sub>20</sub>Cl<sub>2</sub>FN<sub>3</sub>O<sub>4</sub>·1.00H<sub>2</sub>O: C, 58.96; H, 3.89; N, 7.37. Found C, 58.98; H, 3.78; N, 7.06.

**4-{5-(4-Chlorophenyl)-3-[4-(4-chlorophenyl)-6-fluoro-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (50)**

Compound **50** was prepared according to general procedure G using **87** (0.035 g, 0.35 mmol) and **50f** (0.19 g, 0.42 mmol). The title compound was obtained after flash column chromatography using 0–10% MeOH:DCM as a yellow solid. Yield 0.151 g, 65.1%. <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 12.37 (s, 1H), 12.07 (s, 1H), 7.61 (dd, *J* = 8.2, 2.4 Hz, 1H), 7.56 – 7.41 (m, 4H), 7.34 – 7.25 (m, 3H), 6.85 – 6.78 (m, 2H), 6.71 (dd, *J* = 9.7, 2.9 Hz, 1H), 5.36 (dd, *J* = 12.0, 4.5 Hz, 1H), 3.73 (dd, *J* = 18.5, 12.1 Hz, 1H), 2.78 (dd, *J* = 18.5, 4.4 Hz, 1H), 2.62 – 2.54 (m, 1H), 2.53 – 2.40 (m, 1H), 2.33 (t, *J* = 6.7 Hz, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 173.48, 168.69, 159.89, 157.84, 156.25, 152.46, 149.03, 141.01, 135.30, 133.52, 133.45, 131.59, 131.40, 130.47, 128.48, 128.30, 127.28, 124.74, 119.94, 119.78, 119.61, 117.67, 117.62, 111.93, 111.77, 58.24, 45.07, 28.46, 28.18. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ -120.13 – -120.29 (m). HRMS (*m/z*): [M-H]<sup>-</sup> calcd for C<sub>28</sub>H<sub>19</sub>Cl<sub>2</sub>FN<sub>3</sub>O<sub>4</sub>,

550.07421; found, 550.07419. Anal. Calcd for  $C_{28}H_{20}Cl_2FN_3O_4$ : C, 60.88; H, 3.65; N, 7.61. Found C, 60.16; H, 3.98; N, 7.28. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  1.10 min; > 95% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  0.81 min; > 95% purity.

#### 4-{5-(4-Chlorophenyl)-3-[6-fluoro-4-(3-fluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (51)

Compound **51** was prepared according to general procedure G using **87** (0.041 g, 0.41 mmol) and **51f** (0.180 g, 0.41 mmol). The title compound was obtained after flash column chromatography using 0–10% MeOH:DCM as a yellow solid. Yield 0.076 g, 34.3%. <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 12.34 (s, 1H), 12.01 (s, 1H), 7.59 – 7.39 (m, 3H), 7.37 – 7.19 (m, 3H), 7.14 (t, *J* = 7.8 Hz, 1H), 7.07 (t, *J* = 7.2 Hz, 1H), 6.83 – 6.75 (m, 1H), 6.74 – 6.63 (m, 1H), 5.40–5.33 (m, 1H), 3.81 (dd, *J* = 18.5, 12.1 Hz, 2H), 2.85 – 2.75 (m, 1H), 2.46 – 2.36 (m, 2H), 2.27 (t, *J* = 7.0 Hz, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 173.47, 168.66, 162.80, 161.17, 161.11, 159.93, 157.85, 156.26, 152.52, 152.44, 148.77, 141.08, 137.08, 137.02, 136.97, 135.29, 131.62, 131.56, 130.55, 128.39, 127.39, 127.25, 127.21, 127.08, 125.75, 124.69, 124.59, 119.90, 119.83, 117.66, 116.65, 115.50, 115.36, 111.87, 58.36, 58.24, 45.07, 28.46, 28.18. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –112.89 – –113.28 (m), –120.04 – –120.29 (m). HRMS (*m/z*): [M-H]<sup>–</sup> calcd for  $C_{28}H_{19}ClF_2N_3O_4$ , 534.10376; found, 534.10345. Anal. Calcd for  $C_{28}H_{20}ClF_2N_3O_4$ : C, 62.75; H, 3.76; N, 7.84. Found C, 61.88; H, 3.98; N: 7.67.

#### 4-{5-(4-Chlorophenyl)-3-[4-(4-chlorophenyl)-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (52)

Compound **52** was prepared according to general procedure G using **87** (0.039 g, 0.39 mmol) and **52f** (0.180 g, 0.39 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0–10% MeOH:DCM. Yield 0.095 g, 43.4%. <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 12.19 (s, 1H), 12.09 (s, 1H), 7.61 (dd, *J* = 8.2, 2.4 Hz, 1H), 7.52 (dd, *J* = 8.2, 2.4 Hz, 1H), 7.42 (dd, *J* = 8.2, 2.3 Hz, 1H), 7.39 (d, *J* = 8.8 Hz, 1H), 7.32 – 7.25 (m, 4H), 6.82 (d, *J* = 8.5 Hz, 2H), 6.42 (d, *J* = 2.8 Hz, 1H), 5.34 (dd, *J* = 11.9, 4.5 Hz, 1H), 3.73 (dd, *J* = 18.4, 12.0 Hz, 1H), 3.60 (s, 3H), 2.77 (dd, *J* = 18.4, 4.4 Hz, 1H), 2.63 – 2.44 (m, 2H), 2.33 (t, *J* = 6.6 Hz, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 173.52, 168.66, 159.69, 154.24, 152.88, 149.28, 141.09, 134.02, 133.23, 133.15, 131.57, 131.42, 130.51, 128.39, 128.29, 128.12, 127.31, 123.95, 120.17, 119.68, 117.01, 109.05, 58.20, 55.32, 45.15, 28.48, 28.21. HRMS (*m/z*): [M-H]<sup>–</sup> calcd for  $C_{29}H_{22}Cl_2N_3O_5$ , 562.09420; found, 562.09430. Anal. Calcd for  $C_{29}H_{23}Cl_2N_3O_5$ : C, 61.71; H, 4.11; N, 7.44. Found C, 61.47; H, 4.06; N, 7.36.

#### 4-{5-(4-Chlorophenyl)-3-[4-(3-fluorophenyl)-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanoic acid (53)

Compound **53** was prepared according to general procedure G using **87** (0.027 g, 0.27 mmol) and **53f** (0.120 g, 0.27 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0–10% MeOH:DCM. Yield 0.040 g, 27.7%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.21 (s, 1H), 7.64 – 7.48 (m, 1H), 7.43 – 7.07 (m, 6H), 6.87 – 6.77 (m, 2H), 6.45 – 6.40 (m, 1H), 5.40 – 5.30 (m, 1H) 3.87 – 3.74 (m, 2H), 3.60 (s, 3H),

2.83 (dt,  $J = 18.4, 5.2$  Hz, 1H), 2.54 – 2.39 (m, 2H), 2.31 (t,  $J = 6.9$  Hz, 2H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  173.55, 168.62, 162.77, 162.71, 161.15, 161.08, 159.73, 154.23, 152.93, 152.85, 149.03, 141.16, 137.56, 137.51, 133.12, 131.59, 131.52, 130.38, 128.41, 128.32, 127.44, 127.26, 127.07, 125.78, 124.73, 123.87, 123.78, 120.06, 119.63, 116.98, 115.17, 109.18, 58.35, 58.17, 55.38, 55.20, 45.10, 28.49, 28.23.  $^{19}\text{F}$  NMR (376 MHz, DMSO- $d_6$ )  $\delta$  –113.15 – –113.37 (m). HRMS ( $m/z$ ):  $[\text{M}-\text{H}]^-$  calcd for  $\text{C}_{29}\text{H}_{22}\text{ClFN}_3\text{O}_5$ , 546.12375; found, 546.12384. Anal. Calcd for  $\text{C}_{29}\text{H}_{23}\text{ClFN}_3\text{O}_5$ : C, 63.56; H, 4.23; N, 7.67. Found C, 52.15; H, 3.91; N, 5.95. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  0.809 min; > 95% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  0.625 min; > 95% purity.

**(E)-4-[5-(4-Bromophenyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobut-2-enoic acid (54)**

Compound **54** was prepared from **54g** (0.190 g, 0.322 mmol) and 1M NaOH (1.22 mL, 1.22 mmol), which were stirred to give a yellow solution. After four hours, 1M HCl (1.22 mL) was added and a yellow solid precipitated. The solid was filtered and rinsed with water to give the title compound as a yellow solid. Yield 0.170 g, 92.0%.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  13.03 (s, 1H), 12.46 (s, 1H), 7.66 (dd,  $J = 8.8, 2.4$  Hz, 1H), 7.61 – 7.38 (m, 7H), 7.31 (dt,  $J = 5.6, 2.5$  Hz, 1H), 7.26 (d,  $J = 15.7$  Hz, 1H), 6.94 (d,  $J = 2.4$  Hz, 1H), 6.78 (d,  $J = 8.3$  Hz, 2H), 6.45 (d,  $J = 15.7$  Hz, 1H), 5.45 (dd,  $J = 11.8, 4.5$  Hz, 1H), 3.80 (dd,  $J = 18.7, 11.9$  Hz, 1H), 2.88 (dd,  $J = 18.7, 4.5$  Hz, 1H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  167.81, 166.11, 160.30, 159.96, 155.82, 154.41, 150.24, 140.70, 137.39, 134.48, 132.67, 131.83, 131.42, 129.19, 128.63, 128.51, 128.36, 127.93, 127.83, 126.17, 126.05, 124.09, 120.60, 120.44, 117.66, 58.69, 58.63, 45.11. HRMS ( $m/z$ ):  $[\text{M}-\text{H}]^-$  calcd for  $\text{C}_{28}\text{H}_{18}\text{ClBrN}_3\text{O}_4$ , 574.01747; found, 574.01750. Anal. Calcd for  $\text{C}_{28}\text{H}_{19}\text{ClBrN}_3\text{O}_4 \cdot 1.00\text{H}_2\text{O}$ : C, 56.54; H, 3.56; N, 7.06. Found C, 56.66; H, 3.76; N: 6.93.

