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Article

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Isoquinoline-1,3-diones as Selective Inhibitors of Tyrosyl DNA Phosphodiesterase II (TDP2)

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Abstract

Tyrosyl DNA phosphodiesterase II (TDP2) is a recently discovered enzyme that specifically repairs DNA damages induced by topoisomerase II (Top2) poisons and causes resistance to these drugs. Inhibiting TDP2 is expected to enhance the efficacy of clinically important Top2-targeting anticancer drugs. However, TDP2 as a therapeutic target remains poorly understood. We report herein the discovery of isoquinoline-1,3-dione as a viable chemotype for selectively inhibiting TDP2. The initial hit compound **43** was identified by screening our in-house collection of synthetic compounds. Further structure-activity-relationship (SAR) studies identified numerous analogues inhibiting TDP2 in low micromolar range without appreciable inhibition against the homologous TDP1 at the highest testing concentration (111 μM). The best compound **64** inhibited recombinant TDP2 with an IC_{50} of 1.9 μM. The discovery of this chemotype may provide a platform towards understanding TDP2 as a drug target.

Introduction

Many cancer chemotherapies work by inducing excessive DNA damage that causes cell death. The survival of damaged cancer cells hinges on the repair of these DNA lesions through various pathways which ultimately cause therapeutic resistance. Therefore, targeting DNA repair pathways can enhance the efficacy of DNA-damaging anticancer drugs.[1] Among DNA damaging drugs are Top2 poisons, such as etoposide (ETP), teniposide, doxorubicin, epirubicin and idarubicin, which are widely used for treating a broad spectrum of cancers.[2] Mechanistically, Top2 (**1**, Fig. 1) relaxes DNA torsional strains by cutting DNA substrate (**2**), using its tyrosine residue to form the covalent

Top2cc (**4**) along with the released 3' DNA-end (**3**) (Fig. 1). The DNA break is normally resealed when the 3'-end (**3**) attacks back to resolve Top2cc, which allows the continuation of functional DNA transcription and replication. However, the dynamic equilibrium between DNA and Top2cc can shift toward the accumulation of abortive Top2cc (**5**) in which DNA breaks fail to rejoin. Clinical Top2 poisons work by this exact mechanism as they bind to and stabilize the Top2cc to prevent re-ligation (**5**, Fig. 1). TDP2 is the only known human enzyme that can cleave the unique 5' phosphotyrosyl covalent bond featured in abortive Top2cc,[3] demonstrating that TDP2 plays a critical role in the repair of Top2-mediated DNA damage and in resistance development to clinical Top2 poisons. This is supported by observations both in cultured cells and animal models that lack of TDP2 leads to enhanced sensitivity to Top2-induced DNA breaks caused by Top2 poisons.[3-7] Importantly, up-regulation of TDP2 transcription through a gain-of-function p53 mutation has indeed been linked to etoposide resistance in human lung cancer.[8] These observations provide strong rationales for developing TDP2 inhibitors to sensitize cancer cells towards clinical Top2 poisons.

