

Synthetic lethality in pancreatic cancer: discovery of a new RAD51-BRCA2 small molecule disruptor that inhibits homologous recombination and synergizes with olaparib

Greta Bagnolini, Domenico Milano, Marcella Manerba, Fabrizio Schipani, José-Antonio Ortega, Dario Gioia, Federico Falchi, Andrea Balboni, Fulvia Farabegoli, Francesca De Franco, Janet Robertson, Roberto Pellicciari, Isabella Pallavicini, Sebastiano Peri, Saverio Minucci, Stefania Girotto, Giuseppina Di Stefano, Marinella Roberti, and Andrea Cavalli

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.9b01526 • Publication Date (Web): 10 Feb 2020

Downloaded from pubs.acs.org on February 10, 2020

Just Accepted

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.

Synthetic lethality in pancreatic cancer: discovery of a new RAD51-BRCA2 small molecule disruptor that inhibits homologous recombination and synergizes with olaparib

Greta Bagnolini,^{§,†,‡} Domenico Milano,^{§,‡} Marcella Manerba,^{§,‡} Fabrizio Schipani,[§] Jose Antonio Ortega,[§] Dario Gioia,[§] Federico Falchi,[§] Andrea Balboni,^{§,†} Fulvia Farabegoli,[†] Francesca De Franco,[‡] Janet Robertson,[‡] Roberto Pellicciari,[‡] Isabella Pallavicini,[‡] Sebastiano Peri,[‡] Saverio Minucci,^{‡,‡} Stefania Girotto,[§] Giuseppina Di Stefano,[†] Marinella Roberti^{,†} and Andrea Cavalli^{*,§,†}*

[§]Computational & Chemical Biology, Istituto Italiano di Tecnologia, via Morego 30, 16163 Genoa, Italy.

[†]Department of Pharmacy and Biotechnology, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy.

[‡]TES Pharma S.r.l., Via Palmiro Togliatti 22bis, I-06073, Corciano, Perugia, Italy.

[‡]Department of Biosciences, University of Milan, Via Celoria 26, 20100 Milan.

[‡]Department of Experimental Oncology at the IEO, European Institute of Oncology IRCCS, IFOM-IEO Campus, Via Adamello 16, 20100 Milan.

[†]Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Via S. Giacomo 14, 40126 Bologna, Italy.

ABSTRACT

Synthetic lethality is an innovative framework for discovering novel anticancer drug candidates. One example is the use of PARP inhibitors (PARPi) in oncology patients with *BRCA* mutations. Here, we exploit a new paradigm based on the possibility to trigger synthetic lethality using only small organic molecules (dubbed “fully small-molecule-induced synthetic lethality”). We exploited this paradigm to target pancreatic cancer, one of the major unmet needs in oncology. We discovered a dihydroquinolone pyrazoline-based molecule (**35d**), which disrupts the RAD51-BRCA2 protein-protein interaction, thus mimicking the effect of *BRCA2* mutation. **35d** inhibits the homologous recombination in a human pancreatic adenocarcinoma cell line. In addition, it synergizes with olaparib (a PARPi) to trigger synthetic lethality. This strategy aims to widen the use of PARPi in *BRCA*-competent and olaparib-resistant cancers, making fully small-molecule-induced synthetic lethality an innovative approach towards unmet oncological needs.

Keywords: protein-protein interaction, protein-protein inhibitors, DNA repair, anticancer drugs, PARP inhibitors.

INTRODUCTION

Synthetic lethality is a new opportunity for discovering new anticancer molecules for personalized targeted therapies. The concept derives from genetic studies in model organisms.¹⁻⁵ Two genes are synthetically lethal if the perturbation of either gene alone has no effect on cell viability, but the simultaneous impairment of both genes results in cell death. In principle, small organic molecules can target the synthetically lethal partner of an altered gene in cancer cells, but not in normal cells. This creates opportunities to selectively kill cancer cells while sparing normal cells.⁶⁻¹²

The DNA repair and DNA damage response (DDR) pathways are suitable for the application of synthetic lethality as a novel anti-cancer therapeutic strategy.¹³⁻¹⁵ Genome instability is a hallmark of cancer.¹⁶ DNA damage occurs constantly in cells due to the continuous exposition to endogenous and exogenous stressors. Consequently, cells have evolved a complex coordinated DDR, which orchestrates a network of cellular processes to repair DNA damage and preserve genome integrity. DDR thus prevents the transmission of altered genetic material to daughter cells and acts as a tumor-suppressive barrier. Defects in DDR are associated with the accumulation of oncogenic mutations and genome instability, and they contribute to cancer initiation and progression. However, cancer cells with defects in one DDR pathway can become reliant on other pathways for their survival. Targeting these other DDR pathways can potentially cause selective cancer cell death through synthetic lethality. The classic example is the clinical application of poly (ADP-ribose)polymerase (PARP) inhibitors in oncology patients with *BRCA1/2* mutations. PARP is crucial for repairing DNA single-strand breaks (SSBs), whereas BRCA 1/2 are important for repairing DNA double-strand breaks (DSBs) by homologous recombination (HR). The simultaneous impairment of both repair mechanisms results in cell-cycle arrest and apoptosis of cancer cells through synthetic lethality. In 2014, olaparib was the first PARP inhibitor (PARPi) approved to treat advanced ovarian cancer associated with defective *BRCA* genes.¹⁷ In 2018, olaparib was approved to treat metastatic breast tumors associated with germline *BRCA* mutations.¹⁸

1
2
3 In 2019, olaparib gained the FDA approval as first-line maintenance treatment of germline BRCA-
4 mutated metastatic pancreatic cancer. It appears to be a new treatment option for this disease,
5 which is one of the major unmet needs in oncology.¹⁹
6
7

8
9
10 One of BRCA2's key mechanisms in DDR is to recruit RAD51, an evolutionarily conserved
11 recombinase, at the site of DSBs where it performs DSB repair through HR.²⁰ Additionally, the
12 expression of RAD51 and the rate of RAD51-mediated HR are both elevated in a wide variety of
13 cancers (e.g. breast, pancreatic).²¹ Moreover, the cellular amount of RAD51 is positively correlated
14 with resistance to radiotherapies or chemotherapies that induce DNA damage.^{22,23}
15
16
17
18
19

20
21 The RAD51-BRCA2 interaction is mediated by eight well-conserved motifs, known as BRC
22 repeats.²⁴⁻²⁷ The X-ray crystallographic structure of the fourth BRC repeat (BRC4) is available in
23 complex with the catalytic domain of RAD51, making the RAD51-BRCA2 interaction suitable for
24 the structure-based design of small molecule inhibitors of protein-protein interactions (PPI). Indeed,
25 Lee et al. recently identified a small molecule inhibitor of RAD51-BRCA2 for potential cancer
26 treatment.^{28,29}
27
28
29
30
31
32
33
34

35 In this context, we recently proposed a new anticancer drug discovery concept, dubbed “fully
36 small-molecules-induced synthetic lethality”.^{30,31} This concept combines RAD51-BRCA2
37 disruptors with olaparib to simultaneously impair two DNA repair pathways, thus mimicking the
38 synthetic lethality described above. We carried out a successful virtual screening campaign at the
39 FxxA pocket (i.e. zone I), one of the two RAD51 pockets responsible for BRC4 binding. This
40 allowed us to discover a series of triazole-based compounds. Compounds **1** and **2** were selected as
41 initial hit candidates (Figure 1). In line with our hypothesis, **2** increased the sensitivity to olaparib in
42 pancreatic cancer cells (BxPC-3) with fully functional *BRCA2*. Notably, this synergistic effect was
43 not observed in Capan-1, pancreas adenocarcinoma cells that lack *BRCA2*.³⁰ Furthermore, to
44 discover more effective compounds, we conducted a chemical modification campaign around the
45 triazole moiety. We also improved the biological screening cascade with experiments to
46 characterize how the new compounds disrupt the RAD51-BRCA2 interaction and inhibit DSB
47
48
49
50
51
52
53
54
55
56
57
58
59
60

repair. We obtained compound **3** (Figure 1) with an improved profile relative to the initial hits (according to a biochemical ELISA assay) and a clear mechanism of action, allowing synergy with olaparib in cancer cells BxPC3, where olaparib is normally inactive. However, with **3**, we could not fully reproduce the paradigm of synthetic lethality.³¹ This could be due to its low-level potency, which did not cause HR inhibition greater than 40%. Additionally, the inherent resistance to apoptosis of BxPC3 cells, which bear a mutant p53, could have further prevented apoptosis. Therefore, further biological experiments and new classes of RAD51-BRCA2 are needed to confirm this paradigm and to assess its potential as an innovative anticancer strategy.

To this end, we report here on the identification of a new class of RAD51-BRCA2 disruptors based on the dihydroquinolone pyrazoline moiety (**4d-57d**, Table 1, Scheme 2). We attempt to depict general structure-activity relationships (SAR) of this new class of compounds as RAD51-BRCA2 inhibitors, and outline the biological profile of the most promising derivative **35d** (Table 1, Scheme 2).

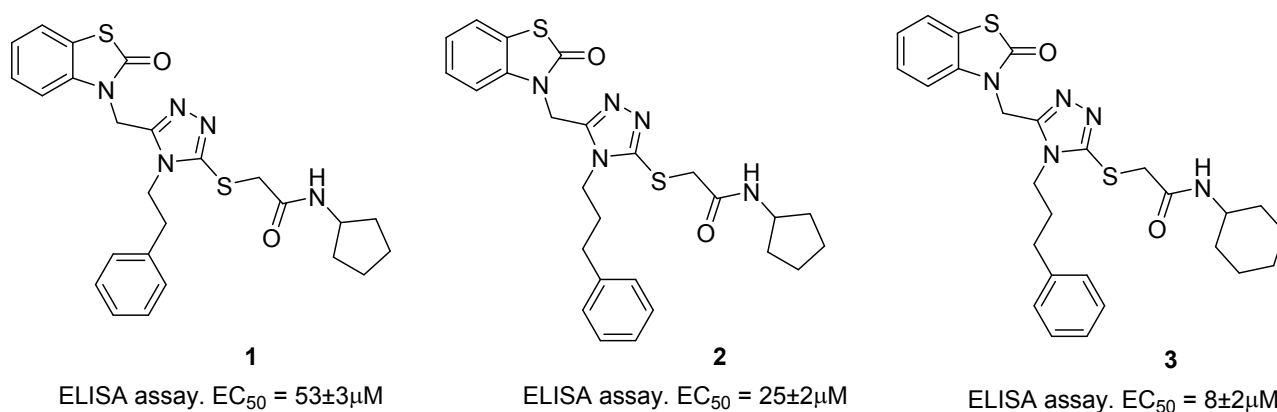


Figure 1. Structure of the previously identify triazoles **1-3**.

RESULTS AND DISCUSSION

Hit identification and optimization

1
2
3 Targeting protein-protein interactions (PPIs) is an attractive strategy for designing innovative
4
5 drugs for complex diseases such as cancer. Indeed, the first PPI inhibitors for cancer are now in
6
7 clinical development.³²
8
9

10 In this context, to identify RAD51-BRCA2 disruptors, we used the available X-ray crystallographic
11
12 structure of the fourth BRC repeat (BRC4) in complex with the catalytic domain of RAD51.²⁴
13
14 BRC4 binds RAD51 in two different hydrophobic pockets (zone I and zone II, respectively). One
15
16 pocket (named zone I) can lodge BRC4's FxxA motif (residues 1524-1527 of BRCA2) and is
17
18 critical for RAD51 multimerization. The other pocket (named zone II) can lodge the BRC4's LFDE
19
20 motif (residues 1545-1548 of BRCA2) far from the oligomerization interface (Figure 2).^{24,33-35}
21
22
23 Recently, we ran a successful virtual screening campaign based on high-throughput docking at the
24
25 FxxA pocket to identify the first RAD51-BRCA2 disruptors.³⁰ To increase the chemical diversity
26
27 and identify a novel class of RAD51-BRCA2 disruptors, we performed a second virtual screening
28
29 campaign targeting the LFDE binding pocket (see the Supporting Information). This binding pocket
30
31 is more evolutionarily conserved than the FxxA. Furthermore, mutation at the LFDE causes
32
33 cellular lethality and failure of RAD51 assembly in nuclear foci at the site of DNA breaks in vivo.
34
35 This further suggests this pocket as a critical site for RAD51's mechanism of action.³³ To the best
36
37 of our knowledge, no inhibitor that binds the LFDE binding pocket has been reported so far in the
38
39 literature. This may open up new possibilities for combining molecules targeting zone I and zone II
40
41 towards a more in depth understanding of the mechanism of inhibition of RAD51-BRCA2
42
43 interaction.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

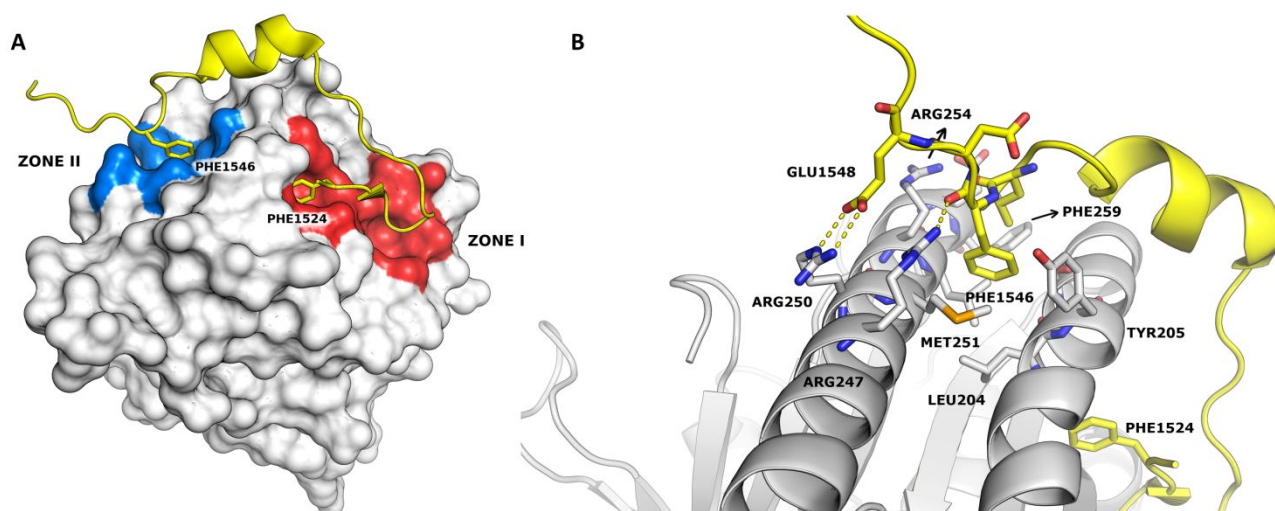


Figure 2. (A) RAD51–BRCA2 BRC repeat complex (PDB code: 1N0W). RAD51 is represented as a surface, BRC4 as a cartoon. The two hot spots of the interaction between the proteins (Phe1524 and Phe1546) are highlighted in sticks; (B) ZONE II magnification showing the interacting residues of BRC4 (yellow) and RAD51 (white).

Here, 42 small molecules were selected, purchased, and tested for their inhibitory activity using a competitive biochemical ELISA assay, as previously described by Rajendra et al.³³ Among the tested compounds, the commercially available dihydroquinolone pyrazoline derivative **4d** (Figure 3 and 4) was the best candidate in terms of EC_{50} and chemical tractability. Its activity was confirmed by retesting the newly synthesized compound **4d** (Scheme 2). Indeed, the dihydroquinolone pyrazoline moiety is a core structure of compounds with different biological targets.^{36,37} The binding mode to RAD51 of both enantiomers of **4d** (Figure 3), as obtained by induced-fit docking simulations, displays some points of interaction similar to those of the crystallographic BRC4–RAD51 complex. Specifically, the docking model suggests that (i) the fluorophenyl ring in position 5 of the pyrazoline lies (similarly to the Phe1549 of BRC4) in a hydrophobic pocket outlined by the side chains of Leu204, Tyr205, Met251, Leu255, and Phe259 of RAD51; (ii) the carboxyl group of the pyrazoline side chain forms an ionic interaction with the Arg250 (or Arg247) of RAD51, as does the side chain Glu1548 of BRC4. In addition, the model suggests that the carbonyl and the nitrogen of the dihydroquinolone moiety, together with the

carbonyl group of the pyrazoline side chain, establish hydrogen bonds with Arg254 and Glu258. Notably, both enantiomers show the same global pattern of interactions.

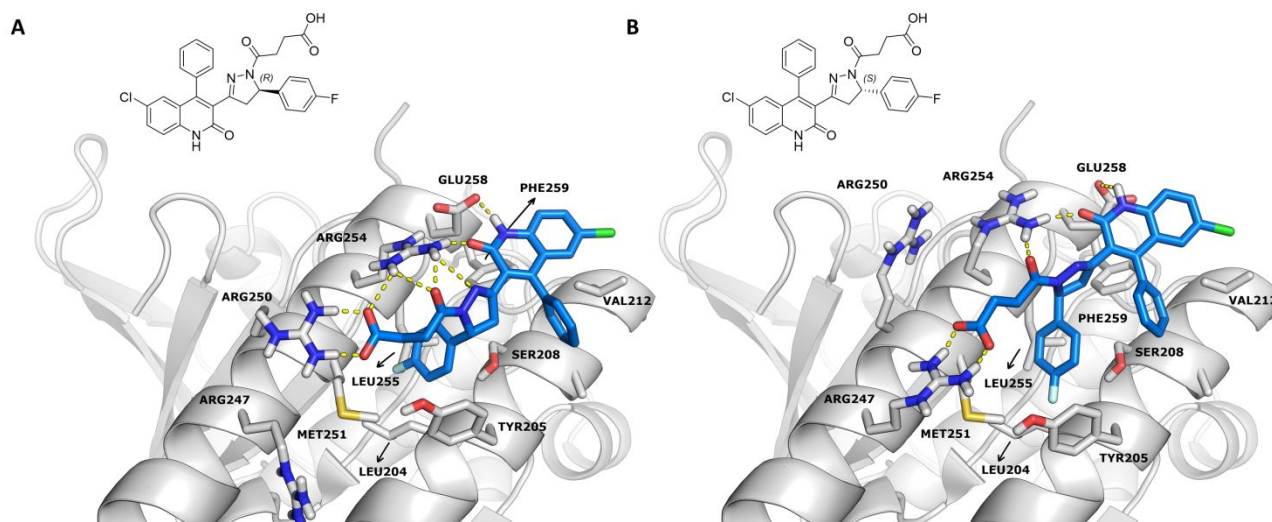


Figure 3. Both enantiomers of compound **4d** docked into the LFDE binding site (site II) of RAD51 (PDB code: 1N0W).

To improve the RAD51-BRCA2 inhibitory activity of **4d**, we conducted a chemical modification campaign around the dihydroquinolone pyrazoline core. We synthesized a chemical library that contained a variety of aromatic substitutions (red and blue regions) in combination with modifications of the acyl chain moiety (green region) (Figure 4). All compounds were synthesized and tested as racemic mixtures, after verifying that the enantiomers of the hit compound **4d** showed the same biochemical activity and the same binding mode (Figure 3, details in the Biological Evaluation section). First, a series of different acyl chains (namely acetyl, propionyl, 3-aminopropionyl, 4-amino-4-oxobutanoyl, 4-methoxy-4-oxobutanoyl, 3-(methylsulfonamido) propanoyl) was introduced on the pyrazoline nitrogen (**5d-10d**, Table 1). Next, the aromatic ring A was modified by replacing the fluorine atom with different substituents, including chlorine and bromine atoms, methoxy, tert-butyl and

trifluoromethyl groups, leaving the succinate acyl chain unchanged (**11d-16d**, Table 1). In addition, the aromatic ring A was replaced by different substituted biphenyl or heterocycle groups in order to probe its role (**17d-32d**, Table 1). The ring A was also modified in combination with the propionyl (**33d-35d**, Table 1) or acetyl substitution on the pyrazoline nitrogen (**36d-51d**, Table 1). Regarding the dihydroquinolone core, the chlorine atom in the C-ring was removed, leaving the acyl chain unchanged and introducing some different substituents in the phenyl ring A (**52d-57d**, Table 1).

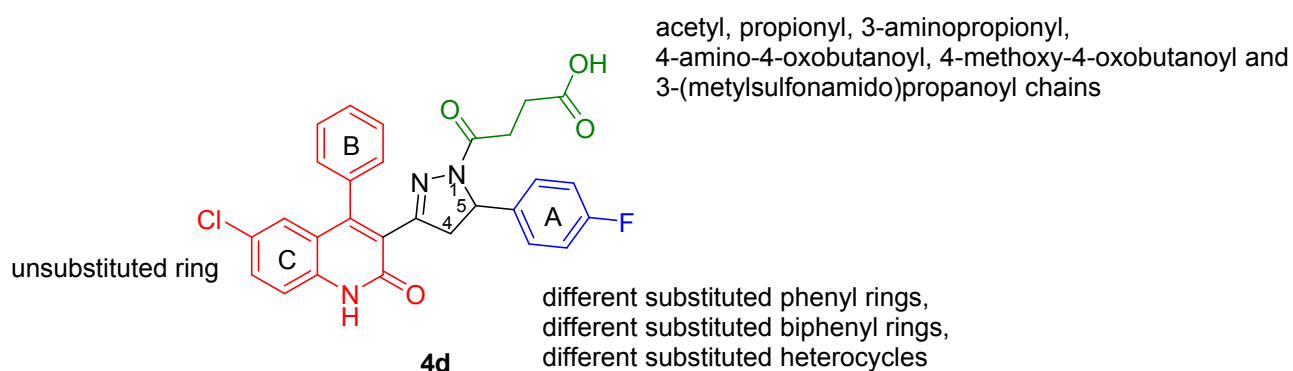


Figure 4. Overview of the optimization strategy of **4d** for SAR exploration.

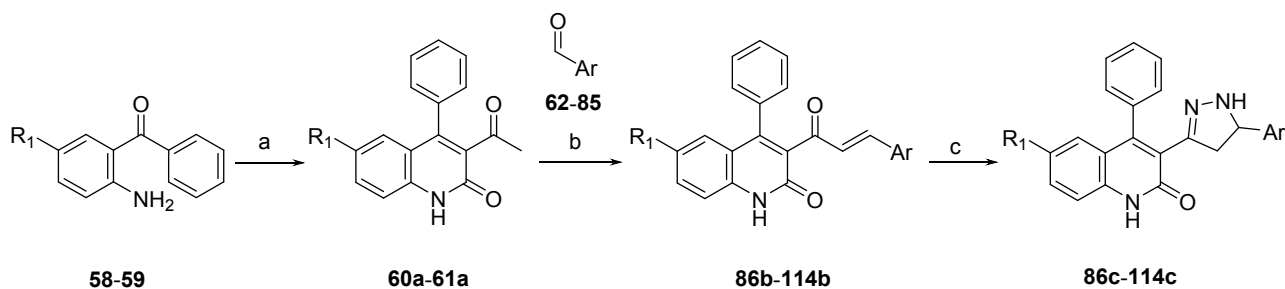
Chemistry

The desired dihydroquinolone pyrazoline derivatives **4d-6d** and **11d-57d** were achieved, taking advantage of a common synthetic strategy previously reported by Acker et al.³⁶ The commercially available 2-aminobenzophenones **58-59** treated with ethyl acetoacetate afforded the corresponding methyl ketones **60a-61a**, which underwent base-catalyzed condensation with the appropriate aryl aldehydes **62-85** yielding the α,β -unsaturated aryl ketones **86b-114b**. In turn, **86b-114b** were treated with hydrazine monohydrate to yield the pyrazoline derivatives **86c-114c** (Scheme 1). The pyrazoline amines **86c-114c** functionalized with succinic anhydride **115**, acetic anhydride **116**, or propionic acid **117** afforded the corresponding desired dihydroquinolone pyrazoline derivatives

4d-6d and **11d-57d** (Scheme 2). **7d** (Scheme 3) was obtained by coupling **86c** with the commercially available 3-((*tert*-butoxycarbonyl)amino)propanoic acid **118** to achieve the Boc-amino pyrazoline derivative **119**. In turn, **119** was Boc-protected to afford the desired **7d**. **8d** (Scheme 4) was obtained by HATU-mediated coupling of **4d** with ammonium chloride. **9d** (Scheme 5) was afforded by coupling **86c** with the commercially available 4-methoxy-4-oxobutanoic acid **120**. The synthesis of **10d** (Scheme 6) began with the commercially available methyl 3-amino-propanoate **121**, which was treated with methansulfonyl chloride **122** to afford the corresponding methyl 3-(methysulfonamido)propanoate **123**, which underwent basic hydrolysis to give 3-(methysulfonamido)propanoic acid **124**. The coupling reaction of **86c** with **124** afforded the desired **10d**.

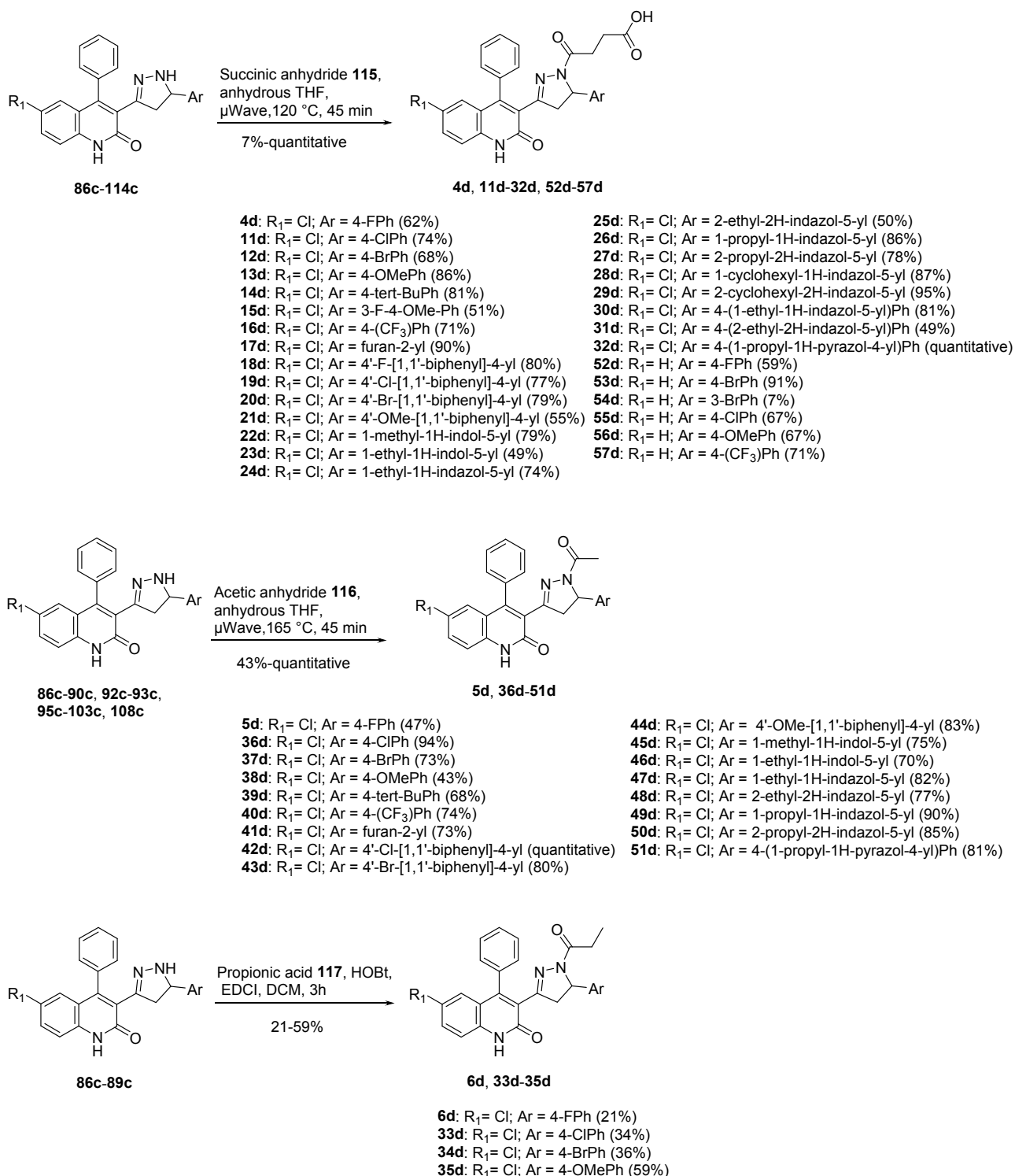
The aldehydes **67**, **70**, **74-84**, not commercially available, were prepared following standard procedures as reported in Supporting Information (Scheme S1- S9).

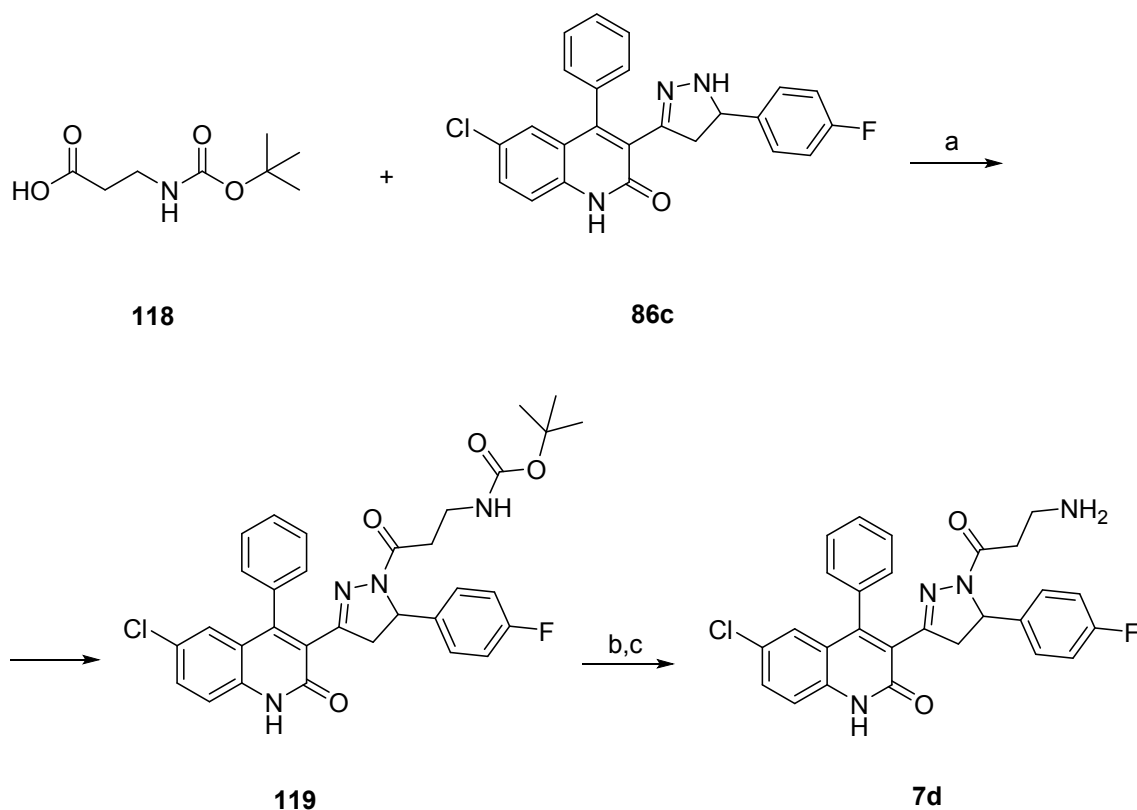
Scheme 1. Synthesis of dihydroquinolone pyrazoline intermediates **86c-114c**.



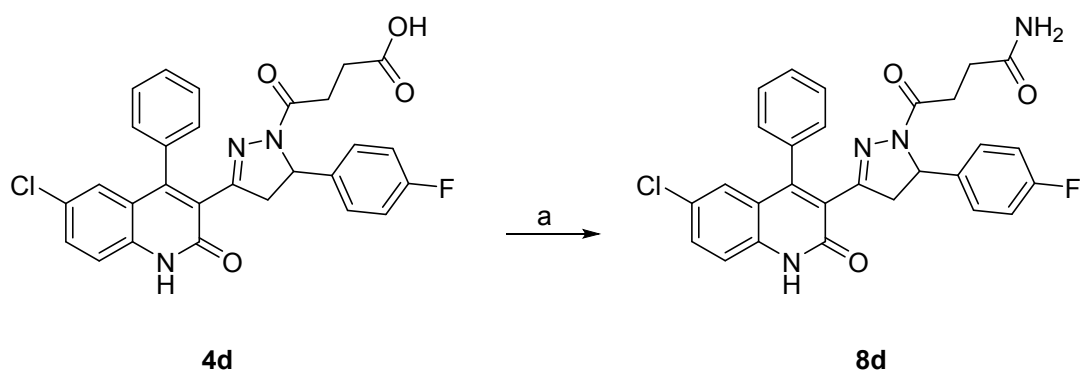
Cp	R ₁	Ar	Cp	R ₁	Ar
58	Cl	-	86b; 86c	Cl	4-FPh
59	H	-	87b; 87c	Cl	4-ClPh
60a	Cl	-	88b; 88c	Cl	4-BrPh
61a	H	-	89b; 89c	Cl	4-OMePh
62	-	4-FPh	90b; 90c	Cl	4-tert-BuPh
63	-	4-ClPh	91b; 91c	Cl	3-F-4-OMePh
64	-	4-BrPh	92b; 92c	Cl	4-(CF ₃)Ph
65	-	4-OMePh	93b; 93c	Cl	furan-2-yl
66	-	4-tert-BuPh	94b; 94c	Cl	4'-F-[1,1'-biphenyl]-4-yl
67	-	3-F-4-OMePh	95b; 95c	Cl	4'-Cl-[1,1'-biphenyl]-4-yl
68	-	4-(CF ₃)Ph	96b; 96c	Cl	4'-Br-[1,1'-biphenyl]-4-y
69	-	furan-2-yl	97b; 97c	Cl	4'-OMe-[1,1'-biphenyl]-4-y
70	-	4'-F-[1,1'-biphenyl]-4-yl	98b; 98c	Cl	1-methyl-1H-indol-5-yl
71	-	4'-Cl-[1,1'-biphenyl]-4-yl	99b; 99c	Cl	1-ethyl-1H-indol-5-yl
72	-	4'-Br-[1,1'-biphenyl]-4-y	100b; 100c	Cl	1-ethyl-1H-indazol-5-yl
73	-	4'-OMe-[1,1'-biphenyl]-4-y	101b; 101c	Cl	2-ethyl-2H-indazol-5-yl
74	-	1-methyl-1H-indol-5-yl	102b; 102c	Cl	1-propyl-1H-indazol-5-yl
75	-	1-ethyl-1H-indol-5-yl	103b; 103c	Cl	2-propyl-2H-indazol-5-yl
76	-	1-ethyl-1H-indazol-5-yl	104b; 104c	Cl	1-cyclohexyl-1H-indazol-5-yl
77	-	2-ethyl-2H-indazol-5-yl	105b; 105c	Cl	2-cyclohexyl-2H-indazol-5-yl
78	-	1-propyl-1H-indazol-5-yl	106b; 106c	Cl	4-(1-ethyl-1H-indazol-5-yl)Ph
79	-	2-propyl-2H-indazol-5-yl	107b; 107c	Cl	4-(2-ethyl-2H-indazol-5-yl)Ph
80	-	1-cyclohexyl-1H-indazol-5-yl	108b; 108c	Cl	4-(1-propyl-1H-pyrazol-4-yl)Ph
81	-	2-cyclohexyl-2H-indazol-5-yl	109b; 109c	H	4-FPh
82	-	4-(1-ethyl-1H-indazol-5-yl)Ph	110b; 110c	H	4-BrPh
83	-	4-(2-ethyl-2H-indazol-5-yl)Ph	111b; 111c	H	3-BrPh
84	-	4-(1-propyl-1H-pyrazol-4-yl)Ph	112b; 112c	H	4-ClPh
85	-	3-BrPh	113b; 113c	H	4-OMePh
			114b; 114c	H	4-(CF ₃)Ph

Reagents and conditions: (a) Ethyl acetoacetate, DMF, 120 °C μ Wave or reflux, **60a** quantitative, **61a** 53%; (b) KOH, EtOH/H₂O 4:3 v/v (0.05 M), 0 °C to rt, 53%-quantitative; (c) hydrazine monohydrate, EtOH, 110 °C μ Wave, 45 min., 64%-quantitative

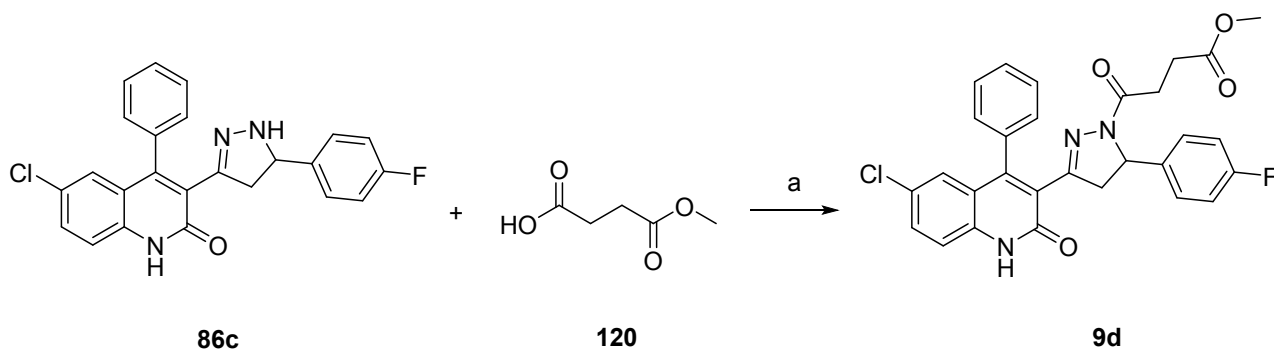
Scheme 2. Synthesis of final dihydroquinolone pyrazoline **4d- 57d**.