**(Z)-4-[5-(4-Bromophenyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobut-2-enoic acid (55)**

Compound **55** was prepared according to general procedure G using furan-2,5-dione (**90**) (0.061 g, 0.63 mmol) and **55f** (0.300 g, 0.63 mmol). The title compound was obtained after filtration from the cooled reaction medium and rinsed with THF. Yield 0.200 g, 55.3%.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.69 (s, 1H), 12.41 (s, 1H), 7.64 (d,  $J = 8.7$  Hz, 1H), 7.60 – 7.36 (m, 7H), 7.25 (d,  $J = 7.2$  Hz, 1H), 6.92 (s, 1H), 6.78 (d,  $J = 8.1$  Hz, 2H), 6.40 (d,  $J = 12.1$  Hz, 1H), 6.16 (d,  $J = 12.1$  Hz, 1H), 5.37 (dd,  $J = 12.0, 4.9$  Hz, 1H), 3.78 (dd,  $J = 18.5, 12.0$  Hz, 1H), 2.79 (dd,  $J = 18.7, 4.8$  Hz, 1H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  166.77, 161.77, 160.01, 153.58, 150.11, 140.93, 137.36, 134.39, 131.31, 129.57, 129.46, 128.64, 128.43, 127.96, 126.16, 126.08, 124.26, 120.62, 120.29, 117.67, 58.43, 45.17. HRMS ( $m/z$ ):  $[\text{M}-\text{H}]^-$  calcd for  $\text{C}_{28}\text{H}_{18}\text{ClBrN}_3\text{O}_4$ , 574.01747; found, 574.01654. Anal. Calcd for  $\text{C}_{28}\text{H}_{19}\text{BrClN}_3\text{O}_4$ : C, 58.30; H, 3.32; N, 7.28. Found C, 43.78; H, 3.00; N: 5.57. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  1.18 min; > 95% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  1.06 min; > 95% purity.

**Methyl 4-[5-(4-bromophenyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-oxobutanoate (56)**

Compound **56** was prepared from **56g** (0.400 g, 0.69 mmol) in the following manner. **56g** (0.400 g, 0.69 mmol) was dissolved in 6.9 mL THF and freshly prepared HCl (Acetyl chloride added to methanol) in MeOH was added dropwise to the reaction vessel with stirring until TLC indicated completion. Upon completion, the THF was removed under vacuum, the residue dissolved in DCM, washed 3X with acidified brine and column chromatographed using a 0–10% MeOH gradient in DCM to give the title compound as a yellow solid. Yield 0.100 g, 24.4%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.42 (s, 1H), 7.64 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.54 – 7.49 (m, 3H), 7.48 – 7.38 (m, 4H), 7.31 – 7.24 (m, 1H), 6.93 (d, *J* = 2.3 Hz, 1H), 6.77 – 6.70 (m, 2H), 5.31 (dd, *J* = 12.0, 4.6 Hz, 1H), 3.76 (dd, *J* = 18.5, 12.2 Hz, 1H), 3.59 (s, 1H), 3.54 (s, 2H), 2.79 (dd, *J* = 18.5, 4.6 Hz, 1H), 2.61 – 2.42 (m, 2H), 2.37 (t, *J* = 6.7 Hz, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 172.44, 168.37, 168.08, 160.09, 152.81, 152.61, 149.98, 141.49, 137.32, 134.58, 131.30, 129.45, 128.48, 128.36, 127.78, 127.67, 126.13, 126.01, 124.50, 120.65, 120.14, 117.65, 58.43, 58.30, 51.35, 51.24, 45.23, 45.08, 28.46, 27.96. HRMS (*m/z*): [M-H]<sup>-</sup> calcd for C<sub>29</sub>H<sub>22</sub>ClBrN<sub>3</sub>O<sub>4</sub>, 590.04877; found, 590.04851. Anal. Calcd for C<sub>29</sub>H<sub>23</sub>ClBrN<sub>3</sub>O<sub>4</sub>: C, 58.75; H, 3.91; N, 7.09. Found C, 58.35; H, 4.08; N, 6.66.

**(E)-4-{3-[4-(4-Bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobut-2-enoic acid (58)**

Compound **58** was prepared from compound **60** using the following method. In a 50 mL round-bottomed flask, ethanol (28.2 mL), **60** (0.500 g, 0.850 mmol) and 1M NaOH (3.22 mL, 3.22 mmol) were stirred to give a yellow solution. After four hours, 1M HCl (3.22 mL) was added and a yellow solid precipitated. The solid was filtered and rinsed with water. The resulting solid was dissolved in DCM, washed with brine and the organics were dried over magnesium sulfate *in vacuo*. The title compound was obtained from column chromatography (0–10% MeOH in DCM) as an off-white solid. Yield 0.320 g, 65.6%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.98 (s, 1H), 12.28 (s, 1H), 7.71 – 7.51 (m, 3H), 7.48 – 7.19 (m, 6H), 7.12 (t, *J* = 7.6 Hz, 1H), 7.02 (d, *J* = 8.2 Hz, 1H), 6.85 (d, *J* = 8.2 Hz, 2H), 6.44 (d, *J* = 15.7 Hz, 1H), 5.45 (dd, *J* = 11.7, 4.4 Hz, 1H), 3.73 (dd, *J* = 18.7, 11.8 Hz, 1H), 2.89 (dd, *J* = 18.6, 4.4 Hz, 1H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 168.08, 166.05, 160.24, 160.04, 154.75, 152.81, 152.77, 150.29, 140.25, 138.65, 134.49, 132.80, 131.95, 131.60, 131.36, 131.26, 130.74, 128.51, 127.55, 127.43, 122.92, 122.47, 121.84, 119.01, 115.61, 58.50, 45.19. HRMS (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>28</sub>H<sub>20</sub>ClBrN<sub>3</sub>O<sub>4</sub>, 576.03202; found, 576.03294. Anal. Calcd for C<sub>28</sub>H<sub>19</sub>ClBrN<sub>3</sub>O<sub>4</sub>·0.40H<sub>2</sub>O: C, 57.58; H, 3.42; N, 7.19. Found C, 57.46; H, 3.50; N, 7.23.

**(Z)-4-{3-[4-(4-Bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobut-2-enoic acid (59)**

Compound **59** was prepared according to general procedure G using **89** (0.061 g, 0.63 mmol) and **22f** (0.300 g, 0.630 mmol). The THF was removed under vacuum and the resultant residue was dissolved in hot EtOAc. A yellow solid was present upon cooling which was filtered and determined to be the desired product. Yield 0.171 g, 47.3%. <sup>1</sup>H NMR

(400 MHz, DMSO- $d_6$ )  $\delta$  12.74 (s, 1H), 12.30 (s, 1H), 7.75 (d,  $J = 8.3$  Hz, 1H), 7.67 (d,  $J = 8.4$  Hz, 1H), 7.58 (t,  $J = 7.9$  Hz, 1H), 7.43 (d,  $J = 8.4$  Hz, 1H), 7.32 (t,  $J = 8.6$  Hz, 3H), 7.22 (d,  $J = 8.2$  Hz, 1H), 7.15 (t,  $J = 7.8$  Hz, 1H), 7.03 (d,  $J = 8.2$  Hz, 1H), 6.87 (d,  $J = 8.1$  Hz, 2H), 6.48 (d,  $J = 12.2$  Hz, 1H), 6.20 (d,  $J = 12.1$  Hz, 1H), 5.47 – 5.38 (m, 1H), 3.76 (dd,  $J = 18.8, 11.9$  Hz, 1H), 2.84 – 2.74 (m, 1H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  166.80, 161.77, 160.05, 153.90, 150.03, 140.50, 138.60, 134.27, 131.76, 131.69, 131.55, 131.28, 130.82, 129.68, 129.41, 128.36, 127.51, 127.32, 123.10, 122.47, 121.89, 119.04, 115.61, 93.88, 67.04, 58.28, 45.27, 25.15. HRMS ( $m/z$ ):  $[\text{M-H}]^-$  calcd for  $\text{C}_{28}\text{H}_{18}\text{ClBrN}_3\text{O}_4$ , 576.03202; found, 576.03371. Anal. Calcd for  $\text{C}_{28}\text{H}_{19}\text{ClBrN}_3\text{O}_4 \cdot 0.60\text{H}_2\text{O}$ : C, 57.23; H, 3.46; N, 7.15. Found C, 57.22; H, 3.54; N, 6.91.

**(E)-Methyl 4-{3-[4-(4-bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobut-2-enoate (60)**

Compound **60** was prepared from **22f** (0.750g, 1.60 mmol) and (*E*)-methyl 4-chloro-4-oxobut-2-enoate (0.280 g, 1.90 mmol) using standard procedure G. The THF was removed under vacuum, the residue dissolved in DCM, washed 3X with brine and the organics concentrated. The title compound was obtained as a yellow solid by flash chromatography using a 0–10% MeOH gradient in DCM. Yield 0.546 g, 59.0%.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.32 (s, 1H), 7.70 (dt,  $J = 8.3, 1.9$  Hz, 1H), 7.67 (dt,  $J = 8.1, 2.0$  Hz, 1H), 7.60 (t,  $J = 7.8$  Hz, 1H), 7.44 (d,  $J = 8.3$  Hz, 1H), 7.37 – 7.32 (m, 3H), 7.30 (dt,  $J = 8.2, 2.0$  Hz, 1H), 7.27 – 7.22 (m, 1H), 7.16 (t,  $J = 7.6$  Hz, 1H), 7.08 (d,  $J = 8.2$  Hz, 1H), 6.95 (d,  $J = 7.6$  Hz, 2H), 6.53 (dd,  $J = 15.5, 1.0$  Hz, 1H), 5.49 (dd,  $J = 11.6, 4.3$  Hz, 1H), 3.86 – 3.73 (m, 4H), 3.03 (dd,  $J = 18.6, 4.2$  Hz, 1H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  165.13, 160.11, 159.92, 154.93, 150.40, 140.16, 138.71, 134.71, 133.36, 132.02, 131.69, 131.37, 131.30, 131.11, 130.80, 129.93, 128.65, 128.56, 127.57, 127.46, 122.77, 122.50, 121.77, 119.01, 115.63, 58.56, 52.16, 45.15. HRMS ( $m/z$ ):  $[\text{M+H}]^+$  calcd for  $\text{C}_{29}\text{H}_{22}\text{ClBrN}_3\text{O}_4$ , 590.04767; found, 590.04887. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  1.56 min; > 90% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  1.39 min; > 90% purity.