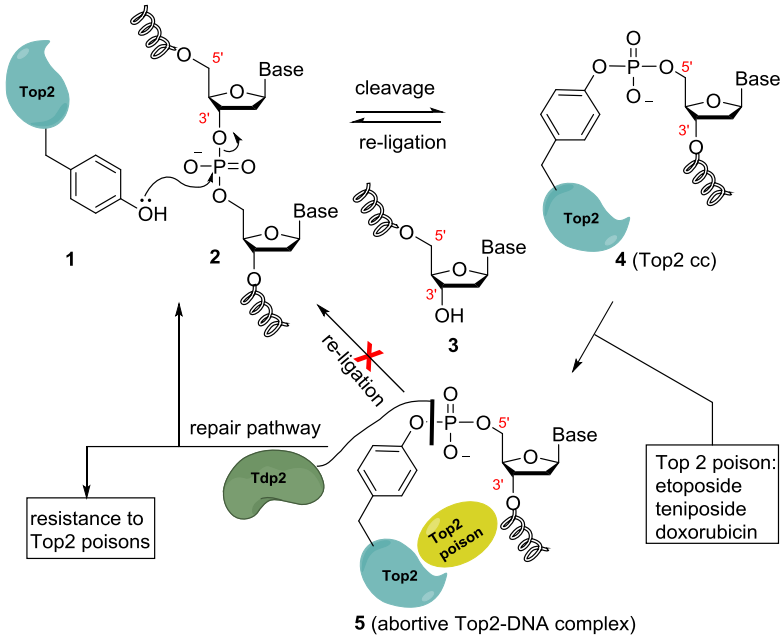


Fig. 1. TDP2 repairs abortive Top2-DNA cleavage complex: **2**, DNA substrate; **4**, transient Top2-DNA complex; **5**, Top2-DNA complex stabilized by a Top2 poison; cyan, Top 2; green, TDP2; yellow, Top2 poison.

In addition, recent studies have also linked the unique function of TDP2 to the replication of picornaviruses,[9] and particularly hepatitis B virus (HBV).[10] HBV chronically infects over 350 million people globally. Current antivirals against HBV can not eradicate the virus to achieve disease cure. This is mainly due to the persistence of viral covalently closed circular DNA (cccDNA)[11, 12] produced from a precursor relaxed circular DNA (RC-DNA) during a key step of HBV replication. A recent report[10] found that this conversion is critically dependent on cellular TDP2 for the cleavage of a protein-DNA adduct similar to the Top2cc. Inhibiting cellular TDP2 thus may lead to the depletion of HBV reservoir cccDNA and may provide a path towards viral eradication, though the role of Tdp2 in HBV cccDNA formation in vivo remains debatable.[13]

Since the discovery of TDP2 in 2009,[3] efforts in biochemistry and crystallography have generated critical knowledge to allow basic understanding on TDP2 active site structure, mode of substrate recognition, enzyme kinetics, and mechanism of catalysis.[14-16] These studies support either a one- or two-metal catalytic mechanism characteristic of the exonuclease-endonuclease-phosphatase (EEP) nuclease superfamily. Based on these mechanisms, TDP2 repairs abortive Top2cc through a hydrolytic reaction promoted by either one or two Mg^{2+} ion(s) and a few key residues at the active site. Particularly important to catalysis are residues D262, E152, H351 and N120, which are highly conserved across species. Knowledge on substrate binding and mechanism of catalysis could assist inhibitor discovery; particularly the structural and biochemical kinship of TDP2 to another DNA repair enzyme, human apurinic/apyrimidinic endonuclease 1 (hAPE1),[17] could allow ligand-based inhibitor design. However, full-fledged medicinal chemistry efforts targeting TDP2 require good lead compounds and the understanding of their inhibition mechanisms, both of which are still lacking. As a result, TDP2 inhibitors remain largely unexplored. Toxoflavins (**6**) and Deazaflavins (**7**) (Fig. 2a) identified through a high-throughput screening (HTS) are the only known inhibitor scaffolds of TDP2.[18] While biochemically inhibiting TDP2, these scaffolds are flawed as drug candidates because **6** poses a redox liability and shows poor in vitro pharmacokinetics (PK) profiles due to its inhibition of metabolic enzyme CYP450, whereas **7** lacks cell permeability.[18] Therefore, there is a pressing need to identify more drug-like TDP2 inhibitors that can also be used as tool compounds towards understanding the mechanism of TDP2 inhibition.