Scheme 3. Synthesis of compounds **7d**.

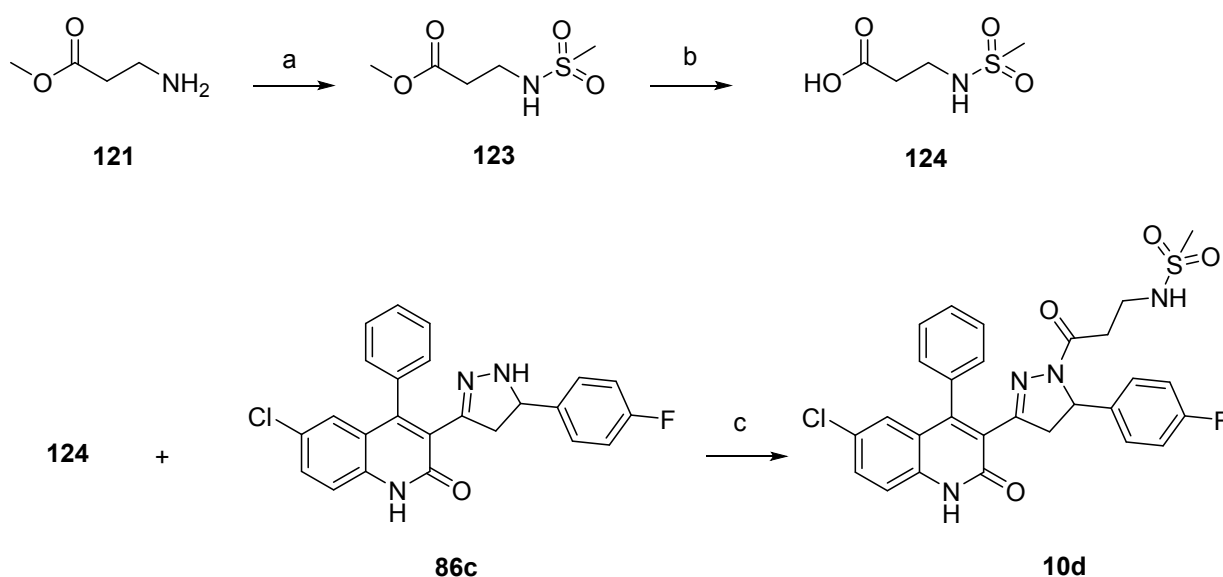
Reagents and conditions: (a) HOBt, EDCI, DCM, overnight, yield 25%; (b) HCl 4 M in dioxane, rt, 15 min; (c) NaOH 0.5 M in EtOAc, rt, 15 min, yield 50%.

Scheme 4. Synthesis of compounds **8d**.

Reagents and conditions: (a) HATU (1.5 equiv), EDC (1.5 equiv), DCM, DMF, ammonium chloride (5.0 equiv), DIPEA (4.0 equiv), rt, 26 h, yield 46%.

Scheme 5: Synthesis of compounds **9d**.

Reagents and conditions: (a) HOBt (1.1 equiv), EDC (1.1 equiv), TEA (2.2. equiv) DCM, rt, 16 h, yield 42%.

Scheme 6: Synthesis of compounds **10d**.

Reagents and conditions: (a) TEA (5.0 equiv), anhydrous DCM, methanesulfonyl chloride **122** (2.0 equiv), rt, 48 h, yield 82%; (b) MeOH/THF (1:1 v/v), 2M LiOH, rt, 16 h, yield 82%; (c) HOBt (1.1 equiv), EDC (1.1 equiv), TEA (2.2. equiv) DCM, rt, 16 h, yield 15%.

Biological evaluation

To investigate the mechanism of action of the new dihydroquinolone pyrazoline derivatives, different biological assays were performed. As a primary screening, the ability of compounds **5d-57d** to inhibit RAD51-BRCA2 interaction was investigated with a competitive biochemical

ELISA assay against the parent compound **4d** (Table 1). This assay is effective in evaluating the ability of new molecules to compete with BRC4 to bind to RAD51.³⁰ Replacing the acyl chain of the pyrazoline nitrogen yielded compounds (**5d-10d**, Table 1), which had reduced inhibitory activity (**5d**, $EC_{50} = 50 \pm 10 \mu M$; **6d**, $EC_{50} = 34 \pm 3 \mu M$) or were completely inactive (**7d-10d**). These data indicate that no improvement in RAD51-BRCA2 inhibitory activity was achieved with this subset of compounds relative to the parent **4d** ($EC_{50} = 16 \pm 4 \mu M$). Replacing the fluorine atom on ring A with bromine led to **12d** ($EC_{50} = 16 \pm 2 \mu M$), which shows the same activity of the initial hit **4d**. The activity was affected when the fluorine was replaced with chlorine (**11d**, $EC_{50} = 59 \pm 8 \mu M$) and trifluoromethyl groups (**16d**, $EC_{50} = 29 \pm 2 \mu M$). The replacement of fluorine with electron-donating groups yielded the inactive compounds (**13d-15d**). For the subset of dihydroquinolone pyrazolines, in which the aromatic ring A was replaced by different substituted biphenyl or heterocycle groups (**17d-32d**, Table 1), the N-methyl indole derivative **22d** showed a fairly good potency with an $EC_{50} = 2 \pm 0.5 \mu M$, eight times higher than that of the parent **4d** ($EC_{50} = 16 \pm 4 \mu M$). **18d-21d**, **23d**, **25d**, **30d**, and **31d** were active at micromolar range, all very similar to the initial hit. A drop in activity was observed with compound **32d**, while derivatives **24d**, **26d-29d** were inactive. The replacement of the aromatic ring A in combination with the substitution of the pyrazoline nitrogen with either propionyl or acetyl chain yielded compounds **33d-51d**. The 1-N-acetyl-5-(1-N-propyl)indazolyl-pyrazoline **49d** showed the best activity of the series with an $EC_{50} = 0.95 \pm 0.05 \mu M$, while **33d-36d**, **39d**, **45d** showed an activity very similar to that of the initial hit. A drop in potency was observed with compounds **37d**, **42d-44d**, **46d-47d** and **51d**, while **38d**, **40d-41d**, **48d** and **50d** were inactive. Finally, removing the chlorine atom on the dihydroquinolone core led to the completely inactive compounds **52d-57d**, suggesting an active role for the halogen. The enantiomers (**4d-I** and **4d-II**) of the racemic hit compound **4d**, separated via reverse phase chiral chromatography, showed a very similar inhibitory activity (**4d-I**, $EC_{50} = 4 \pm 0.5 \mu M$; **4d-II**, $10 \pm 1 \mu M$) to that of the parent **4d**, suggesting

no stereochemical preference of these compounds for the hypothesized molecular target RAD51 (See Experimental Section). As expected for PPI disruptors, the SARs of the new series of dihydroquinolone pyrazoline were rather complex to rationalize, with many cliffs and spikes that were difficult to understand. Nonetheless, the SAR campaign allowed us to identify several compounds with interesting EC₅₀ values ranging from 0.95 to 20 μM. Accordingly, **4d**, **12d**, **18d**, **20d-23d**, **30d-31d**, **33d-36d**, **39d**, **45d**, and **49d** were submitted to cell-based study (Table 2).

Our working hypothesis is that compounds disrupting RAD51-BRCA2 interaction should affect HR repair and increase the efficacy of PARPi in treating breast, ovarian, and pancreatic cancer. For its clinical relevance, pancreatic adenocarcinoma was selected as our final model for cell-based experiments, and BxPC3 cells were selected for a straightforward comparison with the previously reported triazole derivatives.^{30,31} BxPC3 is derived from a human adenocarcinoma that expresses fully functional BRCA2.³⁸ As shown in Table 2 and according to the rationale of our hypothesis, our preliminary screening consisted in verifying the efficacy of compounds in inhibiting cell HR and/or in increasing the antiproliferative effect of olaparib. For each compound, both parameters were verified using only one or two doses, in the range of the EC₅₀ obtained with the ELISA test. HR activity was assessed by evaluating the recombination rate between two transfected plasmids, using a commercially available assay. This preliminary investigation allowed us to rapidly exclude molecules showing i) low or no activity (**4d**, **12d**, **18d**, **20d**, **22d**, **23d**, **33d**, **45d**, **49d**), ii) poor solubility in cell culture media (**21d**, **30d**, **34d**), iii) discrepancy between the data obtained in the two different screening procedures such as HR inhibition not confirmed by increased olaparib efficacy in the viability assay or, on the contrary, increased olaparib efficacy without HR inhibition (**31d**, **36d**), iv) a non-dose-dependent effect (**39d**). As for the emissucinic acid-containing compounds (**4d**, **12d**, **18d**, **20d-23d**, **30d-31d**), one of the reasons of their lower potency in cells might be the general poor permeability, likely related to the ionizable acid moiety. The data reported in Table 2 show that **35d** was the most promising compound in the HR activity test. We then characterized **35d** in additional biophysical and cell-based experiments.

To further assess the physical interaction between **35d** and RAD51, a microscale thermophoresis (MST) assay was performed on the recombinant human RAD51 (see Experimental Section). The binding assay allowed us to determine the dissociation constant (K_d) for the RAD51-**35d** interaction and binding. The final binding curve (Figure 5) shows that **35d** binds to RAD51 with a K_d value of $11 \pm 6 \mu\text{M}$. This is in agreement with the ELISA assay, supporting the initial hypothesis that **35d** could act as a RAD51-BRCA2 disruptor.

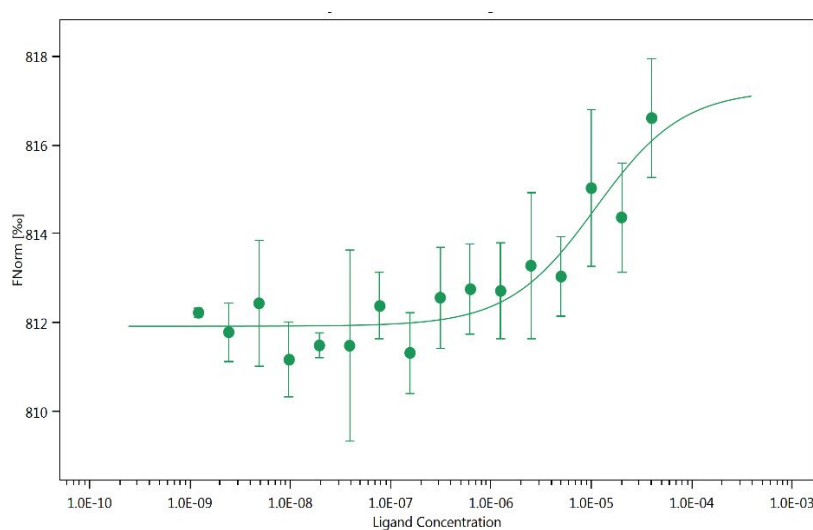


Figure 5. MST analysis of His-hRAD51-**35d** binding. Titration curve of (RED-tris-NTA 2nd Generation)-His-hRAD51 (80 nM) with increasing concentrations of **35d**. Sigmoidal fitting curve was obtained using the Affinity Analysis software of NanoTemper Technologies. MST data are the average of three replicates.

To further characterize the effect of **35d** on HR, the compound was administered at different doses to BxPC3 cells for 5 h simultaneously with plasmid transfection; as shown in Figure 6A, it produced a statistically significant dose-response effect in reducing cell HR. The compound was tested up to $40 \mu\text{M}$ (an upper limit in terms of solubility), and we estimated the dose causing a 50% inhibition of HR by applying the polynomial regression to the collected data. The EC_{50} was $18.4 \mu\text{M}$.

1
2
3 An additional evidence of compromised HR was obtained by assessing the localization of
4
5 RAD51 in BxPC-3 nuclei after DNA damage. The results of this experiment are reported in Figure
6
7 6B,C. To obtain massive DNA damage, BxPC-3 cultures were exposed for 1 h to 50 μ M cisplatin.
8
9 The immunohistochemical staining of RAD51 shown in Figure 6B revealed evident nuclear foci in
10
11 cisplatin-treated cells, which appeared significantly reduced when the drug was administered in
12
13 association with 20 μ M **35d**. Furthermore, the percentage of RAD51-labeled nuclei measured in
14
15 cultures exposed to 20 μ M **35d** was superimposable to that observed in cells exposed to the
16
17 association of cisplatin with 20 μ M **35d** (Figure 6C).
18
19
20
21

22 These results were in good agreement with the ELISA and MST outcomes, ultimately pointing to
23
24 **35d** as a novel and RAD51-BRCA2 disruptor with a clear capability to interfere with HR.
25
26

27 The sustained inhibition of HR in cells should result in increased DNA damage, ultimately
28
29 leading to mutations and chromosome aberrations; these effects are expected to be further amplified
30
31 by PARP inhibition. The extent of DNA damage produced in cells treated for 48 h with 20 μ M **35d**,
32
33 administered singularly or in association with 10 μ M olaparib, was studied by evidencing nuclear γ -
34
35 H2AX foci by immunofluorescence. The experiment was performed on both BxPC-3 and Capan-1
36
37 cultures. Capan-1 cells are derived from a human pancreatic adenocarcinoma (very similar to
38
39 BxPC3cells) and are BRCA2-defective.³⁸ As a consequence, they do not operate RAD51-BRCA2-
40
41 dependent HR. The olaparib dose was selected on the basis of previously obtained results with the
42
43 same cell cultures.^{30,31} Results are reported in Figure 7A,B.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

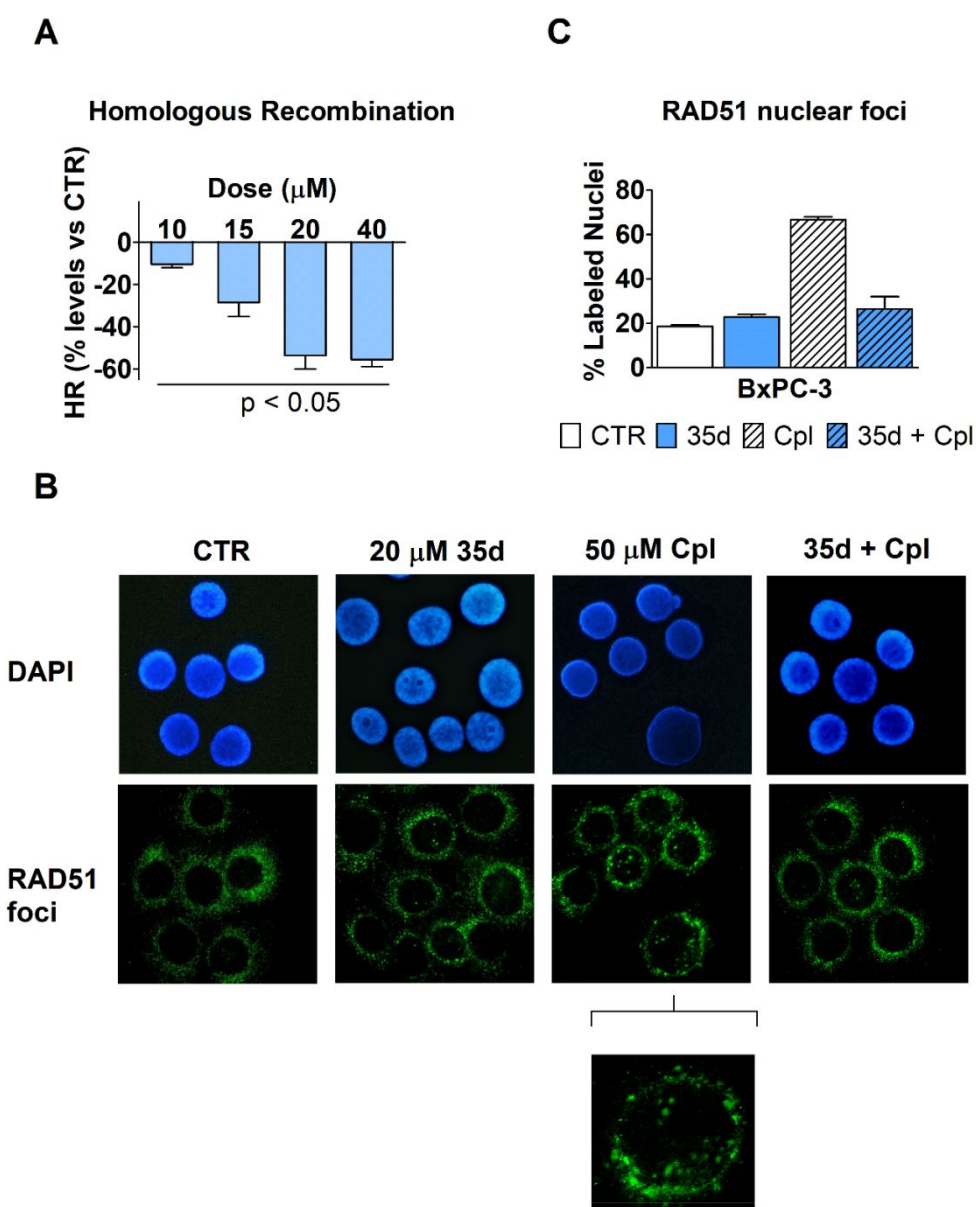
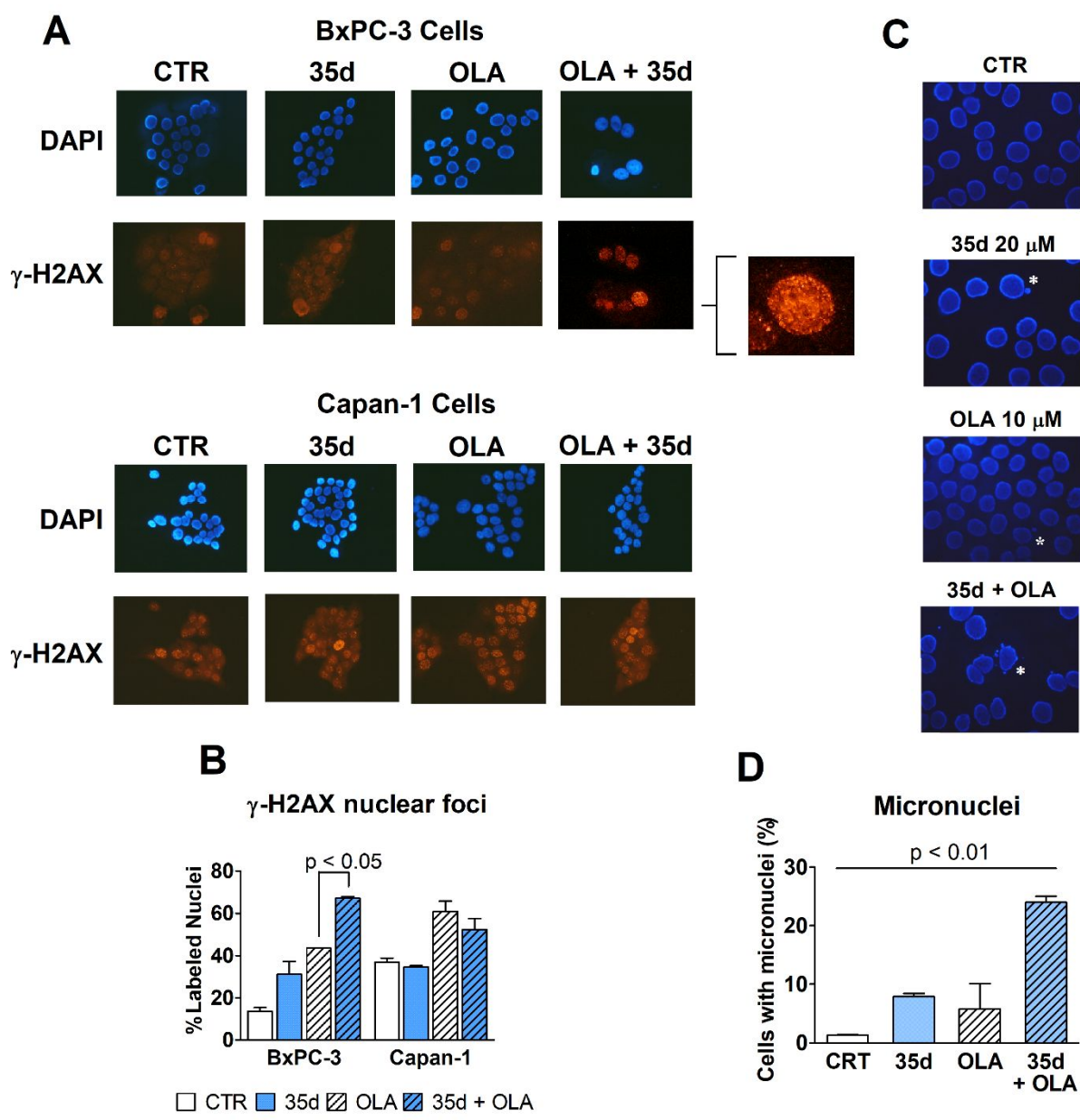


Figure 6. (A): Effect on HR caused by **35d** administered to BxPC3 cells during plasmid transfection (5 h). HR was evaluated by real-time PCR, as described in the Experimental Section. Data were statistically analyzed using the column statistics of Prism 5 software, which applies the inferences analysis and the one-sample t-test. The observed inhibitory effect was significantly different from 0 (the level of untreated cultures) for all tested doses, with $p < 0.05$. (B,C): Immunofluorescence detection of RAD51 in BxPC-3 nuclei after a treatment with 50 μ M Cisplatin (Cpl) given separately or in combination with 20 μ M **35d**. Experimental details are reported in the Experimental Sections. B: Representative pictures showing DAPI-stained cell nuclei and the corresponding immune-labeling of RAD51 localization. In untreated cells (CTR), RAD51 labeling is clearly evident in cytoplasm and does not appear in cell nuclei. In the pictures of Cpl-exposed

cells, nuclear localization of the protein is clearly evident in 3 out of the 6 shown nuclei. A higher magnification detail was included for this sample. C: The bar graph shows the percentage of RAD51-labeled nuclei counted by two independent observers who analyzed the treated cultures. Data were statistically evaluated by applying the one-way ANOVA, which indicated a significantly increased nuclear RAD51 labeling caused by Cpl ($p < 0.05$) and no statistically significant difference between cells treated with **35d** and those exposed to **35d** + Cpl.

The microscope pictures shown in Figure 7A showed increased evidence of γ -H2AX labeling in nuclei of BxPC-3 cells exposed to the compounds' association, compared to the labeling observed in cultures treated with olaparib. In Capan-1 cultures, the constitutive γ -H2AX labeling in nuclei appeared more evident than that observed in BxPC-3 cells.



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 7. (A,B): Evaluation of DNA damage through immune detection of nuclear γ -H2AX foci in BxPC-3 and Capan-1 cells exposed for 48 h to olaparib (10 μ M) or **35d** (20 μ M), given alone or in combination. (A): Representative pictures showing DAPI-stained cell nuclei and the corresponding immune-labeling of γ -H2AX. In BxPC-3 cells, co-administration of **35d** and olaparib produced increased γ -H2AX labeling. A higher magnification detail was included for this sample. As expected, Capan-1 cells showed a constitutive γ -H2AX labeling which was highly increased by olaparib, but was unaffected by **35d** co-administration. (B): The bar graph shows the percentage of γ -H2AX -labeled nuclei counted by two independent observers who analyzed the treated cultures. Data obtained in bxPC-3 cells were statistically evaluated by applying the one-way ANOVA, which indicated a statistically significant difference between the cultures treated with

olaparib and those exposed to olaparib + **35d**. (C,D): Evaluation of micronuclei generation in BxPC3 cells treated (72 h) with **35d** and olaparib, given alone or in combination. C: Representative pictures showing DAPI-stained cell nuclei. White asterisks indicate the presence of micronuclei. D: The percentage of cells bearing micronuclei was estimated by two independent observers, by analyzing 100-250 cells for each treatment sample. The obtained results were statistically analyzed by applying the one-way ANOVA, which indicated a p value < 0.01.

Moreover, in agreement with data showing higher PARPi sensitivity for cells lacking functional HR,¹⁷ these cultures showed increased nuclear labeling when exposed to olaparib. Notably, this labeling was not further enhanced by **35d** co-administration. The percentage of γ -H2AX-labeled nuclei measured in all treated cultures is shown in the bar graphs of Figure 7B.

The sustained and increased DNA damage produced in BxPC-3 nuclei generates chromosomal aberrations which can be visualized through the presence of small DNA-staining bodies outside of the main nucleus (micronuclei). The microscope pictures of Figure 7C show the appearance of this feature in BxPC3 cells exposed for 72 h to 20 μ M **35d**, administered alone or in combination with 10 μ M olaparib. The percentage of cells bearing micronuclei is reported in the graph of Figure 7D. Notably, cells bearing micronuclei were markedly more frequent in cultures treated with the **35d**/olaparib combination.

Taken together, the results reported in Figures 6 and 7 significantly support the requested mechanism of action for **35d**. Therefore, we conducted further experiments to test whether the **35d**/olaparib combination would induce synthetic lethality.

We simultaneously evaluated cell viability and cell death, measured at 72 h in BxPC3 cells exposed to **35d** alone or in combination with 10 μ M olaparib (Figure 8). The statistical analysis (see legend of Figure 8) compared the results for cultures treated with different doses of **35d** or the **35d**/olaparib combination (Figure 8A, upper panel). When applied to the data of the cell viability experiment, this analysis indicated a statistically significant difference produced by **35d** in combination with olaparib in all the treated BxPC3 cultures, with p values ranging from 0.01 (10

1
2
3 μM **35d**) to 0.0001 (15 and 20 μM **35d**). When we applied the same analysis to the data of the cell
4
5 death experiment, we found no statistically significant increase in cell death for olaparib when co-
6
7 administered with 10-15 μM **35d**. Notably, in cultures exposed to olaparib + 20 μM **35d** (the
8
9 concentration producing the highest inhibition of HR), the evidence for cell death was markedly
10
11 increased and statistically significant, with $p < 0.0001$ (Figure 8A, lower panel).
12
13
14

15 When cell death is a consequence of progressive DNA damage accumulation induced by
16
17 simultaneous PARP and HR inhibition, we would expect it to emerge gradually over the time. To
18
19 confirm and better characterize the lethality observed in cultures treated with the **35d**/olaparib
20
21 combination, we therefore considered it inappropriate to conduct a simple evaluation of the
22
23 commonly used markers (e.g. caspase activation), since these can show very transient changes.
24
25 Instead, we observed cell morphology and reaction to vital dyes after the 72-h treatment, when the
26
27 experiment of Figure 8A indicated a significant level of cell death.
28
29
30

31 Figure 8B shows microscope pictures of BxPC3 cells stained with mixed DAPI and PI. The
32
33 simultaneous use of these two dyes can demonstrate cell death and indicate the death pattern.³⁹
34
35 DAPI is cell-permeable and evidences nuclear morphology; healthy cells appear to display normal
36
37 nuclear morphology in the absence of PI staining, since this dye is not cell-permeable. Cells
38
39 undergoing apoptosis display nuclear condensation, which is indicated by increased DAPI staining.
40
41 PI staining indicates compromised membrane integrity, which characterizes necrotic cells and late-
42
43 apoptotic cells maintained in culture.
44
45
46

47 The microscope pictures show that untreated BxPC3 cells display only a moderate DAPI staining
48
49 of their nucleus. Nuclear DAPI staining is slightly increased in cells treated with **35d** alone, but is
50
51 strikingly bright only in cells treated with the **35d**/olaparib combination. Furthermore, PI staining
52
53 appeared only in these cultures, confirming the manifestation of synthetic lethality. The
54
55 simultaneous marked staining of these cells with both dyes could indicate an apoptotic phenomenon
56
57 followed by compromised membrane integrity because of cell persistence in culture.
58
59
60

As expected, the sustained and increased DNA damage observed in BxPC3 cells treated with the **35d**/olaparib combination (Figure 7) reproduced the desired mechanism of synthetic lethality (Figure 8). These results are also relevant given the mutated p53 status of BxPC3 cells, which should make them more resistant to mechanisms that induce cell death.

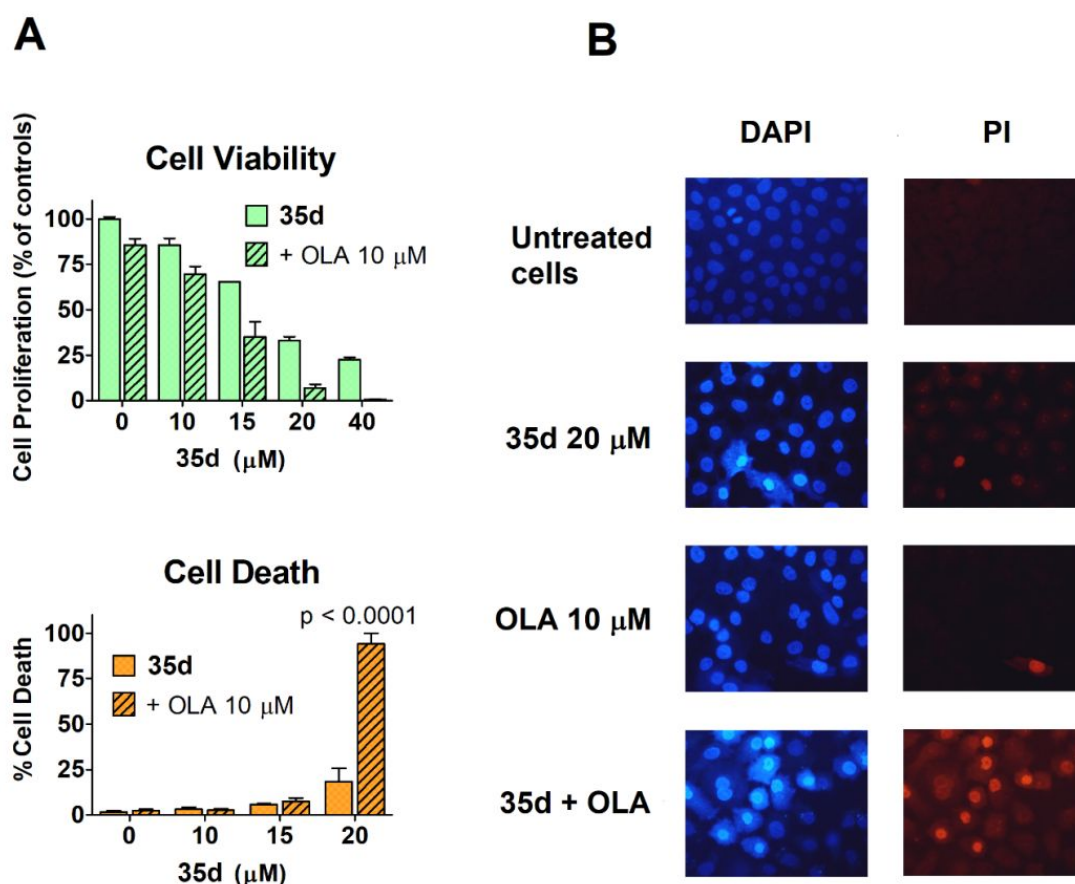


Figure 8. (A): BxPC3 cell viability and death measured after 72 h exposure to **35d** and 10 μM olaparib, given alone or in combination. Data were analyzed by two-way ANOVA using the two treatments (**35d** and olaparib) as variables. In the cell viability experiment, Bonferroni post-test indicated a statistically significant difference produced by olaparib co-administration in all BxPC3 cultures treated with the different **35d** doses, with p values ranging from 0.01 (10 μM **35d**) to 0.0001 (15 and 20 μM **35d**). The same analysis was applied to the data from the cell death experiment and indicated that no statistically significant increase in cell death was produced by olaparib when co-administered with 10-15 μM **35d**. In cultures exposed to olaparib + 20 μM **35d** the evidence for cell death was markedly increased and statistically significant, with p < 0.0001.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

(B): After the 72-h treatment, BxPC3 cells were stained with vital dyes. As shown in the microscope pictures, the only culture displaying sharp evidence of cell death was that exposed to the combination of olaparib/20 μ M **35d**, as demonstrated by PI nuclear staining.

Finally, the antiproliferative effect of the **35d**/olaparib combination was also studied on the HR-defective Capan-1 culture and on a non-neoplastic cell line derived from human kidney (HK-2). Moreover, to evaluate the antineoplastic potency of the **35d**/olaparib association, we also calculated the combination index (CI) of the two compounds, using the procedure previously described^{30,31} (Figure 9).

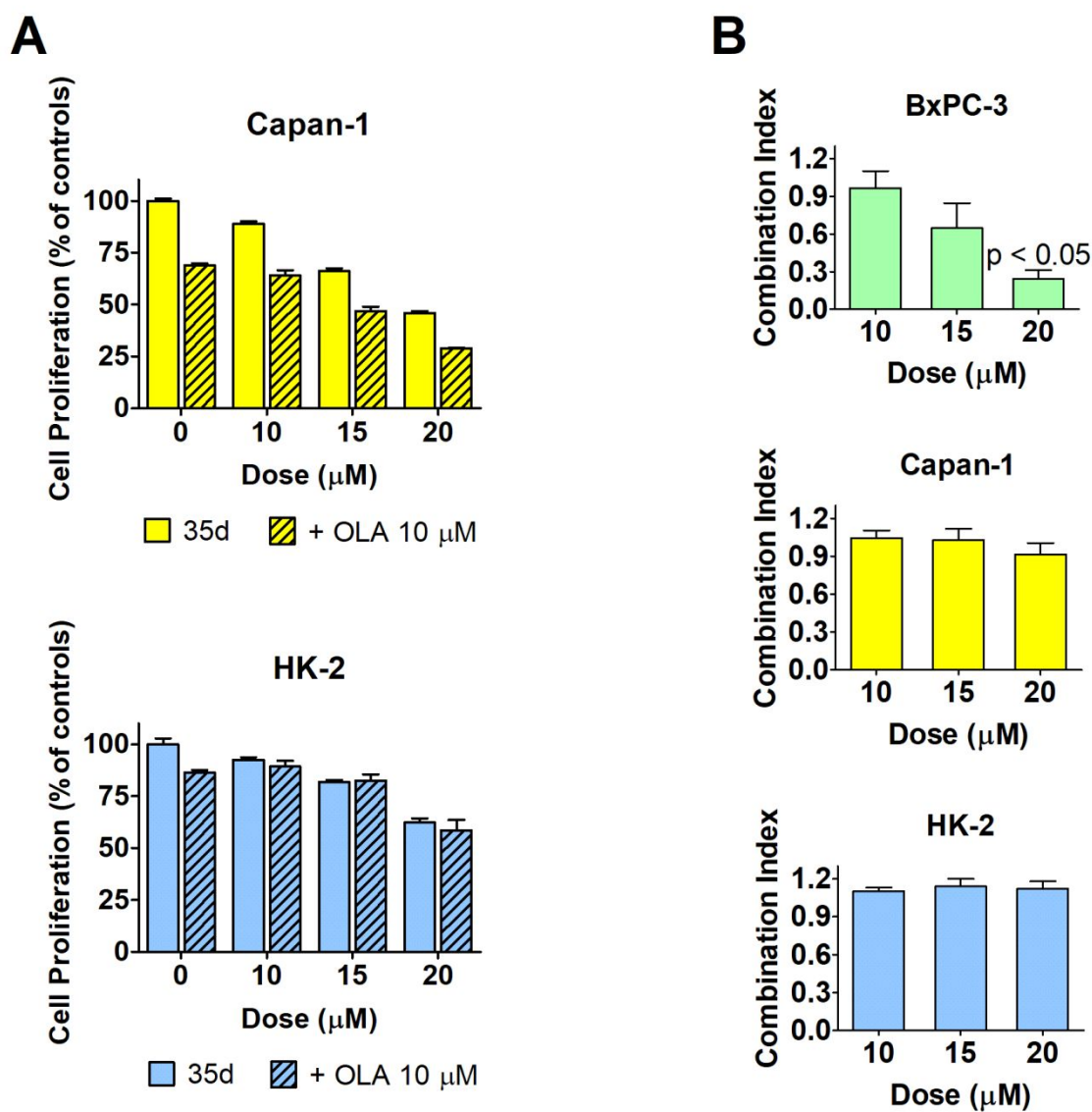


Figure 9. (A): Antiproliferative effect caused by **35d** and its association with olaparib, measured in Capan-1 cells (which do not operate RAD51-BRCA2-dependent HR) and in normal immortalized human renal cells (HK-2). The same procedures described for BxPC3 cells were used here. (B): Combination indexes of the olaparib/**35d** association measured in the three used cell lines, calculated according to the method previously described.^{30,31} For BxPC-3 cells, the data reported in Figure 8A (Cell Proliferation) were used. Data were analyzed using the column statistics and the one-sample t-test of Prism 5 software. For BxPC-3 cells, this test showed a statistically significant difference from 0.8 for the combination of olaparib /20 μ M **35d**. Values < 0.8 indicate synergism between the two compounds.