**5-{3-[4-(4-Bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl}-5-oxopentanoic acid (61)**

Compound **61** was prepared according to general procedure G using **88** (0.071 g, 0.63 mmol) and **22f** (0.30 g, 0.63 mmol). Upon completion, the THF was removed under vacuum and the residue was dissolved in hot EtOAc. Upon cooling, a yellow solid formed which was filtered, dried under vacuum and determined to be the desired product. Yield 0.204 g, 54.9%.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.29 (s, 1H), 12.09 (s, 1H), 7.77 (dd,  $J = 8.2, 2.1$  Hz, 1H), 7.70 (dd,  $J = 8.2, 2.1$  Hz, 1H), 7.58 (t,  $J = 7.7$  Hz, 1H), 7.43 (d,  $J = 8.3$  Hz, 1H), 7.36 (dd,  $J = 8.2, 2.2$  Hz, 1H), 7.34 – 7.28 (m, 2H), 7.23 (dd,  $J = 8.1, 2.3$  Hz, 1H), 7.15 (t,  $J = 7.6$  Hz, 1H), 7.04 (d,  $J = 8.2$  Hz, 1H), 6.86 – 6.76 (m, 2H), 5.36 (dd,  $J = 12.0, 4.4$  Hz, 1H), 3.81 – 3.68 (m, 1H), 2.80 (dd,  $J = 18.7, 4.3$  Hz, 1H), 2.40 (dt,  $J = 15.3, 7.4$  Hz, 1H), 2.29 – 2.06 (m, 3H), 1.68 – 1.51 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  174.11, 169.29, 160.18, 152.73, 149.89, 141.29, 138.57, 134.59, 131.73, 131.64, 131.47, 131.30, 131.25, 130.88, 128.40, 127.31, 127.27, 123.41, 122.44, 121.77, 119.09, 115.60, 58.13, 45.10, 32.96, 32.58, 19.82. HRMS ( $m/z$ ):  $[\text{M+H}]^+$  calcd for  $\text{C}_{29}\text{H}_{24}\text{ClBrN}_3\text{O}_4$ , 592.06332; found,

592.06461. Anal. Calcd for C<sub>29</sub>H<sub>23</sub>ClBrN<sub>3</sub>O<sub>4</sub>: C, 58.75; H, 3.91; N, 7.09. Found C, 58.47; H, 3.96; N, 6.91.

#### 5-[3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-5-oxopentanoic acid (63)

Compound **63** was prepared according to general procedure G using glutaric anhydride (**89**) (0.077 g, 0.67 mmol) and **63f** (0.30 g, 0.67 mmol). The title compound was obtained after being dissolved in hot EtOAc followed by slow addition of hot hexanes to yield the title compound as a yellow solid. Yield 0.220 g, 58.4%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.44 (s, 1H), 12.12 (s, 1H), 8.12 (d, *J* = 8.3 Hz, 2H), 7.70 – 7.51 (m, 4H), 7.51 – 7.38 (m, 2H), 7.28 (d, *J* = 6.6 Hz, 1H), 7.07 (d, *J* = 8.5 Hz, 2H), 6.95 (d, *J* = 2.5 Hz, 1H), 5.50 (dd, *J* = 12.3, 4.8 Hz, 1H), 3.83 (dd, *J* = 18.6, 12.2 Hz, 1H), 2.86 (dd, *J* = 18.6, 4.9 Hz, 1H), 2.45 – 2.30 (m, 1H), 2.29 – 2.17 (m, 1H), 2.13 (t, *J* = 7.4 Hz, 2H), 1.65 – 1.45 (m, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 174.07, 169.49, 160.10, 152.49, 149.59, 146.59, 137.35, 131.30, 129.39, 128.47, 126.68, 126.08, 124.34, 123.82, 120.63, 117.67, 58.32, 44.90, 32.94, 32.53, 19.70. HRMS (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>29</sub>H<sub>24</sub>ClN<sub>4</sub>O<sub>6</sub>, 559.13789; found, 559.13826. Anal. Calcd for C<sub>29</sub>H<sub>23</sub>ClN<sub>4</sub>O<sub>6</sub>: C, 62.31; H, 4.15; N, 10.02. Found C, 62.42; H, 4.39; N, 9.72.

#### 4-(4-Chlorophenyl)-3-[5-(4-chlorophenyl)-1-(4-hydroxybutanoyl)-4,5-dihydro-1H-pyrazol-3-yl]quinolin-2(1H)-one (64)

In a flame dried 25 mL round bottomed flask, **26** (0.300 g, 0.560 mmol) was dissolved in THF (10 mL) and cooled on an ice bath to 0 °C under nitrogen with stirring. BH<sub>3</sub>-Me<sub>2</sub>S (2.0M in Hexanes, 0.561 ml, 2 eq.) was added drop-wise. The reaction was stirred for thirty minutes, quenched with MeOH and the solvent was removed under vacuum. The resultant residue was dissolved in DCM, washed three times with brine and the organics combined, dried over magnesium sulfate, concentrated *in vacuo* and column chromatographed using a 0–8% gradient of MeOH in DCM to give the title compound as a yellow solid. Yield 0.063 g, 21.6%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.29 (s, 1H), 7.65 – 7.49 (m, 3H), 7.46 – 7.24 (m, 5H), 7.20 – 7.00 (m, 2H), 6.89 – 6.78 (m, 2H), 5.35 (dd, *J* = 12.0, 4.4 Hz, 1H), 4.45 (t, *J* = 5.2 Hz, 1H), 3.74 (dd, *J* = 18.5, 12.1 Hz, 1H), 3.41 – 3.27 (m, 2H), 2.80 (dd, *J* = 18.5, 4.5 Hz, 1H), 2.44 – 2.19 (m, 2H), 1.59 – 1.39 (m, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 169.82, 160.17, 152.52, 149.83, 141.31, 138.53, 134.18, 133.14, 131.58, 131.43, 131.39, 130.57, 129.11, 128.33, 127.28, 123.50, 122.41, 119.14, 115.57, 60.19, 58.10, 45.06, 30.14, 27.71. HRMS (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>28</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub>, 520.11892; found, 520.11993. Anal. Calcd for C<sub>28</sub>H<sub>23</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub>·0.40H<sub>2</sub>O: C, 63.74; H, 4.55; N, 7.96. Found C, 63.75; H, 4.33; N, 7.89.

#### 4-{5-(4-Chlorophenyl)-3-[4-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}-4-oxobutanamide (65)

In a flame dried 25 mL round bottomed flask, **26** (0.300 g, 0.560 mmol), 4-dimethylaminopyridine (0.069 g, 0.560 mmol) and N1-((ethylimino)methylene)-N3,N3-dimethylpropane-1,3-diamine (0.096 g, 0.618 mmol) were added to THF (11.23 mL) at 0 °C and stirred for 45 minutes. Ammonia (0.5 M in dioxane, 1.0 eq, 1.1 mL) was added to the flask and the reaction mixture was stirred overnight while being allowed to warm to room



temperature. The reaction was quenched with dilute HCl (0.1 M) and the organics were removed under vacuum. The resultant residue was dissolved in DCM, washed 3X with brine and the organics dried over magnesium sulfate and concentrated *in vacuo* prior to column chromatography using a 0–8% MeOH gradient in DCM (0.1 % Et<sub>3</sub>N). The title compound was obtained as a white solid. Yield 0.019 g, 6.35%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.29 (s, 1H), 7.66 – 7.56 (m, 2H), 7.55 – 7.49 (m, 1H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.28 (dd, *J* = 8.2, 4.4 Hz, 3H), 7.24 (s, 1H), 7.15 (t, *J* = 7.6 Hz, 1H), 7.03 (d, *J* = 8.2 Hz, 1H), 6.79 (d, *J* = 8.5 Hz, 2H), 6.73 (s, 1H), 5.34 (dd, *J* = 12.1, 4.5 Hz, 1H), 3.71 (dd, *J* = 18.4, 12.2 Hz, 1H), 2.72 (dd, *J* = 18.4, 4.5 Hz, 1H), 2.59 – 2.50 (m, 2H), 2.20 (t, *J* = 7.2 Hz, 2H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 173.69, 169.78, 160.79, 153.18, 150.49, 141.85, 139.21, 134.63, 133.87, 132.22, 132.19, 132.09, 131.23, 128.99, 128.95, 127.94, 124.23, 123.07, 119.80, 116.24, 58.83, 46.27, 45.80, 29.94, 29.44. HRMS (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>28</sub>H<sub>23</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>3</sub>, 533.11417; found, 533.11517. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>f</sub> 0.89 min; > 90% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>f</sub> 1.00 min; > 95% purity.

#### Methyl 4-{5-(4-chlorophenyl)-3-[4-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}butanoate (66)

In a 20 mL round-bottomed flask, **26f** (0.500 g, 1.151 mmol) and methyl 4-oxobutanoate (0.121 ml, 1.151 mmol) were dissolved in DCE (11.51 ml). The mixture was allowed to stir at room temperature for four hours and sodium triacetoxyborohydride (0.293 g, 1.381 mmol) was added in one portion. The reaction was monitored by TLC and HPLC-MS. Upon completion, the DCE was removed under vacuum; the residue diluted with DCM and washed 3X with brine. The organics were concentrated and the title compound was obtained from flash column chromatography using 0–10% MeOH in DCM and trituration of the compound from ether as a yellow solid. Yield 0.150 g, 24.4%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 12.22 (s, 1H), 7.55 – 7.45 (m, 3H), 7.40 (s, 1H), 7.34 – 7.26 (m, 5H), 7.22 (s, 2H), 7.13 (t, *J* = 7.6 Hz, 1H), 4.11 (dd, *J* = 13.6, 10.1 Hz, 1H), 3.62 (s, 3H), 3.41 (dd, *J* = 16.4, 10.1 Hz, 1H), 2.91 – 2.78 (m, 1H), 2.70 – 2.53 (m, 2H), 2.30 – 2.08 (m, 2H), 1.84 – 1.66 (m, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 174.18, 162.94, 150.63, 146.34, 139.59, 138.08, 135.07, 134.23, 133.49, 131.12, 130.90, 129.08, 128.89, 128.51, 128.33, 127.82, 124.96, 122.96, 120.65, 116.18, 70.81, 52.20, 51.66, 46.60, 31.30, 23.04. HRMS (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>29</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub>, 534.13457; found, 534.13624. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>f</sub> 2.35 min; > 95% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid) *R*<sub>f</sub> 2.09 min; > 95% purity.

#### 4-{5-(4-Chlorophenyl)-3-[4-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]-4,5-dihydro-1H-pyrazol-1-yl}butanoic acid (67)

In a 10 mL round-bottomed flask, compound **66** (0.100 g, 0.187 mmol) was dissolved in NaOH (0.711 ml, 0.711 mmol), H<sub>2</sub>O (10.00 ml) and Ethanol (6.24 ml). The mixture was stirred at room temperature for four hours and monitored by TLC/LC-MS. Upon completion, HCl (0.711 ml, 0.711 mmol) was added, giving a bright yellow solid which was filtered, dissolved in DCM and washed 3X with acidified (pH 2, HCl) brine. The organics were combined, dried over magnesium sulfate and concentrated *in vacuo*. The title compound was obtained by flash chromatography using 0–8% MeOH in DCM as a yellow solid. Yield 0.050 g, 51.3%. <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 12.16 (s, 1H), 11.92 (s, 1H),

7.59 – 7.49 (m, 3H), 7.42 – 7.37 (m, 3H), 7.35 – 7.29 (m, 4H), 7.13 (t,  $J = 7.6$  Hz, 1H), 7.07 (d,  $J = 8.2$  Hz, 1H), 4.03 (dd,  $J = 13.8, 10.2$  Hz, 1H), 3.33 (dd,  $J = 16.4, 10.2$  Hz, 1H), 2.73 (dd,  $J = 16.5, 13.8$  Hz, 1H), 2.54 – 2.46 (m, 1H), 2.40 – 2.31 (m, 1H), 2.12 – 1.96 (m, 2H), 1.55 – 1.39 (m, 2H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  174.25, 160.57, 148.75, 146.65, 140.21, 138.31, 135.02, 132.63, 131.86, 131.23, 131.19, 130.84, 130.75, 129.09, 128.44, 128.08, 127.81, 127.04, 125.16, 122.15, 119.31, 115.43, 69.48, 52.16, 46.06, 30.83, 22.35. HRMS ( $m/z$ ):  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{28}\text{H}_{24}\text{Cl}_2\text{N}_3\text{O}_3$ , 520.11892; found, 520.11970. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  1.33 min; > 95% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  0.98 min; > 95% purity.