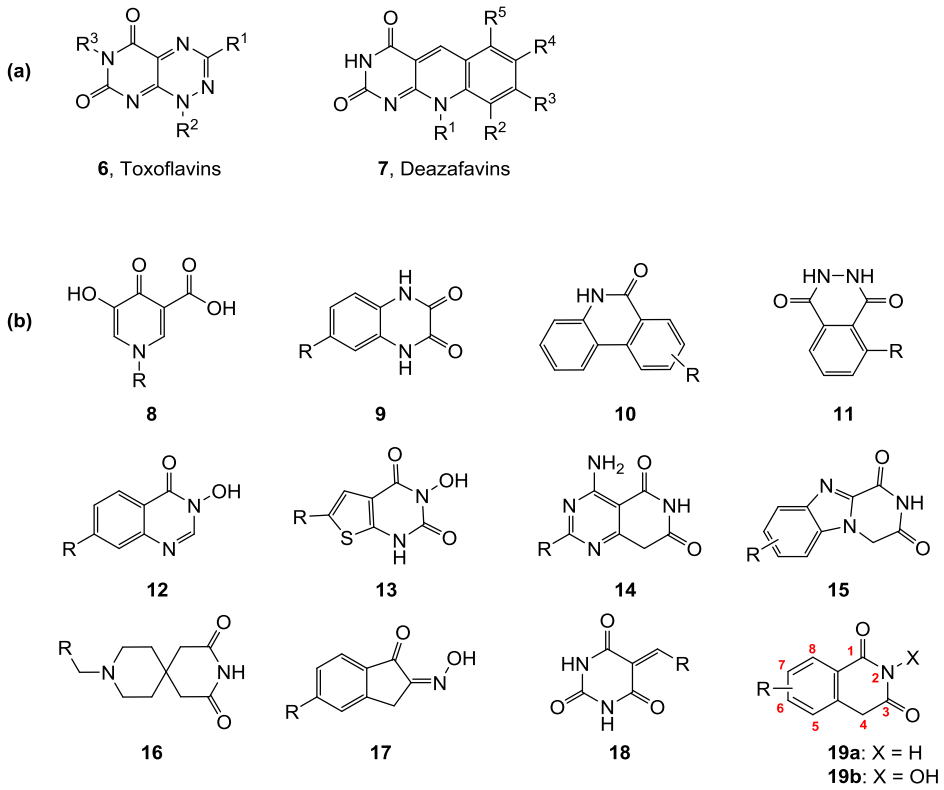
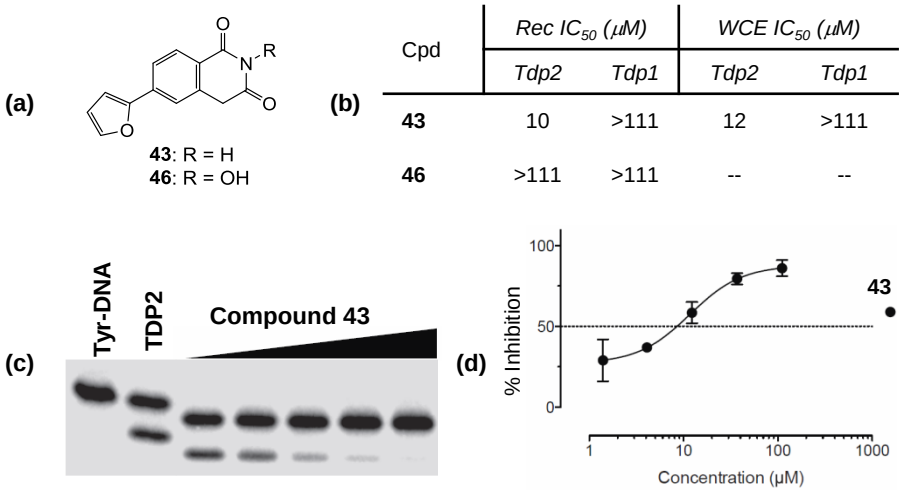


Fig. 2. Chemotypes of interest. (a) Reported inhibitor types of TDP2; (b) in-house synthetic chemotypes screened against TDP2.

Results and Discussion

Hit Identification. Our efforts began with screening a carefully curated library comprising our in-house synthetic compounds. The screen used a biochemical assay with recombinant human TDP2.[14] The library comprises compounds of various chemotypes (Fig. 2b), many featuring a metal chelating functionality, such as **8**–**9**, **12**–**14**, **17** and **19b** (X = OH). The rationale is that the catalysis of TDP2 requires at least one Mg^{2+}. From this initial screening, one analogue of the isoquinoline-1,3-dione chemotype (**19a**), namely 6-furanoisoquinoline-1,3-dione (**43**, Fig. 3a) was found to selectively inhibit TDP2 (Fig. 3c). Dose-response testing revealed that the inhibitory IC_{50} for **43** was 10 μM