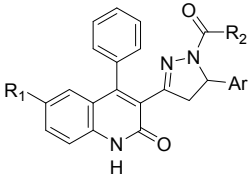
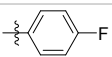
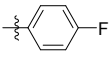
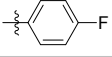
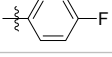
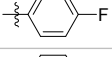
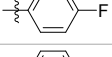
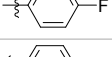
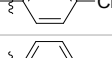
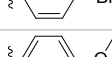
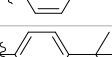
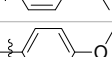
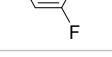
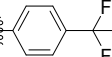
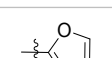
According to this method, a CI < 0.8 indicates synergism, while a result ranging from 0.8 and 1.2 indicates additive effects. This evaluation (Figure 9A,B) was performed for all the three studied cell cultures; for BxPC-3 cells, the data reported in Figure 8A (cell viability experiment) were used for the calculation of CI. Interestingly, in these cells the potency of the compound combination increased in parallel with the **35d** dose, reaching a statistically significant difference from 0.8 at the 20 μ M concentration. The effects observed when 20 μ M **35d** is combined with 10 μ M olaparib may thus indicate a synergism between the two compounds.

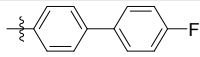
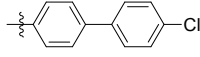
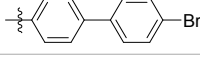
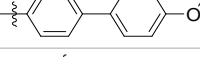
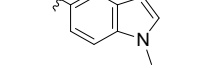
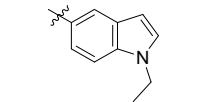
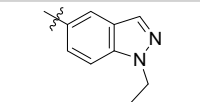
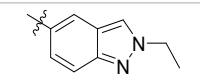
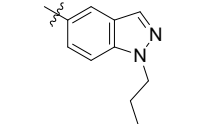
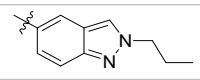
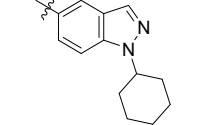
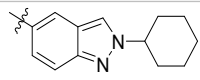
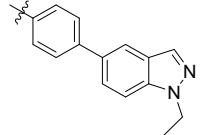
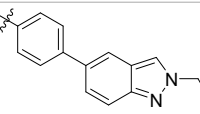
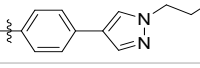
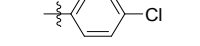
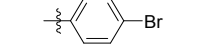
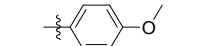
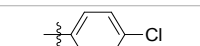
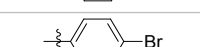
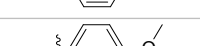
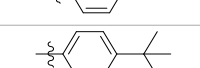
In HK-2 cultures, no dose of **35d** appeared to significantly increase the antiproliferative power of olaparib (two-way ANOVA). In Capan-1 cells, the same statistical evaluation showed significantly increased antiproliferative effects for **35d**/olaparib. However, in these cells, the CI did not show increasing potency of the compound combination with dose escalation. Moreover, at all tested doses, the value of CI was not significantly different from 1, which is the level measured in BxPC3 cells exposed to 10 μ M **35d**, a dose which did not relevantly affect (\approx 10%) HR (Figure 6A). This result further supports the idea that the over-additive effects in the **35d**/olaparib combination arise from and are strictly related to HR inhibition.

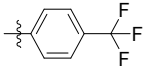

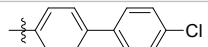
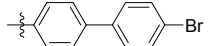
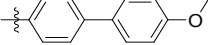
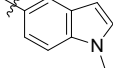
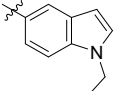
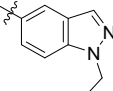
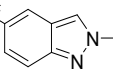
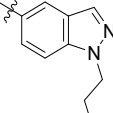
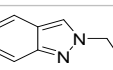
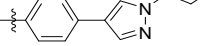
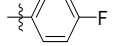
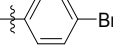
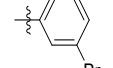
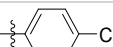
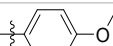
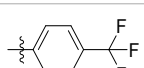
Overall, these data supported our working hypothesis that combining a RAD51-BRCA2 small molecule disruptor with olaparib could be a valuable strategy for inducing synthetic lethality in

cancer cell lines with fully functional *BRCA* genes and homologous recombination. This includes pancreatic cancer, which is a major unmet need in oncology. We believe that this paradigm could be used to discover innovative anticancer therapies based on other lethal gene pairs using a similar medicinal chemistry strategy.

Table 1. Structures and EC₅₀ of compounds **4d-57d** on ELISA Assay.

				
Compound	R ₁	R ₂	Ar	EC ₅₀ (μM)*
4d	Cl	-CH ₂ CH ₂ COOH		16 ± 4
5d	Cl	-CH ₃		50 ± 10
6d	Cl	-CH ₂ CH ₃		34 ± 3
7d	Cl	-CH ₂ CH ₂ NH ₂		NA**
8d	Cl	-CH ₂ CH ₂ C(O)NH ₂		NA**
9d	Cl	-CH ₂ CH ₂ C(O) ₂ CH ₃		NA**
10d	Cl	-CH ₂ CH ₂ NHS(O) ₂ CH ₃		NA**
11d	Cl	-CH ₂ CH ₂ COOH		59 ± 8
12d	Cl	-CH ₂ CH ₂ COOH		16 ± 2
13d	Cl	-CH ₂ CH ₂ COOH		NA**
14d	Cl	-CH ₂ CH ₂ COOH		NA**
15d	Cl	-CH ₂ CH ₂ COOH		NA**
16d	Cl	-CH ₂ CH ₂ COOH		29 ± 2
17d	Cl	-CH ₂ CH ₂ COOH		NA**

18d	Cl	-CH ₂ CH ₂ COOH		13 ± 1
19d	Cl	-CH ₂ CH ₂ COOH		25 ± 5
20d	Cl	-CH ₂ CH ₂ COOH		20 ± 2
21d	Cl	-CH ₂ CH ₂ COOH		20 ± 4
22d	Cl	-CH ₂ CH ₂ COOH		2 ± 0.5
23d	Cl	-CH ₂ CH ₂ COOH		10 ± 2
24d	Cl	-CH ₂ CH ₂ COOH		NA**
25d	Cl	-CH ₂ CH ₂ COOH		28 ± 4
26d	Cl	-CH ₂ CH ₂ COOH		NA**
27d	Cl	-CH ₂ CH ₂ COOH		NA**
28d	Cl	-CH ₂ CH ₂ COOH		NA**
29d	Cl	-CH ₂ CH ₂ COOH		NA**
30d	Cl	-CH ₂ CH ₂ COOH		15 ± 3
31d	Cl	-CH ₂ CH ₂ COOH		17 ± 4
32d	Cl	-CH ₂ CH ₂ COOH		65 ± 5
33d	Cl	-CH ₂ CH ₃		8 ± 2
34d	Cl	-CH ₂ CH ₃		19 ± 1
35d	Cl	-CH ₂ CH ₃		19 ± 1
36d	Cl	-CH ₃		10 ± 0.7
37d	Cl	-CH ₃		38 ± 7
38d	Cl	-CH ₃		NA**
39d	Cl	-CH ₃		15 ± 4

40d	Cl	-CH ₃		NA**
41d	Cl	-CH ₃		NA**
42d	Cl	-CH ₃		45 ± 8
43d	Cl	-CH ₃		70 ± 15
44d	Cl	-CH ₃		70 ± 6
45d	Cl	-CH ₃		18 ± 1
46d	Cl	-CH ₃		50 ± 15
47d	Cl	-CH ₃		40 ± 3
48d	Cl	-CH ₃		NA**
49d	Cl	-CH ₃		0.95 ± 0.05
50d	Cl	-CH ₃		NA**
51d	Cl	-CH ₃		46 ± 9
52d	H	-CH ₂ CH ₂ COOH		NA**
53d	H	-CH ₂ CH ₂ COOH		NA**
54d	H	-CH ₂ CH ₂ COOH		NA**
55d	H	-CH ₂ CH ₂ COOH		NA**
56d	H	-CH ₂ CH ₂ COOH		NA**
57d	H	-CH ₂ CH ₂ COOH		NA**

*: All points were tested in triplicate with error bars indicating the standard deviation.
**NA: not active.

Table 2. Preliminary biological screening of compounds showing EC₅₀ on ELISA assay ranging from 0.95 to 20 μM.

Compound	EC ₅₀ ELISA (μM)	Preliminary Biological Screening
----------	-----------------------------	----------------------------------

4d	16 ± 4	HR inhibition = 10%, 40 μ M
12d	16 ± 2	HR inhibition = not present Olaparib association = not present
18d	13 ± 1	Olaparib association = not present
20d	20 ± 2	Olaparib association = not present
21d	20 ± 4	NE ^a
22d	2 ± 0.5	HR inhibition = not present Olaparib association = not present at 5 μ M
23d	10 ± 2	Olaparib association = not present
30d	15 ± 3	NE ^a
31d	17 ± 4	HR inhibition = NDD ^b Olaparib association = present at 20 μ M
33d	8 ± 2	HR inhibition = not present Olaparib association = not present
34d	19 ± 1	HR inhibition = not present Olaparib association = NE ^a
35d	19 ± 1	HR inhibition = 54%, 20 μ M Olaparib association = present at 15 μ M
36d	10 ± 0.7	HR inhibition = 24%, 10 μ M Olaparib association = not present
39d	15 ± 4	HR inhibition = NDD ^b
45d	18 ± 1	HR inhibition = not present
49d	0.95 ± 0.05	HR inhibition = not present Olaparib association = not present

^a NE, not evaluable; ^b NDD, not dose-dependent

CONCLUSIONS

Continuing our research line, we described a series of dihydroquinolone pyrazoline derivatives as a new class of RAD51-BRCA2 disruptors. Compound **4d** was identified as a promising hit, and

subsequent SAR efforts yielded **35d** with the desired biological profile. As expected, **35d** bound to its target (RAD51) and inhibited the protein-protein interaction between RAD51-BRCA2. Importantly, it synergized and reproduced the paradigm of synthetic lethality in combination with olaparib in pancreatic cancer cells (BxPC3). These effects were strictly related to the extent of HR inhibition in a dose-dependent trend. This is the most promising achievement of the current investigation and supports our working hypothesis that one can trigger synthetic lethality using only small organic molecules. Interestingly, the observed synthetic lethality was triggered by tackling two biochemically different mechanisms: enzyme inhibition (PARP) and protein-protein disruption (RAD51-BRCA2). This highlights how complex and diverse mechanisms of action can synergistically contribute to the same physiological and, in turn, pharmacological activity. We note, however, that **35d**'s low solubility may affect its metabolic and pharmacokinetic profile (DM/PK), preventing it from being studied further in in vivo cancer models. Structural tuning is therefore required (and currently ongoing) to discover more drug-like dihydroquinolone pyrazoline derivatives.

In conclusion, we have further shown that synthetic lethality may be a suitable framework for discovering innovative anticancer therapies. We are confident that this novel concept will open up several new avenues based on other lethal gene pairs to meet the medical needs in oncology.

EXPERIMENTAL SECTION

Chemistry. General Chemical Methods. Solvents and reagents were obtained from commercial suppliers and used without further purification. If required, solvents were distilled prior to use. Automated column chromatography purifications were conducted using a Teledyne ISCO apparatus (CombiFlashTM Rf) with prepacked silica gel columns of different sizes (from 4 g to 120 g). Mixtures of increasing polarity of cyclohexane and ethyl acetate or dichloromethane and methanol/ethanol were used as eluents. Preparative TLCs were performed using Macherey-Nagel precoated 0.05 mm TLC plates (SIL G-50 UV254). Microwave heating was performed using

Explorer-48 positions instrument (CEM). NMR experiments were run on a Bruker Avance III 400 MHz spectrometer (400.13 MHz for ^1H , and 100.62 MHz for ^{13}C), equipped with a BBI probe and Z-gradients, or on a Bruker FT NMR Avance III 600-MHz spectrometer (600.130 MHz for ^1H , and 150.903 MHz for ^{13}C) equipped with a 5-mm CryoProbe QCI quadruple resonance, a shielded Z-gradient coil, and the automatic sample changer SampleJet NMR system. Spectra were acquired at 300 K, using deuterated dimethylsulfoxide ($\text{DMSO-}d_6$) or deuterated chloroform (CDCl_3) as solvents. Chemical shifts for ^1H and ^{13}C spectra were recorded in parts per million using the residual nondeuterated solvent as the internal standard (for CDCl_3 : ^1H 7.26 ppm, ^{13}C 77.16 ppm; for $\text{DMSO-}d_6$: ^1H 2.50 ppm, ^{13}C 39.52 ppm). UPLC-MS analyses were run on a Waters ACQUITY UPLC/MS system consisting of an SQD (Single Quadrupole Detector) Mass Spectrometer equipped with an Electrospray Ionization interface and a Photodiode Array Detector. The PDA range was 210-400 nm. The analyses were performed on either an ACQUITY UPLC HSS T3 C_{18} column (50 x 2.1 mmID, particle size 1.8 μm) with a VanGuard HSS T3 C_{18} pre-column (5 x 2.1 mmID, particle size 1.8 μm) (LogD<1) or an ACQUITY UPLC BEH C_{18} column (50 x 2.1 mmID, particle size 1.7 μm) with a VanGuard BEH C_{18} pre-column (5 x 2.1 mmID, particle size 1.7 μm) (LogD>1). The mobile phase was 10 mM NH_4OAc in H_2O at pH 5 adjusted with AcOH (A) and 10 mM NH_4OAc in $\text{MeCN-H}_2\text{O}$ (95:5) at pH 5 (B). Electrospray ionization in positive and negative mode was applied in the mass scan range 100-500 Da. Methods and gradients used: *Polar method*: Column: Waters ACQUITY UPLC HSS T3 C_{18} , 1.8 μm , 50 x 2.1 mmID; Pre-column: VanGuard HSS T3 C_{18} , 1.8 μm , 5x2.1 mmID; Linear gradient: 0-0.2 min: 0% B, 0.2-2.7 min: 0-50% B, 2.7-2.8 min: 50-100% B, 2.8-3.0 min: 100% B; Flow rate: 0.5 mL/min. *Generic method*: Column: Waters ACQUITY UPLC BEH C_{18} , 1.7 μm , 50 x 2.1 mmID; Linear gradient: 0-0.2 min: 5% B, 0.2-2.7 min: 5-95% B, 2.7-2.8 min: 95-100% B, 2.8-3.0 min: 100% B; Flow rate: 0.5 mL/min. *Apolar method*: Column: Waters ACQUITY UPLC BEH C_{18} , 1.7 μm , 50x2.1 mmID; Pre-column: VanGuard BEH C_{18} , 1.7 μm , 5x2.1 mmID; Gradient: 0-0.2 min: 50% B, 0.2-2.7 min: 50-100% B, 2.7-3.0 min: 100% B; Flow rate: 0.5 mL/min. Compounds were named using the naming algorithm

developed by CambridgeSoft Corporation and used in ChemBioDraw Ultra 15.0. All final compounds displayed $\geq 95\%$ purity as determined by UPLC/MS analysis. All final synthesized compounds were checked for PAINS compliance.

General procedure A for the synthesis of quinolin-2(1H)-one acrolyl intermediates. This procedure has been applied to the preparation of **86b-114b** (Scheme 1). In a round-bottomed flask, the appropriate quinolin-2(1H)-one (**60a-61a**, 1.00 equiv) and potassium hydroxide (25.00 equiv) were stirred in EtOH/H₂O (4:3 v/v, 0.05 M) at 0 °C for 45 min prior to the addition of an appropriately substituted aryl aldehyde (**62-85**, 1.00 equiv). The reaction mixture was stirred overnight as it gradually reached room temperature. The reaction was quenched by slow addition of acetic acid (25.00 equiv). The crude was extracted with DCM/H₂O (3 x 50 mL), the organic layer was then dried over Na₂SO₄, and the solvent removed under reduced pressure. The desired compound was obtained after purification over silica gel, unless otherwise noted.

General procedure B for synthesis of pyrazol-3-yl-quinolin-2(1H)-one intermediates (Scheme 1). This procedure has been applied to the preparation of **86c-114c**. In a microwaveable vessel, the appropriate quinolin-2(1H)-one acrolyl intermediate (**86b-114b**, 1.00 equiv) was dissolved in EtOH absolute (0.2 M) and hydrazine monohydrate (2.00 equiv) was added. The mixture was microwaved with stirring for 45 min at 110 °C (200 W). The EtOH was removed under reduced pressure. Crude was purified over silica gel, unless otherwise noted, to afford desired compounds.

General procedures C₁ to obtain the final desired dihydroquinolone pyrazoline derivatives (Scheme 2). This procedure has been applied to synthesize **4d, 11d-32d, 52d-57d**. In a microwaveable vessel, the appropriate pyrazol-3-ylquinolin-2(1H)-one intermediate (**86c-114c**, 1.00 equiv) was dissolved in anhydrous THF (0.5 M). The succinic anhydride **115** (2.00 equiv) was added. The solution was microwaved (200 W) with stirring for 45 min at the appropriate temperature. The THF was removed under reduced pressure, and the organic layers were dissolved

in DCM, washed with HCl_{aq} pH 2 (3 x 30 mL) and dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude was purified over silica gel.

General procedures C₂ to obtain the final desired dihydroquinolone pyrazoline derivatives (Scheme 2). This procedure has been applied to synthesize **5d**, **36d-51d**. In a microwaveable vessel, the appropriate pyrazol-3-ylquinolin-2(1*H*)-one intermediate (**86c-114c**, 1.00 equiv) was dissolved in anhydrous THF (0.5 M). The acetic anhydride **116** (2.00 equiv) was added. The solution was microwaved (200 W) with stirring for 45 min at the appropriate temperature. The THF was removed under reduced pressure, and the organic layers were dissolved in DCM, washed with HCl_{aq} pH 2 (3 x 30 mL) and dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude was purified over silica gel.

General procedures C₃ to obtain the final dihydroquinolone pyrazoline derivatives (Scheme 2). This procedure has been applied to synthesize **6d**, **33d-35d**. In a round-bottomed flask, propionic acid **117** (1.80 equiv), HOBt (1.80 equiv) and EDCI (1.80 equiv) were stirred in DCM (0.50 M) at room temperature for 1 h. Then a solution of the pyrazol-3-ylquinolin-2(1*H*)-one intermediate **86c-89c** (1.00 equiv) and Et₃N (2.50 equiv) in DCM (0.50 M) was added. The reaction was stirred at room temperature for 3 h. The organic layer was washed with NaHCO₃ (aq) 1M (1 x 50 mL), citric acid 10% (1 x 50 mL) and H₂O (1 x 50 mL), dried over Na₂SO₄, and the solvent removed under reduced pressure. The desired compound was obtained after purification over silica gel, 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 (4d). 6-chloro-3-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1*H*)-one **86c** (270 mg, 0.65 mmol) and succinic anhydride **115** (130 mg, 1.30 mmol) were microwaved (120 °C, 200 W) according to general procedure C₁. The crude was purified by flash column chromatography (SiO₂ gold 24 g; 0-50% EtOH/DCM), to afford the desired **4d** (208 mg, 62% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.38 (s, 1H), 12.02 (s, 1H), 7.64 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.60 – 7.37 (m, 5H), 7.28 (dt, *J* = 6.8, 2.0 Hz, 1H), 7.04 (t, *J* = 8.9

Hz, 2H), 6.94 (d, $J = 2.3$ Hz, 1H), 6.88 – 6.76 (m, 2H), 5.32 (dd, $J = 12.0, 4.5$ Hz, 1H), 3.73 (dd, $J = 18.5, 12.0$ Hz, 1H), 2.79 (dd, $J = 18.4, 4.5$ Hz, 1H), 2.48 – 2.40 (m, 2H), 2.32 – 2.26 (m, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.95, 169.09, 160.56, 152.85, 150.38, 137.78, 135.00, 131.68, 129.88, 128.97, 128.88, 128.81, 127.99, 127.90, 126.57, 126.51, 125.07, 121.14, 118.09, 115.67, 115.46, 95.62, 58.69, 45.66, 29.04, 28.69. In agreement with that previously reported by Acker *et al.*³⁶ R_t 2.04 min (generic method). ESI-MS for $\text{C}_{28}\text{H}_{21}\text{ClFN}_3\text{O}_4$: calculated 517.1, found m/z 518.4, 520.4 $[\text{M}+\text{H}]^+$; 516.4, 518.4 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) 98%.

3-(1-acetyl-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (5d). 6-chloro-3-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one **86c** (200 mg, 0.48 mmol) and acetic anhydride **116** (91 μL , 0.96 mmol) were microwaved (120 $^\circ\text{C}$, 200 W) according to general procedure C₂. The crude was purified by direct phase flash column chromatography (SiO_2 gold 24 g, 0-20% MeOH/DCM) to afford the desired **5d** (207 mg, yield 47%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.38 (s, 1H), 7.64 (dd, $J = 8.8, 2.4$ Hz, 1H), 7.61 – 7.50 (m, 3H), 7.46 (d, $J = 8.8$ Hz, 1H), 7.41 (dt, $J = 6.6, 1.9$ Hz, 1H), 7.29 (dt, $J = 6.4, 1.9$ Hz, 1H), 7.06 (t, $J = 8.9$ Hz, 2H), 6.94 (d, $J = 2.3$ Hz, 1H), 6.84 (dd, $J = 8.6, 5.6$ Hz, 2H), 5.32 (dd, $J = 12.0, 4.5$ Hz, 1H), 3.73 (dd, $J = 18.4, 12.1$ Hz, 1H), 2.84 (dd, $J = 18.5, 4.5$ Hz, 1H), 1.88 (s, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 167.53, 162.80, 160.57, 160.39, 152.67, 150.35, 138.87, 138.84, 137.79, 135.12, 131.64, 129.84, 129.04, 128.96, 128.80, 128.76, 128.00, 127.93, 126.53, 126.49, 125.02, 121.11, 118.09, 115.67, 115.45, 58.49, 45.71, 21.85. R_t 2.38 min (generic method). ESI-MS for $\text{C}_{26}\text{H}_{19}\text{ClFN}_3\text{O}_2$: calculated 459.1, found m/z 460.1, 462.1 $[\text{M}+\text{H}]^+$; 458.1, 460.1 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

6-chloro-3-(5-(4-fluorophenyl)-1-propionyl-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (6d). Compound **6d** was synthesized via general procedure C₃ using **86c** (250 mg, 0.60 mmol). Purification performed by direct phase flash chromatography (SiO_2 gold 24 g, 0-1% MeOH/DCM) to afford **6d** (60 mg, yield 21%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.38

(s, 1H), 7.64 (dd, $J = 2.0, 8.0$ Hz, 1H), 7.58 – 7.50 (m, 3H), 7.45 (d, $J = 8.8$ Hz, 1H), 7.41 – 7.39 (m, 1H), 7.28 (d, $J = 5.6$ Hz, 1H), 7.06 (t, $J = 8.8$ Hz, 2H), 6.93 (d, $J = 2.0$ Hz, 1H), 6.84 (dd, $J = 4.0, 8.0$ Hz, 2H), 5.32 (dd, $J = 8.0, 12.0$ Hz, 1H), 3.73 (dd, $J = 12.0, 16.0$ Hz, 1H), 2.81 (dd, $J = 4.0, 16.0$ Hz, 1H), 2.30 – 2.16 (m, 2H), 0.83 (t, $J = 7.6$ Hz, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 206.89, 170.93, 162.79, 160.57, 160.38, 152.63, 150.30, 139.00, 138.98, 137.75, 135.12, 131.63, 129.79, 128.97, 128.95, 128.86, 128.80, 127.96, 127.88, 126.53, 126.47, 125.10, 121.11, 118.07, 115.67, 115.46, 58.54, 45.54, 29.44, 9.27. ESI-MS for $\text{C}_{27}\text{H}_{21}\text{ClFN}_3\text{O}_2$: calculated 473.1, found m/z 474.2, 476.2 $[\text{M}+\text{H}]^+$; 472.3, 474.3 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) 99%.

3-(1-(3-aminopropanoyl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (7d) (Scheme 3). In round bottom flask **119** (200 mg, 0.34 mmol) was treated with 4M HCl in dioxane (4 mL) and stirred at rt for 15 minutes. The solvent was removed under reduced pressure. In a round bottom flask the solid residue (110 mg, 0.21 mmol) was treated with NaOH 0.5 M (420 μL) in EtOAc (5 mL) stirring at rt for 30 minutes. The mixture was then diluted with further EtOAc and washed twice with H_2O . The organic layer was dried over Na_2SO_4 , filtered and evaporated to dryness. Purification performed by direct phase flash chromatography (0-10% MeOH/DCM, 0-0.1% NH_4OH). Yield 50 mg, 50%. ^1H NMR (400 MHz, DMSO- d_6) δ 7.63 (dd, $J = 12.0, 4.0$ Hz, 1H), 7.57 – 7.50 (m, 3H), 7.54 (d, $J = 4.8$ Hz, 1H), 7.42-7.40 (m, 1H), 7.26 (d, $J = 7.2$ Hz, 1H), 7.03 (t, $J = 8.8$ Hz, 2H), 6.92 (d, $J = 2.0$ Hz, 1H), 6.81 (dd, $J = 2.0, 8.0$ Hz, 2H), 5.32 (dd, $J = 2.0, 12.0$ Hz, 1H), 3.72 (dd, $J = 12.0, 20.0$ Hz, 1H), 2.76 (dd, $J = 4.0, 16.0$ Hz, 1H), 2.57 (t, $J = 6.6$ Hz, 2H), 2.42 – 2.28 (m, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 169.42, 162.77, 160.56, 160.36, 152.76, 150.29, 138.96, 138.93, 137.78, 135.02, 131.63, 129.86, 128.99, 128.92, 128.89, 128.80, 127.95, 127.86, 126.52, 126.45, 125.09, 121.11, 118.10, 115.66, 115.45, 110.00, 58.53, 45.61, 38.06, 38.04. ESI-MS for $\text{C}_{27}\text{H}_{22}\text{ClFN}_4\text{O}_2$: calculated 488.1, found m/z 489.4, 491.4 $[\text{M}+\text{H}]^+$, 487.3, 489.4 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanamide (8d) (Scheme 4). In a round bottomed flask 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 **4d** (258 mg, 0.50 mmol) was dissolved with 3.2 mL of anhydrous DCM, then HATU (285 mg, 0.75 mmol), EDC (144 mg, 0.75 mmol) and 1.8 mL of anhydrous DMF were added. The mixture was stirred at rt for 10 minutes. Ammonium chloride (144 mg 2.50 mmol) and soon after DIPEA (348 μ L, 2.00 mmol) were added. The reaction mixture was thus stirred at r.t. for 26 hours. Purification performed by direct phase chromatography (SiO₂ gold 24 g, 2.5-50% EtOH/DCM). Yield 120 mg, 46%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.38 (s, 1H), 7.64 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.60 – 7.38 (m, 5H), 7.28 (d, *J* = 7.4 Hz, 1H), 7.19 (s, 1H), 7.04 (dd, *J* = 9.9, 7.7 Hz, 2H), 6.93 (d, *J* = 2.3 Hz, 1H), 6.84 – 6.78 (m, 2H), 6.68 (s, 1H), 5.31 (dd, *J* = 12.0, 4.5 Hz, 1H), 3.71 (dd, *J* = 18.5, 12.1 Hz, 1H), 2.76 (dd, *J* = 18.4, 4.5 Hz, 1H), 2.54 – 2.45 (m, 2H), 2.15 (t, *J* = 7.4 Hz, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.51, 169.56, 160.65, 160.38, 152.73, 150.28, 138.90, 137.94, 135.00, 131.61, 129.94, 129.94, 129.02, 128.99, 128.87, 128.79, 127.99, 127.90, 126.48, 125.10, 121.14, 118.19, 115.66, 115.45, 58.66, 45.64, 29.82, 29.38. *R*_t 2.09 min (generic method). ESI-MS for C₂₈H₂₂ClFN₄O₃: calculated 516.1, found *m/z* 517.4, 519.3 [M+H]⁺; 515.4, 517.4 [M-H]⁻. UPLC-MS purity (UV@215 nm) 99%.

Methyl 4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoate (9d) (Scheme 5). In a round-bottomed flask, commercially available 4-methoxy-4-oxobutanoic acid **120** (153 mg, 1.16 mmol), 6-chloro-3-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one **86c** (486 mg, 1.16 mmol), HOBT (173 mg, 1.28 mmol) were dissolved in DCM. Then TEA (355.8 μ L, 2.55 mmol) was added, followed by EDCI (245 mg, 1.28 mmol) suspended in DCM. The mixture was stirred overnight at r.t. The solvent was removed under vacuum, the residue was dissolved in ethyl acetate and washed with H₂O, NaHCO₃ sat. solution and 5% citric acid. The organic phase was dried over Na₂SO₄ and

1
2
3 evaporated to dryness. The title compound was obtained after purification by direct phase flash
4
5 column chromatography (0-30% EtOAc/DCM). Yield 256 mg, 42%. ¹H NMR (400 MHz, DMSO-
6
7 *d*₆) δ 12.36 (s, 1H), 7.60 (dd, *J* = 2.2, 9.0 Hz, 1H), 7.53-7.48 (m, 3 H), 7.41 (d, *J* = 8.8 Hz, 1H), 7.37
8
9 (dd, *J* = 2.2, 4.6 Hz, 1H), 7.23 (d, *J* = 6.8 Hz, 1H), 7.01 (t, *J* = 8.8 Hz, 2H), 6.89 (d, *J* = 2.0, 1H),
10
11 6.77 (dd, *J* = 5.4, 8.6 Hz, 2H), 5.28 (dd, *J* = 12.0, 4.0 Hz, 1H), 3.50 (s, 3H), 3.67 (dd, *J* = 12.0, 18.0
12
13 Hz, 1H), 2.75 (dd, *J* = 4.0, 18.0 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) 172.9, 168.8, 160.0,
14
15 153.1, 150.1, 139.0, 135.0, 131.9, 129.8, 128.9, 127.9, 126.5, 125.0, 120.9, 118.0, 115.6, 115.4,
16
17 58.7, 21.7, 45.0, 28.9, 28.4. UPLC-MS purity (UV@215 nm) 98%.

21
22 **N-(3-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-fluorophenyl)-4,5-**
23
24 **dihydro-1H-pyrazol-1-yl)-3-oxopropyl)methanesulfonamide (10d)** (Scheme 6). In a round
25
26 bottom flask, 6-chloro-3-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-
27
28 2(1H)-one **86c** (500 mg, 1.20 mmol), 3-(methylsulfonamido) propanoic acid **124** (200 mg, 1.20
29
30 mmol), HOBT (178 mg, 1.32 mmol) were stirred in DCM prior to the addition of triethylamine
31
32 (367.7 mL, 2.64 mmol) and EDCI (253 mg, 1.32 mmol) at 0 °C. The reaction was stirred overnight
33
34 while gradually reached rt. The solvent was removed under vacuum and the residue was dissolved
35
36 in DCM. The organic layer was washed with H₂O, NaHCO₃ sat. solution and 5% citric acid, dried
37
38 over Na₂SO₄, filtered and evaporated to dryness. The title compound was obtained after purification
39
40 over direct phase flash column chromatography (0-60% EtOAc/DCM). Yield 100 mg, 15%). ¹H
41
42 NMR (400 MHz, DMSO-*d*₆) δ 12.35 (s, 1H), 7.60 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.55- 7.49 (m, 3H),
43
44 7.41 (d, *J* = 8.0 Hz, 1H), 7.38 (d, *J* = 6.0 Hz, 1H), 7.24 (d, *J* = 6.0 Hz, 1H), 7.00 (t, *J* = 8.0 Hz, 2H),
45
46 6.89 (d, *J* = 2.0 Hz, 2H), 6.77 (dd, *J* = 4.0, 8.0 Hz, 2H), 5.29 (dd, *J* = 4.0, 12.0 Hz, 1H), 3.69 (dd, *J*
47
48 =12.0, 20.0 Hz, 1H), 3.03-2.98 (m, 2H), 2.80 (s, 3H), 2.72 (dd, *J* = 4.0, 20.0 Hz, 1H), 2.51-2.47 (m,
49
50 2H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 168.01, 162.81, 160.49, 160.40, 153.25, 150.39, 138.71,
51
52 137.78, 134.86, 131.68, 129.94, 129.10, 128.94, 128.91, 128.83, 128.02, 127.94, 126.57, 125.01,
53
54 121.07, 118.09, 115.69, 115.48, 58.63, 45.67, 38.61, 34.73. UPLC-MS purity (UV@215 nm) 99%.

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 (11d). Compound **11d** was synthesized via general procedure C₁ using **87c** (253 mg, 0.58 mmol) with succinic anhydride **115** (116 mg, 1.16 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-60% EtOH/DCM) to afford **11d** (231 mg, yield 74%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.38 (s, 1H), 12.03 (s, 1H), 7.64 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.59 – 7.48 (m, 3H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.41 (dt, *J* = 6.3, 2.0 Hz, 1H), 7.31 – 7.23 (m, 3H), 6.93 (d, *J* = 2.4 Hz, 1H), 6.83 – 6.76 (m, 2H), 5.32 (dd, *J* = 12.0, 4.6 Hz, 1H), 3.74 (dd, *J* = 18.5, 12.1 Hz, 1H), 2.78 (dd, *J* = 18.5, 4.6 Hz, 1H), 2.48 – 2.41 (m, 2H), 2.29 (t, *J* = 7.1 Hz, 2H). In agreement with that previously reported by Acker *et al.*³⁶ R_t 2.16 min (generic method). ESI-MS for C₂₈H₂₁Cl₂N₃O₄: calculated 533.1, found *m/z* 534.4, 536.4, 538.3 [M+H]⁺; 532.4, 534.4, 536.4 [M-H]⁻. UPLC-MS purity (UV@215 nm) 99%.

4-(5-(4-bromophenyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (12d). Compound **12d** was synthesized via general procedure C₁ using **88c** (330 mg, 0.69 mmol) with succinic anhydride **115** (138 mg, 1.38 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-50% EtOH/DCM) to afford **12d** (275 mg, yield 68%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.38 (s, 1H), 12.03 (s, 1H), 7.63 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.59 – 7.47 (m, 3H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.27 (dd, *J* = 6.5, 1.8 Hz, 1H), 6.93 (d, *J* = 2.3 Hz, 1H), 6.73 (d, *J* = 8.5 Hz, 2H), 5.30 (dd, *J* = 12.0, 4.6 Hz, 1H), 3.74 (dd, *J* = 18.5, 12.1 Hz, 1H), 2.77 (dd, *J* = 18.5, 4.6 Hz, 1H), 2.48 – 2.36 (m, 2H), 2.28 (t, *J* = 7.1 Hz, 2H). In agreement with that previously reported by Acker *et al.*³⁶ R_t 2.18 min (generic method). ESI-MS for C₂₈H₂₁BrClN₃O₄: calculated 577.0, found *m/z* 578.2, 580.2, 582.3 [M+H]⁺; 576.1, 578.1, 580.0 [M-H]⁻. UPLC-MS purity (UV@215 nm) >99.5%.

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 (13d). Compound **13d** was synthesized via general

procedure C₁ using **89c** (320 mg, 0.74 mmol) with succinic anhydride **115** (148 mg, 1.48 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-15% EtOH/DCM) to afford **13d** (339 mg, yield 86%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.19 (s, 1H), 7.57 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.51 (m, 3H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.36 – 7.31 (m, 1H), 7.27 – 7.21 (m, 1H), 7.02 (dd, *J* = 8.9, 5.9 Hz, 3H), 6.90 (d, *J* = 2.3 Hz, 1H), 6.80 (d, *J* = 8.7 Hz, 2H), 4.52 (td, *J* = 10.4, 2.9 Hz, 1H), 3.73 (s, 3H), 3.18 (dd, *J* = 16.4, 10.9 Hz, 1H), 2.58 – 2.52 (m, 5H). In agreement with that previously reported by Acker *et al.*³⁶ R_t 1.99 min (generic method). ESI-MS for C₂₉H₂₄ClN₃O₅: calculated 529.1, found *m/z* 530.5, 532.4 [M+H]⁺; 528.4, 530.4 [M-H]⁻. UPLC-MS purity (UV@215 nm) >99.5%.