#### 4-(4-Chlorophenyl)-3-[5-(4-chlorophenyl)-1-(4-fluorobutanoyl)-4,5-dihydro-1H-pyrazol-3-yl]quinolin-2(1H)-one (68)

In a 10 mL round-bottomed flask, 4-fluorobutanoic acid **68g** (0.100 g, 0.943 mmol), DMAP (0.127 g, 1.037 mmol) and EDCI (0.199 g, 1.037 mmol) were dissolved in DCM (9.43 ml) which had been pre-cooled to 0°C. The mixture was stirred for 45 minutes prior to the addition of the **26f** (0.409 g, 0.943 mmol). The reaction was allowed to warm to room temperature, and monitored by TLC. The reaction was quenched with 0.2 N HCl and extracted into DCM. The organics were washed 3X with brine, dried over magnesium sulfate and concentrated. The title compound was obtained as a yellow solid after column chromatography using a gradient of 0–50% EtOAc in DCM as a yellow solid. Yield 0.050 g, 21.6%.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  13.16 (s, 1H), 7.62 – 7.43 (m, 4H), 7.38 (d,  $J = 8.2$  Hz, 1H), 7.34 – 7.16 (m, 5H), 7.02 (d,  $J = 8.3$  Hz, 2H), 5.42 (dd,  $J = 11.8, 4.0$  Hz, 1H), 4.44 (dtd,  $J = 47.3, 5.8, 1.7$  Hz, 2H), 3.70 (dd,  $J = 18.2, 11.8$  Hz, 1H), 3.15 (dd,  $J = 18.3, 4.1$  Hz, 1H), 2.68 – 2.45 (m, 2H), 2.18 – 1.82 (m, 2H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  170.45, 162.91, 152.52, 151.98, 151.92, 140.41, 138.42, 135.43, 134.99, 133.97, 133.56, 132.00, 130.81, 130.72, 130.68, 130.36, 129.05, 128.96, 128.85, 128.73, 128.70, 128.04, 127.60, 127.52, 126.86, 123.47, 123.09, 120.36, 116.41, 84.06, 82.97, 59.35, 45.44, 29.91, 29.88, 25.69, 25.62, 25.56.  $^{19}\text{F}$  NMR (282 MHz,  $\text{CDCl}_3$ )  $\delta$  -220.53 (tt,  $J = 47.3, 26.3$  Hz). HRMS ( $m/z$ ):  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{28}\text{H}_{23}\text{Cl}_2\text{N}_3\text{O}_2\text{F}$ , 522.11459; found, 522.11462. HPLC 85% MeOH:H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  1.46 min; > 95% purity; 75% ACN: H<sub>2</sub>O (0.1% Formic Acid)  $R_t$  1.32 min; > 95% purity.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations Used

<b>Ca<sup>2+</sup></b>	calcium ion
<b>Mg<sup>2+</sup></b>	magnesium ion
<b>ATD</b>	amino-terminal domain
<b>LBD</b>	ligand-binding domain
<b>DQP</b>	dihydroquinolone-pyrazoline
<b>BH<sub>3</sub>-DMS</b>	borane dimethylsulfide
<b>NH<sub>3</sub></b>	ammonia
<b>μM</b>	micromolar
<b>mV</b>	millivolt
<b>QNZ</b>	quinazoline-4-one
<b>S2</b>	segment two
<b>TPSA</b>	topological polar surface area
<b>P<sub>app</sub></b>	permeability coefficient
<b>MDR1-MDCK</b>	multidrug resistance gene 1-Madin-Darby canine kidney cells
<b>cRNA</b>	complementary RNA
<b>NaCl</b>	sodium chloride
<b>KCl</b>	potassium chloride
<b>BaCl<sub>2</sub></b>	barium chloride
<b>NaOH</b>	sodium hydroxide
<b>MΩ</b>	megaohm
<b>NIMH PDSP</b>	National Institute of Mental Health Psychoactive Drug Screening Program
<b>ACN</b>	acetonitrile
<b>EtOAc</b>	ethyl acetate

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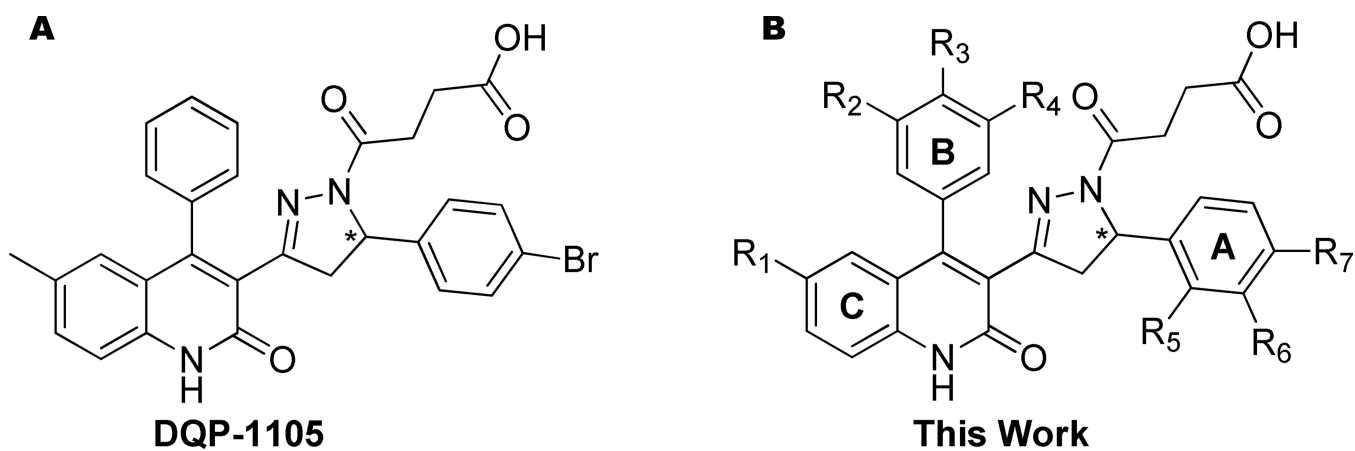
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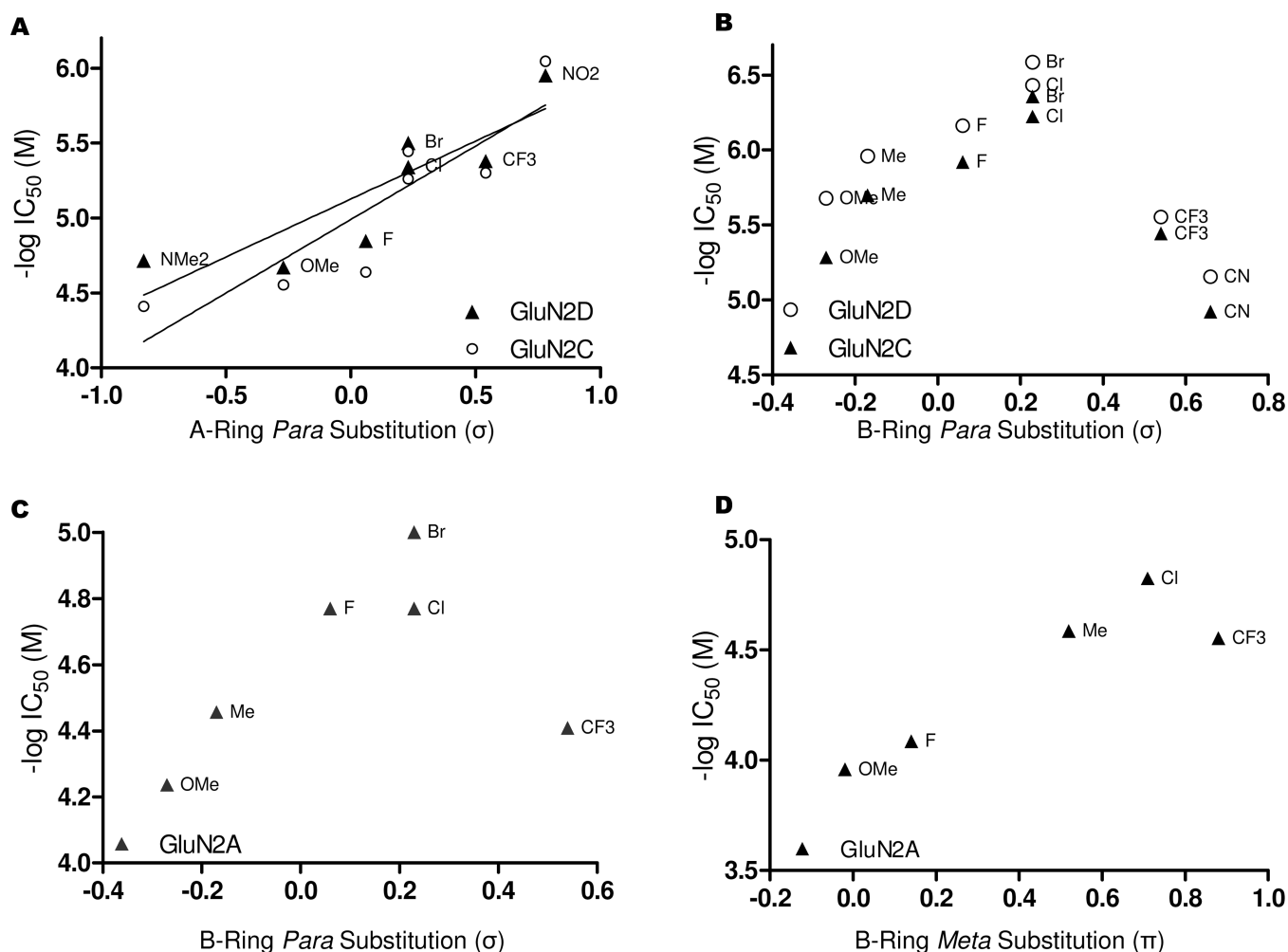
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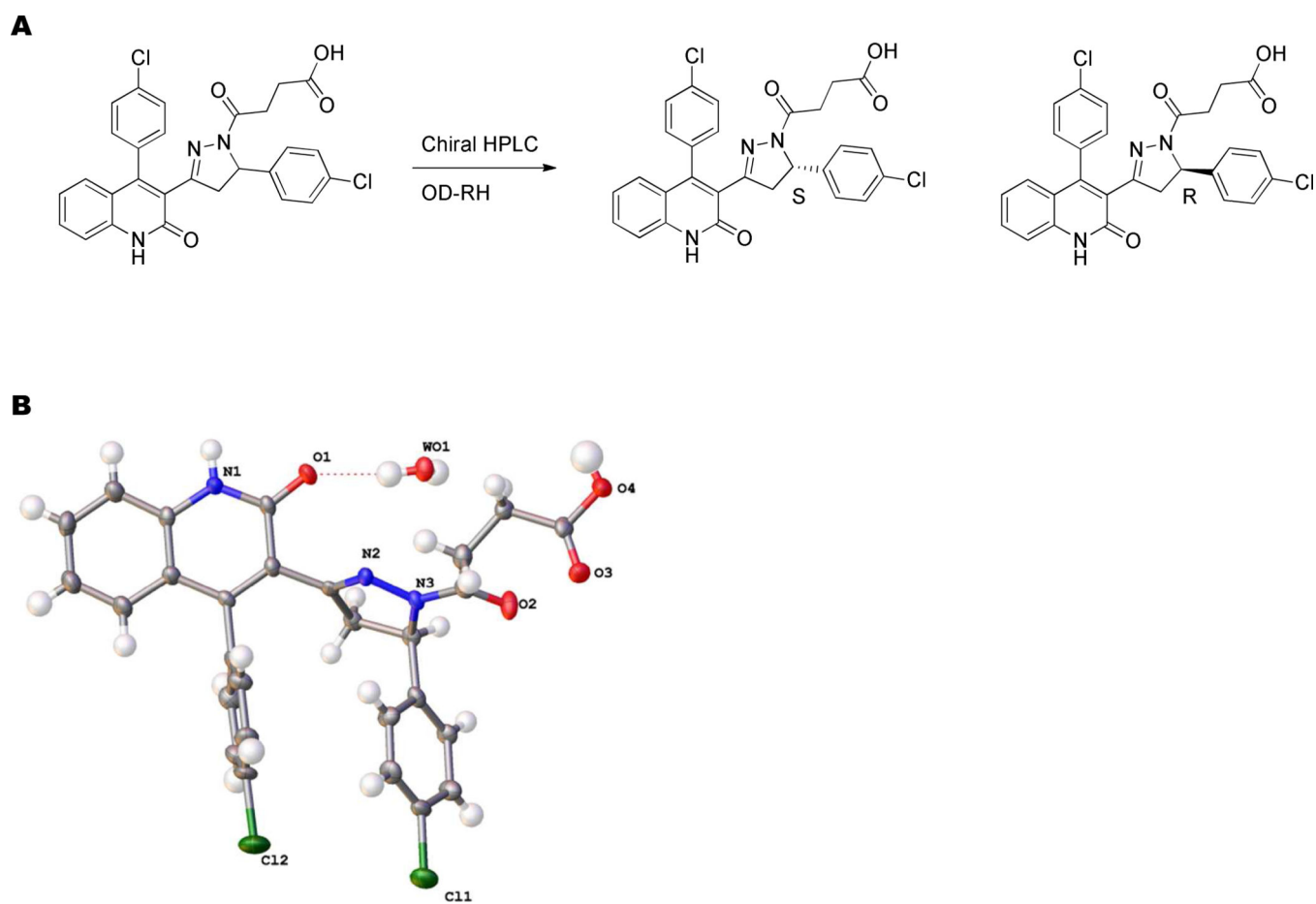