(Fig. 3d). This potency was confirmed through a secondary assay using a more robust assay where endogenous TDP2 from whole cell extracts was used (WCE, IC_{50} = 12 μM, Fig. 3b). Remarkably, none of any other screened chemotypes showed significant TDP2 inhibition, including the highly homologous **19b** where the only structural difference is the presence of the N-2 OH group. That the 2-hydroxyisoquinoline-1,3-dione (HID) analogue **46** did not inhibit TDP2 at concentrations up to 111 μM was remarkable. It implies that unlike the inhibition of some other Mg^{2+} dependent DNA processing enzymes, TDP2 inhibition may not require a chelating functionality. On the other hand, counter assays against the homologous TDP1 showed no inhibition by **43** (Fig. 3b), strongly suggesting that the observed TDP2 inhibition was highly specific. In addition, the small size (mw = 227) and the lack of apparent PK liabilities render **43** an attractive hit, which is amenable to further SAR.



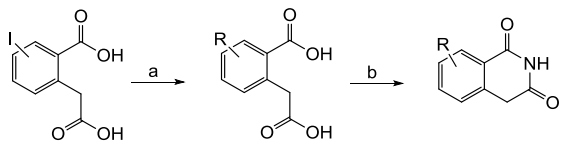
Cpd	Rec IC_{50} (μM)		WCE IC_{50} (μM)	
	Tdp2	*Tdp1*	*Tdp2*	*Tdp1*
43	10	>111	12	>111
46	>111	>111	--	--

Fig. 3. SAR and selective TDP2 inhibition of the isoquinolinedione chemotype: (a) structure of isoquinoline-1,3-dione **43** and HID **46**; (b) inhibition profile: **43** selectively inhibits TDP2 in assays using either recombinant (Rec) or endogenous TDP2 from whole cell extracts (WCE), while **46** was inactive in either condition; (c) gel images of dose

response testing of **43** in Rec. TDP2 biochemical assays. Conc. (μM):1.4, 4.1, 12.3, 37, 111; (d) dose response curve for **43** in Rec. TDP2 assay expressed as mean ± SD from at least three independent experiments.

Chemistry. The SAR of compound **43** concerns primarily three distinct series of compounds: 1) isoquinoline-1,3-diones with a simple functional group (Br, I, CO_2H, CF_3, NO_2) around the left ring (**33**–**42**, Table 1); 2) isoquinoline-1,3-diones with an aromatic ring substituted around the left ring (**43**–**88**, Table 1); and 3) scaffold analogues (**89**–**96**, Table 1). Synthetically, all isoquinoline-1,3-dione analogues of the first two series (**43**–**88)** were accessed by either of the two routes delineated in Schemes 1–2. The first approach (Scheme 1) was adapted based on our previously reported route for the synthesis of HID.[19] This approach features key iodo homophthalic acid intermediates (**20**) which were synthesized using a Hurtley reaction.[19] The structural diversity for analogues of series two was subsequently introduced via a Suzuki coupling reaction to afford intermediates **21**, which were condensed with urea in 1,2-dichlorobenzene to yield desired isoquinoline-1,3-dione analogues (Scheme 1).[20]

Scheme 1[a].