4-(5-(4-(tert-butyl) phenyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (14d). Compound **14d** was synthesized via general procedure C₁ using **90c** (124 mg, 0.27 mmol) with succinic anhydride **115** (54 mg, 0.54 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-20% EtOH/DCM) to afford **14d** (121 mg, yield 81%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.27 (d, *J* = 78.0 Hz, 1H), 12.08 (s, 1H), 7.64 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.59 – 7.47 (m, 3H), 7.47 – 7.43 (m, 1H), 7.40 (dd, *J* = 4.6, 2.1 Hz, 1H), 7.28 (d, *J* = 7.5 Hz, 1H), 7.23 (d, *J* = 8.3 Hz, 2H), 6.93 (d, *J* = 2.3 Hz, 1H), 6.73 (d, *J* = 8.3 Hz, 2H), 5.26 (dd, *J* = 11.9, 4.5 Hz, 1H), 3.69 (dt, *J* = 24.9, 12.5 Hz, 1H), 2.85 (ddd, *J* = 23.0, 18.2, 4.3 Hz, 1H), 2.47 (dd, *J* = 6.7, 4.0 Hz, 2H), 2.36 – 2.22 (m, 2H), 1.27 (s, 9H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.52, 168.56, 160.09, 152.41, 149.87, 149.19, 139.24, 137.30, 134.59, 131.15, 129.30, 128.57, 128.47, 128.33, 126.06, 126.03, 125.13, 125.09, 124.67, 120.69, 117.60, 58.62, 45.26, 34.12, 31.11, 28.57, 28.21, 26.32. R_t 2.39 min (generic method). ESI-MS for C₃₂H₃₀ClN₃O₄: calculated 555.2, found *m/z* 556.5, 558.4 [M+H]⁺; 554.4, 556.4 [M-H]⁻. UPLC-MS purity (UV@215 nm) 97%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(3-fluoro-4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (15d). Compound **15d** was synthesized via

general procedure C₁ using **91c** (184 mg, 0.41 mmol) with succinic anhydride **115** (82 mg, 0.82 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-10% EtOH/DCM) to afford **15d** (115 mg, yield 51%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.38 (s, 1H), 12.06 (s, 1H), 7.64 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.59 – 7.43 (m, 4H), 7.43 – 7.38 (m, 1H), 7.29 (dd, *J* = 7.1, 1.9 Hz, 1H), 7.01 (t, *J* = 8.8 Hz, 1H), 6.94 (d, *J* = 2.4 Hz, 1H), 6.69 – 6.61 (m, 2H), 5.27 (dd, *J* = 12.0, 4.5 Hz, 1H), 3.82 (s, 3H), 3.70 (dd, *J* = 18.5, 12.0 Hz, 1H), 2.82 (dd, *J* = 18.5, 4.5 Hz, 1H), 2.46 (dd, *J* = 14.3, 7.0 Hz, 2H), 2.29 (t, *J* = 7.2 Hz, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.96, 169.09, 160.59, 152.89, 150.37, 146.62, 137.79, 135.43, 135.05, 131.68, 129.76, 129.01, 128.94, 128.84, 128.74, 126.57, 125.06, 123.56, 122.25, 121.13, 118.10, 114.22, 113.68, 113.50, 58.52, 56.48, 45.52, 29.02, 28.67. R_t 2.00 min (generic method). ESI-MS for C₂₉H₂₃ClFN₃O₅: calculated 547.1, found *m/z* 548.3, 550.3 [M+H]⁺; 546.3, 548.2 [M-H]⁻. UPLC-MS purity (UV@215 nm) >99.5%.

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 (16d). Compound **16d** was synthesized via general procedure C₁ using **92c** (309 mg, 0.66 mmol) with succinic anhydride **115** (132 mg, 1.32 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 40 g, 0-10% EtOH/DCM) to afford **16d** (266 mg, yield 71%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.38 (s, 1H), 12.05 (s, 1H), 7.63 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.60 – 7.39 (m, 7H), 7.31 – 7.19 (m, 1H), 7.00 (d, *J* = 8.1 Hz, 2H), 6.93 (d, *J* = 2.3 Hz, 1H), 5.42 (dd, *J* = 12.1, 4.7 Hz, 1H), 3.78 (dd, *J* = 18.5, 12.2 Hz, 1H), 2.80 (dd, *J* = 18.5, 4.7 Hz, 1H), 2.59 – 2.38 (m, 2H), 2.36 – 2.26 (m, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.45, 168.78, 160.05, 152.42, 150.01, 146.65, 137.33, 134.55, 131.24, 129.42, 128.54, 128.44, 128.38, 128.35, 127.85, 127.53, 127.22, 126.20, 126.12, 126.04, 125.54, 125.39, 125.35, 124.45, 122.83, 120.63, 117.63, 58.51, 45.05, 28.49, 28.18, 26.32. In agreement with that previously reported by Acker *et al.*³⁶ R_t 2.22 min (generic method). ESI-MS for

C₂₉H₂₁ClF₃N₃O₄: calculated 567.1, found *m/z* 568.5, 570.4, [M+H]⁺; 566.4, 568.4 [M-H]⁻. UPLC-MS purity (UV@215 nm) 99%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (17d). Compound **17d** was synthesized via general procedure C₁ using **93c** (201 mg, 0.51 mmol) with succinic anhydride **115** (102 mg, 1.02 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-10% EtOH/DCM) to afford **17d** (226 mg, yield 90%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.38 (s, 1H), 12.06 (s, 1H), 7.64 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.54 – 7.43 (m, 5H), 7.32 (t, *J* = 6.4 Hz, 2H), 6.96 (d, *J* = 2.3 Hz, 1H), 6.33 (dd, *J* = 3.1, 1.9 Hz, 1H), 5.99 (d, *J* = 3.2 Hz, 1H), 5.39 (dd, *J* = 11.8, 4.5 Hz, 1H), 3.58 (dd, *J* = 18.2, 11.9 Hz, 1H), 3.17 (dd, *J* = 18.2, 4.6 Hz, 1H), 2.41 – 2.18 (m, 4H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.58, 173.49, 168.82, 160.10, 152.84, 152.49, 150.04, 142.05, 137.37, 134.77, 131.19, 128.81, 128.78, 128.29, 128.26, 128.24, 126.12, 126.07, 124.33, 120.63, 117.60, 110.33, 106.27, 56.01, 52.62, 41.72, 28.84, 28.52, 28.15. In agreement with that previously reported by Acker *et al.*³⁶ R_t 1.90 min (generic method). ESI-MS for C₂₆H₂₀ClN₃O₅: calculated 489.1, found *m/z* 490.4, 492.4 [M+H]⁺; 488.4, 490.4 [M-H]⁻. UPLC-MS purity (UV@215 nm) >99.5%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4'-fluoro-[1,1'-biphenyl]-4-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (18d). Compound **18d** was synthesized via general procedure C₁ using **94c** (171 mg, 0.35 mmol) with succinic anhydride **115** (70 mg, 0.70 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-10% EtOH/DCM) to afford **18d** (167 mg, yield 80%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.38 (s, 1H), 12.08 (s, 1H), 7.71 – 7.61 (m, 3H), 7.55 (ddt, *J* = 10.6, 9.5, 4.0 Hz, 3H), 7.50 – 7.40 (m, 4H), 7.33 – 7.25 (m, 3H), 6.94 (d, *J* = 2.3 Hz, 1H), 6.87 (d, *J* = 8.3 Hz, 2H), 5.35 (dd, *J* = 12.0, 4.6 Hz, 1H), 3.77 (dd, *J* = 18.5, 12.1 Hz, 1H), 2.84 (dd, *J* = 18.4, 4.7 Hz, 1H), 2.57 – 2.44 (m, 2H), 2.30 (t, *J* = 6.8 Hz, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.63, 173.50, 168.63, 163.05, 160.62,

160.11, 152.46, 149.94, 141.37, 137.96, 137.31, 136.34, 136.31, 134.60, 131.19, 129.41, 128.64, 128.56, 128.47, 128.33, 126.73, 126.08, 124.64, 120.69, 117.62, 115.78, 115.57, 58.66, 45.22, 28.96, 28.60, 28.23. R_t 2.36 min (generic method). ESI-MS for $C_{34}H_{25}ClFN_3O_4$: calculated 593.1, found m/z 594.1, 596.1 $[M+H]^+$, 592.2, 594.2 $[M-H]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4'-chloro-[1,1'-biphenyl]-4-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (19d). Compound **19d** was synthesized via general procedure C₁ using **95c** (227 mg, 0.44 mmol) with succinic anhydride **115** (88 mg, 0.88 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-10% EtOH/DCM) to afford **19d** (205 mg, yield 77%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.38 (s, 1H), 12.04 (s, 1H), 7.67 (d, J = 8.6 Hz, 2H), 7.63 (dd, J = 8.8, 2.4 Hz, 1H), 7.59 – 7.48 (m, 7H), 7.47 – 7.39 (m, 2H), 7.29 (dd, J = 6.5, 2.0 Hz, 1H), 6.94 (d, J = 2.4 Hz, 1H), 6.88 (d, J = 8.3 Hz, 2H), 5.35 (dd, J = 12.0, 4.6 Hz, 1H), 3.77 (dd, J = 18.5, 12.1 Hz, 1H), 2.84 (dd, J = 18.5, 4.6 Hz, 1H), 2.50 (m, 2H), 2.29 (t, J = 6.8 Hz, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.58, 173.49, 168.64, 160.10, 152.44, 149.94, 141.77, 138.63, 137.62, 137.31, 134.60, 132.26, 131.18, 129.40, 128.84, 128.52, 128.45, 128.37, 128.32, 126.72, 126.14, 126.10, 126.05, 124.62, 120.68, 117.61, 58.66, 45.21, 28.60, 28.23. R_t 2.51 min (generic method). ESI-MS for $C_{34}H_{25}Cl_2N_3O_4$: calculated 609.1, found m/z 610.0, 612.0, 614.2 $[M+H]^+$; 608.1, 610.1, 612.0 $[M-H]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-[3-[4-(4-4-(5-(4'-bromo-[1,1'-biphenyl]-4-yl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (20d). Compound **20d** was synthesized via general procedure C₁ using **96c** (214 mg, 0.38 mmol) with succinic anhydride **115** (76 mg, 0.76 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-20% EtOH/DCM) to afford **20d** (197 mg, yield 79%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.37 (s, 1H), 12.08 (s, 1H), 7.68 – 7.38 (m, 12H), 7.30 – 7.26 (m, 1H), 6.94 (d, J = 2.4 Hz, 1H), 6.89 (d, J = 8.4 Hz, 2H), 5.35 (dd, J = 12.0, 4.6 Hz, 1H), 3.78 (dd, J =

18.5, 12.1 Hz, 1H), 2.84 (dd, $J = 18.5, 4.7$ Hz, 1H), 2.58 – 2.43 (m, $J = 3.7$ Hz, 2H), 2.30 (t, $J = 6.9$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.50, 168.66, 160.11, 152.44, 149.95, 141.81, 139.00, 137.67, 137.31, 134.60, 131.76, 131.18, 129.40, 128.71, 128.52, 128.45, 128.33, 126.68, 126.16, 126.11, 126.05, 124.62, 120.84, 120.68, 117.61, 58.67, 45.20, 28.61, 28.24. R_t 2.52 min (generic method). ESI-MS for $\text{C}_{34}\text{H}_{25}\text{BrClN}_3\text{O}_4$: calculated 653.1, found m/z 653.9, 655.9, 657.9 $[\text{M}+\text{H}]^+$; 652.0, 654.0, 655.8 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4'-methoxy-[1,1'-biphenyl]-4-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (21d). Compound **21d** was synthesized via general procedure C₁ using **97c** (545 mg, 1.10 mmol) with succinic anhydride **115** (220 mg, 2.20 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 40 g, 0-10% EtOH/DCM) to afford **21d** (359 mg, yield 55%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.38 (s, 1H), 12.03 (s, 1H), 7.67 – 7.50 (m, 6H), 7.44 (dt, $J = 7.7, 5.9$ Hz, 4H), 7.29 (dd, $J = 6.7, 2.1$ Hz, 1H), 7.02 (d, $J = 8.8$ Hz, 2H), 6.94 (d, $J = 2.3$ Hz, 1H), 6.84 (d, $J = 8.3$ Hz, 2H), 5.33 (dd, $J = 12.0, 4.6$ Hz, 1H), 3.79 (s, 4H), 2.84 (dd, $J = 18.4, 4.6$ Hz, 1H), 2.30 (t, $J = 6.9$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.51, 168.62, 160.12, 158.85, 152.44, 149.92, 140.63, 138.67, 137.31, 134.60, 132.23, 131.17, 129.40, 128.54, 128.44, 128.32, 127.68, 126.23, 126.10, 126.00, 124.66, 120.69, 117.61, 114.34, 58.70, 55.14, 45.24, 28.62, 28.25. R_t 2.30 min (generic method). ESI-MS for $\text{C}_{35}\text{H}_{28}\text{ClN}_3\text{O}_5$: calculated 605.2, found m/z 606.0, 608.0 $[\text{M}+\text{H}]^+$; 604.0, 606.0 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(1-methyl-1H-indol-5-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (22d). Compound **22d** was synthesized via general procedure C₁ using **98c** (153 mg, 0.34 mmol) with succinic anhydride **115** (68 mg, 0.68 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-20% EtOH/DCM) to afford **22d** (150 mg, yield 79%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.37 (s, 1H), 12.18 (s, 1H), 7.63 (dd, $J = 8.8, 2.3$ Hz, 1H), 7.60 – 7.55 (m, 2H), 7.55 – 7.49 (m, 1H), 7.44 (dd, $J =$

7.7, 4.3 Hz, 2H), 7.31 – 7.20 (m, 3H), 6.98 (d, $J = 1.7$ Hz, 1H), 6.93 (d, $J = 2.3$ Hz, 1H), 6.61 (dd, $J = 8.5, 1.7$ Hz, 1H), 6.34 (dd, $J = 3.0, 0.8$ Hz, 1H), 5.35 (dd, $J = 12.0, 4.6$ Hz, 1H), 3.84 – 3.69 (m, 4H), 2.85 (dd, $J = 18.4, 4.6$ Hz, 1H), 2.53 – 2.45 (m, 2H), 2.26 (t, $J = 6.9$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.72, 173.52, 168.43, 160.17, 152.27, 149.80, 137.29, 135.60, 134.66, 133.04, 131.11, 129.90, 129.47, 128.52, 128.43, 128.25, 127.73, 126.06, 124.84, 120.75, 118.98, 117.58, 117.22, 109.64, 100.28, 59.51, 45.76, 32.46, 29.17, 28.70, 28.27. R_t 2.09 min (generic method). ESI-MS for $\text{C}_{31}\text{H}_{25}\text{ClN}_4\text{O}_4$: calculated 552.2, found m/z 553.1, 555.2 $[\text{M}+\text{H}]^+$; 551.2, 553.2 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(1-ethyl-1H-indol-5-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (23d). Compound **23d** was synthesized via general procedure C₁ using **99c** (153 mg, 0.33 mmol) with succinic anhydride **115** (66 mg, 0.66 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-10% EtOH/DCM) to afford **23d** (92 mg, yield 49%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.37 (s, 1H), 12.02 (s, 1H), 7.63 (dd, $J = 8.8, 2.4$ Hz, 1H), 7.58 (dd, $J = 5.5, 3.3$ Hz, 2H), 7.52 (ddd, $J = 8.9, 5.2, 3.1$ Hz, 1H), 7.44 (dd, $J = 8.7, 4.4$ Hz, 2H), 7.35 (d, $J = 3.1$ Hz, 1H), 7.29 (dd, $J = 8.5, 2.2$ Hz, 2H), 6.95 (dd, $J = 15.5, 2.0$ Hz, 2H), 6.60 (dd, $J = 8.5, 1.7$ Hz, 1H), 6.34 (d, $J = 3.1$ Hz, 1H), 5.34 (dd, $J = 12.0, 4.7$ Hz, 1H), 4.17 (q, $J = 7.2$ Hz, 2H), 3.77 (dd, $J = 18.4, 12.1$ Hz, 1H), 2.86 (dd, $J = 18.4, 4.7$ Hz, 1H), 2.48 – 2.42 (m, 2H), 2.26 (t, $J = 6.9$ Hz, 2H), 1.34 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.53, 168.45, 160.17, 152.28, 149.80, 137.29, 134.66, 134.56, 133.01, 131.11, 129.45, 128.53, 128.42, 128.28, 127.89, 126.05, 124.84, 120.74, 118.93, 117.58, 117.32, 109.62, 100.49, 59.51, 45.75, 40.26, 28.72, 28.30, 15.53. R_t 2.20 min (generic method). ESI-MS for $\text{C}_{32}\text{H}_{27}\text{ClN}_4\text{O}_4$: calculated 566.2, found m/z 567.2, 569.2 $[\text{M}+\text{H}]^+$; 565.3, 567.3 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(1-ethyl-1H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (24d). Compound **24d** was synthesized via general

procedure C₁ using **100c** (196 mg, 0.42 mmol) with succinic anhydride **115** (84 mg, 0.84 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-10% EtOH/DCM) to afford **24d** (177 mg, yield 74%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.39 (s, 1H), 12.03 (s, 1H), 7.98 (s, 1H), 7.64 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.59 (dd, *J* = 5.4, 3.5 Hz, 2H), 7.52 (dd, *J* = 10.0, 6.6 Hz, 2H), 7.48 – 7.43 (m, 2H), 7.29 (d, *J* = 7.5 Hz, 1H), 7.14 (d, *J* = 1.5 Hz, 1H), 6.94 (d, *J* = 2.3 Hz, 1H), 6.83 (dd, *J* = 8.7, 1.6 Hz, 1H), 5.42 (dd, *J* = 12.0, 4.6 Hz, 1H), 4.42 (q, *J* = 7.2 Hz, 2H), 3.80 (dd, *J* = 18.5, 12.1 Hz, 1H), 2.85 (dd, *J* = 18.5, 4.6 Hz, 1H), 2.54 – 2.48 (m, 2H), 2.28 (t, *J* = 6.9 Hz, 2H), 1.38 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.99, 169.05, 160.62, 152.86, 150.36, 138.51, 137.79, 135.07, 134.85, 132.81, 131.65, 130.00, 129.09, 128.97, 128.93, 128.78, 126.57, 126.54, 125.22, 124.57, 123.78, 121.19, 118.09, 117.79, 110.22, 59.57, 45.96, 43.53, 29.13, 28.72, 15.43. R_t 1.95 min (generic method). ESI-MS for C₃₁H₂₆ClN₅O₄: calculated 567.2, found *m/z* 568.5, 570.4 [M+H]⁺, 566.4, 568.4 [M-H]⁻. UPLC-MS purity (UV@215 nm) >99.5%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(2-ethyl-2H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (25d). Compound **25d** was synthesized via general procedure C₁ using **101c** (198 mg, 0.42 mmol) with succinic anhydride **115** (84 mg, 0.84 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-20% EtOH/DCM) to afford **25d** (120 mg, yield 50%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.37 (s, 1H), 12.04 (s, 1H), 8.29 (s, 1H), 7.66 – 7.55 (m, 3H), 7.51 (td, *J* = 6.6, 5.5, 3.3 Hz, 1H), 7.44 (dd, *J* = 10.0, 6.8 Hz, 3H), 7.28 (d, *J* = 7.5 Hz, 1H), 7.07 (d, *J* = 1.6 Hz, 1H), 6.93 (d, *J* = 2.4 Hz, 1H), 6.61 (dd, *J* = 8.9, 1.7 Hz, 1H), 5.35 (dd, *J* = 12.0, 4.6 Hz, 1H), 4.43 (q, *J* = 7.3 Hz, 2H), 3.77 (dd, *J* = 18.4, 12.1 Hz, 1H), 2.82 (dd, *J* = 18.4, 4.6 Hz, 1H), 2.54 – 2.44 (m, 2H), 2.28 (t, *J* = 6.9 Hz, 2H), 1.49 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.50, 168.55, 160.12, 152.37, 149.84, 147.32, 137.29, 134.57, 134.39, 131.15, 129.53, 128.57, 128.49, 128.42, 128.28, 126.07, 126.04, 124.75, 123.57, 123.00, 120.94, 120.72, 117.60, 117.32, 116.68, 59.31, 47.68, 45.24, 28.67, 28.26,

15.84. R_t 1.85 min (generic method). ESI-MS for $C_{31}H_{26}ClN_5O_4$: calculated 567.2, found m/z 568.4, 570.4 $[M+H]^+$, 566.5, 568.4 $[M-H]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(1-propyl-1H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (26d). Compound **26d** was synthesized via general procedure C₁ using **102c** (176 mg, 0.36 mmol) with succinic anhydride **115** (72 mg, 0.72 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-25% EtOH/DCM) to afford **26d** (178 mg, yield 86%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.36 (s, 1H), 12.05 (s, 1H), 7.97 (d, J = 0.9 Hz, 1H), 7.62 (dd, J = 8.8, 2.4 Hz, 1H), 7.57 (qd, J = 4.0, 1.0 Hz, 2H), 7.53 – 7.47 (m, 2H), 7.47 – 7.41 (m, 2H), 7.30 – 7.25 (m, 1H), 7.16 – 7.11 (m, 1H), 6.94 (d, J = 2.3 Hz, 1H), 6.84 (dd, J = 8.8, 1.6 Hz, 1H), 5.42 (dd, J = 12.0, 4.6 Hz, 1H), 4.33 (t, J = 6.9 Hz, 2H), 3.78 (dd, J = 18.5, 12.0 Hz, 1H), 2.85 (dd, J = 18.5, 4.6 Hz, 1H), 2.54 – 2.48 (m, 2H), 2.28 (t, J = 6.9 Hz, 2H), 1.83 (h, J = 7.2 Hz, 2H), 0.83 (t, J = 7.4 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.53, 168.61, 160.15, 152.41, 149.89, 138.59, 137.31, 134.60, 134.31, 132.32, 131.17, 129.50, 128.60, 128.49, 128.43, 128.32, 126.11, 126.06, 124.73, 124.13, 123.19, 120.71, 117.62, 117.28, 109.80, 59.09, 49.64, 45.49, 28.67, 28.26, 22.83, 11.17. R_t 2.06 min (generic method). ESI-MS for $C_{32}H_{28}ClN_5O_4$: calculated 581.2, found m/z 582.1, 584.1 $[M+H]^+$, 580.4, 582.0 $[M-H]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(2-propyl-2H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (27d). Compound **27d** was synthesized via general procedure C₁ using **103c** (173 mg, 0.36 mmol) with succinic anhydride **115** (72 mg, 0.72 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-20% EtOH/DCM) to afford **27d** (162 mg, yield 78%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.37 (s, 2H), 8.28 (d, J = 0.9 Hz, 1H), 7.63 – 7.54 (m, 3H), 7.50 (td, J = 6.6, 5.5, 3.3 Hz, 1H), 7.47 – 7.40 (m, 3H), 7.30 – 7.24 (m, 1H), 7.08 (t, J = 1.2 Hz, 1H), 6.93 (d, J = 2.4 Hz, 1H), 6.62 (dd, J = 8.9, 1.7 Hz, 1H), 5.36 (dd, J = 12.0, 4.6 Hz, 1H), 4.35 (t, J = 6.9 Hz, 2H), 3.77 (dd, J = 18.5, 12.0 Hz,

1H), 2.83 (dd, $J = 18.5, 4.6$ Hz, 1H), 2.54 – 2.50 (m, 2H), 2.29 (t, $J = 6.9$ Hz, 2H), 1.96 - 1.87 (m, 2H), 0.83 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.57, 168.64, 160.16, 152.44, 149.89, 147.41, 137.31, 134.59, 134.40, 131.16, 129.56, 128.60, 128.52, 128.43, 128.31, 126.13, 126.07, 124.77, 123.75, 123.60, 120.86, 120.74, 117.62, 117.39, 116.71, 59.36, 54.27, 45.27, 28.73, 28.32, 23.44, 10.91. R_t 2.00 min (generic method). ESI-MS for $\text{C}_{32}\text{H}_{28}\text{ClN}_5\text{O}_4$: calculated 581.2, found m/z 582.2, 584.3 $[\text{M}+\text{H}]^+$, 580.3, 582.4 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(1-cyclohexyl-1H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (28d). Compound **28d** was synthesized via general procedure C₁ using **104c** (164 mg, 0.31 mmol) with succinic anhydride **115** (62 mg, 0.62 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-12% EtOH/DCM) to afford **28d** (169 mg, yield 87%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.38 (s, 1H), 12.07 (s, 1H), 7.96 (s, 1H), 7.65 – 7.49 (m, 5H), 7.48 – 7.41 (m, 2H), 7.28 (dd, $J = 7.5, 1.8$ Hz, 1H), 7.11 (d, $J = 1.6$ Hz, 1H), 6.94 (d, $J = 2.3$ Hz, 1H), 6.84 (dd, $J = 8.8, 1.6$ Hz, 1H), 5.41 (dd, $J = 12.0, 4.6$ Hz, 1H), 4.54 (tt, $J = 10.0, 5.5$ Hz, 1H), 3.80 (dd, $J = 18.5, 12.1$ Hz, 1H), 2.85 (dd, $J = 18.5, 4.7$ Hz, 1H), 2.53 – 2.46 (m, 2H), 2.28 (t, $J = 6.9$ Hz, 2H), 2.01 – 1.81 (m, 6H), 1.70 (dt, $J = 12.8, 3.4$ Hz, 1H), 1.58 – 1.43 (m, 2H), 1.26 (qt, $J = 12.7, 3.5$ Hz, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.74, 173.51, 168.59, 160.16, 152.36, 149.90, 137.70, 137.31, 134.64, 134.41, 132.08, 131.16, 129.51, 128.59, 128.48, 128.45, 128.30, 126.12, 126.07, 124.73, 123.92, 123.18, 120.71, 117.61, 117.24, 109.77, 59.12, 56.63, 45.50, 32.34, 28.67, 28.26, 25.08. R_t 2.33 min (generic method). ESI-MS for $\text{C}_{35}\text{H}_{32}\text{ClN}_5\text{O}_4$: calculated 621.2, found m/z 622.3, 624.2 $[\text{M}+\text{H}]^+$, 620.3, 622.4 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(2-cyclohexyl-2H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (29d). Compound **29d** was synthesized via general procedure C₁ using **105c** (100 mg, 0.19 mmol) with succinic anhydride **115** (38 mg, 0.38 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold

24 g, 0-20% EtOH/DCM) to afford **29d** (113 mg, yield 95%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.37 (s, 1H), 12.10 (s, 1H), 8.29 (d, *J* = 1.0 Hz, 1H), 7.65 – 7.55 (m, 3H), 7.53 – 7.48 (m, 1H), 7.44 (t, *J* = 8.7 Hz, 3H), 7.30 – 7.24 (m, 1H), 7.10 – 7.05 (m, 1H), 6.93 (d, *J* = 2.4 Hz, 1H), 6.60 (dd, *J* = 9.0, 1.7 Hz, 1H), 5.35 (dd, *J* = 12.0, 4.6 Hz, 1H), 4.44 (tt, *J* = 11.2, 3.8 Hz, 1H), 3.77 (dd, *J* = 18.4, 12.1 Hz, 1H), 2.82 (dd, *J* = 18.4, 4.7 Hz, 1H), 2.55 – 2.46 (m, 2H), 2.28 (t, *J* = 6.9 Hz, 2H), 2.14 – 2.02 (m, 2H), 1.95 – 1.81 (m, 4H), 1.70 (dd, *J* = 12.6, 3.7 Hz, 1H), 1.45 (qt, *J* = 12.5, 3.4 Hz, 2H), 1.25 (tdd, *J* = 15.6, 11.7, 7.8 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.72, 173.52, 168.56, 160.14, 152.38, 149.87, 146.88, 137.30, 134.58, 134.35, 131.15, 129.52, 128.59, 128.51, 128.44, 128.30, 126.09, 126.05, 124.76, 123.49, 121.57, 120.73, 120.62, 117.61, 117.47, 116.76, 61.63, 59.36, 45.25, 33.29, 29.13, 28.69, 28.28, 24.92, 24.89. *R*_t 2.20 min (generic method). ESI-MS for C₃₅H₃₂ClN₅O₄: calculated 621.2, found *m/z* 622.2, 624.2 [M+H]⁺, 620.3, 622.3 [M-H]⁻. UPLC-MS purity (UV@215 nm) 99%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-(1-ethyl-1H-indazol-5-yl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (30d). Compound **30d** was synthesized via general procedure C₁ using **106c** (170 mg, 0.31 mmol) with succinic anhydride **115** (62 mg, 0.62 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-20% EtOH/DCM) to afford **30d** (163 mg, yield 81%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.38 (s, 1H), 12.07 (s, 1H), 8.10 (d, *J* = 0.8 Hz, 1H), 7.99 (d, *J* = 1.6 Hz, 1H), 7.74 (d, *J* = 8.8 Hz, 1H), 7.67 (dd, *J* = 8.8, 1.7 Hz, 1H), 7.63 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.60 – 7.51 (m, 5H), 7.48 – 7.40 (m, 2H), 7.30 (dd, *J* = 5.9, 1.8 Hz, 1H), 6.94 (d, *J* = 2.3 Hz, 1H), 6.89 (d, *J* = 8.3 Hz, 2H), 5.35 (dd, *J* = 12.0, 4.6 Hz, 1H), 4.46 (q, *J* = 7.2 Hz, 2H), 3.78 (dd, *J* = 18.5, 12.1 Hz, 1H), 2.86 (dd, *J* = 18.5, 4.6 Hz, 1H), 2.59 – 2.44 (m, 2H), 2.31 (t, *J* = 6.8 Hz, 2H), 1.41 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.53, 168.65, 160.13, 152.47, 149.95, 140.77, 139.50, 138.23, 137.32, 134.63, 132.92, 132.54, 131.18, 129.43, 128.55, 128.47, 128.34, 126.90, 126.11, 126.07, 125.52, 124.67, 124.25, 120.70, 118.46, 117.62, 110.02, 58.72, 45.26, 43.15, 28.64, 28.26, 14.94. *R*_t

2.24 min (generic method). ESI-MS for $C_{37}H_{30}ClN_5O_4$: calculated 643.2, found m/z 644.1, 646.1 $[M+H]^+$, 642.2, 644.1 $[M-H]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-(2-ethyl-2H-indazol-5-yl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (31d). Compound **31d** was synthesized via general procedure C₁ using **107c** (117 mg, 0.21 mmol) with succinic anhydride **115** (42 mg, 0.42 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-20% EtOH/DCM) to afford **31d** (66 mg, yield 49%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.38 (s, 1H), 12.15 (s, 1H), 8.42 (d, *J* = 0.9 Hz, 1H), 7.92 (t, *J* = 1.3 Hz, 1H), 7.68 (d, *J* = 8.9 Hz, 1H), 7.63 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.60 – 7.50 (m, 6H), 7.48 – 7.41 (m, 2H), 7.30 (dd, *J* = 6.1, 1.9 Hz, 1H), 6.94 (d, *J* = 2.3 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 2H), 5.35 (dd, *J* = 12.0, 4.6 Hz, 1H), 4.47 (q, *J* = 7.3 Hz, 2H), 3.78 (dd, *J* = 18.5, 12.1 Hz, 1H), 2.86 (dd, *J* = 18.5, 4.7 Hz, 1H), 2.57 – 2.43 (m, 2H), 2.30 (t, *J* = 6.9 Hz, 2H), 1.52 (t, *J* = 7.3 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.63, 173.52, 168.64, 160.13, 152.47, 149.95, 147.41, 140.72, 137.33, 132.68, 128.56, 128.47, 128.35, 126.74, 126.11, 126.06, 126.02, 125.23, 124.68, 123.68, 121.91, 120.71, 117.89, 117.63, 117.45, 58.72, 47.80, 28.93, 28.63, 28.26, 15.76. R_t 2.10 min (generic method). ESI-MS for $C_{37}H_{30}ClN_5O_4$: calculated 643.2, found m/z 644.2, 646.2 $[M+H]^+$, 642.3, 644.3 $[M-H]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-(1-propyl-1H-pyrazol-4-yl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (32d). Compound **32d** was synthesized via general procedure C₁ using **108c** (155 mg, 0.30 mmol) with succinic anhydride **115** (60 mg, 0.60 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-30% EtOH/DCM) to afford **32d** (189 mg, quantitative yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.37 (s, 1H), 12.10 (s, 1H), 8.14 (s, 1H), 7.84 (s, 1H), 7.62 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.59 – 7.50 (m, 3H), 7.48 – 7.36 (m, 4H), 7.31 – 7.25 (m, 1H), 6.94 (d, *J* = 2.3 Hz, 1H), 6.77 (d, *J* = 8.0 Hz, 2H), 5.28 (dd, *J* = 12.0, 4.6 Hz, 1H), 4.07 (t, *J* = 7.0 Hz, 2H), 3.75 (dd, *J* = 18.4, 12.1

Hz, 1H), 2.82 (dd, $J = 18.5, 4.6$ Hz, 1H), 2.48 – 2.41 (m, 2H), 2.28 (t, $J = 6.9$ Hz, 2H), 1.96 – 1.87 (m, 2H), 0.85 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 173.50, 168.56, 160.12, 152.39, 149.89, 139.75, 137.30, 135.88, 134.62, 131.56, 131.16, 129.42, 128.53, 128.44, 128.29, 126.88, 126.09, 125.98, 124.96, 124.68, 121.24, 120.70, 117.60, 58.75, 53.00, 45.20, 28.63, 28.26, 23.17, 10.93. R_t 2.09 min (generic method). ESI-MS for $\text{C}_{34}\text{H}_{30}\text{ClN}_5\text{O}_4$: calculated 607.2, found m/z 608.0, 610.0 $[\text{M}+\text{H}]^+$, 606.1, 608.0 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) 99%.

6-chloro-3-(5-(4-chlorophenyl)-1-propionyl-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (33d). Compound **33d** was synthesized via general procedure C₃ using **87c** (200 mg, 0.46 mmol). Purification performed by direct phase flash chromatography (SiO_2 gold 24 g, 0-2 % MeOH/DCM) to afford **33d** (76 mg, yield 34%). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 12.38 (s, 1H), 7.63 (dd, $J = 9.0, 2.4$ Hz, 1H), 7.54 – 7.49 (m, 3H), 7.44 (d, $J = 8.8$ Hz, 1H), 7.41 – 7.39 (m, 1H), 7.30 – 7.27 (m, 3H), 6.92 (d, $J = 2.4$ Hz, 1H), 6.80 (d, $J = 8.8$ Hz, 2H), 5.31 (dd, $J = 12.2, 4.6$ Hz, 1H), 3.74 (dd, $J = 18.4, 12.0$ Hz, 1H), 2.80 (dd, $J = 18.4, 4.0$ Hz, 1H), 2.31 – 2.14 (m, 2H), 0.82 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 206.88, 170.96, 160.55, 152.65, 150.33, 141.76, 137.75, 135.13, 132.03, 131.64, 129.80, 128.95, 128.87, 128.82, 128.79, 127.81, 126.55, 126.48, 125.04, 121.10, 118.07, 110.00, 58.61, 45.45, 31.11, 27.06, 9.26. ESI-MS for $\text{C}_{27}\text{H}_{21}\text{Cl}_2\text{N}_3\text{O}_2$: calculated 489.1, found m/z 490.2, 492.1, 494.1 $[\text{M}+\text{H}]^+$; 488.3, 490.3, 492.1 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) 98%.

3-(5-(4-bromophenyl)-1-propionyl-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (34d). Compound **34d** was synthesized via general procedure C₃ using **88c** (150 mg, 0.31 mmol). Purification performed by direct phase flash chromatography (SiO_2 gold 24 g, 0-0.5 % MeOH/DCM) to afford **34d** (60 mg, yield 36%). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 12.41 (s, 1H), 7.64 (dd, $J = 12.8, 1.6$ Hz, 1H), 7.55 – 7.50 (m, 3H), 7.46-7.41 (m, 4H), 7.28 (d, $J = 6.8$ Hz, 1H), 6.93 (d, $J = 2.0$ Hz, 1H), 6.75 (d, $J = 8.0$ Hz, 2H), 5.30 (dd, $J = 12.4, 4.0$ Hz, 1H), 3.75 (dd, $J = 18.4, 12.0$ Hz, 1H), 2.80 (dd, $J = 18.2, 4.6$ Hz, 1H), 2.31 – 2.14 (m, 2H), 0.83 (t, $J = 7.6$ Hz,

3H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 206.78, 171.02, 160.54, 152.60, 150.33, 142.18, 137.77, 135.16, 131.72, 131.61, 129.79, 128.97, 128.92, 128.84, 128.78, 128.18, 126.56, 126.49, 125.03, 121.13, 120.51, 118.06, 58.73, 45.40, 31.08, 27.05, 9.23. ESI-MS for $\text{C}_{27}\text{H}_{21}\text{BrClN}_3\text{O}_2$: calculated 533.0, found m/z 534.2, 536.1, 538.1 $[\text{M}+\text{H}]^+$; 532.2, 534.1, 536.2 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) 97%.

6-chloro-3-(5-(4-methoxyphenyl)-1-propionyl-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (35d). Compound **35d** was synthesized via general procedure C₃ using **89c** (150 mg, 0.36 mmol). The crude was purified by direct phase flash column chromatography (SiO_2 gold 24 g; 0-2% MeOH/DCM), to afford **35d** (100 mg, yield 59%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 12.36 (s, 1H), 7.64 (dd, J = 8.6, 2.4 Hz, 1H), 7.56 – 7.52 (m, 3H), 7.45 (d, J = 8.4 Hz, 1H), 7.40 – 7.38 (m, 1H), 7.29 – 7.27 (m, 1H), 6.92 (d, J = 2.0 Hz, 1H), 6.75 (dd, J = 15.2, 9.2 Hz, 4H), 5.24 (dd, J = 12.0, 4.8 Hz, 1H), 3.72 (s, 3H), 3.70 (dd, J = 16.0, 12.0 Hz, 1H), 2.81 (dd, J = 20.0, 4.4 Hz, 1H), 2.29 – 2.12 (m, 2H), 0.82 (t, J = 8.0 Hz, 3H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 170.80, 160.60, 158.67, 152.56, 150.23, 137.74, 135.16, 134.94, 131.58, 129.76, 129.03, 128.89, 128.84, 128.77, 127.20, 126.51, 126.47, 125.22, 121.15, 118.05, 114.15, 58.73, 55.49, 45.59, 27.12, 9.31. ESI-MS for $\text{C}_{28}\text{H}_{24}\text{ClN}_3\text{O}_3$: calculated 485.1, found m/z 486.4, 488.4 $[\text{M}+\text{H}]^+$; 484.3, 486.3 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) 98%.