**Figure 1. Previously reported best in class compound and representative structure for SAR**  
A. The structure of the previously reported best in class compound, **DQP-1105**<sup>30</sup> B. The structure of a general analogue with numbered substituents is shown.



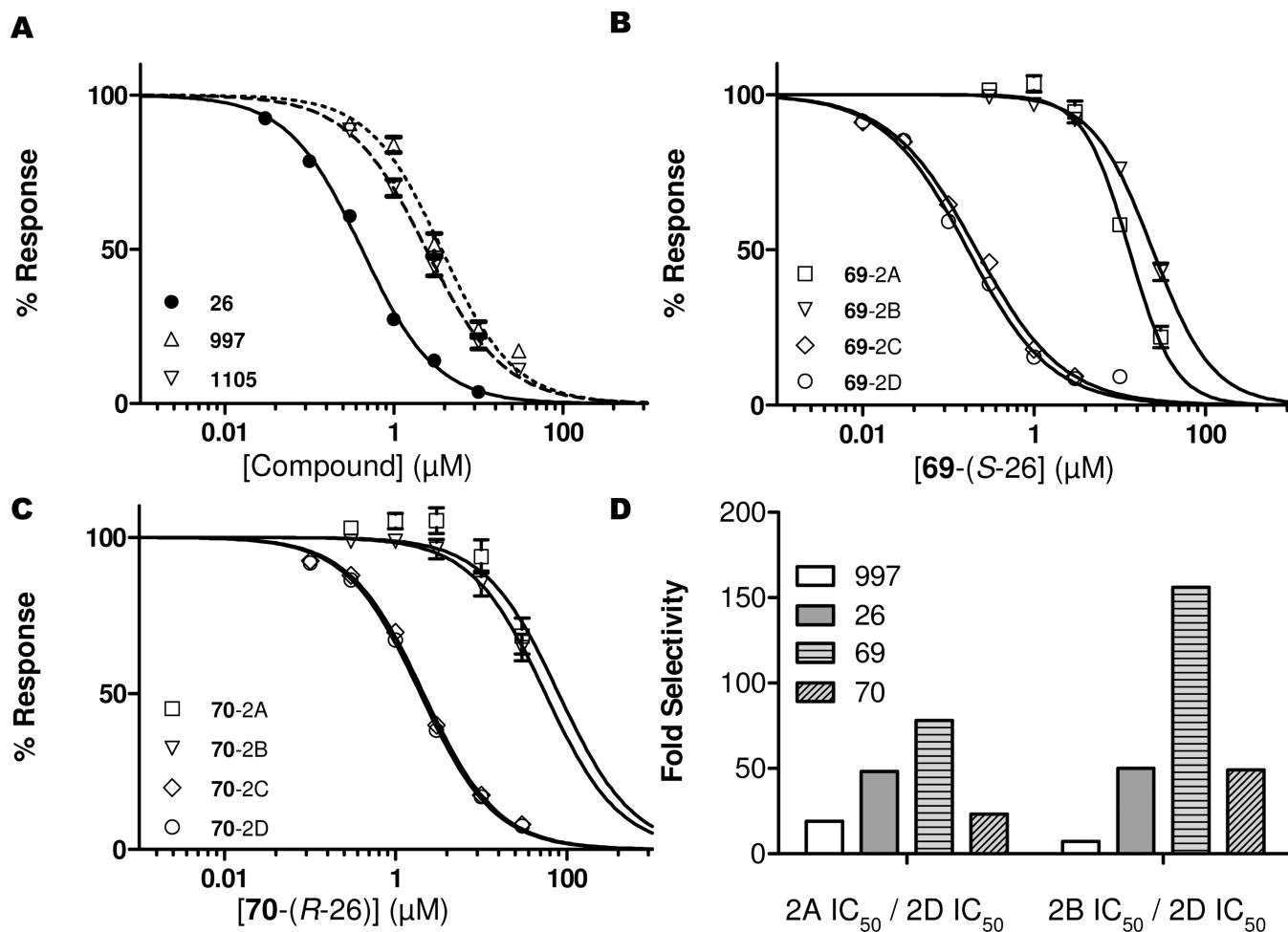
**Figure 2. Evaluation of substituent effects for A- and B-ring modifications**

A. The  $\sigma$  substituent constants of the *para*-substituted A-ring analogs vs. activity show a correlation for GluN2C- and GluN2D-containing receptors, when the R<sub>1</sub> position of the C-ring is substituted with chloro. (GluN2D  $r^2=0.82$ ,  $p < 0.05$  Pearson two-tailed correlation analysis; GluN2C  $r^2=0.84$ ,  $p < 0.05$  Pearson two-tailed correlation analysis; **Compounds 2–5 and 8–10**). B. The analysis of the *para*-substituents on the B-ring as a function of activity at GluN2C- and GluN2D-containing receptors appears parabolic with respect to the  $\sigma$  substituent constants, with an optimal value close to that of the chloro- and bromo-substitutions (**Compounds 29–33**). C. The analysis of the *para*-substituents at GluN2A-containing receptors shows a similar parabolic relationship as observed at the GluN2C- and GluN2D-containing receptors when the activity is plotted as a function of the  $\sigma$  substituent constants (**Compounds 29–33**). D. The analysis of the substituent effects appears parabolic with respect to the  $\pi$  substituent constants for B-ring meta-substituted compounds at GluN2A-containing receptors, suggesting an optimal hydrophobicity close to that of the chloro-substitution. Substituent constants were obtained from the same source (**Compounds 34–39**).<sup>35</sup>



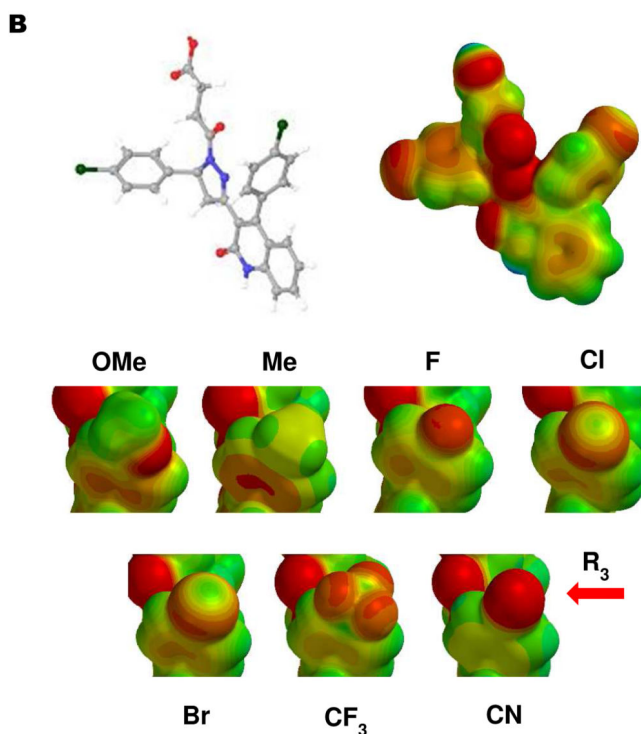
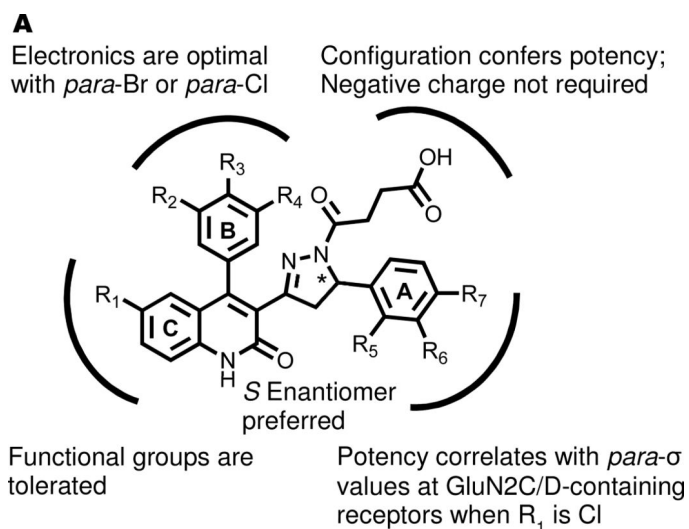
**Figure 3. Separation of enantiomers**

A. The enantiomers of the final compound, **26**, could be separated using reverse phase chiral chromatography (see *Methods*). B. The crystal structure of the inactive enantiomer, **70** (Table 6) was solved using X-ray diffraction and has the *R* configuration.