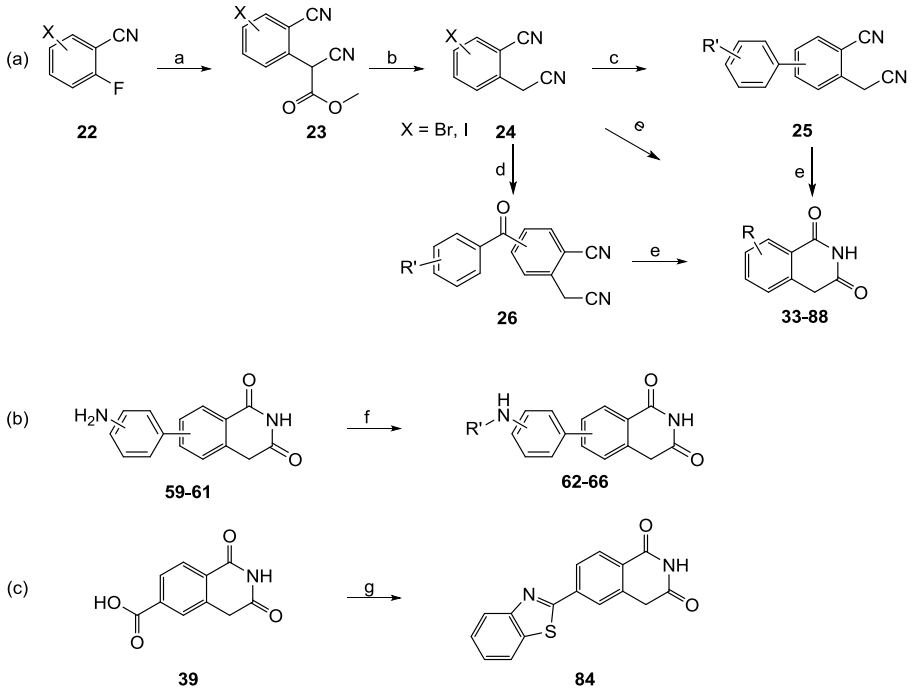
20 **21** **33-88**

[a] Reagents and conditions: a) arylboronic acid, K_2CO_3, $Pd(PPh_3)_4$, $EtOH/H_2O$ (1:1), 150 °C, 30 min, MW, 60-85%; b) urea, 1,2-dichlorobenzene, 170 °C, 45 min, MW, 55-80%.

To limit the usage of 1,2-dichlorobenzene and to produce a range of substituted isoquinoline-1,3-diones, an alternative method was employed (Scheme 2). This method

began with the S_NAr reaction of substituted-2-fluorobenzonitrile (**22**) with methyl cyanoacetate at 90 °C to produce the ester intermediate (**23**) which upon heating in a mixture of DMSO/H_2O (9:1) at 120 °C resulted in the smooth decarboxylation to furnish the halogen (I or Br) substituted dinitrile compounds (**24**). At this stage structural diversity was introduced via either a Suzuki coupling to afford various aryl substituted dinitrile intermediates (**25**) or a palladium assisted arylation and carbonyl insertion to produce benzoyl substituted dinitrile compounds (**26)**. Treatment of dinitrile intermediates **24**–**26** with con. HCl at 70 °C afforded the desired cyclized products (**33-88**, Scheme 2a). The sulfonamide (**62**–**64**) or acrylamide (**65**–**66**) analogues were synthesized by derivatizing the aniline analogues (**59**–**61**) with sulfonyl or acryloyl chloride (Scheme 2b). The benzothiazole derivative (**84**) was synthesized using a T3P mediated coupling of **39** with 2-aminothiophenol (Scheme 2c).

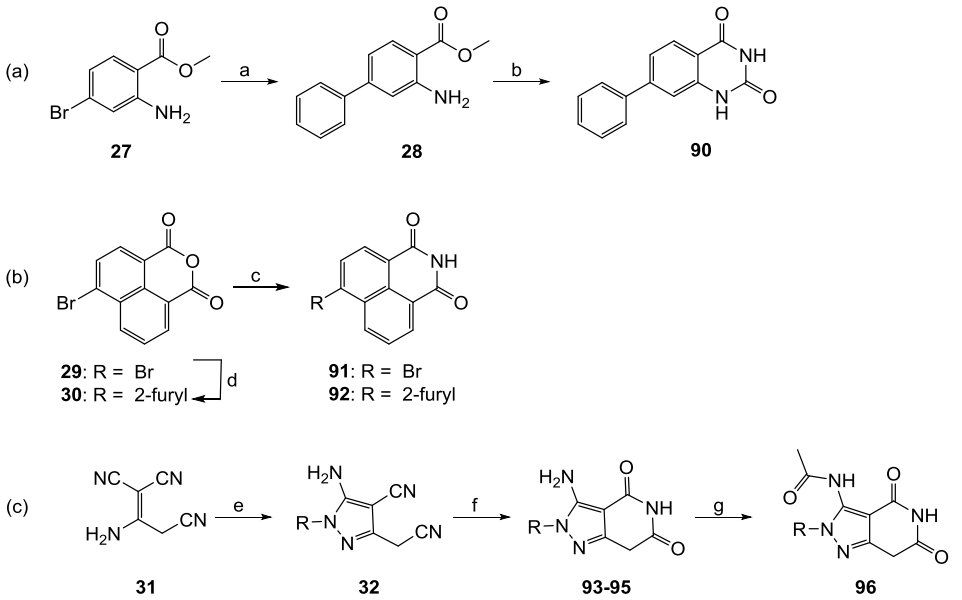
Scheme 2[a].