3-(1-acetyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (36d). Compound **36d** was synthesized via general procedure C₂ using **87c** (210 mg, 0.48 mmol) with acetic anhydride **116** (888 μL , 0.96 mmol) (165 $^\circ\text{C}$, 200 W). Purification performed by direct phase flash chromatography (SiO_2 gold 24 g, 0-1.5% MeOH/DCM) to afford **36d** (200 mg, yield 94%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 12.41 (s, 1H), 7.64 (dt, J = 8.0, 1.2 Hz, 1H), 7.58 – 7.53 (m, 3H), 7.44 (d, J = 8.0 Hz, 1H), 7.41-7.39 (m, 1H), 7.30 – 7.28 (m, 3H), 6.93 (d, J = 0.8 Hz, 1H), 6.80 (d, J = 7.6 Hz, 2H), 5.32 (dd, J = 12.0, 8.0 Hz, 1H), 3.74 (dd, J = 20.0, 12.0 Hz, 1H), 2.82 (dd, J = 16.0, 4.0 Hz, 1H), 1.87 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 167.56,

160.54, 152.67, 150.39, 141.60, 137.75, 135.11, 132.05, 131.65, 129.86, 129.01, 128.96, 128.81, 128.75, 127.83, 126.55, 126.49, 124.96, 121.10, 118.06, 58.56, 45.62, 21.82. ESI-MS for $C_{26}H_{19}Cl_2N_3O_2$: calculated 475.1, found m/z 476.3, 478.4, 480.4 $[M+H]^+$; 474.3, 476.3, 478.1 $[M-H]^-$. UPLC-MS purity (UV@215 nm) 99%.

3-(1-acetyl-5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (37d). Compound **37d** was synthesized via general procedure C₂ using **88c** (100 mg, 0.21 mmol) with acetic anhydride **116** (40 μ L, 0.42 mmol) (165 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 12 g, 0-2% MeOH/DCM) to afford **37d** (80 mg, yield 73%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.39 (s, 1H), 7.64 (dd, J = 8.0, 4.0 Hz, 1H), 7.57 – 7.53 (m, 3H), 7.45-7.39 (m, 4H), 7.28 (dd, J = 6.0, 1.6 Hz, 1H), 6.93 (d, J = 2.0 Hz, 1H), 6.75 (d, J = 8.8 Hz, 2H), 5.30 (dd, J = 12.0, 4.4 Hz, 1H), 3.74 (dd, J = 18.5, 12.0 Hz, 1H), 2.82 (dd, J = 4.0, 20.0 Hz, 1H), 1.86 (s, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 167.58, 160.54, 152.64, 150.39, 142.02, 137.78, 135.15, 131.72, 131.63, 129.83, 129.03, 128.94, 128.78, 128.74, 128.20, 126.56, 126.50, 124.96, 121.13, 120.54, 118.06, 58.67, 45.58, 21.78. ESI-MS for $C_{26}H_{19}BrClN_3O_2$: calculated 519.0, found m/z 520.1, 522.1, 524.1 $[M+H]^+$; 518.1, 520.1, 522.2 $[M-H]^-$. UPLC-MS purity (UV@215 nm) 99%.

3-(1-acetyl-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (38d). Compound **38d** was synthesized via general procedure C₂ using **89c** (65 mg, 0.15 mmol) with acetic anhydride **116** (28 μ L, 0.30 mmol) (165 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 12 g, 0-1.5% MeOH/DCM) to afford **38d** (30 mg, yield 43%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.37 (s, 1H), 7.63 (dd, J = 8.8, 2.0 Hz, 1H), 7.55 – 7.52 (m, 3H), 7.45 (d, J = 8.8 Hz, 1H), 7.40 – 7.39 (m, 1H), 7.29 (d, J = 6.8 Hz, 1H), 6.93 (d, J = 2.0 Hz, 1H), 6.75 (dd, J = 8.8, 16.0 Hz, 4H), 5.24 (dd, J = 12.0, 4.0 Hz, 1H), 3.72 (s, 3H), 3.70 – 3.65 (m, 1H), 2.83 (dd, J = 4.4, 18.4 Hz, 2H), 1.84 (s, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 167.42, 160.60, 158.70, 152.63, 150.29, 137.75, 135.14, 134.79, 131.60, 129.80, 129.09, 128.92,

128.78, 128.75, 127.24, 126.52, 126.49, 125.13, 121.14, 118.07, 114.15, 58.67, 55.49, 45.74, 21.90.

ESI-MS for $C_{27}H_{22}ClN_3O_3$: calculated 471.1, found m/z 472.2, 474.2, $[M+H]^+$; 470.3, 472.3 $[M-H]^-$.

UPLC-MS purity (UV@215 nm) 99%.

3-(1-acetyl-5-(4-(tert-butyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-

phenylquinolin-2(1H)-one (39d) Compound **39d** was synthesized via general procedure C₂ using **90c** (141 mg, 0.31 mmol) with acetic anhydride **116** (59 μ L, 0.62 mmol) (165 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-5% EtOH/ DCM) to afford **39d** (105 mg, yield 68%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.36 (s, 1H), 7.63 (dt, J = 9.8, 4.9 Hz, 1H), 7.60 – 7.48 (m, 3H), 7.44 (t, J = 9.5 Hz, 1H), 7.41 – 7.36 (m, 1H), 7.30 (d, J = 7.1 Hz, 1H), 7.24 (d, J = 8.3 Hz, 2H), 6.94 (d, J = 2.3 Hz, 1H), 6.75 (d, J = 8.3 Hz, 2H), 5.32 – 5.17 (m, 1H), 3.69 (dt, J = 28.5, 14.3 Hz, 1H), 2.86 (dd, J = 18.4, 4.6 Hz, 1H), 1.86 (s, 3H), 1.27 (s, 9H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 167.01, 160.12, 152.20, 149.87, 149.25, 139.30, 137.30, 134.72, 131.14, 129.30, 128.66, 128.44, 128.31, 128.29, 126.07, 126.04, 125.16, 125.13, 124.65, 120.69, 117.60, 58.45, 45.30, 34.12, 31.10, 21.42. *R*_t 1.83 min (apolar method). ESI-MS for $C_{30}H_{28}ClN_3O_2$: calculated 497.2, found m/z 498.5, 500.5 $[M+H]^+$; 494.4, 496.4 $[M-H]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

3-(1-acetyl-5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-

phenylquinolin-2(1H)-one (40d). Compound **40d** was synthesized via general procedure C₂ using **92c** (326 mg, 0.70 mmol) with acetic anhydride **116** (132 μ L, 1.40 mmol) (165 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-5% EtOH/DCM) to afford **40d** (268 mg, yield 74%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.39 (s, 1H), 7.67 – 7.49 (m, 6H), 7.46 (d, J = 8.8 Hz, 1H), 7.44 – 7.39 (m, 1H), 7.32 – 7.25 (m, 1H), 7.03 (d, J = 8.0 Hz, 2H), 6.95 (d, J = 2.4 Hz, 1H), 5.42 (dd, J = 12.1, 4.7 Hz, 1H), 3.79 (dd, J = 18.5, 12.1 Hz, 1H), 2.87 (dd, J = 18.5, 4.8 Hz, 1H), 1.89 (s, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 167.21, 160.08, 152.27, 150.01, 146.70, 137.34, 134.69, 131.23, 129.39, 128.54, 128.51, 128.33, 127.90, 127.58, 126.24,

126.11, 126.05, 125.52, 125.43, 125.39, 125.35, 124.41, 122.82, 120.62, 117.63, 58.36, 45.14, 21.30. R_t 2.55 min (generic method). ESI-MS for $C_{27}H_{19}ClF_3N_3O_2$: calculated 509.1, found m/z 510.5, 513.5 $[M+H]^+$; 508.4, 510.4 $[M-H]^-$. UPLC-MS purity (UV@215 nm) 99%.

3-(1-acetyl-5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (41d). Compound **41d** was synthesized via general procedure C₂ using **93c** (202 mg, 0.52 mmol) with acetic anhydride **116** (98 μ L, 1.04 mmol) (165 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-5% EtOH/DCM) to afford **41d** (162 mg, yield 73%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.36 (s, 1H), 7.63 (dd, J = 8.8, 2.3 Hz, 1H), 7.52 – 7.42 (m, 5H), 7.35 – 7.29 (m, 2H), 6.96 (d, J = 2.3 Hz, 1H), 6.33 (dd, J = 3.3, 1.9 Hz, 1H), 6.03 (d, J = 3.2 Hz, 1H), 5.38 (dd, J = 11.9, 4.6 Hz, 1H), 3.57 (dd, J = 18.2, 11.9 Hz, 1H), 3.20 (dd, J = 18.2, 4.7 Hz, 1H), 1.75 (s, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 167.26, 160.12, 152.98, 152.28, 150.04, 142.02, 137.37, 134.92, 131.18, 128.87, 128.80, 128.25, 128.19, 126.12, 126.07, 124.31, 120.62, 117.59, 110.33, 106.33, 52.35, 41.84, 21.33. R_t 0.95 min (apolar method). ESI-MS for $C_{24}H_{18}ClN_3O_3$: calculated 431.1, found m/z 432.4, 434.4 $[M+H]^+$; 430.4, 432.5 $[M-H]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

3-(1-acetyl-5-(4'-chloro-[1,1'-biphenyl]-4-yl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (42d). Compound **42d** was synthesized via general procedure C₂ using **95c** (224 mg, 0.44 mmol) with acetic anhydride **116** (83 μ L, 0.88 mmol) (165 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-10% EtOH/DCM) to afford **42d** (245 mg, quantitative yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.37 (s, 1H), 7.71 – 7.48 (m, 10H), 7.48 – 7.39 (m, 2H), 7.29 (dt, J = 5.2, 2.0 Hz, 1H), 6.94 (d, J = 2.4 Hz, 1H), 6.90 (d, J = 8.3 Hz, 2H), 5.35 (dd, J = 12.1, 4.6 Hz, 1H), 3.78 (dd, J = 18.4, 12.1 Hz, 1H), 2.88 (dd, J = 18.5, 4.6 Hz, 1H), 1.88 (s, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 167.09, 160.12, 152.23, 149.93, 141.80, 138.63, 137.66, 137.30, 134.73, 132.26, 131.16, 129.39, 128.82, 128.59, 128.49, 128.40, 128.37, 128.29, 126.74, 126.15, 126.10, 126.05, 124.60, 120.67, 117.60, 58.48, 45.29, 21.41. R_t

2.00 min (apolar method). ESI-MS for $C_{32}H_{23}Cl_2N_3O_2$: calculated 551.1, found m/z 552.0, 554.0, 556.1 $[M+H]^+$; 550.1, 552.1, 554.0 $[M-H]^-$. UPLC-MS purity (UV@215 nm) 99%.

3-(1-acetyl-5-(4'-bromo-[1,1'-biphenyl]-4-yl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (43d). Compound **43d** was synthesized via general procedure C₂ using **96c** (203 mg, 0.36 mmol) with acetic anhydride **116** (68 μ L, 0.72 mmol) (165 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-10% EtOH/DCM) to afford **43d** (175 mg, yield 80%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.37 (s, 1H), 7.66 – 7.36 (m, 12H), 7.32 – 7.24 (m, 1H), 6.98 – 6.85 (m, 3H), 5.35 (dd, J = 12.0, 4.6 Hz, 1H), 3.78 (dd, J = 18.4, 12.1 Hz, 1H), 2.90 (dd, J = 18.4, 4.6 Hz, 1H), 1.88 (s, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 167.10, 160.12, 152.20, 149.92, 141.81, 138.97, 137.70, 137.30, 134.73, 131.71, 131.12, 129.36, 128.67, 128.58, 128.46, 128.36, 128.27, 126.67, 126.17, 126.12, 126.04, 124.57, 120.84, 120.65, 117.58, 79.15, 58.51, 45.28, 21.39. R_t 2.52 min (apolar method). ESI-MS for $C_{32}H_{23}BrClN_3O_2$: calculated 595.1, found m/z 595.9, 597.9, 600.0 $[M+H]^+$; 594.0, 596.0, 597.9 $[M-H]^-$. UPLC-MS purity (UV@215 nm) 99%.

3-(1-acetyl-5-(4'-methoxy-[1,1'-biphenyl]-4-yl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (44d). Compound **44d** was synthesized via general procedure C₂ using **97c** (179 mg, 0.35 mmol) with acetic anhydride **116** (66 μ L, 0.70 mmol) (165 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-8% EtOH/DCM) to afford **44d** (158 mg, yield 83%). ¹H NMR (600 MHz, DMSO-*d*₆) δ 12.39 (s, 1H), 7.63 (dd, J = 8.8, 2.4 Hz, 1H), 7.60 – 7.53 (m, 5H), 7.45 (d, J = 8.5 Hz, 3H), 7.43 – 7.39 (m, 1H), 7.31 – 7.27 (m, 1H), 7.03 – 6.99 (m, 2H), 6.94 (d, J = 2.4 Hz, 1H), 6.86 (d, J = 8.3 Hz, 2H), 5.33 (dd, J = 12.1, 4.6 Hz, 1H), 3.79 (s, 4H), 2.88 (dd, J = 18.5, 4.6 Hz, 1H), 1.88 (s, 3H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 167.09, 160.17, 158.87, 152.28, 149.95, 140.70, 138.74, 137.33, 134.75, 132.24, 131.20, 129.43, 128.64, 128.53, 128.43, 128.33, 127.72, 126.28, 126.12, 126.08, 126.04, 124.66, 120.71, 117.64, 114.35, 58.53, 55.16, 45.34, 21.47. R_t 1.65 min (apolar method). ESI-MS for

$C_{33}H_{26}ClN_3O_3$: calculated 547.2, found m/z 548.0, 550.0 $[M+H]^+$; 546.0, 548.0 $[M-H]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

3-(1-acetyl-5-(1-methyl-1H-indol-5-yl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (45d). Compound **45d** was synthesized via general procedure C₂ using **98c** (127 mg, 0.28 mmol) with acetic anhydride **116** (53 μ L, 0.56 mmol) (165 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-10% EtOH/DCM) to afford **45d** (107 mg, yield 75%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.36 (s, 1H), 7.64 – 7.56 (m, 3H), 7.53 (ddd, J = 8.9, 5.6, 3.3 Hz, 1H), 7.47 – 7.40 (m, 2H), 7.31 – 7.23 (m, 3H), 6.99 (d, J = 1.7 Hz, 1H), 6.94 (d, J = 2.3 Hz, 1H), 6.65 (dd, J = 8.5, 1.7 Hz, 1H), 6.35 (dd, J = 3.1, 0.8 Hz, 1H), 5.36 (dd, J = 12.0, 4.5 Hz, 1H), 3.75 (s, 4H), 2.90 (dd, J = 18.4, 4.6 Hz, 1H), 1.86 (s, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 166.93, 160.20, 152.06, 149.80, 137.30, 135.61, 134.79, 133.10, 131.09, 129.92, 129.46, 128.59, 128.47, 128.37, 128.23, 127.74, 126.06, 124.81, 120.74, 119.02, 117.58, 117.16, 109.63, 100.26, 59.32, 45.86, 32.45, 21.54. R_t 2.41 min (generic method). ESI-MS for $C_{29}H_{23}ClN_4O_2$: calculated 494.1, found m/z 495.2, 497.2 $[M+H]^+$; 493.3, 495.3 $[M-H]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

3-(1-acetyl-5-(1-ethyl-1H-indol-5-yl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (46d). Compound **46d** was synthesized via general procedure C₂ using **99c** (168 mg, 0.36 mmol) with acetic anhydride **116** (68 μ L, 0.72 mmol) (165 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-10% EtOH/DCM) to afford **46d** (128 mg, yield 70%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.36 (s, 1H), 7.64 – 7.50 (m, 4H), 7.47 – 7.40 (m, 2H), 7.35 (d, J = 3.1 Hz, 1H), 7.32 – 7.27 (m, 2H), 6.97 (dd, J = 22.0, 2.0 Hz, 2H), 6.64 (dd, J = 8.5, 1.7 Hz, 1H), 6.35 (dd, J = 3.1, 0.8 Hz, 1H), 5.35 (dd, J = 12.0, 4.6 Hz, 1H), 4.16 (q, J = 7.2 Hz, 2H), 3.78 (dd, J = 18.4, 12.0 Hz, 1H), 2.91 (dd, J = 18.4, 4.6 Hz, 1H), 1.86 (s, 3H), 1.33 (t, J = 7.2 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 166.95, 160.20, 152.07, 149.81, 137.29, 134.80, 134.57, 133.07, 131.09, 129.43, 128.61, 128.47, 128.36, 128.31, 128.24, 127.90,

126.07, 124.82, 120.74, 118.97, 117.58, 117.29, 109.62, 100.48, 59.33, 45.85, 40.28, 21.55, 21.18, 15.52. R_t 2.51 min (generic method). ESI-MS for $C_{30}H_{25}ClN_4O_2$: calculated 508.2, found m/z 509.3, 511.2 $[M+H]^+$; 507.3, 509.3 $[M-H]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

3-(1-acetyl-5-(1-ethyl-1H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-

phenylquinolin-2(1H)-one (47d). Compound **47d** was synthesized via general procedure C₂ using **100c** (131 mg, 0.28 mmol) with acetic anhydride **116** (53 μ L, 0.56 mmol) (165 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 12 g, 0-5% EtOH/DCM) to afford **47d** (118 mg, yield 82%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.37 (s, 1H), 7.98 (d, J = 0.9 Hz, 1H), 7.66 – 7.57 (m, 3H), 7.57 – 7.49 (m, 2H), 7.48 – 7.40 (m, 2H), 7.29 (ddt, J = 7.6, 2.0, 1.0 Hz, 1H), 7.15 (t, J = 1.1 Hz, 1H), 6.94 (d, J = 2.3 Hz, 1H), 6.84 (dd, J = 8.7, 1.6 Hz, 1H), 5.41 (dd, J = 12.0, 4.5 Hz, 1H), 4.41 (q, J = 7.2 Hz, 2H), 3.79 (dd, J = 18.5, 12.1 Hz, 1H), 2.88 (dd, J = 18.4, 4.6 Hz, 1H), 1.88 (s, 3H), 1.37 (t, J = 7.2 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 167.05, 160.18, 152.21, 149.88, 138.03, 137.33, 134.72, 134.44, 132.33, 131.16, 129.51, 128.57, 128.41, 128.29, 126.08, 124.71, 124.11, 123.28, 120.72, 117.63, 117.30, 109.78, 58.90, 45.57, 43.06, 21.50, 14.95. R_t 2.29 min (generic method). ESI-MS for $C_{29}H_{24}ClN_5O_2$: calculated 509.2, found m/z 510.2, 512.2 $[M+H]^+$, 508.3, 510.4 $[M-H]^-$. UPLC-MS purity (UV@215 nm) 99%.

3-(1-acetyl-5-(2-ethyl-2H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-

phenylquinolin-2(1H)-one (48d). Compound **48d** was synthesized via general procedure C₂ using **101c** (80 mg, 0.17 mmol) with acetic anhydride **116** (32 μ L, 0.34 mmol) (165 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-10% EtOH/DCM) to afford **48d** (67 mg, yield 77%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.37 (s, 1H), 8.31 (d, J = 0.9 Hz, 1H), 7.67 – 7.50 (m, 4H), 7.48 – 7.42 (m, 3H), 7.36 – 7.26 (m, 1H), 7.13 – 7.05 (m, 1H), 6.94 (d, J = 2.4 Hz, 1H), 6.65 (dd, J = 9.0, 1.7 Hz, 1H), 5.36 (dd, J = 12.0, 4.6 Hz, 1H), 4.44 (q, J = 7.3 Hz, 2H), 3.78 (dd, J = 18.4, 12.0 Hz, 1H), 2.87 (dd, J = 18.4, 4.6 Hz, 1H), 1.89 (s, 3H), 1.50 (t, J = 7.2 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 167.04, 160.18, 152.20, 149.83, 147.32, 137.35,

134.71, 134.46, 131.13, 129.52, 128.57, 128.54, 128.37, 128.27, 126.04, 124.73, 123.59, 123.01, 120.93, 120.72, 117.63, 117.34, 116.65, 59.13, 47.69, 45.35, 21.52, 15.84. R_t 2.14 min (generic method). ESI-MS for $C_{29}H_{24}ClN_5O_2$: calculated 509.2, found m/z 510.2, 512.2 $[M+H]^+$, 508.2, 510.3 $[M-H]^-$. UPLC-MS purity (UV@215 nm) 99%.

3-(1-acetyl-5-(1-propyl-1H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (49d). Compound **49d** was synthesized via general procedure C₂ using **102c** (188 mg, 0.39 mmol) with acetic anhydride **116** (74 μ L, 0.78 mmol) (165 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-10% EtOH/DCM) to afford **49d** (184 mg, yield 90%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.37 (s, 1H), 7.99 (d, J = 0.9 Hz, 1H), 7.65 – 7.57 (m, 3H), 7.56 – 7.50 (m, 2H), 7.47 – 7.41 (m, 2H), 7.36 – 7.25 (m, 1H), 7.17 – 7.09 (m, 1H), 6.94 (d, J = 2.3 Hz, 1H), 6.85 (dd, J = 8.8, 1.7 Hz, 1H), 5.41 (dd, J = 12.0, 4.6 Hz, 1H), 4.33 (t, J = 6.9 Hz, 2H), 3.78 (dd, J = 18.5, 12.0 Hz, 1H), 2.89 (dd, J = 18.5, 4.6 Hz, 1H), 1.90 – 1.77 (m, 5H), 0.82 (t, J = 7.4 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 167.06, 160.14, 152.20, 149.85, 147.37, 137.29, 134.69, 134.43, 131.13, 129.52, 128.53, 128.35, 128.26, 126.07, 124.73, 123.73, 123.58, 120.81, 120.71, 117.59, 117.37, 116.65, 59.13, 54.23, 45.34, 23.40, 21.51, 10.86. R_t 1.21 min (generic method). ESI-MS for $C_{30}H_{26}ClN_5O_2$: calculated 523.2, found m/z 524.1, 526.2 $[M+H]^+$, 522.3, 524.2 $[M-H]^-$. UPLC-MS purity (UV@215 nm) 94%.

3-(1-acetyl-5-(2-propyl-2H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (50d). Compound **50d** was synthesized via general procedure C₂ using **103c** (132 mg, 0.27 mmol) with acetic anhydride **116** (51 μ L, 0.54 mmol) (165 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 0-10% EtOH/DCM) to afford **50d** (120 mg, yield 85%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.37 (s, 1H), 8.29 (d, J = 1.0 Hz, 1H), 7.65 – 7.56 (m, 3H), 7.56 – 7.49 (m, 1H), 7.48 – 7.40 (m, 3H), 7.32 – 7.26 (m, 1H), 7.08 (t, J = 1.2 Hz, 1H), 6.93 (d, J = 2.3 Hz, 1H), 6.64 (dd, J = 8.9, 1.7 Hz, 1H), 5.35 (dd, J = 12.0, 4.5 Hz, 1H), 4.36 (t, J = 6.9 Hz, 2H), 3.77 (dd, J = 18.4, 12.0 Hz, 1H), 2.87 (dd, J = 18.4, 4.6 Hz, 1H),

1.97 – 1.83 (m, 5H), 0.83 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 167.07, 160.15, 152.21, 149.85, 147.38, 137.31, 134.69, 134.43, 131.13, 129.52, 128.57, 128.53, 128.36, 128.26, 126.07, 126.04, 124.74, 123.74, 123.58, 120.81, 120.72, 117.61, 117.38, 116.65, 59.13, 54.24, 45.35, 23.41, 21.52, 10.87. R_t 1.01 min (apolar method). ESI-MS for $\text{C}_{30}\text{H}_{26}\text{ClN}_5\text{O}_2$: calculated 523.2, found m/z 524.2, 526.2 $[\text{M}+\text{H}]^+$, 522.3, 524.3 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) 99%.

3-(1-acetyl-5-(4-(1-propyl-1H-pyrazol-4-yl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (51d). Compound **51d** was synthesized via general procedure C_2 using **108c** (146 mg, 0.31 mmol) with acetic anhydride **116** (59 μL , 0.62 mmol) (165 $^\circ\text{C}$, 200 W). Purification performed by direct phase flash chromatography (SiO_2 gold 24 g, 0-8% EtOH/DCM) to afford **51d** (138 mg, yield 81%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.36 (s, 1H), 8.13 (s, 1H), 7.83 (d, $J = 0.7$ Hz, 1H), 7.62 (dd, $J = 8.8, 2.4$ Hz, 1H), 7.60 – 7.52 (m, 3H), 7.45 (d, $J = 8.8$ Hz, 1H), 7.43 – 7.38 (m, 3H), 7.30 (dt, $J = 6.2, 1.7$ Hz, 1H), 6.94 (d, $J = 2.3$ Hz, 1H), 6.79 (d, $J = 8.3$ Hz, 2H), 5.28 (dd, $J = 12.0, 4.5$ Hz, 1H), 4.07 (t, $J = 7.0$ Hz, 2H), 3.75 (dd, $J = 18.4, 12.0$ Hz, 1H), 2.86 (dd, $J = 18.4, 4.6$ Hz, 1H), 1.91 – 1.75 (m, 5H), 0.85 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 167.01, 160.14, 152.19, 149.89, 139.79, 137.30, 135.89, 134.74, 131.59, 131.15, 129.41, 128.60, 128.49, 128.38, 128.26, 126.88, 126.08, 126.00, 124.96, 124.66, 121.22, 120.69, 117.59, 58.57, 52.99, 45.27, 23.17, 21.42, 10.92. R_t 1.22 min (apolar method). ESI-MS for $\text{C}_{32}\text{H}_{28}\text{ClN}_5\text{O}_2$: calculated 549.2, found m/z 550.0, 552.0 $[\text{M}+\text{H}]^+$, 548.1, 550.0 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) 99%.

4-(5-(4-fluorophenyl)-3-(2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (52d). Compound **52d** was synthesized via general procedure C_1 using **109c** (150 mg, 0.39 mmol) with succinic anhydride **115** (78 mg, 0.78 mmol) (120 $^\circ\text{C}$, 200 W). Purification performed by direct phase flash chromatography (SiO_2 gold 24 g, 20-50% EtOH/DCM) to afford **52d** (114 mg, yield 59%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.22 (s, 1H), 12.01 (s, 1H), 7.63 – 7.34 (m, 6H), 7.25 (dt, $J = 6.8, 1.9$ Hz, 1H), 7.14 (ddd, $J = 8.2, 7.0, 1.2$ Hz,

1H), 7.07 – 6.98 (m, 3H), 6.86 – 6.78 (m, 2H), 5.31 (dd, $J = 12.0, 4.5$ Hz, 1H), 3.72 (dd, $J = 18.4, 12.0$ Hz, 1H), 2.78 (dd, $J = 18.4, 4.6$ Hz, 1H), 2.47 (m, 2H), 2.32 – 2.25 (m, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.48, 168.56, 160.25, 152.77, 151.05, 141.11, 138.53, 138.42, 135.16, 131.27, 129.38, 128.53, 128.22, 128.20, 128.13, 127.54, 127.45, 127.38, 123.30, 122.25, 119.35, 115.51, 115.16, 114.95, 58.16, 45.27, 28.58, 28.23. R_t 1.90 min (generic method). ESI-MS for $\text{C}_{28}\text{H}_{22}\text{FN}_3\text{O}_4$: calculated 483.2, found m/z 484.5 $[\text{M}+\text{H}]^+$, 482.5 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-(5-(4-Bromophenyl)-3-(2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydropyrazol-1-yl)-4-oxobutanoic acid (53d). Compound **53d** was synthesized via general procedure C_1 using **110c** (41 mg, 0.09 mmol) with succinic anhydride **115** (18 mg, 0.18 mmol) (120 °C, 200 W). Title compound **53d** was obtained after the acidic work up (46 mg, yield 91%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.22 (s, 1H), 12.01 (s, 1H), 7.61 – 7.45 (m, 4H), 7.45 – 7.36 (m, 4H), 7.25 (dt, $J = 6.8, 1.9$ Hz, 1H), 7.14 (ddd, $J = 8.3, 7.0, 1.2$ Hz, 1H), 7.04 (dd, $J = 8.2, 1.4$ Hz, 1H), 6.74 (d, $J = 8.4$ Hz, 2H), 5.29 (dd, $J = 12.0, 4.6$ Hz, 1H), 3.74 (dd, $J = 18.4, 12.1$ Hz, 1H), 2.77 (dd, $J = 18.5, 4.6$ Hz, 1H), 2.50 (m, 2H), 2.28 (t, $J = 7.1$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.46, 168.60, 160.25, 152.78, 151.09, 141.60, 138.54, 135.17, 131.29, 131.23, 129.41, 128.51, 128.21, 128.14, 127.75, 127.39, 123.23, 122.27, 121.42, 120.03, 119.35, 115.53, 58.30, 45.12, 28.55, 28.22. R_t 2.03 min (generic method). ESI-MS for $\text{C}_{28}\text{H}_{22}\text{BrN}_3\text{O}_4$: calculated 543.1, found m/z 544.5, 546.5 $[\text{M}+\text{H}]^+$; 542.5, 544.4. UPLC-MS purity (UV@215 nm) >99.5%.

4-(5-(3-bromophenyl)-3-(2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (54d). Compound **54d** was synthesized via general procedure C_1 using **111c** (121 mg, 0.27 mmol) with succinic anhydride **115** (54 mg, 0.54 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO_2 gold 24 g, 20-90% EtOH/DCM) to afford **54d** (11 mg, yield 7%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.24 (s, 1H), 12.13 (s, 1H), 7.61 – 7.34 (m, 7H), 7.26 (dt, $J = 7.8, 1.8$ Hz, 1H), 7.23 – 7.11 (m, 3H), 7.06 (dd, $J = 8.2, 1.4$ Hz,

1H), 6.76 (dt, $J = 7.8, 1.3$ Hz, 1H), 5.32 (dd, $J = 12.1, 4.8$ Hz, 1H), 3.74 (dd, $J = 18.5, 12.1$ Hz, 1H), 2.84 (dd, $J = 18.5, 4.8$ Hz, 1H), 2.51 (m, 2H), 2.33 – 2.26 (m, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 174.08, 173.94, 169.19, 160.76, 153.36, 151.60, 145.30, 139.04, 135.72, 131.79, 131.18, 130.45, 129.75, 128.92, 128.71, 128.56, 127.90, 124.94, 123.65, 122.75, 122.09, 119.80, 116.01, 58.80, 45.64, 29.39, 29.01, 28.67. R_t 2.00 min (generic method). ESI-MS for $\text{C}_{28}\text{H}_{22}\text{BrN}_3\text{O}_4$: calculated 543.1, found m/z 544.4, 546.4 $[\text{M}+\text{H}]^+$; 542.4, 544.4 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-(5-(4-chlorophenyl)-3-(2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (55d). Compound **55d** was synthesized via general procedure C₁ using **112c** (121 mg, 0.27 mmol) with succinic anhydride **115** (54 mg, 0.54 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 4-20% EtOH/DCM) to afford **55d** (130 mg, yield 67%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.23 (s, 1H), 12.05 (s, 1H), 7.61 – 7.46 (m, 4H), 7.43 (dd, $J = 8.3, 1.1$ Hz, 1H), 7.39 (dt, $J = 6.0, 2.0$ Hz, 1H), 7.30 – 7.23 (m, 3H), 7.15 (ddd, $J = 8.3, 7.0, 1.2$ Hz, 1H), 7.05 (dd, $J = 8.2, 1.4$ Hz, 1H), 6.81 (d, $J = 8.5$ Hz, 2H), 5.31 (dd, $J = 12.0, 4.6$ Hz, 1H), 3.75 (dd, $J = 18.5, 12.1$ Hz, 1H), 2.78 (dd, $J = 18.5, 4.6$ Hz, 1H), 2.58-2.44 (m, 2H), 2.30 (t, $J = 7.1$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.95, 169.08, 160.73, 153.27, 151.57, 141.65, 139.02, 135.65, 132.01, 131.77, 129.89, 129.00, 128.79, 128.69, 128.62, 127.88, 123.73, 122.74, 119.83, 116.01, 58.72, 45.66, 29.04, 28.70. R_t 1.97 min (generic method). ESI-MS for $\text{C}_{28}\text{H}_{22}\text{ClN}_3\text{O}_4$: calculated 499.1, found m/z 500.4, 502.4 $[\text{M}+\text{H}]^+$; 498.4, 500.3 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-(5-(4-methoxyphenyl)-3-(2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (56d). Compound **56d** was synthesized via general procedure C₁ using **113c** (120 mg, 0.30 mmol) with succinic anhydride **115** (60 mg, 0.60 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 5-35% EtOH/DCM) to afford **56d** (99 mg, yield 67%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.21 (s, 1H), 12.00 (s, 1H),

7.61 – 7.34 (m, 6H), 7.29 – 7.23 (m, 1H), 7.14 (ddd, $J = 8.2, 7.0, 1.2$ Hz, 1H), 7.04 (dd, $J = 8.2, 1.4$ Hz, 1H), 6.79 – 6.66 (m, 4H), 5.23 (dd, $J = 11.9, 4.5$ Hz, 1H), 3.72 (s, 4H), 2.79 (dd, $J = 18.4, 4.5$ Hz, 1H), 2.46 (dd, $J = 6.8, 2.4$ Hz, 2H), 2.27 (t, $J = 6.9$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.99, 168.91, 160.78, 158.68, 153.22, 151.47, 139.00, 135.70, 134.85, 131.71, 129.84, 129.08, 128.66, 128.60, 127.87, 127.27, 123.91, 122.71, 119.88, 115.99, 114.16, 58.85, 55.51, 45.80, 29.11, 28.74. R_t 1.83 min (generic method). ESI-MS for $\text{C}_{29}\text{H}_{25}\text{N}_3\text{O}_5$: calculated 495.1, found m/z 496.5 $[\text{M}+\text{H}]^+$; 494.5 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

4-oxo-4-(3-(2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)butanoic acid (57d). Compound **57d** was synthesized via general procedure C₁ using **114c** (189 mg, 0.45 mmol) with succinic anhydride **115** (90 mg, 0.90 mmol) (120 °C, 200 W). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g, 2-50% EtOH/DCM) to afford **57d** (173 mg, yield 71%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.23 (s, 1H), 12.02 (s, 1H), 7.64 – 7.36 (m, 8H), 7.25 (d, $J = 7.3$ Hz, 1H), 7.15 (ddd, $J = 8.3, 7.1, 1.2$ Hz, 1H), 7.08 – 6.98 (m, 3H), 5.42 (dd, $J = 12.1, 4.7$ Hz, 1H), 3.79 (dd, $J = 18.5, 12.1$ Hz, 1H), 2.81 (dd, $J = 18.5, 4.7$ Hz, 1H), 2.59-2.48 (m, 2H), 2.36 – 2.26 (m, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.94, 169.21, 160.71, 153.30, 151.65, 147.20, 139.03, 135.67, 131.80, 129.88, 128.96, 128.70, 128.65, 128.29, 127.88, 126.70, 126.02, 125.84, 125.80, 123.63, 123.31, 122.76, 119.81, 116.02, 58.95, 45.63, 28.99, 28.68. R_t 2.06 min (generic method). ESI-MS for $\text{C}_{29}\text{H}_{22}\text{F}_3\text{N}_3\text{O}_4$: calculated 533.2, found m/z 534.5 $[\text{M}+\text{H}]^+$; 532.5 $[\text{M}-\text{H}]^-$. UPLC-MS purity (UV@215 nm) >99.5%.

3-Acetyl-6-chloro-4-phenylquinolin-2(1H)-one (60a) (Scheme 1). 2-amino-5-chlorophenyl-phenyl-methanone **58** (1.460 g, 6.3 mmol) and the ethyl acetoacetate (2.4 mL, 18.9 mmol) were dissolved in DMF (1.00 M) in an appropriately sized screw-capped pressure tube. The mixture was heated at 153 °C and stirred for 19 h. The solvent was removed under reduced pressure to afford **60a** (1.875 g, quantitative yield). ^1H NMR (400 MHz, DMSO- d_6) δ 12.38 (s, 1H), 7.64 (dd, $J = 8.8, 2.4$ Hz, 1H), 7.59 – 7.48 (m, 3H), 7.44 (d, $J = 8.8$ Hz, 1H), 7.39 – 7.28 (m, 2H), 6.95 (d, $J = 2.3$ Hz,

1H), 2.22 (s, 3H). R_t 2.06 min (generic method). ESI-MS for $C_{17}H_{12}ClNO_2$: calculated 297.1, found m/z 298.3, 300.3 $[M+H]^+$; 296.3, 298.3 $[M-H]^-$.

3-acetyl-4-phenylquinolin-2(1H)-one (61a) (Scheme 1). (2-aminophenyl)-phenyl-methanone **59** (2.00 g, 10.1 mmol) and the ethyl acetoacetate (1.9 mL, 15.2 mmol) were dissolved in DMF (1.00 M) in an appropriately sized microwaveable vessel and microwaved at 120 °C (200 W) for 1.5 h. The solvent was concentrated under reduced pressure, the residue was diluted with DCM and washed (3 x 100 mL) with H_2O . The organic layer was dried over Na_2SO_4 , filtered and the solvent removed under reduced pressure. **61a** was obtained after precipitation from EtOAc (1.44 g, 53% yield). 1H NMR (400 MHz, $DMSO-d_6$) δ 12.22 (s, 1H), 7.56 (ddd, J = 8.4, 7.1, 1.5 Hz, 1H), 7.54 – 7.47 (m, 3H), 7.41 (dd, J = 8.4, 1.2 Hz, 1H), 7.34 – 7.28 (m, 2H), 7.14 (ddd, J = 8.3, 7.1, 1.2 Hz, 1H), 7.05 (dd, J = 8.2, 1.4 Hz, 1H), 2.21 (s, 3H). ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 201.60, 159.26, 146.96, 138.45, 134.19, 133.29, 131.20, 128.71, 128.64, 128.46, 127.03, 122.33, 118.89, 115.64, 31.47. R_t 1.86 min (generic method). ESI-MS for $C_{17}H_{13}NO_2$: calculated 263.1, found m/z 264.5 $[M+H]^+$; 262.4 $[M-H]^-$.