**Figure 4. Improvements in selectivity and potency**

A. The potency of the racemic compounds at GluN2D-containing receptors was improved 10-fold over the previous members in the class. B. The potency of the *S*-enantiomer of compound **26**, compound **69**, is two-fold more potent than the racemic mixture at GluN2D-containing receptors while the potency at GluN2A- and GluN2B-containing receptors is unaffected, making it more selective for GluN2C- and GluN2D-containing receptors. C. The potency of the *R*-enantiomer of compound **26**, compound **70**, at GluN2C- and GluN2D-containing receptors is diminished as compared to the racemate **26**, making it less selective over GluN2A- and GluN2B-containing receptors. D. Bar graph showing the fold-selectivity improvements attained through SAR. Data for compounds **997** and **DQP-1105** (Panels A and D) are previously published and shown here for comparison.<sup>30</sup>



**Figure 5. Pharmacophore model and electrostatic potential maps of *para*-B-ring modifications**  
 A. The *para*-substitution of the A-ring shows correlation between the  $\sigma$  substituent constants and activity at GluN2C/D-containing receptors, when R<sub>1</sub> is a chlorine. The length and configuration of the acyl-chain is flexible, with the *trans*-configuration improving potency; B-ring modification shows an optimal *para*-sigma coefficient close to that of chloro- and bromo-substitutions for GluN2A-, GluN2C- and GluN2D-containing receptors, suggesting a conserved nature of the binding interaction at each of the three receptors. The C-ring substitutions explored are consistent with this portion of the molecule interacting with a

hydrophobic pocket and could allow for improvements in selectivity. B. The electrostatic potential maps of the *para*-B-ring modifications evaluated are shown. Only the Cl- and Br-substituents show significant electron deficiency at the termini of the substituents, suggesting a potential halogen bond could be responsible for the improved potency of these compounds.

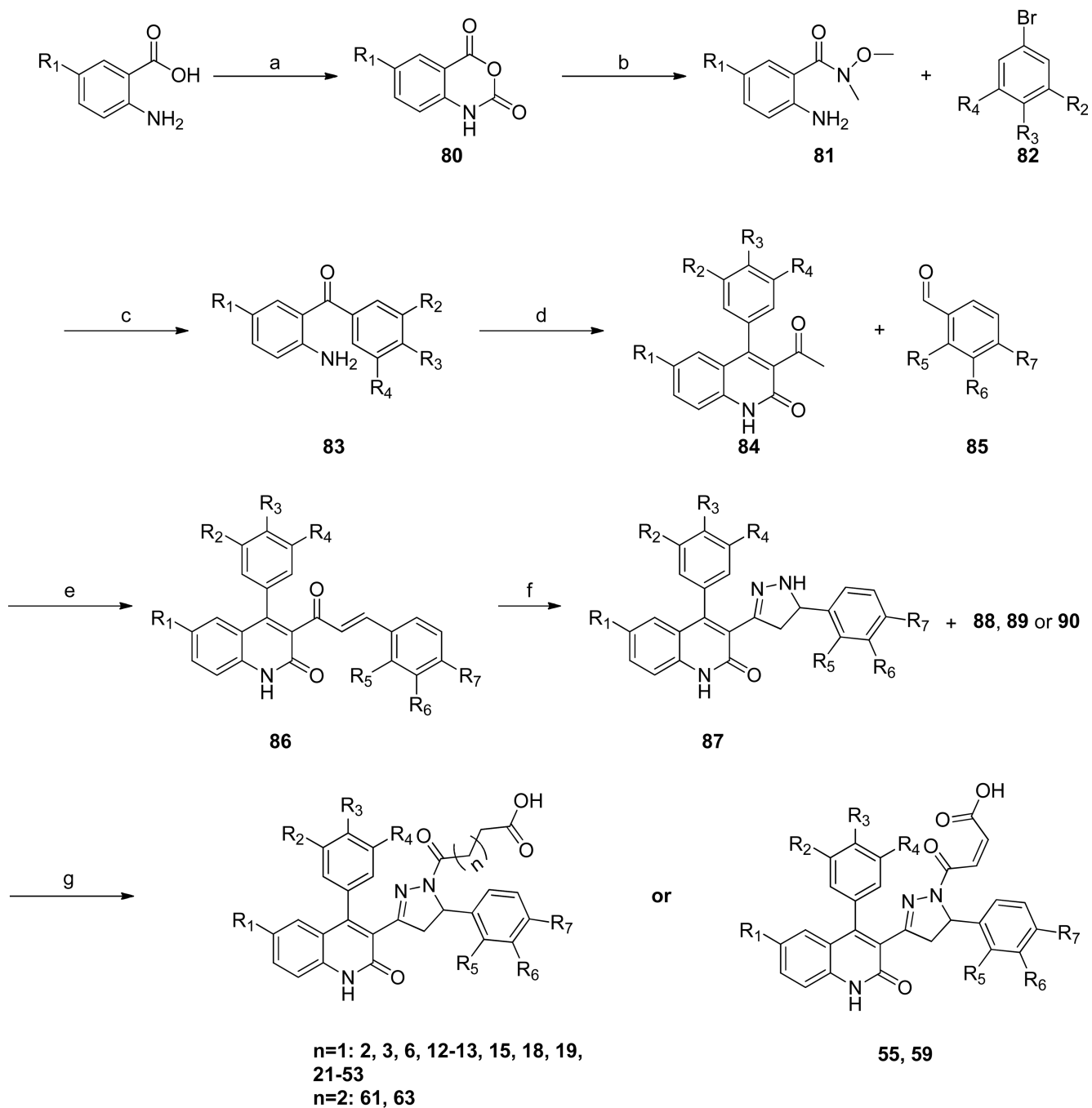
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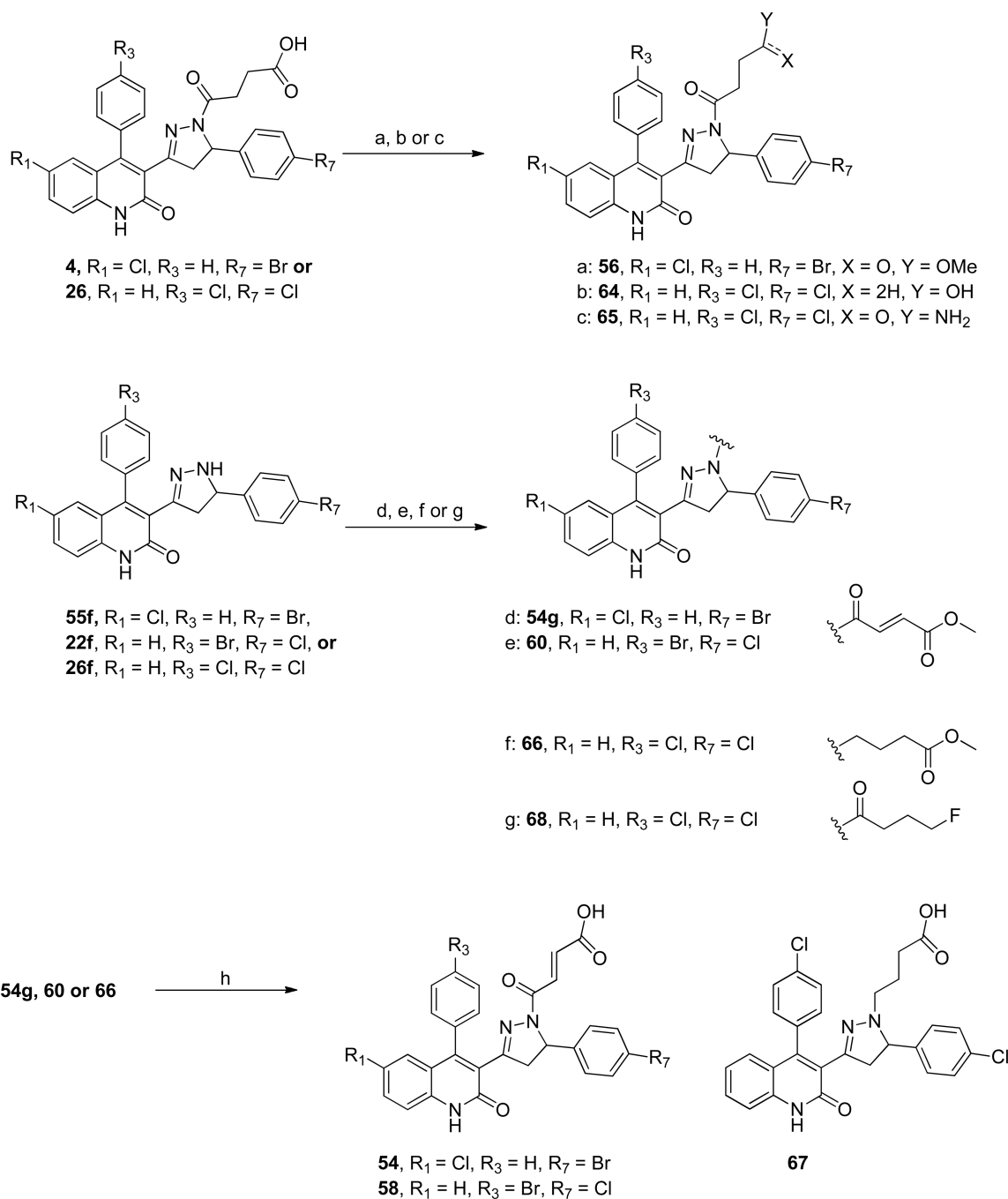
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### Scheme 1. Synthesis of dihydro-quinolone-pyrazoline derivatives

(a) Anhydrous THF, Triphosgene (warning, triphosgene is toxic, see *Methods*), reflux. (b) EtOH, Weinreb's HCl salt, reflux. (c) Anhydrous THF, *n*-Butyllithium,  $-78^{\circ}\text{C}$ . (d) Ethylacetoacetate, DMF, 4Å molecular sieves,  $180^{\circ}\text{C}$ ,  $\mu\text{W}$ . (e) 4:3 EtOH:H<sub>2</sub>O (0.05 M),  $0^{\circ}\text{C}$  to r.t. (f) hydrazine monohydrate, EtOH,  $110^{\circ}\text{C}$ ,  $\mu\text{W}$ . (g) Anhydrous THF, 4Å molecular sieves,  $165^{\circ}\text{C}$ ,  $\mu\text{W}$ .