[a] Reagents and conditions: a) Methyl cyanoacetate, NaH, DMSO, 90 °C; b) DMSO/H_2O (9:1), 120 °C, 16 h, 70-85%; c) arylboronic acid, K_2CO_3, $Pd(PPh_3)_4$, DME/H_2O (4:1), 110 °C, 40 min, MW, 55-87%; d) $Pd(OAc)_2$, $Mo(CO)_6$, K_2CO_3, anisole (0.2M), 140 °C, 30 min, MW, 50-62%; e) Con HCl, 70 °C, 4 h, 60-90%; f) sulfonyl or acryloyl chloride, pyridine, dioxane, r.t; 65-79%; g) 2-aminothiophenol, T3P (50% in EtOAc), DIPEA, 100 °C, 30 min, MW, 69%.

The methods for synthesizing scaffold analogues are depicted in Scheme 3. The synthesis of quinazoline-2,4-dione (**90**) involved a Suzuki coupling reaction of the starting 4-bromomethyl anthranilate **27**. The resulting intermediate **28** was then converted to the desired cyclized product **90** via the treatment with potassium cyanate and the subsequent saponification (Scheme 3a).[21]The 1,8-naphthalimide derivatives (**91**–**92**) were synthesized by the condensation of 1,8-naphthalic anhydrides (**29**–**30**) with ammonium hydroxide (Scheme 3b).[22] The 5,6-fused heterocyclic core was obtained by treating 2-amino-1-propene-1,1,3-tricarbonitrile (**31**) with various substituted hydrazine in refluxing ethanol to yield dinitrile compounds (**32**), which were then cyclized with the aid of acid to furnish compounds **93**–**95**. Compound **95** was acetylated to produce **96** (Scheme 3c).

Scheme 3[a].

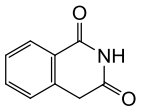
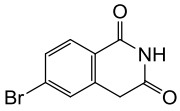


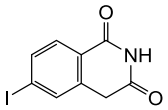
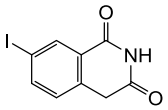
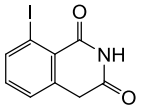
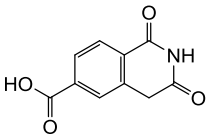
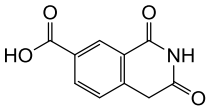
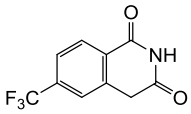
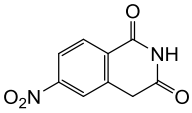
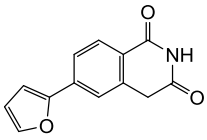
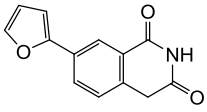
[a] Reagents and conditions: a) phenylboronic acid, K_2CO_3, $Pd(PPh_3)_4$, DME/H_2O (4:1), 110 °C, 45 min, MW, 50%; b) i) KOCN, toluene, r.t; ii) NaOH, EtOH, reflux, 68%; c) NH_4OH, 40 °C, 4 h, 70-90%; d) 2-(tributylstannyl)furan, $Pd(PPh_3)_4$, toluene, 150 °C, 30 min, 72%; e) R-$NHNH_2$, EtOH, reflux, 70-95%; f) Con HCl, 70 °C, 4 h, 65-85%; g) acetyl chloride, AcOH, 70 °C, 62%.