(E)-6-chloro-3-(3-(4-fluorophenyl) acryloyl)-4-phenylquinolin-2(1H)-one (86b). 3-Acetyl-6-chloro-4-phenylquinolin-2(1H)-one **60a** (2.517 g, 8.45 mmol) and 4-fluorobenzaldehyde **62** (906 μ L, 8.45 mmol) were allowed to react overnight according to general procedure A. Purification performed by direct phase flash chromatography (SiO_2 gold 40 g; 0-20% EtOH/DCM), to afford **86b** (2.548 g, 75% yield). 1H NMR (400 MHz, $DMSO-d_6$) δ 12.35 (s, 1H), 7.81 – 7.70 (m, 2H), 7.66 (dd, J = 8.8, 2.4 Hz, 1H), 7.54 – 7.41 (m, 5H), 7.36 – 7.30 (m, 2H), 7.28 – 7.19 (m, 2H), 6.98 (d, J = 2.4 Hz, 1H), 6.73 (d, J = 16.4 Hz, 1H). R_t 2.42 min (generic method). ESI-MS for $C_{24}H_{15}ClFNO_2$: calculated 403.1, found m/z 404.1, 406.1 $[M+H]^+$; 402.1, 404.1 $[M-H]^-$.

6-chloro-3-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (86c). **(E)-6-chloro-3-(3-(4-fluorophenyl) acryloyl)-4-phenylquinolin-2(1H)-one 86b** (317 mg, 0.78

mmol) and hydrazine monohydrate (76 μ L, 1.56 mmol) were allowed to react according to general procedure B. Crude compound was purified by flash column chromatography (SiO₂ gold 40 g; 0-20% EtOH/DCM) to afford **86c** (303 mg, yield 92%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.19 (s, 1H), 7.57 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.50 (m, 2H), 7.41 (d, *J* = 8.8 Hz, 1H), 7.33 (dt, *J* = 6.9, 1.5 Hz, 1H), 7.23 (dt, *J* = 6.8, 2.0 Hz, 1H), 7.14 (d, *J* = 3.2 Hz, 1H), 7.13 – 7.02 (m, 4H), 6.89 (d, *J* = 2.3 Hz, 1H), 4.59 (td, *J* = 10.9, 10.2, 3.1 Hz, 1H), 3.23 (dd, *J* = 16.5, 11.0 Hz, 1H), 2.59 – 2.52 (m, 1H). *R*_t 2.31 min (generic method). ESI-MS for C₂₄H₁₇ClFN₃O: calculated 417.1, found *m/z* 418.4, 420.4 [M+H]⁺; 416.4, 418.3 [M-H]⁻.

(E)-6-chloro-3-(3-(4-chlorophenyl)acryloyl)-4-phenylquinolin-2(1H)-one (87b). Compound **87b** was synthesized via general procedure A using **60a** (304 mg, 1.00 mmol) and 4-chlorobenzaldehyde **63** (140 mg, 1.00 mmol). Title compound **87b** was obtained by precipitation and filtration from the reaction crude (429 mg, quantitative yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.70 (d, *J* = 8.6 Hz, 2H), 7.63 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.55 – 7.37 (m, 7H), 7.32 (dd, *J* = 7.6, 1.9 Hz, 2H), 6.96 (d, *J* = 2.4 Hz, 1H), 6.78 (d, *J* = 16.4 Hz, 1H). *R*_t 2.55 min (generic method). ESI-MS for C₂₄H₁₅Cl₂NO₂: calculated 419.0, found *m/z* 420.4, 422.4, 424.4 [M+H]⁺; 418.5, 420.4, 422.3 [M-H]⁻.

6-Chloro-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (87c). Compound **87c** was synthesized via general procedure B using **87b** (429 mg, 1.00 mmol) with hydrazine hydrate (97 μ L, 2.00 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-10% EtOH/DCM) to afford **87c** (301 mg, yield 68%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.20 (s, 1H), 7.58 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.50 (tt, *J* = 7.5, 2.9 Hz, 3H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.34 (dt, *J* = 7.0, 1.7 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.23 (dt, *J* = 7.3, 2.0 Hz, 1H), 7.18 (d, *J* = 3.2 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 2H), 6.90 (d, *J* = 2.3 Hz, 1H), 4.59 (td, *J* = 11.6, 9.3, 3.1 Hz, 1H), 3.25 (dd, *J* = 16.5, 11.1 Hz, 1H), 2.58 – 2.53 (m, 1H). *R*_t 2.45 min (generic

method). ESI-MS for $C_{24}H_{17}Cl_2N_3O$: calculated 433.1, found m/z 434.4, 436.4, 438.5 $[M+H]^+$; 432.4, 434.4, 436.4 $[M-H]^-$.

(E)-3-(3-(4-bromophenyl)acryloyl)-6-chloro-4-phenylquinolin-2(1H)-one (88b). Compound **88b** was synthesized via general procedure A using **60a** (328 mg, 1.10 mmol) and 4-bromobenzaldehyde **64** (204 mg, 1.10 mmol). Title compound **88b** was obtained by precipitation and filtration from the reaction crude (511 mg, quantitative yield). 1H NMR (400 MHz, DMSO- d_6) δ 7.67 – 7.47 (m, 6H), 7.46 – 7.35 (m, 4H), 7.30 (dd, J = 7.7, 1.8 Hz, 2H), 6.93 (d, J = 2.3 Hz, 1H), 6.80 (d, J = 16.4 Hz, 1H). R_t 2.59 min (generic method). ESI-MS for $C_{24}H_{15}BrClNO_2$: calculated 463.0, found m/z 464.3, 466.2, 468.2 $[M+H]^+$; 462.2, 464.2, 466.2 $[M-H]^-$.

3-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (88c). Compound **88c** was synthesized via general procedure B using **88b** (510 mg, 1.10 mmol) with hydrazine hydrate (110 μ L, 2.20 mmol). Purification performed by direct phase flash chromatography (0-8% EtOH/DCM), to afford **88c** (349 mg, yield 64%). 1H NMR (400 MHz, DMSO- d_6) δ 12.20 (s, 1H), 7.58 (dd, J = 8.8, 2.4 Hz, 1H), 7.56 – 7.45 (m, 3H), 7.42 (dd, J = 8.6, 5.5 Hz, 3H), 7.34 (dt, J = 7.2, 1.6 Hz, 1H), 7.23 (dt, J = 7.2, 2.0 Hz, 1H), 7.18 (d, J = 3.2 Hz, 1H), 7.07 – 6.99 (m, 2H), 6.89 (d, J = 2.3 Hz, 1H), 4.58 (td, J = 11.6, 9.3, 3.2 Hz, 1H), 3.25 (dd, J = 16.5, 11.1 Hz, 1H), 2.57 – 2.53 (m, 1H). R_t 2.50 min (generic method). ESI-MS for $C_{24}H_{17}BrClN_3O$: calculated 477.0, found m/z 478.2, 480.2, 482.2 $[M+H]^+$; 476.3, 478.2, 480.3 $[M-H]^-$.

(E)-6-chloro-3-(3-(4-methoxyphenyl)acryloyl)-4-phenylquinolin-2(1H)-one (89b). 3-Acetyl-6-chloro-4-phenylquinolin-2(1H)-one **60a** (611 mg, 2.00 mmol) and *p*-anisaldehyde **65** (243 μ L, 2.00 mmol) were allowed to react overnight according to general procedure A. Purification performed by direct phase flash chromatography (SiO₂ gold 40 g; 0-5% EtOH/DCM), affording the desired **89b** (867 mg, quantitative yield). 1H NMR (400 MHz, DMSO- d_6) δ 12.31 (s, 1H), 7.68 –

7.58 (m, 3H), 7.49 – 7.38 (m, 5H), 7.34 – 7.29 (m, 2H), 6.99 – 6.89 (m, 3H), 6.60 (d, $J = 16.3$ Hz, 1H), 3.78 (s, 3H). R_t 1.19 min (apolar method). ESI-MS for $C_{25}H_{18}ClNO_3$: calculated 415.1, found m/z 416.4, 418.4 $[M+H]^+$; 414.4, 416.4 $[M-H]^-$.

6-Chloro-3-(5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (89c). (*E*)-6-chloro-3-(3-(4-methoxyphenyl)acryloyl)-4-phenylquinolin-2(1H)-one **89b** (780 mg, 1.9 mmol) and hydrazine monohydrate (185 μ L, 3.8 mmol) were allowed to react according to general procedure B. Crude compound was purified by flash column chromatography (SiO_2 gold 40 g; 0-10% EtOH/DCM), to afford **89c** (611 mg, yield 75%). 1H NMR (400 MHz, $DMSO-d_6$) δ 12.19 (s, 1H), 7.57 (dd, $J = 8.8, 2.3$ Hz, 1H), 7.53 – 7.47 (m, 3H), 7.42 (d, $J = 8.8$ Hz, 1H), 7.34 (dd, $J = 10.3, 3.8$ Hz, 1H), 7.27 – 7.19 (m, 1H), 7.02 (dd, $J = 8.9, 5.9$ Hz, 3H), 6.90 (d, $J = 2.3$ Hz, 1H), 6.80 (d, $J = 8.7$ Hz, 2H), 4.52 (td, $J = 10.4, 2.9$ Hz, 1H), 3.73 (s, 3H), 3.18 (dd, $J = 16.4, 10.9$ Hz, 1H), 2.58 – 2.53 (m, 1H). ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 166.84, 160.17, 152.10, 149.77, 149.57, 137.28, 134.76, 131.10, 129.96, 129.28, 128.71, 128.42, 128.30, 128.28, 126.41, 126.04, 124.80, 120.72, 117.58, 112.23, 58.37, 45.22, 40.19, 21.49. R_t 2.24 min (generic method). ESI-MS for $C_{25}H_{20}ClN_3O_2$: calculated 429.1, found m/z 430.4, 432.4 $[M+H]^+$; 428.4, 430.5 $[M-H]^-$.

(*E*)-3-(3-(4-(tert-butyl)phenyl)acryloyl)-6-chloro-4-phenylquinolin-2(1H)-one (90b).

Compound **90b** was synthesized via general procedure A using **60a** (543 mg, 1.80 mmol) and 4-tert-butylbenzaldehyde **66** (301 μ L, 1.80 mmol). Purification performed by direct phase flash chromatography (SiO_2 gold 40 g; 0-10% EtOH/DCM) to afford **90b** (441 mg, yield 55%). 1H NMR (400 MHz, $DMSO-d_6$) δ 12.33 (s, 1H), 7.64 (dd, $J = 8.8, 2.4$ Hz, 1H), 7.58 (d, $J = 8.5$ Hz, 2H), 7.50 – 7.37 (m, 7H), 7.32 (dd, $J = 7.7, 1.9$ Hz, 2H), 6.97 (d, $J = 2.4$ Hz, 1H), 6.69 (d, $J = 16.4$ Hz, 1H), 1.26 (s, 9H). ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 193.82, 159.40, 153.81, 146.61, 146.19, 137.50, 133.60, 132.56, 131.52, 130.89, 128.86, 128.80, 128.50, 128.43, 126.64, 126.05, 125.71, 125.56, 120.56, 117.68, 79.15, 34.62, 30.97, 30.82. R_t 2.18 min (generic method). ESI-MS for $C_{28}H_{24}ClNO_2$: calculated 441.1, found m/z 442.4, 444.4 $[M+H]^+$; 440.4, 442.4 $[M-H]^-$.

3-[5-(4-*tert*-Butylphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-6-chloro-4-phenylquinolin-2(1*H*)-

one (90c). Compound **90c** was synthesized via general procedure B using **90b** (369 mg, 0.83 mmol) with hydrazine hydrate (81 μ L, 1.66 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-90% EtOAc/CHX) to afford **90c** (351 mg, yield 93%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.20 (s, 1H), 7.56 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.49 (dtd, *J* = 9.6, 7.2, 5.3 Hz, 3H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.33 (dt, *J* = 7.2, 1.7 Hz, 1H), 7.25 (d, *J* = 8.3 Hz, 3H), 7.06 (d, *J* = 3.0 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 2H), 6.90 (d, *J* = 2.3 Hz, 1H), 4.54 (t, *J* = 10.4 Hz, 1H), 3.19 (dd, *J* = 16.4, 10.9 Hz, 1H), 2.58 (dd, *J* = 16.4, 9.6 Hz, 1H), 1.26 (s, 9H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 160.54, 149.21, 148.30, 145.49, 140.31, 136.97, 135.21, 130.32, 129.19, 128.72, 128.26, 128.21, 128.14, 127.79, 127.18, 126.88, 126.33, 126.18, 125.75, 125.62, 124.92, 120.92, 117.33, 79.16, 62.69, 44.34, 34.09, 31.13, 31.05, 30.74. *R*_t 1.82 min (generic method). ESI-MS for C₂₈H₂₆ClN₃O: calculated 455.2, found *m/z* 456.5, 458.4, [M+H]⁺; 454.5 456.5 [M-H]⁻.

(*E*)-6-chloro-3-(3-(3-fluoro-4-methoxyphenyl)acryloyl)-4-phenylquinolin-2(1*H*)-one (91b).

Compound **91b** was synthesized via general procedure A using **60a** (473 mg, 1.60 mmol) and 3-fluoro-4-methoxy-benzaldehyde **67** (247 mg, 1.60 mmol). Title compound **91b** was obtained after precipitation and filtration from the reaction crude (547 mg, yield 79%). ¹H NMR (600 MHz, DMSO-*d*₆) δ 12.38 (s, 1H), 7.70 – 7.62 (m, 2H), 7.52 – 7.40 (m, 6H), 7.35 – 7.30 (m, 2H), 7.18 (t, *J* = 8.7 Hz, 1H), 6.97 (d, *J* = 2.4 Hz, 1H), 6.69 (d, *J* = 16.3 Hz, 1H), 3.88 (s, 3H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 194.19, 159.89, 153.13, 150.69, 149.82, 149.71, 147.06, 145.83, 138.00, 134.10, 133.01, 131.35, 129.34, 129.27, 128.90, 127.92, 127.85, 127.10, 126.87, 126.52, 126.02, 121.07, 118.17, 115.70, 115.52, 114.26, 56.62. *R*_t 2.35 min (generic method). ESI-MS for C₂₅H₁₇ClFNO₃: calculated 433.1, found *m/z* 434.4, 436.4 [M+H]⁺; 432.3, 434.3 [M-H]⁻.

6-chloro-3-(5-(3-fluoro-4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-4-phenylquinolin-

2(1*H*)-one (91c). Compound **91c** was synthesized via general procedure B using **91b** (203 mg, 0.50 mmol) with hydrazine hydrate (49 μ L, 1.00 mmol). Purification performed by direct phase flash

chromatography (SiO₂ gold 24 g; 0-5% EtOH/DCM) to afford **91c** (213 mg, quantitative yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.20 (s, 1H), 7.63 – 7.39 (m, 5H), 7.35 – 7.29 (m, 1H), 7.23 (dt, *J* = 7.0, 2.0 Hz, 1H), 7.12 (d, *J* = 3.1 Hz, 1H), 7.03 (t, *J* = 8.6 Hz, 1H), 6.97 – 6.85 (m, 3H), 4.55 (ddd, *J* = 11.5, 9.0, 2.9 Hz, 1H), 3.81 (s, 3H), 3.19 (dd, *J* = 16.5, 11.0 Hz, 1H), 2.60 – 2.53 (m, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 161.01, 152.92, 150.50, 148.84, 146.52, 145.91, 137.45, 137.04, 135.64, 130.85, 129.61, 129.14, 128.70, 127.20, 126.24, 126.10, 123.00, 121.36, 117.82, 114.42, 114.23, 114.02, 62.31, 56.48, 44.83. *R*_t 2.27 min (generic method). ESI-MS for C₂₅H₁₉ClFN₃O₂: calculated 447.1, found *m/z* 448.4, 450.3 [M+H]⁺; 446.3, 448.2 [M-H]⁻.

(*E*)-6-chloro-4-phenyl-3-(3-(4-(trifluoromethyl)phenyl)acryloyl)quinolin-2(1H)-one (92b).

Compound **92b** was synthesized via general procedure A using **60a** (760 mg, 2.50 mmol) and 4-(trifluoromethyl) benzaldehyde **68** (341 μL, 2.50 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 40 g; 0-5% EtOH/DCM) to afford **92b** (820 mg, yield 71%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.38 (s, 1H), 7.89 (d, *J* = 8.2 Hz, 2H), 7.74 (d, *J* = 8.4 Hz, 2H), 7.67 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.62 – 7.57 (m, 1H), 7.53 – 7.40 (m, 4H), 7.36 – 7.31 (m, 2H), 6.99 (d, *J* = 2.3 Hz, 1H), 6.91 (d, *J* = 16.5 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 193.90, 159.39, 147.09, 144.03, 138.29, 137.58, 133.52, 132.23, 131.05, 130.34, 130.07, 130.02, 129.59, 129.19, 128.88, 128.48, 126.12, 125.68, 125.65, 125.62, 125.30, 122.59, 120.55, 117.74. *R*_t 1.65 min (apolar method). ESI-MS for C₂₅H₁₅ClF₃NO₂: calculated 453.1, found *m/z* 454.4, 456.4 [M+H]⁺; 452.4, 454.4 [M-H]⁻.

6-chloro-4-phenyl-3-(5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-

2(1H)-one (92c). Compound **92c** was synthesized via general procedure B using **92b** (752 mg, 1.70 mmol) with hydrazine hydrate (165 μL, 3.40 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 40 g; 0-5% EtOH/DCM) to afford **92c** (696 mg, yield 90%) ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.21 (s, 1H), 7.63 – 7.56 (m, 3H), 7.56 – 7.44 (m, 3H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.37 – 7.32 (m, 1H), 7.29 (d, *J* = 8.5 Hz, 3H), 7.25 – 7.20 (m, 1H), 6.90 (d, *J* = 2.3 Hz,

1H), 4.75 – 4.64 (m, 1H), 3.39 – 3.25 (m, 1H), 2.63 – 2.54 (m, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 160.49, 148.49, 148.37, 145.45, 136.99, 135.13, 130.43, 129.25, 128.63, 128.29, 128.23, 128.20, 127.68, 127.22, 126.58, 125.79, 125.64, 125.11, 125.08, 122.95, 120.88, 117.37, 62.16, 44.50. R_t 2.52 min (generic method). ESI-MS for C₂₅H₁₇ClF₃N₃O: calculated 467.1, found *m/z* 468.4, 469.5 [M+H]⁺, 466.4, 468.4 [M-H]⁻.

(*E*)-6-chloro-3-(3-(furan-2-yl)acryloyl)-4-phenylquinolin-2(1H)-one (93b). Compound **93b** was synthesized via general procedure A using **60a** (503 mg, 1.70 mmol) and furan-2-carbaldehyde **69** (147 μL, 1.70 mmol). Purified Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-5% EtOH/DCM) to afford **93b** (471 mg, yield 74%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.34 (s, 1H), 7.85 (d, *J* = 1.7 Hz, 1H), 7.64 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.51 – 7.40 (m, 4H), 7.35 (s, 1H), 7.33 – 7.26 (m, 2H), 6.96 (dd, *J* = 6.9, 2.9 Hz, 2H), 6.63 (dd, *J* = 3.5, 1.8 Hz, 1H), 6.37 (d, *J* = 16.1 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 193.04, 159.41, 150.27, 146.93, 146.60, 137.52, 133.55, 132.45, 132.30, 130.98, 128.84, 128.46, 126.08, 125.58, 123.96, 120.51, 117.69, 117.67, 113.14, 40.19, 39.98. R_t 2.24 min (generic method). ESI-MS for C₂₂H₁₄ClNO₃: calculated 375.1, found *m/z* 376.3, 378.3 [M+H]⁺; 374.3, 376.4 [M-H]⁻.

6-chloro-3-(5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (93c). Compound **93c** was synthesized via general procedure B using **93b** (442 mg, 1.20 mmol) with hydrazine hydrate (117 μL, 2.40 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 40 g; 0-7.5% EtOH/DCM) to afford **93c** (422 mg, yield 91%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.21 (s, 1H), 7.57 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.54 – 7.51 (m, 1H), 7.50 – 7.38 (m, 4H), 7.31 – 7.23 (m, 2H), 7.07 (d, *J* = 2.8 Hz, 1H), 6.90 (d, *J* = 2.3 Hz, 1H), 6.34 (dd, *J* = 3.1, 1.9 Hz, 1H), 6.17 (d, *J* = 3.2 Hz, 1H), 4.61 – 4.53 (m, 1H), 3.04 (dd, *J* = 16.2, 10.6 Hz, 1H), 2.87 (dd, *J* = 16.2, 9.2 Hz, 1H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 160.55, 155.04, 148.48, 146.34, 142.11, 137.05, 135.23, 130.50, 128.95, 128.90, 128.34, 128.26, 128.18, 126.54, 125.83, 125.74,

120.91, 117.42, 110.33, 105.94, 40.63, 40.06. R_t 2.14 min (generic method). ESI-MS for $C_{22}H_{16}ClN_3O_2$: calculated 389.1, found m/z 390.4, 392.3 $[M+H]^+$; 388.4, 390.4 $[M-H]^-$.

(*E*)-6-chloro-3-(3-(4'-fluoro-[1,1'-biphenyl]-4-yl)acryloyl)-4-phenylquinolin-2(1H)-one

(**94b**). Compound **94b** was synthesized via general procedure A using **60a** (214 mg, 0.72 mmol) and 4'-fluoro-[1,1'-biphenyl]-4-carbaldehyde **70** (144 mg, 0.72 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-7% EtOH/CHCl₃) to afford **94b** (305 mg, yield 89%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.35 (s, 1H), 7.82 – 7.62 (m, 7H), 7.57 – 7.40 (m, 5H), 7.36 – 7.26 (m, 4H), 6.98 (d, J = 2.3 Hz, 1H), 6.80 (d, J = 16.4 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 193.83, 163.40, 160.96, 159.45, 146.77, 145.64, 141.15, 137.55, 135.57, 135.54, 133.63, 133.34, 132.54, 131.52, 131.42, 130.93, 129.33, 128.90, 128.84, 128.80, 128.72, 128.46, 127.29, 126.98, 126.10, 125.60, 120.59, 117.71, 115.91, 115.70. R_t 1.90 min (apolar method). ESI-MS for $C_{30}H_{19}ClFNO_2$: calculated 479.1, found m/z 480.2, 482.2 $[M+H]^+$, 478.2, 480.5 $[M-H]^-$.

6-chloro-3-(5-(4'-fluoro-[1,1'-biphenyl]-4-yl)-4,5-dihydro-1H-pyrazol-3-yl)-4-

phenylquinolin-2(1H)-one (94c). Compound **94c** was synthesized via general procedure B using **94b** (273 mg, 0.57 mmol) with hydrazine hydrate (55 μ L, 1.14 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-10% EtOH/DCM) to afford **94c** (188 mg, yield 67%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.18 (s, 1H), 7.71 – 7.64 (m, 2H), 7.60 – 7.46 (m, 6H), 7.42 (d, J = 8.8 Hz, 1H), 7.37 – 7.32 (m, 1H), 7.31 – 7.21 (m, 3H), 7.19 – 7.13 (m, 3H), 6.90 (d, J = 2.3 Hz, 1H), 4.67 – 4.57 (m, 1H), 3.26 (dd, J = 16.6, 11.1 Hz, 1H), 2.60 (dd, J = 16.4, 9.5 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 162.98, 160.55, 160.52, 148.35, 145.48, 142.66, 137.78, 136.96, 136.44, 136.41, 135.19, 130.33, 129.22, 128.69, 128.51, 128.43, 128.25, 128.17, 127.06, 126.78, 126.48, 125.75, 125.62, 120.91, 117.32, 115.73, 115.52, 62.49, 44.43. R_t 1.72 min (apolar method). ESI-MS for $C_{30}H_{21}ClFN_3O$: calculated 493.1, found m/z 494.2, 496.1 $[M+H]^+$, 492.2, 494.2 $[M-H]^-$.

(*E*)-6-chloro-3-(3-(4'-chloro-[1,1'-biphenyl]-4-yl)acryloyl)-4-phenylquinolin-2(1H)-one

(95b). Compound **95b** was synthesized via general procedure A using **60a** (339 mg, 1.10 mmol) and 4'-chloro-[1,1'-biphenyl]-4-carbaldehyde **71** (238 mg, 1.10 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-5% EtOH/DCM) to afford **95b** (567 mg, quantitative yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.35 (s, 1H), 7.78 – 7.68 (m, 6H), 7.65 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.57 – 7.48 (m, 3H), 7.48 – 7.40 (m, 4H), 7.34 (dd, *J* = 7.8, 1.8 Hz, 2H), 6.99 (d, *J* = 2.3 Hz, 1H), 6.81 (d, *J* = 16.4 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 193.80, 159.41, 146.75, 145.51, 140.78, 137.87, 137.53, 133.70, 133.61, 132.90, 132.51, 130.92, 129.34, 128.93, 128.88, 128.82, 128.45, 127.42, 126.98, 126.07, 125.58, 120.57, 117.69, 79.15. R_t 2.16 min (apolar method). ESI-MS for C₃₀H₁₉Cl₂NO₂: calculated 495.1, found *m/z* 496.0, 497.9, 499.9, [M+H]⁺; 494.1, 496.0, 498.0 [M-H]⁻.

6-chloro-3-(5-(4'-chloro-[1,1'-biphenyl]-4-yl)-4,5-dihydro-1H-pyrazol-3-yl)-4-

phenylquinolin-2(1H)-one (95c). Compound **95c** was synthesized via general procedure B using **95b** (558 mg, 1.10 mmol) with hydrazine hydrate (107 μL, 2.20 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 40 g; 0-15% EtOH/DCM) to afford **95c** (501 mg, yield 87%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.20 (s, 1H), 7.69 – 7.63 (m, 2H), 7.59 – 7.46 (m, 8H), 7.41 (d, *J* = 8.7 Hz, 1H), 7.37 – 7.31 (m, 1H), 7.26 – 7.20 (m, 1H), 7.17 (d, *J* = 8.3 Hz, 2H), 6.90 (d, *J* = 2.3 Hz, 1H), 4.63 (ddd, *J* = 11.3, 9.5, 3.0 Hz, 1H), 3.27 (dd, *J* = 16.4, 11.1 Hz, 1H), 2.60 (dd, *J* = 16.4, 9.4 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 160.53, 148.37, 145.49, 143.11, 138.74, 137.45, 136.97, 135.20, 132.16, 130.35, 129.23, 128.82, 128.69, 128.28, 128.18, 127.14, 126.77, 126.49, 125.77, 125.63, 120.91, 117.34, 62.49, 44.45. R_t 1.99 min (apolar method). ESI-MS for C₃₀H₂₁Cl₂N₃O: calculated 509.1, found *m/z* 510.0, 511.9, 514.0 [M+H]⁺; 508.0, 510.0, 511.9 [M-H]⁻.

(*E*)-3-(3-(4'-bromo-[1,1'-biphenyl]-4-yl)acryloyl)-6-chloro-4-phenylquinolin-2(1H)-one

(96b). Compound **96b** was synthesized via general procedure A using **60a** (337 mg, 1.10 mmol)

and 4'-bromo-[1,1'-biphenyl]-4-carbaldehyde **72** (287 mg, 1.10 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-5% EtOH/DCM) to afford **96b** (576 mg, yield 89%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.35 (s, 1H), 7.80 – 7.68 (m, 4H), 7.68 – 7.62 (m, 5H), 7.57 – 7.39 (m, 5H), 7.34 (dd, *J* = 7.7, 1.8 Hz, 2H), 6.99 (d, *J* = 2.3 Hz, 1H), 6.81 (d, *J* = 16.4 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 193.81, 159.41, 146.76, 145.52, 140.83, 138.24, 137.53, 133.74, 133.60, 132.51, 131.85, 130.92, 129.36, 128.88, 128.82, 128.77, 128.44, 127.43, 126.94, 126.07, 125.58, 121.53, 120.57, 117.69, 79.15. *R*_t 2.20 min (apolar method). ESI-MS for C₃₀H₁₉BrClNO₂: calculated 539.0, found *m/z* 539.9, 541.9, 543.9 [M+H]⁺; 538.0, 540.0, 542.0 [M-H]⁻.

3-(5-(4'-bromo-[1,1'-biphenyl]-4-yl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (96c). Compound **96c** was synthesized via general procedure B using **96b** (570 mg, 1.00 mmol) with hydrazine hydrate (97 μL, 2.00 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-10% EtOH/DCM) to afford **96c** (472 mg, yield 81%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.20 (s, 1H), 7.68 – 7.46 (m, 10H), 7.41 (d, *J* = 8.8 Hz, 1H), 7.37 – 7.33 (m, 1H), 7.26 – 7.21 (m, 1H), 7.19 – 7.15 (m, 3H), 6.90 (d, *J* = 2.4 Hz, 1H), 4.63 (ddd, *J* = 11.3, 9.5, 3.1 Hz, 1H), 3.27 (dd, *J* = 16.4, 11.0 Hz, 1H), 2.60 (dd, *J* = 16.4, 9.4 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 160.53, 148.37, 145.48, 143.17, 139.11, 137.48, 136.97, 135.20, 131.74, 130.36, 129.23, 128.69, 128.63, 128.27, 128.18, 127.16, 126.77, 126.45, 125.76, 125.63, 120.91, 120.72, 117.34, 79.15, 62.49, 44.45, 40.15, 39.99, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89. *R*_t 2.05 min (apolar method). ESI-MS for C₃₀H₂₁BrClN₃O: calculated 553.0, found *m/z* 553.9, 555.9, 557.9 [M+H]⁺, 551.9, 554.0, 556.0 [M-H]⁻.

(*E*)-6-chloro-3-(3-(4'-methoxy-[1,1'-biphenyl]-4-yl)acryloyl)-4-phenylquinolin-2(1H)-one (97b). Compound **97b** was synthesized via general procedure A using **60a** (430 mg, 1.40 mmol) and 4'-methoxy-[1,1'-biphenyl]-4-carbaldehyde **73** (297 mg, 1.40 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-10% EtOH/DCM) to afford **97b** (528 mg,

yield 74%). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 12.35 (s, 1H), 7.75 – 7.62 (m, 6H), 7.55 – 7.40 (m, 6H), 7.33 (ddt, $J = 6.7, 3.2, 1.8$ Hz, 2H), 7.05 – 7.00 (m, 2H), 6.98 (d, $J = 2.4$ Hz, 1H), 6.77 (d, $J = 16.4$ Hz, 1H), 3.79 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 193.75, 159.42, 159.38, 159.07, 146.68, 145.85, 141.92, 137.52, 133.63, 132.58, 131.32, 131.13, 130.90, 129.30, 128.88, 128.81, 128.69, 128.65, 128.43, 127.84, 126.86, 126.37, 126.22, 126.06, 125.69, 125.57, 120.58, 117.69, 114.43, 55.19, 31.34. R_t 1.81 min (apolar method). ESI-MS for $\text{C}_{31}\text{H}_{22}\text{ClNO}_3$: calculated 491.1, found m/z 492.0, 494.0 $[\text{M}+\text{H}]^+$; 490.0, 492.1 $[\text{M}-\text{H}]^-$.

6-chloro-3-(5-(4'-methoxy-[1,1'-biphenyl]-4-yl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (97c). Compound **97c** was synthesized via general procedure B using **97b** (522 mg, 1.10 mmol) with hydrazine hydrate (107 μL , 2.20 mmol). Purification performed by direct phase flash chromatography (SiO_2 gold 40 g; 0-10% EtOH/DCM) to afford **97c** (412 mg, yield 77%). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 12.20 (s, 1H), 7.57 (dd, $J = 8.9, 2.4$ Hz, 3H), 7.54 – 7.44 (m, 5H), 7.41 (d, $J = 8.8$ Hz, 1H), 7.38 – 7.31 (m, 1H), 7.24 (td, $J = 4.2, 3.7, 1.8$ Hz, 1H), 7.16 – 7.11 (m, 3H), 7.01 (d, $J = 8.8$ Hz, 2H), 6.90 (d, $J = 2.3$ Hz, 1H), 4.60 (ddd, $J = 11.0, 9.5, 3.1$ Hz, 1H), 3.79 (s, 3H), 3.25 (dd, $J = 16.4, 11.0$ Hz, 1H), 2.60 (dd, $J = 16.4, 9.5$ Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 160.54, 158.79, 148.34, 145.49, 141.88, 138.51, 136.97, 135.21, 132.34, 130.35, 129.24, 128.71, 128.27, 128.18, 127.60, 126.99, 126.82, 126.01, 125.75, 125.63, 120.93, 117.33, 114.32, 62.58, 55.14, 44.44. R_t 2.60 min (generic method). ESI-MS for $\text{C}_{31}\text{H}_{24}\text{ClN}_3\text{O}_2$: calculated 505.2, found m/z 506.0, 508.0 $[\text{M}+\text{H}]^+$; 504.1, 506.1 $[\text{M}-\text{H}]^-$.

(E)-6-chloro-3-(3-(1-methyl-1H-indol-5-yl)acryloyl)-4-phenylquinolin-2(1H)-one (98b). Compound **98b** was synthesized via general procedure A using **60a** (545 mg, 1.80 mmol) and 1-methyl-1H-indole-5-carbaldehyde **74** (287 mg, 1.80 mmol). Purification performed by direct phase flash chromatography (SiO_2 gold 24 g; 0-10% EtOH/DCM) to afford **98b** (427 mg, yield 53%). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 12.31 (s, 1H), 7.86 (d, $J = 1.5$ Hz, 1H), 7.65 (dd, $J = 8.8, 2.3$ Hz, 1H), 7.56 (d, $J = 16.3$ Hz, 1H), 7.51 – 7.30 (m, 9H), 6.97 (d, $J = 2.3$ Hz, 1H), 6.65 (d, $J = 16.3$ Hz,

1H), 6.44 (dd, $J = 3.2, 0.7$ Hz, 1H), 3.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 193.46, 159.48, 148.78, 146.31, 137.77, 137.46, 133.77, 132.92, 131.03, 130.76, 128.89, 128.72, 128.37, 128.15, 125.99, 125.53, 125.36, 124.31, 123.22, 121.09, 120.65, 117.63, 110.33, 101.50, 32.61. R_t 2.45 min (generic method). ESI-MS for $\text{C}_{27}\text{H}_{19}\text{ClN}_2\text{O}_2$: calculated 438.1, found m/z 439.2, 441.1 $[\text{M}+\text{H}]^+$; 437.3, 439.3 $[\text{M}-\text{H}]^-$.

6-chloro-3-(5-(1-methyl-1H-indol-5-yl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (98c). Compound **98c** was synthesized via general procedure B using **98b** (370 mg, 0.84 mmol) with hydrazine hydrate (82 μL , 1.68 mmol). Purification performed by direct phase flash chromatography (SiO_2 gold 24 g; 0-5% EtOH/DCM) to afford **98c** (325 mg, yield 85%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.20 (s, 1H), 7.57 – 7.46 (m, 4H), 7.41 (d, $J = 8.8$ Hz, 1H), 7.35 (dt, $J = 7.2, 1.7$ Hz, 1H), 7.31 – 7.21 (m, 4H), 7.02 (s, 1H), 6.91 (td, $J = 3.9, 1.7$ Hz, 2H), 6.34 (dd, $J = 3.0, 0.7$ Hz, 1H), 4.66 (t, $J = 10.5$ Hz, 1H), 3.75 (s, 3H), 3.22 (dd, $J = 16.4, 11.0$ Hz, 1H), 2.63 (dd, $J = 16.4, 9.9$ Hz, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 160.60, 148.21, 145.51, 136.97, 135.70, 135.30, 133.87, 130.28, 129.72, 129.24, 128.74, 128.27, 128.17, 127.79, 127.03, 125.76, 125.62, 120.96, 120.01, 118.09, 117.32, 109.44, 100.21, 63.57, 44.92, 32.45. R_t 2.38 min (generic method). ESI-MS for $\text{C}_{27}\text{H}_{21}\text{ClN}_4\text{O}$: calculated 452.1, found m/z 453.2, 455.2 $[\text{M}+\text{H}]^+$; 451.3, 453.3 $[\text{M}-\text{H}]^-$.

(E)-6-chloro-3-(3-(1-ethyl-1H-indol-5-yl)acryloyl)-4-phenylquinolin-2(1H)-one (99b). Compound **99b** was synthesized via general procedure A using **60a** (402 mg, 1.30 mmol) and 1-ethyl-1H-indole-5-carbaldehyde **75** (225 mg, 1.30 mmol). Purification performed by direct phase flash chromatography (SiO_2 gold 40 g; 0-5% EtOH/DCM) to afford **99b** (395 mg, yield 65%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.31 (s, 1H), 7.85 (d, $J = 1.4$ Hz, 1H), 7.65 (dd, $J = 8.8, 2.4$ Hz, 1H), 7.56 (d, $J = 16.3$ Hz, 1H), 7.50 – 7.45 (m, 3H), 7.45 – 7.38 (m, 4H), 7.33 (dd, $J = 7.7, 1.8$ Hz, 2H), 6.97 (d, $J = 2.3$ Hz, 1H), 6.65 (d, $J = 16.2$ Hz, 1H), 6.45 (d, $J = 3.1$ Hz, 1H), 4.20 (q, $J = 7.2$ Hz, 2H), 1.34 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 193.47, 159.48, 148.79, 146.30, 137.46, 136.80, 133.77, 132.92, 130.76, 129.42, 128.98, 128.89, 128.72, 128.66, 128.37,

128.30, 125.99, 125.53, 125.35, 124.27, 123.35, 121.02, 120.65, 117.63, 110.32, 101.75, 79.15, 15.44. R_t 2.56 min (generic method). ESI-MS for $C_{28}H_{21}ClN_2O_2$: calculated 452.1, found m/z 453.2, 455.2 $[M+H]^+$; 451.3, 453.3 $[M-H]^-$.