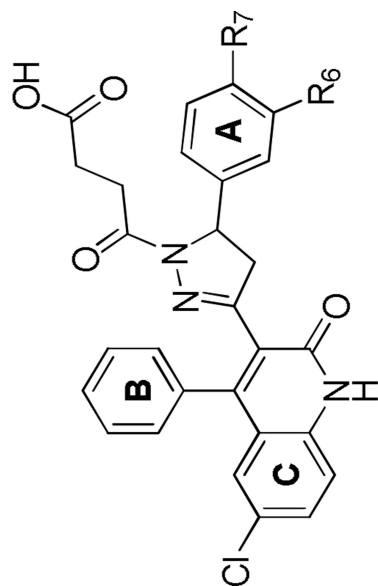


### Scheme 2. Modifications to the acyl-chain

(a) HCl, MeOH. (b)  $\text{BH}_3\text{-Me}_2\text{S}$ , Anhydrous THF,  $0^\circ\text{C}$ . (c) EDCl, DMAP,  $\text{NH}_3$  in dioxane (0.5M), THF. (d) EDCl, DMAP, (*E*)-4-methoxy-4-oxobut-2-enoic acid. (e) Anhydrous THF, (*E*)-methyl 4-chloro-4-oxobut-2-enoate, 4Å molecular sieves,  $165^\circ\text{C}$ ,  $\mu\text{W}$ . (f) methyl 4-oxobutanoate,  $\text{BH}_3\text{-Me}_2\text{S}$ , THF. (g) EDCl, DMAP, 4-fluorobutanoic acid, DCM (h) NaOH,  $\text{EtOH:H}_2\text{O}$ .

Table 1

Evaluation of A-Ring *para*- and ether substitutions.



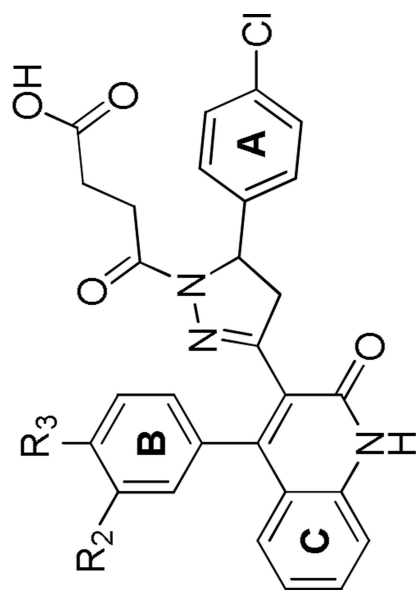
DQP-	R <sub>6</sub>	R <sub>7</sub>	$\frac{2AIC_{50}}{2DIC_{50}}$	$\frac{2BIC_{50}}{2DIC_{50}}$	GluN2A IC <sub>50</sub> (μM)	GluN2B IC <sub>50</sub> (μM)	GluN2C IC <sub>50</sub> (μM)	GluN2D IC <sub>50</sub> (μM)
1	H	H	-	-	NE	NE	86	88
2	H	F	9	6	128	87	23	14
3	H	Cl	-	-	NE	NE	5.5	4.5
4	H	Br	-	7	NE	22	3.6	3.1
5	H	NO <sub>2</sub>	85	42	91	45	0.9	1.1
6	H	COOH	-	-	NE	NE	NE	NE
7	H	COOMe	-	-	NE	NE	93	32
8	H	CF <sub>3</sub>	20	13	80	54	5	4.1
9	H	OMe	9	9	197	187	28	21
10	H	NMe <sub>2</sub>	-	-	NE	NE	39	19
11	H	-OCH <sub>2</sub> O-	-	4	NE	90	23	23

IC<sub>50</sub> values were obtained by fitting the Hill equation to the average composite concentration-effect curves (see Methods). Data are from 7–18 oocytes between 2–4 frogs; NE indicates less than 30% inhibition at 100 μM.



Table 3

B-Ring Modifications.

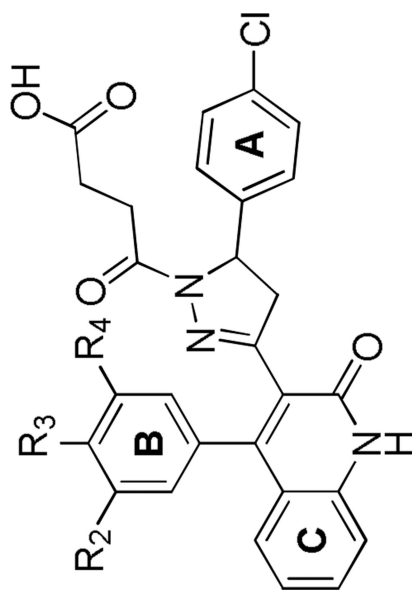


DQP-	R <sub>2</sub>	R <sub>3</sub>	2AIC <sub>50</sub>		2BIC <sub>50</sub>		GluN2A		GluN2B		GluN2C		GluN2D	
			IC <sub>50</sub>	2DIC <sub>50</sub>	IC <sub>50</sub>	2DIC <sub>50</sub>	IC <sub>50</sub> (nM)	IC <sub>50</sub> (nM)	IC <sub>50</sub> (nM)	IC <sub>50</sub> (nM)	IC <sub>50</sub> (nM)	IC <sub>50</sub> (nM)		
29	H	F	36	98	21	57	21	57	21	57	1.0	1.0	0.58	0.58
26	H	Cl	48	50	21	22	21	22	21	22	0.77	0.77	0.44	0.44
30	H	Me	24	30	33	42	33	42	33	42	2.5	2.5	1.4	1.4
31	H	OMe	27	33	62	75	62	75	62	75	5	5	2.3	2.3
32	H	CN	22	-	156	NE	156	NE	156	NE	12	12	7	7
33	H	CF <sub>3</sub>	10	14	29	39	29	39	29	39	3.6	3.6	2.8	2.8
34	F	H	67	101	46	70	46	70	46	70	1.1	1.1	0.69	0.69
35	Cl	H	20	43	20	43	20	43	20	43	2.1	2.1	1.0	1.0
36	Me	H	13	37	24	71	24	71	24	71	4.0	4.0	1.9	1.9
37	OMe	H	24	34	110	152	110	152	110	152	7.8	7.8	4.5	4.5
38	CN	H	-	-	NE	NE	NE	NE	NE	NE	19	19	13	13
39	CF <sub>3</sub>	H	11	18	28	47	28	47	28	47	3.4	3.4	2.6	2.6

IC<sub>50</sub> values were obtained by fitting the Hill equation to the average composite concentration-effect curves (see Methods). Data are from 6–14 oocytes between 2–3 frogs; NE indicates less than 30% inhibition at 100 μM. Data for **26** is presented in Table 2 and repeated here to facilitate comparisons with data.

Table 4

B-Ring Di-substitution.



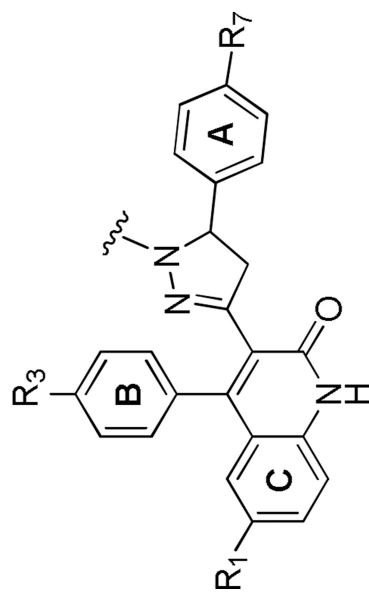
DQP-	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	2AIC <sub>50</sub>	2BIC <sub>50</sub>	GluNZA IC <sub>50</sub> (μM)	GluN2B IC <sub>50</sub> (μM)	GluN2C IC <sub>50</sub> (μM)	GluN2D IC <sub>50</sub> (μM)
<b>40</b>	F	Cl	H	23	53	12	28	0.91	0.53
<b>41</b>	Cl	F	H	19	34	19	34	1.4	1.0
<b>42</b>	Cl	Cl	H	8	22	7.7	20	0.79	0.91
<b>43</b>	F	F	H	32	108	21	71	0.78	0.66
<b>44</b>	F	H	F	26	123	21	100	1.1	0.81
<b>45</b>	Cl	H	Cl	8	19	5.5	13	0.78	0.70







IC<sub>50</sub> values were obtained by fitting the Hill equation to the average composite concentration-effect curves from oocyte recordings (see *Methods*). Data are from 8–15 oocytes between 2 frogs.

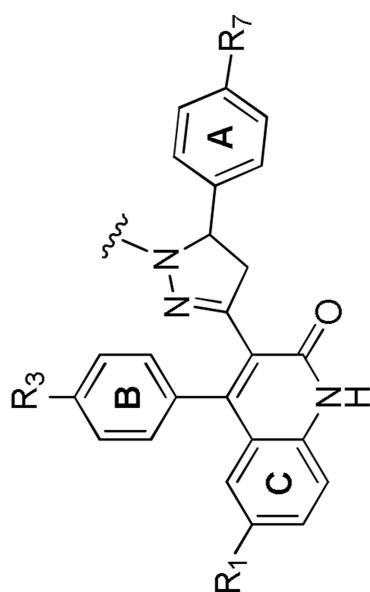


Table 5

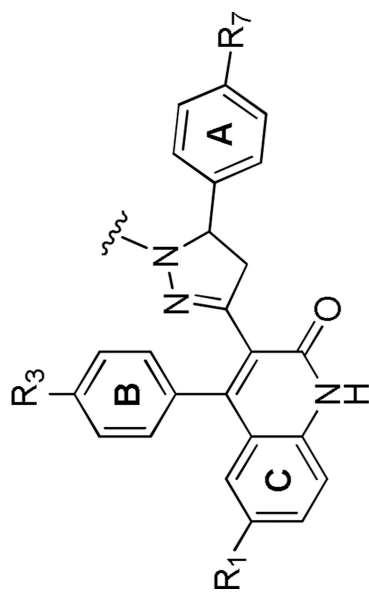
Acyl chain perturbations.