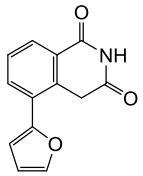
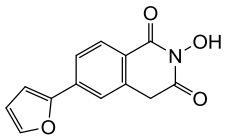
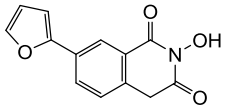
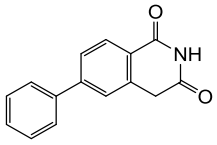
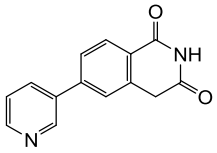
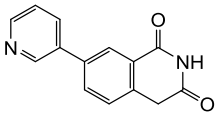
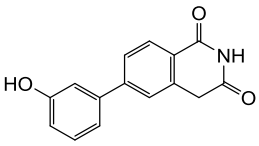
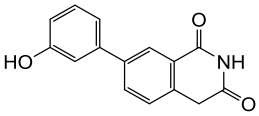
Biology. All final compounds **33**–**96** were evaluated biochemically for inhibition of human TDP2. The primary assay used recombinant TDP2 and all compounds were tested in dose response fashion at concentrations up to 111 μM. A counter assay was also run with each compound against the homologous TDP1 and none of the tested compounds showed significant inhibition at the highest concentration (111μM). The testing results are summarized in Table 1. The most striking SAR trend was that while the vast majority of the quinolone-1,3-dione analogues (**33**-**89**) potently inhibited recombinant human TDP2 in the low micromolar range, none of the scaffold analogues (**90**-**96**) showed considerable TDP2 inhibition in the same assay. This observation strongly validates quinolone-1,3-dione as an important TDP2 inhibitor type. In addition, the dramatic OH

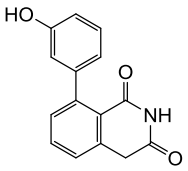
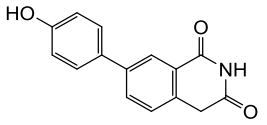
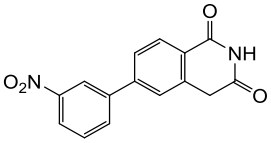
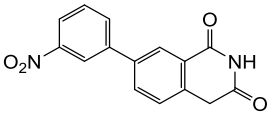
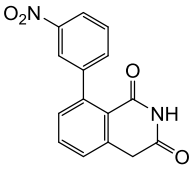
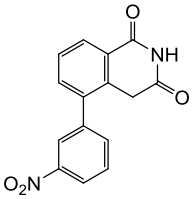
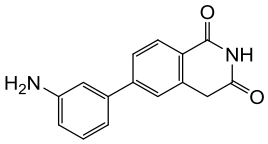
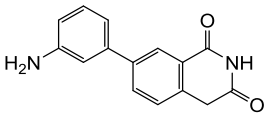
effect at the N-2 position initially observed with compound **46** was confirmed with an additional pair of analogues (**44** and **47**). Consistent with the SAR trend, the 2-NH analogue **44** inhibited TDP2 with an IC_{50} of 13 μM whereas the 2-NOH analogue **47** was completely inactive (IC_{50} > 111μM). This confirmed SAR trend suggests that compounds with a chleting triad, such as the HID chemotype, should not be considered as favorable TDP2 inhibitor types. The third major SAR observation concerns the substitution position on the left ring of the isoquinoline-1,3-dione core. Notably, most of the C6 or C7 substituted analogues potently inhibited recombinant TDP2, whereas C8 analogues (**53** and **57**) showed substantially reduced inhibitory activity (compared to **51-52** and **55-56**), and more dramatically, C5 substituted analogues (**34**, **45** and **58**, Table 1) were all inactive regardless of the nature of the substituent.

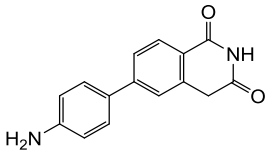
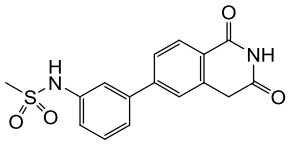
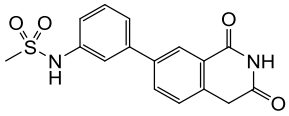
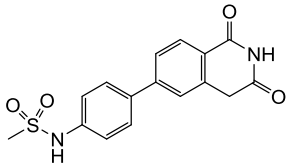
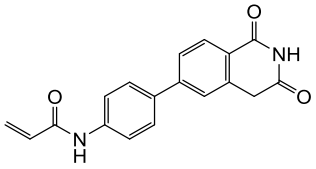
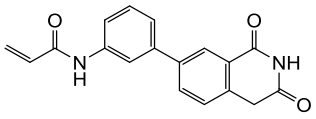
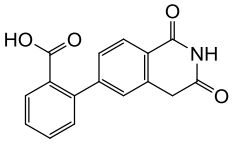
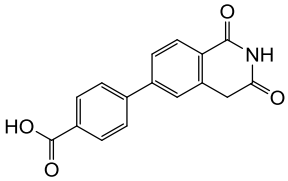
Table 1. Biochemical inhibition of synthetic isoquinolinedione analogues against recombinant hTDP2.