6-chloro-3-(5-(1-ethyl-1H-indol-5-yl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-

2(1H)-one (99c). Compound **99c** was synthesized via general procedure B using **99b** (336 mg, 0.79 mmol) with hydrazine hydrate (77 μ L, 1.58 mmol). Purification performed by direct phase flash chromatography (SiO_2 gold 24 g; 0-10% EtOH/DCM) to afford **99c** (322 mg, yield 87%). 1H NMR (400 MHz, $DMSO-d_6$) δ 12.18 (s, 1H), 7.60 – 7.48 (m, 4H), 7.41 (d, J = 8.8 Hz, 1H), 7.38 – 7.30 (m, 3H), 7.25 (dt, J = 6.6, 2.1 Hz, 2H), 7.00 (d, J = 3.2 Hz, 1H), 6.89 (dd, J = 9.7, 2.0 Hz, 2H), 6.34 (dd, J = 3.2, 0.8 Hz, 1H), 4.64 (td, J = 10.4, 3.2 Hz, 1H), 4.17 (q, J = 7.2 Hz, 2H), 3.21 (dd, J = 16.4, 11.0 Hz, 1H), 2.63 (dd, J = 16.4, 9.9 Hz, 1H), 1.33 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 160.59, 148.22, 145.47, 136.97, 135.29, 134.66, 133.82, 130.30, 129.23, 128.75, 128.27, 128.18, 128.12, 127.94, 127.04, 125.74, 125.63, 120.97, 119.94, 118.22, 117.33, 109.48, 100.42, 63.56, 56.02, 44.87, 18.54, 15.49. R_t 2.51 min (generic method). ESI-MS for $C_{28}H_{23}ClN_4O$: calculated 466.2, found m/z 467.2, 469.2 $[M+H]^+$; 465.3, 467.4 $[M-H]^-$.

(E)-6-chloro-3-(3-(1-ethyl-1H-indazol-5-yl)acryloyl)-4-phenylquinolin-2(1H)-one (100b)

Compound **100b** was synthesized via general procedure A using **60a** (292 mg, 0.98 mmol) and 1-ethyl-1H-indazole-5-carbaldehyde **76** (171 mg, 0.98 mmol). Purification performed by direct phase flash chromatography (SiO_2 gold 24 g; 0-5% EtOH/DCM) to afford **100b** (275 mg, yield 61%). 1H NMR (400 MHz, $DMSO-d_6$) δ 12.33 (s, 1H), 8.09 (s, 1H), 8.07 (d, J = 1.4 Hz, 1H), 7.72 (dd, J = 8.9, 1.6 Hz, 1H), 7.69 – 7.57 (m, 3H), 7.48 (d, J = 8.8 Hz, 1H), 7.46 – 7.36 (m, 3H), 7.34 (dd, J = 7.8, 1.8 Hz, 2H), 6.98 (d, J = 2.3 Hz, 1H), 6.74 (d, J = 16.3 Hz, 1H), 4.43 (q, J = 7.2 Hz, 2H), 1.38 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 193.64, 159.45, 147.35, 146.51, 139.48, 137.50, 133.76, 133.70, 132.72, 130.84, 128.89, 128.76, 128.40, 126.97, 126.03, 125.64, 125.55,

125.18, 123.97, 123.80, 120.63, 117.66, 110.26, 43.20, 14.85. R_t 2.35 min (generic method). ESI-MS for $C_{27}H_{20}ClN_3O_2$: calculated 453.1, found m/z 454.4, 456.4 $[M+H]^+$, 452.4, 454.4 $[M-H]^-$.

6-chloro-3-(5-(1-ethyl-1H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (100c). Compound **100c** was synthesized via general procedure B using **100b** (260 mg, 0.57 mmol) with hydrazine hydrate (55 μ L, 1.14 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-10% EtOH/DCM) to afford **100c** (228 mg, yield 86%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.21 (s, 1H), 7.97 (d, J = 0.9 Hz, 1H), 7.59 – 7.46 (m, 5H), 7.42 (d, J = 8.8 Hz, 2H), 7.38 – 7.34 (m, 1H), 7.27 – 7.21 (m, 1H), 7.17 – 7.08 (m, 2H), 6.91 (d, J = 2.4 Hz, 1H), 4.71 (ddd, J = 11.3, 9.3, 2.5 Hz, 1H), 4.41 (q, J = 7.2 Hz, 2H), 3.27 (dd, J = 16.5, 11.1 Hz, 1H), 2.62 (dd, J = 16.5, 9.4 Hz, 1H), 1.38 (t, J = 7.2 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 161.06, 148.81, 145.93, 138.63, 137.46, 136.04, 135.71, 134.85, 132.74, 130.82, 129.75, 129.17, 128.76, 128.70, 127.36, 126.93, 126.25, 126.11, 125.62, 124.49, 123.84, 121.42, 118.50, 117.82, 110.00, 109.89, 63.43, 45.22, 43.52, 15.40. R_t 2.19 min (generic method). ESI-MS for $C_{27}H_{22}ClN_5O$: calculated 467.1, found m/z 468.5, 470.5 $[M+H]^+$, 466.5, 468.5 $[M-H]^-$.

(E)-6-chloro-3-(3-(2-ethyl-2H-indazol-5-yl)acryloyl)-4-phenylquinolin-2(1H)-one (101b). Compound **101b** was synthesized via general procedure A using **60a** (307 mg, 1.00 mmol) and 2-ethyl-2H-indazole-5-carbaldehyde **77** (174 mg, 1.00 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-5% EtOH/DCM) to afford **101b** (386 mg, yield 82%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.33 (s, 1H), 8.48 (s, 1H), 8.02 (s, 1H), 7.65 (dd, J = 8.8, 2.4 Hz, 1H), 7.61 – 7.51 (m, 3H), 7.51 – 7.38 (m, 4H), 7.34 (dd, J = 7.7, 1.9 Hz, 2H), 6.98 (d, J = 2.3 Hz, 1H), 6.68 (d, J = 16.3 Hz, 1H), 4.44 (q, J = 7.3 Hz, 2H), 1.49 (t, J = 7.2 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 193.61, 159.46, 148.47, 147.59, 146.48, 137.49, 133.71, 132.78, 130.83, 128.88, 128.76, 128.40, 127.38, 126.02, 125.55, 125.30, 125.16, 123.56, 121.42, 120.63, 117.65, 117.56, 47.87, 15.57. R_t 2.22 min (generic method). ESI-MS for $C_{27}H_{20}ClN_3O_2$: calculated 453.1, found m/z 454.4, 456.4 $[M+H]^+$, 452.4, 454.4 $[M-H]^-$.

6-chloro-3-(5-(2-ethyl-2H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-

2(1H)-one (101c). Compound **101c** was synthesized via general procedure B using **101b** (302 mg, 0.60 mmol) with hydrazine hydrate (58 μ L, 1.20 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-10% EtOH/DCM) to afford **101c** (236 mg, yield 76%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.19 (s, 1H), 8.27 (d, *J* = 0.9 Hz, 1H), 7.59 – 7.38 (m, 6H), 7.35 (td, *J* = 3.1, 1.5 Hz, 2H), 7.28 – 7.20 (m, 1H), 7.08 (d, *J* = 3.2 Hz, 1H), 6.98 – 6.86 (m, 2H), 4.65 (td, *J* = 10.3, 3.1 Hz, 1H), 4.42 (q, *J* = 7.3 Hz, 2H), 3.24 (dd, *J* = 16.5, 11.1 Hz, 1H), 2.60 (dd, *J* = 16.5, 9.6 Hz, 1H), 1.48 (t, *J* = 7.3 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 160.56, 148.29, 147.50, 145.53, 136.97, 135.54, 135.22, 130.32, 129.28, 128.68, 128.27, 128.20, 126.91, 125.76, 125.62, 124.73, 122.79, 121.02, 120.94, 117.33, 117.28, 117.07, 63.27, 47.64, 44.41, 15.77. *R*_t 2.06 min (generic method). ESI-MS for C₂₇H₂₂ClN₅O: calculated 467.1, found *m/z* 468.5, 470.4 [M+H]⁺, 466.4, 468.5 [M-H]⁻.

(E)-6-chloro-4-phenyl-3-(3-(1-propyl-1H-indazol-5-yl)acryloyl)quinolin-2(1H)-one (102b).

Compound **102b** was synthesized via general procedure A using **60a** (402 mg, 1.35 mmol) and 1-propyl-1H-indazole-5-carbaldehyde **78** (254 mg, 1.35 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-7% EtOH/DCM) to afford **102b** (401 mg, yield 64%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.33 (s, 1H), 8.10 (d, *J* = 0.8 Hz, 1H), 8.07 (d, *J* = 1.4 Hz, 1H), 7.72 (dd, *J* = 9.0, 1.6 Hz, 1H), 7.70 – 7.57 (m, 3H), 7.48 (d, *J* = 8.8 Hz, 1H), 7.44 – 7.38 (m, 3H), 7.34 (dd, *J* = 7.7, 1.8 Hz, 2H), 6.98 (d, *J* = 2.4 Hz, 1H), 6.74 (d, *J* = 16.3 Hz, 1H), 4.36 (t, *J* = 6.9 Hz, 2H), 1.82 (h, *J* = 7.2 Hz, 2H), 0.80 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 193.66, 159.46, 147.36, 146.52, 140.12, 137.50, 133.80, 133.70, 132.74, 130.85, 128.89, 128.77, 128.41, 126.94, 126.03, 125.65, 125.55, 125.20, 123.98, 123.65, 120.64, 117.67, 110.36, 49.71, 22.82, 11.04. *R*_t 2.51 min (generic method). ESI-MS for C₂₈H₂₂ClN₃O₂: calculated 467.1, found *m/z* 468.2, 470.2 [M+H]⁺, 466.3, 468.3 [M-H]⁻.

6-chloro-4-phenyl-3-(5-(1-propyl-1H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (102c). Compound **102c** was synthesized via general procedure B using **102b** (395 mg, 0.84 mmol) with hydrazine hydrate (82 μ L, 1.68 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-10% EtOH/DCM) to afford **102c** (381 mg, yield 94%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.19 (s, 1H), 8.00 – 7.91 (m, 1H), 7.59 – 7.45 (m, 6H), 7.42 (d, *J* = 8.7 Hz, 2H), 7.38 – 7.31 (m, 1H), 7.24 (dd, *J* = 6.8, 1.9 Hz, 1H), 7.12 (dd, *J* = 8.7, 1.6 Hz, 1H), 6.90 (d, *J* = 2.3 Hz, 1H), 4.70 (t, *J* = 10.3 Hz, 1H), 4.33 (t, *J* = 6.9 Hz, 2H), 3.25 (dd, *J* = 16.5, 11.1 Hz, 1H), 2.62 (dd, *J* = 16.5, 9.5 Hz, 1H), 1.88 – 1.77 (m, 2H), 0.81 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 160.54, 148.29, 145.42, 138.73, 136.96, 135.44, 135.21, 132.22, 130.31, 129.23, 128.68, 128.25, 128.22, 128.18, 126.87, 125.74, 125.60, 125.13, 123.18, 120.92, 117.98, 117.32, 109.56, 62.94, 49.59, 44.66, 22.81, 11.09. *R*_t 2.34 min (generic method). ESI-MS for C₂₈H₂₄ClN₅O: calculated 481.2, found *m/z* 482.1, 484.2 [M+H]⁺, 480.2, 482.3 [M-H]⁻.

(*E*)-6-chloro-4-phenyl-3-(3-(2-propyl-2H-indazol-5-yl)acryloyl)quinolin-2(1H)-one (103b). Compound **103b** was synthesized via general procedure A using **60a** (292 mg, 0.98 mmol) and 2-propyl-2H-indazole-5-carbaldehyde **79** (184 mg, 0.98 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-7% EtOH/DCM) to afford **103b** (372 mg, yield 81%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.33 (s, 1H), 8.47 (s, 1H), 8.04 – 8.00 (m, 1H), 7.65 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.60 – 7.51 (m, 3H), 7.47 (d, *J* = 8.8 Hz, 1H), 7.44 – 7.38 (m, 3H), 7.36 – 7.32 (m, 2H), 6.97 (d, *J* = 2.3 Hz, 1H), 6.68 (d, *J* = 16.2 Hz, 1H), 4.36 (t, *J* = 6.9 Hz, 2H), 1.96-1.87 (m, 2H), 0.83 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 193.63, 159.46, 148.52, 147.59, 146.47, 137.50, 133.71, 132.79, 130.83, 128.88, 128.77, 128.41, 127.39, 126.02, 125.87, 125.55, 125.31, 123.56, 121.32, 120.63, 117.66, 117.59, 54.36, 23.22, 10.84. *R*_t 2.37 min (generic method). ESI-MS for C₂₈H₂₂ClN₃O₂: calculated 467.1, found *m/z* 468.2, 470.2 [M+H]⁺, 466.3, 468.3 [M-H]⁻.

6-chloro-4-phenyl-3-(5-(2-propyl-2H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (103c). Compound **103c** was synthesized via general procedure B using **103b** (342 mg,

0.73 mmol) with hydrazine hydrate (71 μ L, 1.46 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-10% EtOH/DCM) to afford **103c** (316 mg, yield 89%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.19 (s, 1H), 8.27 (d, *J* = 0.9 Hz, 1H), 7.57 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.55 – 7.44 (m, 4H), 7.41 (d, *J* = 8.8 Hz, 1H), 7.38 – 7.33 (m, 2H), 7.24 (dt, *J* = 6.6, 1.9 Hz, 1H), 7.08 (d, *J* = 3.2 Hz, 1H), 6.92 (dd, *J* = 8.9, 1.6 Hz, 1H), 6.90 (d, *J* = 2.4 Hz, 1H), 4.70 – 4.59 (m, 1H), 4.35 (t, *J* = 6.9 Hz, 2H), 3.24 (dd, *J* = 16.5, 11.1 Hz, 1H), 2.60 (dd, *J* = 16.4, 9.6 Hz, 1H), 1.96-1.87 (m, 2H), 0.82 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 160.58, 148.31, 147.56, 145.50, 136.98, 135.55, 135.23, 130.37, 129.31, 128.70, 128.30, 128.28, 128.23, 126.93, 125.76, 125.64, 124.75, 123.59, 120.95, 120.89, 117.36, 117.31, 117.11, 63.26, 54.20, 44.40, 23.40, 10.89. R_t 2.23 min (generic method). ESI-MS for C₂₈H₂₄ClN₅O: calculated 481.2, found *m/z* 482.2, 484.2 [M+H]⁺, 480.3, 482.3 [M-H]⁻.

(*E*)-6-chloro-3-(3-(1-cyclohexyl-1H-indazol-5-yl)acryloyl)-4-phenylquinolin-2(1H)-one

(104b). Compound **104b** was synthesized via general procedure A using **60a** (134 mg, 0.46 mmol) and 1-cyclohexyl-1H-indazole-5-carbaldehyde **80** (105 mg, 0.46 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-5% EtOH/DCM) to afford **104b** (193 mg, yield 83%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.33 (s, 1H), 8.08 (s, 1H), 8.06 (d, *J* = 1.4 Hz, 1H), 7.72 – 7.69 (m, 2H), 7.65 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.60 (d, *J* = 16.3 Hz, 1H), 7.48 (d, *J* = 8.8 Hz, 1H), 7.45 – 7.37 (m, 3H), 7.33 (dd, *J* = 7.7, 1.8 Hz, 2H), 6.98 (d, *J* = 2.4 Hz, 1H), 6.73 (d, *J* = 16.3 Hz, 1H), 4.60 (tt, *J* = 9.6, 4.8 Hz, 1H), 1.98 – 1.80 (m, 6H), 1.70 (d, *J* = 13.0 Hz, 1H), 1.48 (td, *J* = 12.4, 11.1, 4.6 Hz, 2H), 1.25 (dtd, *J* = 12.9, 9.7, 3.6 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 193.66, 159.47, 147.44, 146.53, 139.15, 137.51, 133.72, 133.57, 132.73, 130.86, 128.90, 128.79, 128.42, 127.00, 126.05, 125.59, 125.56, 125.00, 123.98, 123.66, 120.65, 117.68, 110.37, 56.77, 32.26, 24.98. R_t 1.94 min (apolar method). ESI-MS for C₃₁H₂₆ClN₃O₂: calculated 507.2, found *m/z* 508.2, 510.2 [M+H]⁺, 506.3, 508.3 [M-H]⁻.

6-chloro-3-(5-(1-cyclohexyl-1H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-3-yl)-4-

phenylquinolin-2(1H)-one (104c). Compound **104c** was synthesized via general procedure B using **104b** (187 mg, 0.37 mmol) with hydrazine hydrate (36 μ L, 0.74 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-10% EtOH/DCM) to afford **104c** (172 mg, yield 86%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.20 (s, 1H), 7.95 (d, *J* = 0.8 Hz, 1H), 7.62 – 7.55 (m, 2H), 7.50 (dddd, *J* = 13.9, 7.1, 5.1, 4.0 Hz, 3H), 7.44 – 7.39 (m, 2H), 7.38 – 7.32 (m, 1H), 7.28 – 7.20 (m, 1H), 7.15 – 7.08 (m, 2H), 6.90 (d, *J* = 2.3 Hz, 1H), 4.69 (ddd, *J* = 11.0, 9.5, 3.2 Hz, 1H), 4.55 (tt, *J* = 10.0, 5.3 Hz, 1H), 3.25 (dd, *J* = 16.5, 11.1 Hz, 1H), 2.61 (dd, *J* = 16.5, 9.5 Hz, 1H), 1.88 (ddd, *J* = 20.4, 10.2, 3.5 Hz, 6H), 1.71 (dt, *J* = 13.0, 3.2 Hz, 1H), 1.51 (qd, *J* = 12.1, 11.7, 5.9 Hz, 2H), 1.26 (tdd, *J* = 12.8, 9.5, 4.4 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 160.56, 148.32, 145.45, 137.82, 136.98, 135.55, 135.24, 132.00, 130.35, 129.24, 128.70, 128.29, 128.26, 128.21, 126.89, 125.76, 125.63, 124.92, 123.21, 120.93, 118.01, 117.35, 109.60, 62.97, 56.62, 44.74, 32.30, 25.06. *R*_t 2.65 min (generic method). ESI-MS for C₃₁H₂₈ClN₅O: calculated 521.2, found *m/z* 522.3, 524.3 [M+H]⁺, 520.3, 522.4 [M-H]⁻.

(*E*)-6-chloro-3-(3-(2-cyclohexyl-2H-indazol-5-yl)acryloyl)-4-phenylquinolin-2(1H)-one

(105b). Compound **105b** was synthesized via general procedure A using **60a** (89 mg, 0.30 mmol) and 2-cyclohexyl-2H-indazole-5-carbaldehyde **81** (68 mg, 0.30 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 12 g; 0-5% EtOH/DCM) to afford **105b** (136 mg, yield 90%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.33 (s, 1H), 8.49 (s, 1H), 8.00 (d, *J* = 1.5 Hz, 1H), 7.65 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.59 – 7.52 (m, 3H), 7.47 (d, *J* = 8.8 Hz, 1H), 7.45 – 7.38 (m, 3H), 7.35 – 7.30 (m, 2H), 6.97 (d, *J* = 2.3 Hz, 1H), 6.66 (d, *J* = 16.3 Hz, 1H), 4.45 (tt, *J* = 11.3, 3.8 Hz, 1H), 2.09 (dd, *J* = 13.0, 4.0 Hz, 2H), 1.86 (ddt, *J* = 14.4, 9.8, 5.6 Hz, 4H), 1.76 – 1.63 (m, 1H), 1.51 – 1.37 (m, 2H), 1.25 (qt, *J* = 13.1, 3.4 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 193.63, 159.47, 148.04, 147.67, 146.47, 137.49, 133.72, 132.78, 130.84, 128.89, 128.78, 128.41, 127.34, 126.03, 125.55, 125.42, 125.24, 123.78, 123.45, 121.13, 120.65, 117.70, 61.84, 33.12, 24.85, 24.82. *R*_t 2.60

min (generic method). ESI-MS for $C_{31}H_{26}ClN_3O_2$: calculated 507.2, found m/z 508.3, 510.2 $[M+H]^+$, 506.3, 508.4 $[M-H]^-$.

6-chloro-3-(5-(2-cyclohexyl-2H-indazol-5-yl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (105c). Compound **105c** was synthesized via general procedure B using **105b** (130 mg, 0.26 mmol) with hydrazine hydrate (25 μ L, 0.52 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-10% EtOH/DCM) to afford **104c** (117 mg, yield 86%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.19 (s, 1H), 8.28 (d, J = 0.9 Hz, 1H), 7.59 – 7.44 (m, 5H), 7.41 (d, J = 8.8 Hz, 1H), 7.38 – 7.31 (m, 2H), 7.23 (dt, J = 6.5, 2.0 Hz, 1H), 7.07 (d, J = 3.2 Hz, 1H), 6.94 – 6.87 (m, 2H), 4.64 (td, J = 9.9, 3.1 Hz, 1H), 4.43 (tt, J = 11.3, 3.8 Hz, 1H), 3.23 (dd, J = 16.5, 11.1 Hz, 1H), 2.59 (dd, J = 16.4, 9.6 Hz, 1H), 2.15 – 2.03 (m, 2H), 1.90 – 1.80 (m, 4H), 1.69 (dt, J = 12.5, 3.3 Hz, 1H), 1.44 (qt, J = 13.0, 3.5 Hz, 2H), 1.35 – 1.17 (m, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 160.58, 148.32, 147.05, 145.53, 136.99, 135.52, 135.24, 130.37, 129.30, 128.70, 128.31, 128.29, 128.24, 126.93, 125.77, 125.65, 124.65, 121.38, 120.96, 120.70, 117.37, 117.34, 117.22, 63.29, 61.58, 44.46, 33.30, 24.93, 24.90. R_t 2.47 min (generic method). ESI-MS for $C_{31}H_{28}ClN_5O$: calculated 521.2, found m/z 522.2, 524.2 $[M+H]^+$, 520.2, 522.3 $[M-H]^-$.

(E)-6-chloro-3-(3-(4-(1-ethyl-1H-indazol-5-yl)phenyl)acryloyl)-4-phenylquinolin-2(1H)-one (106b). Compound **106b** was synthesized via general procedure A using **60a** (137 mg, 0.46 mmol) and 4-(1-ethyl-1H-indazol-5-yl)benzaldehyde **82** (115 mg, 0.46 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-2% EtOH/DCM) to afford **106b** (244 mg, quantitative yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.35 (s, 1H), 8.10 (d, J = 7.3 Hz, 2H), 7.74 (s, 5H), 7.65 (dd, J = 8.8, 2.4 Hz, 1H), 7.59 – 7.39 (m, 6H), 7.34 (dd, J = 7.8, 1.8 Hz, 2H), 6.99 (d, J = 2.4 Hz, 1H), 6.80 (d, J = 16.3 Hz, 1H), 4.46 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 193.80, 159.46, 146.73, 145.88, 142.84, 138.45, 137.54, 133.65, 133.16, 132.74, 132.59, 131.71, 130.93, 129.35, 128.91, 128.47, 127.09, 126.97, 126.09, 125.60,

125.35, 124.25, 120.61, 118.93, 117.71, 110.15, 43.17, 14.92. R_t 2.64 min (generic method). ESI-MS for $C_{33}H_{24}ClN_3O_2$: calculated 529.2, found m/z 530.2, 532.2 $[M+H]^+$, 528.3, 530.3 $[M-H]^-$.

6-chloro-3-(5-(4-(1-ethyl-1H-indazol-5-yl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (106c). Compound **106c** was synthesized via general procedure B using **106b** (233 mg, 0.44 mmol) with hydrazine hydrate (43 μ L, 0.88 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-8% EtOH/DCM) to afford **106c** (181 mg, yield 75%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.20 (s, 1H), 8.10 (d, J = 0.9 Hz, 1H), 7.98 (t, J = 1.2 Hz, 1H), 7.74 (d, J = 8.8 Hz, 1H), 7.67 (dd, J = 8.8, 1.7 Hz, 1H), 7.60 – 7.47 (m, 6H), 7.42 (d, J = 8.8 Hz, 1H), 7.36 (dt, J = 6.4, 1.8 Hz, 1H), 7.28 – 7.24 (m, 1H), 7.20 – 7.14 (m, 3H), 6.90 (d, J = 2.4 Hz, 1H), 4.63 (td, J = 10.9, 10.3, 3.2 Hz, 1H), 4.46 (q, J = 7.2 Hz, 2H), 3.29 – 3.20 (m, 1H), 2.62 (dd, J = 16.4, 9.4 Hz, 1H), 1.41 (t, J = 7.2 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 160.55, 148.36, 145.50, 142.03, 139.35, 138.19, 136.98, 135.22, 132.90, 132.66, 130.37, 129.25, 128.73, 128.29, 128.20, 127.06, 126.83, 126.69, 125.76, 125.64, 125.48, 124.24, 120.94, 118.34, 117.35, 110.00, 62.58, 44.46, 43.14, 14.93. R_t 1.49 min (apolar method). ESI-MS for $C_{33}H_{26}ClN_5O$: calculated 543.2, found m/z 544.1, 546.1 $[M+H]^+$, 542.2, 544.1 $[M-H]^-$.

(E)-6-chloro-3-(3-(4-(2-ethyl-2H-indazol-5-yl)phenyl)acryloyl)-4-phenylquinolin-2(1H)-one (107b). Compound **107b** was synthesized via general procedure A using **60a** (92 mg, 0.31 mmol) and 4-(2-ethyl-2H-indazol-5-yl)benzaldehyde **83** (78 mg, 0.31 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 12 g; 0-10% EtOH/DCM) to afford **107b** (150 mg, yield 91%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.35 (s, 1H), 8.44 (d, J = 0.8 Hz, 1H), 8.04 (t, J = 1.3 Hz, 1H), 7.73 (s, 4H), 7.70 – 7.63 (m, 2H), 7.59 (dd, J = 9.1, 1.8 Hz, 1H), 7.57 – 7.42 (m, 5H), 7.34 (dd, J = 7.8, 1.8 Hz, 2H), 6.99 (d, J = 2.3 Hz, 1H), 6.79 (d, J = 16.4 Hz, 1H), 4.47 (q, J = 7.3 Hz, 2H), 1.52 (t, J = 7.3 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 193.79, 159.45, 147.56, 146.70, 145.88, 143.10, 137.54, 133.65, 132.67, 132.59, 131.84, 130.91, 129.31, 128.90, 128.83, 128.45, 126.92, 126.07, 125.58, 124.89, 124.04, 121.88, 120.60, 118.56, 117.70, 117.56, 47.83,

15.72. R_t 2.48 min (generic method). ESI-MS for $C_{33}H_{24}ClN_3O_2$: calculated 529.2, found m/z 530.1, 532.1 $[M+H]^+$, 528.2, 530.2 $[M-H]^-$.

6-chloro-3-(5-(4-(2-ethyl-2H-indazol-5-yl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (107c). Compound **107c** was synthesized via general procedure B using **107b** (141 mg, 0.27 mmol) with hydrazine hydrate (26 μ L, 0.54 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-15% EtOH/DCM) to afford **107c** (123 mg, yield 83%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.41 (d, J = 0.9 Hz, 1H), 7.92 – 7.88 (m, 1H), 7.67 (d, J = 9.0 Hz, 1H), 7.59 – 7.48 (m, 7H), 7.42 (d, J = 8.8 Hz, 1H), 7.37 – 7.33 (m, 1H), 7.25 (dq, J = 4.9, 1.9 Hz, 1H), 7.16 (dd, J = 9.3, 2.7 Hz, 3H), 6.90 (d, J = 2.4 Hz, 1H), 4.62 (ddd, J = 10.9, 9.4, 3.1 Hz, 1H), 4.47 (q, J = 7.3 Hz, 2H), 3.27 (dd, J = 16.5, 11.1 Hz, 1H), 2.62 (dd, J = 16.4, 9.5 Hz, 1H), 1.52 (t, J = 7.3 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 160.67, 148.34, 147.41, 145.61, 141.99, 139.64, 137.15, 135.26, 132.81, 130.34, 129.28, 128.75, 128.31, 128.21, 127.04, 126.81, 126.55, 125.72, 125.64, 125.23, 123.65, 121.93, 120.96, 117.77, 117.44, 62.61, 47.80, 44.49, 15.76. R_t 2.35 min (generic method). ESI-MS for $C_{33}H_{26}ClN_5O$: calculated 543.2, found m/z 544.2, 546.2 $[M+H]^+$, 542.3, 544.3 $[M-H]^-$.

(E)-6-chloro-4-phenyl-3-(3-(4-(1-propyl-1H-pyrazol-4-yl)phenyl)acryloyl)quinolin-2(1H)-one (108b). Compound **108b** was synthesized via general procedure A using **60a** (324 mg, 1.10 mmol) and 4-(1-propyl-1H-pyrazol-4-yl)benzaldehyde **84** (236 mg, 1.10 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 0-8% EtOH/DCM) to afford **108b** (387 mg, yield 72%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.33 (s, 1H), 8.26 (d, J = 0.8 Hz, 1H), 7.94 (d, J = 0.8 Hz, 1H), 7.68 – 7.55 (m, 5H), 7.50 – 7.38 (m, 5H), 7.33 (dd, J = 7.7, 1.8 Hz, 2H), 6.98 (d, J = 2.3 Hz, 1H), 6.73 (d, J = 16.4 Hz, 1H), 4.06 (t, J = 7.0 Hz, 2H), 1.96-1.87 (m, 2H), 0.84 (t, J = 7.4 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 193.72, 159.43, 146.62, 146.14, 137.50, 136.32, 135.30, 133.65, 132.61, 131.69, 130.87, 129.40, 128.88, 128.79, 128.42, 127.62, 126.26,

126.05, 125.56, 125.07, 120.85, 120.59, 117.67, 53.07, 23.07, 10.90. R_t 2.46 min (generic method).
ESI-MS for $C_{30}H_{24}ClN_3O_2$: calculated 493.2, found m/z 494.0, 496.0 $[M+H]^+$, 492.0, 494.0 $[M-H]^-$.

6-chloro-4-phenyl-3-(5-(4-(1-propyl-1H-pyrazol-4-yl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (108c). Compound **108c** was synthesized via general procedure B using **108b** (358 mg, 0.72 mmol) with hydrazine hydrate (70 μ L, 1.44 mmol). Purification performed by direct phase flash chromatography (SiO_2 gold 24 g; 0-10% EtOH/DCM) to afford **108c** (326 mg, yield 89%). 1H NMR (400 MHz, $DMSO-d_6$) δ 12.18 (s, 1H), 8.13 (d, J = 0.8 Hz, 1H), 7.83 (d, J = 0.8 Hz, 1H), 7.57 (dd, J = 8.8, 2.4 Hz, 1H), 7.51 (tdd, J = 6.6, 3.8, 1.8 Hz, 3H), 7.42 (dd, J = 8.5, 6.2 Hz, 3H), 7.34 (dt, J = 6.8, 1.7 Hz, 1H), 7.26 – 7.22 (m, 1H), 7.09 (d, J = 3.2 Hz, 1H), 7.08 – 7.03 (m, 2H), 6.90 (d, J = 2.3 Hz, 1H), 4.61 – 4.49 (m, 1H), 4.06 (t, J = 7.0 Hz, 2H), 3.22 (dd, J = 16.4, 11.0 Hz, 1H), 2.56 (dd, J = 16.4, 9.6 Hz, 1H), 1.96-1.87 (m, 2H), 0.85 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 160.53, 148.31, 145.48, 140.99, 136.97, 135.81, 135.21, 131.39, 130.34, 129.22, 128.71, 128.27, 128.19, 126.92, 126.85, 126.79, 125.75, 125.62, 124.76, 121.34, 120.92, 117.33, 62.66, 52.98, 44.43, 23.16, 10.94. R_t 2.34 min (generic method). ESI-MS for $C_{30}H_{26}ClN_5O$: calculated 507.2, found m/z 508.0, 510.1 $[M+H]^+$, 506.1, 508.1 $[M-H]^-$.

(E)-3-(3-(4-fluorophenyl)acryloyl)-4-phenylquinolin-2(1H)-one (109b). Compound **109b** was synthesized via general procedure A using **61a** (265 mg, 1.00 mmol) and 4-fluorobenzaldehyde **62** (107 μ L, 1.00 mmol). Title compound **109b** was obtained after precipitation and filtration from the reaction crude (345 mg, yield 93%). 1H NMR (400 MHz, $DMSO-d_6$) δ 12.30 (s, 1H), 7.73 (d, J = 7.2 Hz, 2H), 7.65 – 7.00 (m, 12H), 6.73 (d, J = 16.4 Hz, 1H). ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 194.31, 159.77, 147.76, 144.37, 134.36, 131.36, 130.92, 130.92, 128.92, 128.46, 128.23, 127.52, 126.86, 126.86, 124.54, 122.05, 119.21, 116.00, 115.92, 115.90, 115.88, 115.79. R_t 2.25 min (generic method). ESI-MS for $C_{24}H_{16}FNO_2$: calculated 369.1, found m/z 370.5 $[M+H]^+$; 368.4 $[M-H]^-$.

3-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (109c).

Compound **109c** was synthesized via general procedure B using **109b** (300 mg, 0.81 mmol) with hydrazine hydrate (79 μ L, 1.62 mmol). Title compound **109c** was obtained after precipitation from DCM (292 mg, yield 94%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.11 (s, 1H), 7.54 – 7.44 (m, 4H), 7.40 (dd, J = 8.3, 1.2 Hz, 1H), 7.32 (dt, J = 7.4, 1.7 Hz, 1H), 7.21 (dt, J = 6.8, 1.9 Hz, 1H), 7.14 – 7.02 (m, 6H), 6.99 (dd, J = 8.1, 1.4 Hz, 1H), 4.58 (td, J = 10.9, 3.1 Hz, 1H), 3.23 (dd, J = 16.4, 11.0 Hz, 1H), 2.56 – 2.52 (m, 1H). R_t 2.13 min (generic method). ESI-MS for $\text{C}_{24}\text{H}_{18}\text{FN}_3\text{O}$: calculated 383.1, found m/z 384.5 $[\text{M}+\text{H}]^+$, 382.5 $[\text{M}-\text{H}]^-$.

(E)-3-(3-(4-bromophenyl)acryloyl)-4-phenylquinolin-2(1H)-one (110b). Compound **110b**

was synthesized via general procedure A using **61a** (265 mg, 1.00 mmol) and 4-bromobenzaldehyde **64** (185 mg, 1.00 mmol). Title compound **110b** was obtained after precipitation and filtration from the reaction crude (396 mg, yield 92%). ^1H NMR (400 MHz, DMSO- d_6) δ 7.67 – 7.50 (m, 5H), 7.45 – 7.36 (m, 5H), 7.29 (dd, J = 7.7, 1.8 Hz, 2H), 7.15 – 7.02 (m, 2H), 6.79 (d, J = 16.4 Hz, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 210.70, 133.70, 131.89, 131.23, 130.49, 129.00, 128.43, 128.25, 126.84, 124.03, 121.73, 119.29, 116.57, 99.54. R_t 2.43 min (generic method). ESI-MS for $\text{C}_{24}\text{H}_{16}\text{BrNO}_2$: calculated 429.0, found m/z 430.4, 432.4 $[\text{M}+\text{H}]^+$; 428.4, 430.4 $[\text{M}-\text{H}]^-$.

3-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (110c).

Compound **110c** was synthesized via general procedure B using **110b** (300 mg, 0.70 mmol) with hydrazine hydrate (68 μ L, 1.40 mmol). Purification performed by direct phase flash chromatography (SiO_2 gold 24 g; 0.2-3% EtOH/DCM) to afford **110c** (213 mg, yield 69%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.04 (s, 1H), 7.58 – 7.36 (m, 7H), 7.31 (dt, J = 7.3, 1.6 Hz, 1H), 7.20 (dt, J = 7.3, 2.0 Hz, 1H), 7.13 – 6.95 (m, 5H), 4.56 (ddd, J = 11.0, 9.3, 3.3 Hz, 1H), 3.30 – 3.14 (m, 1H), 2.57 – 2.51 (m, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 160.71, 149.63, 145.96, 143.13, 138.24, 135.77, 131.05, 130.51, 129.28, 128.73, 128.69, 128.10, 128.06, 127.90, 126.94, 125.39,

121.92, 119.82, 119.57, 115.31, 62.01, 44.62. R_t 2.31 min (generic method). ESI-MS for $C_{24}H_{18}BrN_3O$: calculated 443.1, found m/z 444.4, 446.5 $[M+H]^+$.