DQP-	R <sub>1</sub>	R <sub>3</sub>	R <sub>7</sub>	Acyl Chain	2AIC <sub>50</sub> 2DIC <sub>50</sub>	2BIC <sub>50</sub> 2DIC <sub>50</sub>	GluN2A IC <sub>50</sub> (μM)	GluN2B IC <sub>50</sub> (μM)	GluN2C IC <sub>50</sub> (μM)	GluN2D IC <sub>50</sub> (μM)
4	Cl	H	Br		-	7	NE	22	3.6	3.1
54	Cl	H	Br		-	-	NE	NE	2.1	1.4
55	Cl	H	Br		15	7	74	37	8.9	5
56	Cl	H	Br		-	-	NE	NE	NE	NE
57	Cl	H	Br		20	23	78	90	6.4	4.0
22	H	Br	Cl		34	79	10	23	0.56	0.29



DQP-	R <sub>1</sub>	R <sub>3</sub>	R <sub>7</sub>	Acyl Chain	$\frac{2AIC_{50}}{2DIC_{50}}$	$\frac{2BIC_{50}}{2DIC_{50}}$	GluN2A IC <sub>50</sub> (μM)	GluN2B IC <sub>50</sub> (μM)	GluN2C IC <sub>50</sub> (μM)	GluN2D IC <sub>50</sub> (μM)
58	H	Br	Cl		23	63	4.3	12	0.20	0.19
59	H	Br	Cl		21	30	12	17	1.0	0.57
60	H	Br	Cl		-	-	NE	NE	59	95
61	H	Br	Cl		33	91	10	29	0.6	0.32
5	Cl	H	NO <sub>2</sub>		85	42	91	45	0.9	1.1
63	Cl	H	NO <sub>2</sub>		10	8	109	92	10	11
26	H	Cl	Cl		48	50	21	22	0.77	0.44
64	H	Cl	Cl		90	48	62	33	1.7	0.69

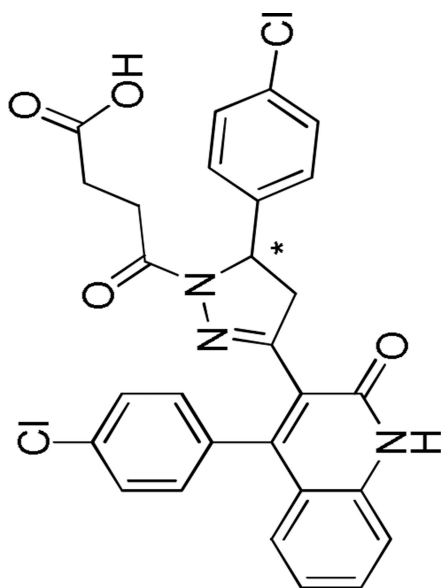


DQP-	R <sub>1</sub>	R <sub>3</sub>	R <sub>7</sub>	Acyl Chain	$\frac{2AIC_{50}}{2DIC_{50}}$	$\frac{2BIC_{50}}{2DIC_{50}}$	GluN2A IC <sub>50</sub> (μM)	GluN2B IC <sub>50</sub> (μM)	GluN2C IC <sub>50</sub> (μM)	GluN2D IC <sub>50</sub> (μM)
65	H	Cl	Cl		31	18	34	20	2.0	1.1
66	H	Cl	Cl		-	-	NE	NE	NE	NE
67	H	Cl	Cl		34	31	58	53	3	1.7
68	H	Cl	Cl		-	-	NE	NE	NE	NE

IC<sub>50</sub> values were obtained by fitting the Hill equation to the average composite concentration-effect curves (see methods). Data are from 5–24 oocytes between 2–4 frogs; NE indicates less than 30% inhibition at 100 μM. Data for compounds **4**, **22**, **5** and **26** were presented in preceding tables, and are shown here for comparison.

Table 6

Stereo-selectivity for the purified enantiomers of compound **26**.



DQP-	R <sub>2</sub>	R <sub>4</sub>	2AIC <sub>50</sub>		2BIC <sub>50</sub>		GluN2A		GluN2B		GluN2C		GluN2D	
			2AIC <sub>50</sub>	2BIC <sub>50</sub>	2BIC <sub>50</sub>	2DIC <sub>50</sub>	IC <sub>50</sub> (μM)	IC <sub>50</sub> (μM)	IC <sub>50</sub> (μM)	IC <sub>50</sub> (μM)	IC <sub>50</sub> (μM)	IC <sub>50</sub> (μM)	IC <sub>50</sub> (μM)	IC <sub>50</sub> (μM)
<b>26</b>	Cl	Cl	48	50	50	21	22	0.77	0.44					
<b>69 (S-26)</b>	Cl	Cl	78	156	13	26	0.22	0.17						
<b>70 (R-26)</b>	Cl	Cl	23	49	45	52	2.1	1.9						

IC<sub>50</sub> values were obtained by fitting the Hill equation to the average composite concentration-effect curves (see methods). Data are from 8–17 oocytes between 2–4 frogs; data for compound **26**, which was presented in preceding tables, are shown here for comparison.

Table 7

Off-target responses for compound **26** and **58**.

Receptor	Agonist ( $\mu$ M)	$I_{\text{TEST}}/I_{\text{CONTROL}}$ (mean $\pm$ SEM, %)	N (26)	$I_{\text{TEST}}/I_{\text{CONTROL}}$ (mean $\pm$ SEM, %)	N (58)
GluN1/GluN2A	100 glutamate, 30 glycine	89 $\pm$ 4.2*	14	89 $\pm$ 2.2*	12
GluN1/GluN2B	100 glutamate, 30 glycine	79 $\pm$ 2.9*	12	98 $\pm$ 1.9	12
GluN1/GluN2C	100 glutamate, 30 glycine	21 $\pm$ 1.2*	14	26 $\pm$ 1.5*	11
GluN1/GluN2D	100 glutamate, 30 glycine	14 $\pm$ 1.6*	13	14 $\pm$ 1.6*	13
GluA1	100 glutamate	97 $\pm$ 2.2	6	99 $\pm$ 1.1	6
GluA2	100 glutamate	98 $\pm$ 0.8	3	96 $\pm$ 0.9*	4
GluA3	100 glutamate	99 $\pm$ 0.3	4	100 $\pm$ 1.1	4
GluA4	100 glutamate	96 $\pm$ 1.3	3	97 $\pm$ 1.5	4
GluK1	100 glutamate	97 $\pm$ 1.0	3	100 $\pm$ 4.4	3
GluK2	100 glutamate	97 $\pm$ 1.1	4	97 $\pm$ 0.6*	4
GluK2/GluK5	100 glutamate	97 $\pm$ 1.3	3	95 $\pm$ 1.8	3
Serotonin 5-HT <sub>3A</sub>	3 serotonin	95 $\pm$ 1.5*	4	95 $\pm$ 1.2*	4
GABAA $\alpha\beta_1\beta_2\gamma_2s$	20 GABA	97 $\pm$ 2.7	4	95 $\pm$ 3.1	4
GABA $\alpha$ (p1) (human)	2 GABA	99 $\pm$ 2.1	4	97 $\pm$ 0.6*	4
Glycine $\alpha_1$	50 glycine	101 $\pm$ 1.6	4	99 $\pm$ 1.0	4
Nicotinic $\alpha_1\beta_1\gamma\delta$ (mouse)	1 acetylcholine	94 $\pm$ 1.2*	6	98 $\pm$ 0.7	7
Nicotinic $\alpha_4\beta_2$ (human)	10 acetylcholine	79 $\pm$ 4.2*	6	82 $\pm$ 1.7*	5
Nicotinic $\alpha_3\beta_4$ (human)	10 acetylcholine	77 $\pm$ 2.7*	7	87 $\pm$ 2.2*	5
Nicotinic $\alpha_7$ (human)	300 acetylcholine	82 $\pm$ 9.4	3	64 $\pm$ 7.3*	3
Nicotinic $\alpha_9\alpha_{10}$	100 acetylcholine	67 $\pm$ 4.6*	3	72 $\pm$ 4.7*	4
Purinergic P <sub>2X2</sub> (human)	9 ATP	113 $\pm$ 1.8*	5	100 $\pm$ 0.9	4
Purinergic P <sub>2X2</sub> (rat)	9 ATP	97 $\pm$ 1.2*	5	96 $\pm$ 1.0*	4

Agonist-evoked currents were recorded from the receptors listed using the *Xenopus laevis* oocyte expression system under two-electrode voltage clamp (VHOLD=-30 to -60 mV) in the absence and presence of 3  $\mu$ M **26** or 3  $\mu$ M **58**. The cDNA origin used was rat unless otherwise indicated (paired t-test).

\*  
 $p < 0.05$

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Table 8

MDR1-MDCK permeability.

Test Compound	Direction	Recovery (%)	P <sub>app</sub> (10 <sup>-6</sup> cm/s)			Efflux Ratio
			1	2	Avg	
64	A-to-B	43	2.45	2.48	2.46	26
	B-to-A	73	66.2	62.7	64.5	
26	A-to-B	73	0.47	0.47	0.47	55
	B-to-A	76	33.4	17.9	25.6	
68	A-to-B	43	4.51	3.24	3.88	2.5
	B-to-A	67	9.32	9.72	9.52	

The P<sub>app</sub> and efflux ratio were calculated as described in the Methods. Compounds displaying a P<sub>app</sub> < 3.0 X 10<sup>-6</sup> cm/s and an efflux ratio > 10 are interpreted to have a low potential for crossing the BBB. Compounds with P<sub>app</sub> > 3.0 × 10<sup>-6</sup> cm/s and an efflux ratio < 10 are expected to have high brain penetration.<sup>38</sup>

Table 9

Human microsomal stability.

Test Compound	% Remaining of initial					Half-life	CL <sub>int</sub> <sup>b</sup> (ml/min/mg protein)
	0 min	10 min	20 min	30 min	60 min		
64	100	52	31	24	6.5	13	0.110
26	100	101	100	115	86	>60	<0.02
68	100	67	49	49	39	35	0.040

<sup>a</sup> Half-life was calculated based on  $t_{1/2} = 0.693/k$ , where  $k$  is the elimination rate constant based on the slope of the natural logarithm percent remaining versus incubation time.

<sup>b</sup> Intrinsic clearance (CL<sub>int</sub>) was calculated on  $CL_{int} = k/P$ , where  $k$  is the elimination rate constant and  $P$  is the protein concentration in the incubation.