(*E*)-3-(3-(3-bromophenyl)acryloyl)-4-phenylquinolin-2(1H)-one (111b). Compound **111b** was synthesized via general procedure A using **61a** (265 mg, 1.00 mmol) and 3-bromobenzaldehyde **85** (117 μ L, 1.00 mmol). Title compound **111b** was obtained after precipitation and filtration from the reaction crude (380 mg, yield 88%). 1H NMR (400 MHz, DMSO- d_6) δ 12.18 (s, 1H), 7.93 (s, 1H), 7.69 (d, J = 7.8 Hz, 1H), 7.59 (ddt, J = 7.2, 3.9, 1.6 Hz, 2H), 7.51 – 7.37 (m, 5H), 7.37 – 7.27 (m, 3H), 7.16 (td, J = 7.6, 7.1, 1.2 Hz, 1H), 7.08 (dd, J = 8.2, 1.5 Hz, 1H), 6.84 (d, J = 16.4 Hz, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 194.29, 159.58, 148.05, 143.93, 138.80, 136.83, 134.24, 133.07, 131.37, 131.26, 131.06, 130.87, 128.92, 128.85, 128.52, 128.26, 127.14, 126.93, 122.24, 122.19, 119.23, 115.66. R_t 2.40 min (generic method). ESI-MS for $C_{24}H_{16}BrNO_2$: calculated 429.0, found m/z 430.4, 432.4 $[M+H]^+$; 428.3, 430.3 $[M-H]^-$.

3-(5-(3-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (111c). Compound **111c** was synthesized via general procedure B using **111b** (300 mg, 0.70 mmol) with hydrazine hydrate (68 μ L, 1.40 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 1-5% EtOH/DCM) to afford **111c** (256 mg, yield 84%). 1H NMR (400 MHz, DMSO- d_6) δ 12.06 (s, 1H), 7.56 – 7.36 (m, 7H), 7.36 – 7.29 (m, 1H), 7.26 – 7.18 (m, 2H), 7.18 – 7.06 (m, 3H), 7.01 (dd, J = 8.2, 1.4 Hz, 1H), 4.59 (ddd, J = 11.1, 9.3, 3.4 Hz, 1H), 3.24 (dd, J = 16.6, 11.2 Hz, 1H), 2.56 (dd, J = 16.6, 9.4 Hz, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 160.67, 149.59, 146.52, 145.91, 138.21, 135.73, 130.47, 130.39, 129.66, 129.25, 129.10, 128.64, 128.02, 128.00, 127.87, 126.89, 125.61, 125.30, 121.87, 121.52, 119.47, 115.27, 61.94, 44.57. R_t 2.30 min (generic method). ESI-MS for $C_{24}H_{18}BrN_3O$: calculated 443.0, found m/z 444.5, 446.4 $[M+H]^+$; 442.6, 444.4 $[M-H]^-$.

(E)-3-(3-(4-chlorophenyl)acryloyl)-4-phenylquinolin-2(1H)-one (112b). Compound **112b** was synthesized via general procedure A using **61a** (169 mg, 0.64 mmol) and 4-chlorobenzaldehyde **63** (90 mg, 0.64 mmol). Title compound **112b** was obtained after precipitation and filtration from the reaction crude (246 mg, quantitative yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.18 (s, 1H), 7.71 (d, *J* = 8.3 Hz, 2H), 7.59 (td, *J* = 7.6, 7.0, 1.5 Hz, 1H), 7.52 – 7.37 (m, 7H), 7.34 – 7.29 (m, 2H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.12 – 7.05 (m, 1H), 6.79 (d, *J* = 16.4 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 194.66, 160.05, 148.50, 144.66, 139.25, 135.67, 134.74, 133.75, 131.78, 131.55, 130.77, 129.39, 129.00, 128.73, 128.65, 127.42, 122.69, 119.69, 116.13. *R*_t 2.37 min (generic method). ESI-MS for C₂₄H₁₆ClNO₂: calculated 385.1, found *m/z* 386.4, 388.4 [M+H]⁺; 384.4, 386.4 [M-H]⁻.

3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (112c). Compound **112c** was synthesized via general procedure B using **112b** (225 mg, 0.58 mmol) with hydrazine hydrate (56 μL, 1.16 mmol). Purification performed by direct phase flash chromatography (SiO₂ gold 24 g; 1-10% EtOH/DCM) to afford **112c** (185 mg, yield 79%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.05 (s, 1H), 7.56 – 7.43 (m, 4H), 7.40 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.35 – 7.26 (m, 3H), 7.21 (dt, *J* = 7.3, 2.0 Hz, 1H), 7.13 – 7.06 (m, 4H), 7.00 (dd, *J* = 8.2, 1.4 Hz, 1H), 4.58 (ddd, *J* = 10.9, 9.2, 3.2 Hz, 1H), 3.25 (dd, *J* = 16.5, 11.1 Hz, 1H), 2.58 – 2.52 (m, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 161.18, 150.09, 146.43, 143.16, 138.71, 136.24, 131.79, 130.97, 129.76, 129.16, 128.82, 128.60, 128.56, 128.53, 128.36, 127.41, 125.88, 122.39, 120.04, 115.78, 62.43, 45.12. *R*_t 2.26 min (generic method). ESI-MS for C₂₄H₁₈ClN₃O: calculated 399.1, found *m/z* 400.4, 402.4 [M+H]⁺; 398.4, 400.5 [M-H]⁻.

(E)-3-(3-(4-methoxyphenyl)acryloyl)-4-phenylquinolin-2(1H)-one (113b). Compound **113b** was synthesized via general procedure A using **61a** (265 mg, 1.00 mmol) and *p*-anisaldehyde **65** (122 μL, 1.00 mmol). Title compound **113b** was obtained after precipitation and filtration from the reaction crude (304 mg, yield 80%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.16 (s, 1H), 7.66 – 7.51

(m, 3H), 7.47 – 7.34 (m, 5H), 7.32 – 7.27 (m, 2H), 7.14 (t, $J = 7.7$ Hz, 1H), 7.06 (dd, $J = 8.2, 1.4$ Hz, 1H), 6.93 (d, $J = 8.8$ Hz, 2H), 6.60 (d, $J = 16.3$ Hz, 1H), 3.78 (s, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 194.07, 161.37, 159.72, 147.51, 145.75, 138.92, 134.40, 131.59, 130.85, 130.48, 128.91, 128.41, 128.19, 126.83, 125.41, 122.04, 119.23, 115.76, 114.36, 55.33. R_t 2.19 min (generic method). ESI-MS for $\text{C}_{25}\text{H}_{19}\text{NO}_3$: calculated 381.1, found m/z 382.5 $[\text{M}+\text{H}]^+$, 380.5 $[\text{M}-\text{H}]^+$.

3-(5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (113c).

Compound **113c** was synthesized via general procedure B using **113b** (300 mg, 0.79 mmol) with hydrazine hydrate (77 μL , 1.58 mmol). Purification performed by direct phase flash chromatography (SiO_2 gold 24 g; 0-5% EtOH/DCM) to afford **113c** (212 mg, yield 69%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.02 (s, 1H), 7.54 – 7.43 (m, 4H), 7.39 (dd, $J = 8.3, 1.2$ Hz, 1H), 7.31 (dq, $J = 7.6, 1.4$ Hz, 1H), 7.21 (ddd, $J = 5.3, 4.0, 2.0$ Hz, 1H), 7.09 (ddd, $J = 8.2, 7.0, 1.2$ Hz, 1H), 7.00 (dt, $J = 8.2, 2.2$ Hz, 3H), 6.82 – 6.76 (m, 2H), 4.51 (t, $J = 10.3$ Hz, 1H), 3.72 (s, 3H), 3.16 (dd, $J = 16.4, 10.9$ Hz, 1H), 2.57 – 2.51 (m, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 160.73, 158.22, 149.48, 146.05, 138.21, 135.81, 135.45, 130.43, 129.25, 128.74, 128.08, 128.05, 127.86, 127.59, 126.91, 125.62, 121.88, 119.59, 115.28, 113.57, 62.36, 55.02, 44.63. R_t 2.08 min (generic method). ESI-MS for $\text{C}_{25}\text{H}_{21}\text{N}_3\text{O}_2$: calculated 395.1, found m/z 396.5 $[\text{M}+\text{H}]^+$; 394.6 $[\text{M}-\text{H}]^-$.

(E)-4-phenyl-3-(3-(4-(trifluoromethyl)phenyl)acryloyl)quinolin-2(1H)-one (114b).

Compound **114b** was synthesized via general procedure A using **61a** (301 mg, 1.10 mmol) and 4-trifluoromethylbenzaldehyde **68** (150 μL , 1.10 mmol). Title compound **114b** was obtained after precipitation and filtration from the reaction crude (414 mg, yield 87%). ^1H NMR (400 MHz, DMSO- d_6) δ 12.23 (s, 1H), 7.81 (dd, $J = 62.7, 7.9$ Hz, 4H), 7.67 – 7.24 (m, 8H), 7.25 – 7.05 (m, 2H), 6.91 (d, $J = 16.2$ Hz, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 194.74, 160.08, 148.71, 143.97, 139.35, 138.85, 134.71, 131.63, 130.31, 129.64, 129.40, 129.04, 128.76, 127.45, 126.14, 122.71,

119.67, 116.20. R_t 2.41 min (generic method). ESI-MS for $C_{25}H_{16}F_3NO_2$: calculated 419.1, found m/z 420.5 $[M+H]^+$; 418.5 $[M-H]^-$.

4-phenyl-3-(5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (114c). Compound **114c** was synthesized via general procedure B using **114b** (256 mg, 0.60 mmol) with hydrazine hydrate (58 μ L, 1.20 mmol). Purification performed by direct phase flash chromatography (SiO_2 gold 24 g; 0.8-6% EtOH/DCM) to afford **114c** (214 mg, yield 80%). 1H NMR (400 MHz, $DMSO-d_6$) δ 12.05 (s, 1H), 7.60 (d, J = 8.1 Hz, 2H), 7.55 – 7.37 (m, 5H), 7.32 (dd, J = 11.5, 7.6 Hz, 3H), 7.24 – 7.17 (m, 2H), 7.10 (ddd, J = 8.2, 7.0, 1.2 Hz, 1H), 7.00 (dd, J = 8.2, 1.4 Hz, 1H), 4.69 (ddd, J = 11.6, 9.1, 2.8 Hz, 1H), 3.33 (m, 1H), 2.63 – 2.52 (m, 1H). ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 161.17, 150.18, 148.95, 146.44, 138.72, 136.22, 131.00, 129.76, 129.22, 129.13, 128.78, 128.57, 128.51, 128.37, 128.02, 127.72, 127.42, 126.14, 125.77, 125.62, 125.57, 125.53, 122.40, 120.02, 115.79, 62.59, 45.15. R_t 2.30 min (generic method). ESI-MS for $C_{25}H_{18}F_3N_3O$: calculated 433.1, found m/z 434.4 $[M+H]^+$; 432.5 $[M-H]^-$.

tert-butyl (3-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-oxopropyl)carbamate (119). In round bottom flask, the commercially available 3-((tert-butoxycarbonyl)-amino)-propanoic acid **118** (182 mg, 0.96 mmol), HOBt (156 mg, 1.16 mmol) and EDCI (222 mg, 1.16 mmol) were stirred in DCM (15 mL) at rt for 1 hour. Then a solution of the **86c** (400 mg, 0.96 mmol) and Et_3N (296 μ L, 2.12 mmol) in DCM (5 mL) was added. The mixture was stirred at rt overnight. The solvent was removed under reduced pressure, the residue re-dissolved with EtOAc and then washed with H_2O , 1M $NaHCO_3$ and finally 10% citric acid. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Purification performed by direct phase flash chromatography (0- 30% EtOAc/DCM). Yield 72 mg, 25%. 1H NMR (400 MHz, $DMSO-d_6$) δ 12.40 (s, 1H), 7.65 (dd, J = 4.0, 8.0 Hz, 1H), 7.59-7.50 (m, 4H), 7.46 (d, J = 8.0 Hz, 1H), 7.43-7.42 (m, 1H), 7.28 (d, J = 8.0 Hz, 1H), 7.04 (t, J = 8.0 Hz, 2H), 6.93 (d, J = 2.0 Hz, 1H), 6.80 (dd, J = 4.0, 8.0 Hz, 2H), 6.66 (t, J = 8.0 Hz, 1H), 5.32 (dd, J = 4.0, 12.0 Hz,

1H), 3.73 (dd, $J = 12.0, 16.0$ Hz, 1H), 2.99 (dd, $J = 8.0, 12.0$ Hz, 2H), 2.76 (dd, $J = 4.0, 20.0$ Hz, 1H) 2.41 (q, $J = 8.0$ Hz, 2H), 1.38 (s, 9H).

Methyl 3-(methylsulfonamido) propanoate (123). In a dried round bottom flask, commercially available methyl 3-amino-propanoate **121** (745 mg, 5.34 mmol) and triethylamine (3.7 mL, 26.68 mmol) were stirred in anhydrous DCM (5.6 mL) prior to the addition of methansulfonyl chloride **122** (1.65 mL, 10.67 mmol). After stirring at rt for 2 days, the reaction was quenched with NaHCO₃ sat. solution and extracted three times with CHCl₃. The organic layer was dried over Na₂SO₄ and evaporated to dryness. The title compound was obtained after purification over direct phase flash column chromatography (0-40% EtOAc/petroleum ether). Yield 791 mg, 82%. ¹H NMR (400 MHz, CDCl₃-*d*) δ 4.94 (s, 1H), 3.72 (s, 3H), m (3.42-3.37, 2H), 2.97 (s, 3H), 2.64 (t, $J = 4$ Hz, 2H). ¹³C NMR (101 MHz, CDCl₃-*d*) δ 172.63, 52.18, 40.60, 38.89, 34.58.

3-(Methylsulfonamido) propanoic acid (124). In round bottom flask, methyl 3-(methylsulfonamido) propanoate **123** (791 mg, 4.36 mmol) was stirred in MeOH/THF (1:1 v/v) prior to the addition of 2 M LiOH (2.5 mL). The reaction was stirred at rt overnight. The reaction mixture was acidified with 1M HCl_{aq} (pH 2) and extracted three times with ethyl acetate. The organic layer was dried over Na₂SO₄, filtered and evaporated to dryness to obtain the title compound. Yield 600 mg, 82%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.29 (s, 1H), 7.02 (t, $J = 4.0$ Hz, 1H), 7.15 (dd, $J = 7.0, 12.6$, 2H), 2.43 (t, $J = 7.2$ Hz, 2H).

Biology. ELISA assay. Competitive ELISA screening assay using biotinylated BRC4 peptide to disrupt the BRC4–RAD51 interaction was performed by modifying the method described by Rajendra et al.³³ BRC4-biotinylated peptide (N-term BiotinKEPTLLGFHTASGKKVKIAKESLDKVKNLFDKEQ from Life Technologies) was used to coat 384-well plates (Nunc). After washing with PBS containing 0.05% Tween-20 (PBST), and blocking with the solution BSA 1% /PBST, overnight hybridization with human RAD51 protein (NP_002866 Creative Biomart, NY) was performed. Test

compounds were added in dose response from 0.01 to 100 μ M in triplicate with constant DMSO 1%. Antibody raised against RAD51 (Millipore) and HRP-secondary antibody staining to develop the 3,3',5,5'-tetramethylbenzidine signal (Sigma) quenched with 1M HCl was used as the assay readout. Colorimetric measure was read on Victor5 (PerkinElmer) plate Reader. BRC4 and Rad51 included in the assay as positive control. Results were analysed by using GraphPad Software.

Protocol for the expression and purification of His-hRAD51. hRAD51 was expressed in E. coli Rosetta2(DE3)pLysS cells. A saturated overnight culture of Rosetta2(DE3)pLysS/pET15b-His-hRAD51 was diluted (1:1000) into a fresh TB-5052 auto-induction medium containing ampicillin (100 μ g/mL). The flasks were shaken at 200 rpm at 20 °C for 72 h. Pellet was subsequently resuspended in an appropriate volume of buffer A (20 mM Tris-HCl (pH 8.00), 500 mM NaCl, 10 mM Imidazole, 5 mM DTT, 10% (v/v) Glycerol) supplemented with protease inhibitor cocktail (SIGMAFAST Protease Inhibitor Cocktail Tablets, EDTA-50 Free). The cell suspension was lysed on ice through sonication (24 rounds of 30"; Amplitude 85%; Tip MS72; Bandelin Sonoplus HD2070 sonicator). The disrupted cell suspension was centrifuged for 1 h at 13000 rpm. The supernatant fraction was filtered with a 0.45 μ m (MiniSart Syringe filter 0.45 μ m) membrane to remove residual particulates before chromatography. The supernatant was applied onto a His-Trap column (His-TrapTM FF 5mL, GE Healthcare) equilibrated with buffer A. A wash step was performed using 10% of buffer B (20 mM Tris- HCl (pH8.00), 500 mM NaCl, 500 mM Imidazole, 10% (v/v) Glycerol). The protein was then eluted with a linear gradient from 10% to 100% of buffer B over 10 column volumes. Fractions (0.5 mL) were collected and analyzed by SDS-PAGE. Collected fractions corresponding to the recombinant protein were dialyzed overnight at 4 °C against buffer C (50 mM Tris-HCl (pH 8.00), 200 mM KCl, 0.25 mM EDTA, 2 mM DTT, 10% (v/v) glycerol). Dialyzed protein was loaded onto an anion exchange column (ResQ, GE Healthcare) equilibrated in buffer C. The elution was performed with a linear gradient of buffer B (50 mM Tris-HCl (pH 8.00), 1 M KCl, 0.25 mM EDTA, 2 mM DTT, 10% (v/v) glycerol). Fractions (0.5 mL) were collected and analyzed by SDS-PAGE. Fractions containing His-hRAD51 were

pooled and dialyzed against the storage buffer (20 mM Hepes (pH 8.00), 250 mM KCl, 0.1 mM EDTA, 2 mM DTT, 10% (v/v) glycerol). The protein yield was determined from the optical absorption at 280 nm (extinction coefficient 14900 M⁻¹cm⁻¹) of the final sample.

Microscale thermophoresis. The recombinant protein hRAD51 was labelled with the Monolith His-Tag Labeling Kit RED-tris-NTA 2nd Generation kit (NanoTemper Technologies). MST measurements were simultaneously performed on 16 capillaries containing a constant concentration (25 nM) of -labelled- RED-tris-NTA 2nd Generation His-hRAD51 protein and 16 different concentrations of **35d** in order to determine a concentration-dependent MST binding curve. The highest **35d** concentration tested was 40 μM. Measurements were carried out in MST buffer (20 mM Hepes (pH 8.00), 250 mM KCl, 0.1 Mm EDTA, 5% (v/v) Glycerol, 5% DMSO).

Cell culture and treatments. BxPC-3 and Capan-1 cells were grown in RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin/streptomycin, 2 mM glutamine. All media and supplements were from Sigma-Aldrich. Non-neoplastic, immortalized cells from human kidney (HK-2, ATCC® CRL2190™) were grown in DMEM:F12 medium containing 40 ng/ml dexamethasone and supplemented as described above.

All cultures were routinely tested for Mycoplasma contamination. Treatments (olaparib and BRCA2-RAD51 disruptors) were administered in culture medium supplemented with 0.6% DMSO. The same amount of DMSO was added to the control, untreated cultures.

Homologous Recombination assay. Homologous recombination (HR) was assessed by using a commercially available assay (Norgen). This assay is based on cell transfection with two plasmids that, upon cell entry, recombine. The efficiency of HR can be assessed by real-time PCR, using primer mixtures included in the assay kit. Different primer mixtures allow one to discriminate between the original plasmid backbones and their recombination product.

BxPC3 cells (2 × 10⁵ per well) were seeded in a 24-well plate and allowed to adhere overnight. Co-transfection with the two plasmids was performed in Lipofectamine 2000 (Invitrogen), according to the manufacturer's instructions. During transfection (5 h), cells were exposed to different doses of

1
2
3 RAD51-BRCA2 disruptors, dissolved in RPMI in the presence of 0.6% DMSO. After washing with
4
5 PBS, cells were harvested, and DNA was isolated using Illustra Tissue and Cell Genomic Prep Mini
6
7 Spin kit (GE Healthcare). Sample concentration was measured using an ONDA Nano Genius
8
9 photometer. The efficiency of HR was assessed by real-time PCR, using 25 ng of template, the
10
11 primer mixtures included in the assay kit and following the protocol indicated by the manufacturer.
12
13 Data analysis was based on the $\Delta\Delta C_t$ method: [Recombination Product / Backbone Plasmids]^{treated}
14
15 versus [Recombination Product / Backbone Plasmids]^{control}.
16
17

18
19 **Immunofluorescence.** Immunofluorescence was used for studying RAD51 nuclear translocation
20
21 and for evaluating DNA damage through the detection of γ H2AX nuclear foci.
22

23
24 To visualize RAD51 in cell nuclei, BxPC-3 cells were seeded on glass coverslips placed in a 6-well
25
26 culture plate (2×10^5 cells/well) and allowed to adhere overnight. Cultures were then pre-incubated
27
28 with 20 μ M **35d** for 1 h and subsequently exposed to 50 μ M cisplatin for an additional 1.5 h.
29
30 Medium was removed and cells were maintained in the presence of 20 μ M **35d** for 4 h. After this
31
32 time, cultures growing on coverslips were fixed in PBS containing 1% formalin for 20 min,
33
34 permeabilized in 70% ethanol, air-dried and washed twice with PBS. Samples were incubated in
35
36 10% Bovine Serum Albumin (BSA) in PBS for 30 min at 37 °C and subsequently exposed to an
37
38 anti-RAD51 mouse monoclonal antibody (Santa Cruz Biotechnology, 1:1000 in 5% BSA / PBS),
39
40 overnight at 4 °C. After washing, coverslips were incubated with an anti-mouse FITC-conjugated
41
42 secondary antibody (1:1000 in 1% BSA / PBS) for 1 h at 37 °C, washed, air-dried, and mounted
43
44 with a solution of DAPI (2 μ g/ml) and DABCO.
45
46

47
48 To evaluate DNA damage through γ H2AX nuclear foci, BxPC3 and Capan1 cells were seeded on
49
50 glass coverslips in 6-well tissue culture plate (2×10^5 cells/well) and allowed to adhere overnight.
51
52 After 48 h treatment with olaparib (10 μ M) or **35d** (20 μ M) given alone or in combination, cultures
53
54 growing on coverslips were fixed and treated as described above. For this experiment, the used
55
56 antibodies were a rabbit polyclonal anti- γ H2AX (Abcam, 1:1000 in 5% BSA / PBS) and a
57
58 secondary anti-rabbit rhodamine-labeled (Novus Biologicals, 1:1000 in 1% BSA / PBS). For both
59
60

experiments, images were acquired using a Nikon fluorescent microscope equipped with filters for FITC, TRITC and DAPI. The percentage of cells bearing nuclear foci was estimated by two independent observers, by analyzing 100-250 cells for each treatment sample.

Cell viability assay. Cell viability was assessed with the CellTiter-Glo Luminescent Cell Viability Assay from Promega. For this experiment, 1×10^4 cells in 200 μ l of culture medium were seeded into each well of a 96-multiwell white body plate and allowed to adhere overnight. After 72-h incubation in the presence of olaparib (10 μ M) and the RAD51-BRCA2 disruptors alone or in combination, the plate was allowed to equilibrate at room temperature for 30 min and the CellTiter-Glo reactive was directly added to each well. The plate was kept on a shaker for 10 min to induce cell lysis and its luminescence was measured with a Fluoroskan Ascent FL reader (Labsystems).

Cytotoxicity assay. Cell death was assessed by applying the CellTox Green Cytotoxicity assay (Promega). Briefly, BxPC-3 (1×10^4 /well) were plated in 96-well plates and treated for 72 h with olaparib (10 μ M) and the BRCA2-RAD51 disruptors administered alone or in combination. At the end of treatment, the CellTox dye was added to cell cultures and the green fluorescence signal, which is produced by the binding interaction with dead cell DNA, was measured following the manufacturer's instructions.

Assessment of cell death with vital dyes. BxPC-3 cells were grown on coverslips placed in a 6-multiwell plate (5×10^5 cells/well). After a 72-h treatment with olaparib (10 μ M) or **35d** (20 μ M) given alone or in combination, wells were rapidly washed with PBS and filled with 500 μ L of a PBS solution containing DAPI (4.6 μ g/mL) and PI (50 μ g/mL). After a 10-min incubation at room temperature under light-shielded condition, they were washed with PBS, fixed (10 min) with 10% neutral buffered formalin solution, and washed again to eliminate the fixative. Coverslips were then applied on glass slides using two drops of mounting media (DABCO). Images were acquired on a Nikon fluorescent microscope equipped with filters for DAPI and PI.

Micronuclei visualization. For micronuclei visualization, cells were grown on coverslips placed in a 6-multiwell plate (5×10^5 cells/well). After a 72-h treatment with olaparib (10 μ M) or

35d (20 μ M) given alone or in combination, wells were rapidly washed with PBS and then fixed for 10 min with cold methanol. Coverslips were then airdried and mounted on glass slides using a solution of DAPI (5 μ g/mL) / DABCO. Images were acquired on a Nikon fluorescent microscope equipped with filters for DAPI. A cell was considered to contain micronuclei if the following criteria were met: (i) one or more round fluorescent bodies were present in the cytoplasm which did not touch the main nucleus; (ii) they were $< 1/3$ of the main nucleus diameter; and (iii) they were nonrefractile, to exclude foreign bodies. The percentage of cells bearing micronuclei was estimated by two independent observers, by analyzing 100-250 cells for each treatment sample.

ASSOCIATED CONTENT

Supporting Information

Computational methods

Separation and ELISA assay results of enantiomers **4d-I** and **4d-II**

Experimental procedure and characterization data of aldehydes **67**, **70**, **74-84**

^1H -NMR spectrum, ^{13}C -NMR spectrum of final compounds **4d-10d**, **14d-15d**, **18d-57d** and

HPLC-MS analysis of selected final compounds **4d-10d**, **14d-15d**, **18-25d**, **35d**, **49d**, **57d**

PDB file for figure 3A and PDB file for figure 3B.

Molecular formula strings and some data for all final (CSV).

AUTHOR INFORMATION

Corresponding Author

*Marinella Roberti: Phone: +39 051 2099738; E-mail address: marinella.roberti@unibo.it

*Andrea Cavalli: Phone: +39 010 71781530; E-mail address: andrea.cavalli@iit.it

ORCID

Marinella Roberti: [0000-0001-9807-2886](https://orcid.org/0000-0001-9807-2886)

Andrea Cavalli: [0000-0002-6370-1176](https://orcid.org/0000-0002-6370-1176)

Author Contributions

[†]These authors contributed equally to this work

Present Address

For Federico Falchi: Molecular Horizon srl, Via Montelino 30, 06084 Bettona (PG), Italy.

Notes

The authors declare the following competing financial interest(s): One patent application protecting the class of compounds disclosed in this article has been filed by the following authors: Greta Bagnolini, Domenico Milano, Marcella Manerba, Jose Antonio Ortega, Francesca De Franco, Roberto Pellicciari, Saverio Minucci, Giuseppina Di Stefano, Marinella Roberti, and Andrea Cavalli.

ACKNOWLEDGMENTS

This work was supported by the Associazione Italiana per la Ricerca sul Cancro AIRC (Progetto IG 2018, id 21386), the Italian Institute of Technology (IIT), and the University of Bologna. We thank Prof. Maurizio Recanatini for fruitful discussions.

ABBREVIATIONS USED

acetonitrile (MeCN); di-*tert*-butyl dicarbonate (Boc₂O); cyclohexane (CHX); dichloromethane (DCM); *N,N*-Diisopropylethylamine (DIPEA); dimethylsulfoxide (DMSO); *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC); 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI); ethanol (EtOH); electrospray ionization (ESI); ethyl acetate (EtOAc); 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU); hydroxybenzotriazole (HOBt); mass spectrometry (MS); microwave (MW/ μ Wave); iodomethane (MeI); *N,N*-dimethylformamide (DMF); methanol (MeOH); nuclear magnetic resonance (NMR); room temperature (rt); tetrahydrofuran (THF); thin layer chromatography (TLC); triethylamine (Et₃N), 4',6-diamidino-2-phenylindole (DAPI);

propidium iodide (PI).

REFERENCES

- (1) Bridges, C. B. The origin and variations in sexual and sex limited characters. *Amer. Natur.* **1922**, *56*, 51-63.
- (2) Dobzhansky, T. Genetics of natural populations; recombination and variability in populations of *Drosophila pseudoobscura*. *Genetics* **1946**, *31*, 269-290.
- (3) Lucchesi, J. C. Synthetic lethality and semi-lethality among functionally related mutants of *Drosophila melanogaster*. *Genetics* **1968**, *59*, 37-44.
- (4) Kaiser, C. A.; Schekman, R. Distinct sets of SEC genes govern transport vesicle formation and fusion early in the secretory pathway. *Cell* **1990**, *61*, 723-733.
- (5) Hennessy, K. M.; Lee, A.; Chen, E.; Botstein, D. A group of interacting yeast DNA replication genes. *Genes Dev.* **1991**, *5*, 958-969.
- (6) Kaelin, W. G., Jr. The concept of synthetic lethality in the context of anticancer therapy. *Nat. Rev. Cancer* **2005**, *5*, 689-698.
- (7) Gupta, A.; Ahmad, A.; Dar, A. I.; Khan, R. Synthetic Lethality: From research to precision cancer nanomedicine. *Curr. Cancer Drug Targets* **2017**, *18*, 337-346.
- (8) Chan, D. A.; Giaccia, A. J. Harnessing synthetic lethal interactions in anticancer drug discovery. *Nat. Rev. Drug Discov.* **2017**, *10*, 351-364.
- (9) Beijersbergen, R.; Wessel, L.; Bernards, R. Synthetic lethality in cancer therapeutics. *Annu. Rev. Cancer Biol.* **2017**, *1*, 141-161.
- (10) Chen, E. Targetin epigenetics using synthetic lethality in precision medicine. *Cellular and Molecular Life Science* **2018**, *75*, 3381-3392.
- (11) Ashworth, A.; Lord, C. J. Synthetic lethal therapies for cancer: what's next after PARP inhibitors? *Nat. Rev. Clin. Oncol.* **2018**, *15*, 564-576.
- (12) Parameswaran, S.; Kundapur, D.; Vizeacoumar, F. S.; Freywald, A.; Uppalapati, M. Vizeacoumar, F.J. A road map to personalizing targeted cancer therapies using synthetic lethality. *Trends Cancer* **2019**, *5*, 11-29.
- (13) Bhattacharjee, S.; Nandi, S. Synthetic lethality in DNA repair network: a novel avenue in targeted cancer therapy and combination therapeutics. *IUBMB Life* **2017**, *69*, 929-937.
- (14) Minchom, A.; Aversa, C.; Lopez, J. Dancing with the DNA damage response: next-generation anti-cancer therapeutic strategies. *Ther. Adv. Med. Oncol.* **2018**, *10*, 1-18.

- (15) Pilie, P. G.; Tang, C.; Mills, G. B.; Yap, T. A. State-of-the-art strategies for targeting the DNA damage response in cancer. *Nat. Rev. Clin. Oncol.* **2019**, *16*, 81-104.
- (16) Hanahan, D.; Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **2011**, *144*, 646-674.
- (17) Deeks, E. D. Olaparib: first global approval. *Drugs* **2015**, *75*, 231-240.
- (18) www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm592357.htm (accessed Sept 15, 2019).
- (19) Golan, T.; Hammel, P.; Reni, M.; Van Cutsem, E.; Macarulla, T.; Hall, M.J.; Park, J.; Hochhauser, D.; Arnold, D.; Oh, D.; Reinacher-Schick, A.; Tortora, G.; Algul, H.; O'Reilly, E.M.; McGuinness, D.; Cui, K.Y.; Schielinger, K.; Locker, G.Y.; Kindler, H.L. Maintenance olaparib for germline BRCA-mutated metastatic pancreatic cancer. *N. Engl. J. Med.* **2019**, *381*, 317-327.
- (20) Davies, A. A.; Masson, J. Y.; McIlwraith, M. J.; Stasiak, A. Z.; Stasiak, A.; Venkitaraman, A.R.; West, S.C. Role of BRCA2 in control of the RAD51 recombination and DNA repair protein. *Mol. Cell* **2001**, *7*, 273-282.
- (21) Klein, H. L. The consequences of Rad51 overexpression for normal and tumor cells. *DNA Repair (Amst)* **2008**, *7*, 686-693.
- (22) Henning, W.; Sturzbecher, H. W. Homologous recombination and cell cycle checkpoints: Rad51 in tumour progression and therapy resistance. *Toxicology* **2003**, *193*, 91-109.
- (23) Yanagisawa, T.; Urade, M.; Yamamoto, Y.; Furuyama, J. Increased expression of human DNA repair genes, XRCC1, XRCC3 and RAD51, in radioresistant human KB carcinoma cell line N10. *Oral Oncol.* **1998**, *34*, 524-528.
- (24) Pellegrini, L.; Yu, D. S.; Lo, T.; Anand, S.; Lee, M.; Blundell, T.M.; Venkitaraman, A.R. Insights into DNA recombination from the structure of a RAD51-BRCA2 complex. *Nature* **2002**, *420*, 287-293.
- (25) Bork, P.; Blomberg, N.; Nilges, M. Internal repeats in the BRCA2 protein sequence. *Nat. Genet.* **1996**, *13*, 22-23.
- (26) Wong, A. K.; Pero, R.; Ormonde, P. A.; Tavtigian, S. V.; Bartel, P. L. RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene *brca2*. *J. Biol. Chem.* **1997**, *272*, 31941-31944.
- (27) Bignell, G.; Micklem, G.; Stratton, M. R.; Ashworth, A.; Wooster, R. The BRC repeats are conserved in mammalian BRCA2 proteins. *Hum. Mol. Genet.* **1997**, *6*, 53-58.
- (28) Zhu, J.; Zhou, L.; Wu, G.; Konig, H.; Lin, X.; Li, G.; Qiu, X.; Chen, C.; Hu, C.; Goldblatt, E.; Bhatia, R.; Chamberlin, A.R.; Chen, P.; Lee, W. A novel small molecule RAD51

- inactivator overcomes imatinib-resistance in chronic myeloid leukaemia. *EMBO Mol. Med.* **2013**, *5*, 353-365.
- (29) Lee, W. H.; Chen, P. L.; Zhu, J. Composition and Methods for the Disruption of BRCA2-RAD51 Interaction, 2006, PCT Int. Appl. WO 2006/044933.
- (30) Falchi, F.; Giacomini, E.; Masini, T.; Boutard, N.; Di Ianni, L.; Manerba, M.; Farabegoli, F.; Rossini, L.; Robertson, J.; Minucci, S.; Pallavicini, I.; Di Stefano, G.; Roberti, M.; Pellicciari, R.; Cavalli, A. Synthetic lethality triggered by combining olaparib with BRCA2-Rad51 disruptors. *ACS Chem. Biol.* **2017**, *12*, 2491-2497.
- (31) Roberti, M.; Schipani, F.; Bagnolini, G.; Milano, D.; Giacomini, E.; Falchi, F.; Balboni, A.; Manerba, M.; Farabegoli, F.; De Franco, F.; Robertson, J.; Minucci, S.; Pallavicini, I.; Di Stefano, G.; Girotto, S.; Pellicciari, R.; Cavalli, A. Rad51/BRCA2 disruptors inhibit homologous recombination and synergize with olaparib in pancreatic cancer cells. *Eur. J. Med. Chem.* **2019**, *165*, 80-92.
- (32) Scott, D. E.; Bayly, A. R.; Abell, C.; Skidmore, J. Small molecules, big targets: drug discovery faces the protein-protein interaction challenge. *Nat. Rev. Drug Discov.* **2016**, *15*, 533-550.
- (33) Rajendra, E.; Venkitaraman, A. R. Two modules in the BRC repeats of BRCA2 mediate structural and functional interactions with the RAD51 recombinase. *Nucleic Acids Res.* **2010**, *38*, 82-96.
- (34) Nomme, J.; Takizawa, Y.; Martinez, S. F.; Renodon-Corniere, A.; Fleury, F.; Weigel, P.; Yamamoto, K.; Kurumizaka, H.; Takahashi, M. Inhibition of filament formation of human Rad51 protein by a small peptide derived from the BRC-motif of the BRCA2 protein. *Genes Cells* **2008**, *13*, 471-481.
- (35) Nomme, J.; Renodon-Corniere, A.; Asanomi, Y.; Sakaguchi, K.; Stasiak, A. Z.; Stasiak, A.; Norden, B.; Tran, V.; Takahashi, M. Design of potent inhibitors of human RAD51 recombinase based on BRC motifs of BRCA2 protein: modeling and experimental validation of a chimera peptide. *J. Med. Chem.* **2010**, *53*, 5782-5791.
- (36) Acker, T. M.; Khatri, A.; Vance, K. M.; Slabber, C.; Bacsá, J.; Snider, J.P.; Traynelis, S.F., Iiotta, D.C. Structure-activity relationships and pharmacophore model of a noncompetitive pyrazoline containing class of GluN2C/GluN2D selective antagonists. *J. Med. Chem.* **2013**, *56*, 6434-6456.
- (37) Sankaran, M.; Uvarani, C.; Kumarasamy Chandraprakash; Mani Umamaheswari; Mohan, P. S. An Expedient Approach for the Synthesis of Bioactive Pyrazole, Isoxazole and

- 1
2
3 Benzodiazepine-Substituted Quinolin-2(1H)-one Derivatives. *J. Heterocyclic Chem.* **2015**,
4 52, 1082-1092.
5
6
7 (38) Abbott, D. W.; Freeman, M. L.; Holt, J. T. Double-strand break repair deficiency and
8 radiation sensitivity in BRCA2 mutant cancer cells. *J. Natl. Cancer. Inst.* **1998**, 90, 978-985.
9
10 (39) Cummings, B. S.; Schnellmann, R. G. Measurement of cell death in mammalian cells.
11 *Curr. Protoc. Pharmacol.* **2004**, 25, 12.8.1-12.8.22.
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table of Content Graphic