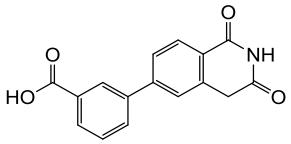
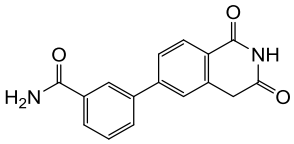
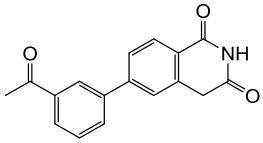
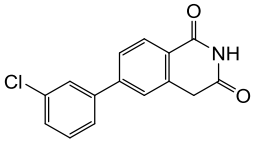
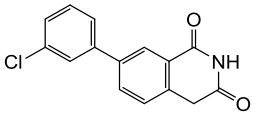
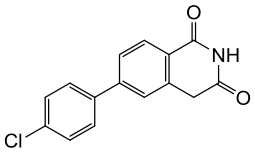
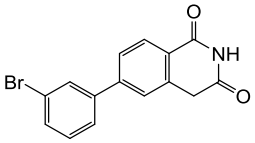
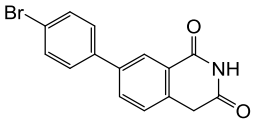
Compd	*Structure*	*IC_{50} (μM)* [a]
33		25±8.2
34		>37
35		11±8.3

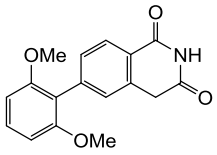
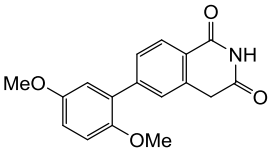
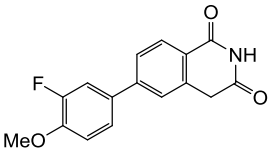
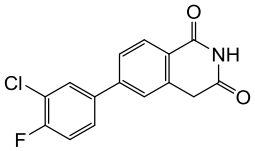
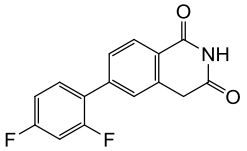
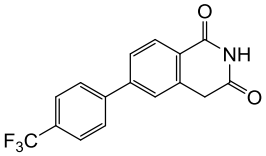
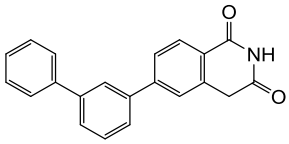
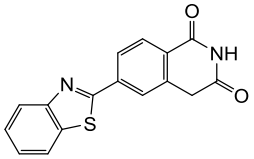
36		7.6±4.1
37		9.8±3.7
38		7.5±3.0
39		>111
40		3.0±1.0
41		7.0±1.7
42		19±9.5
43		10±2.5
44		13, 26[b]

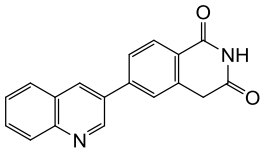
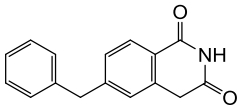
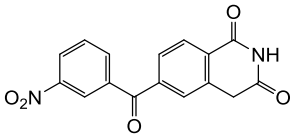
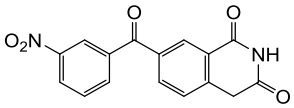
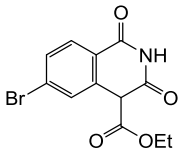
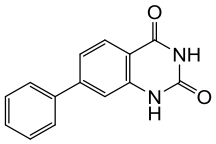
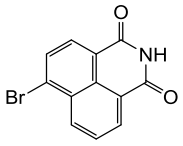
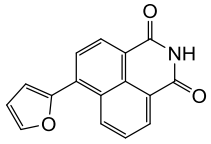
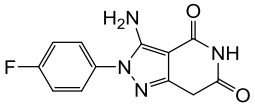
45		>111
46		>111
47		>111
48		23, 71[b]
49		16±5.0
50		20±6.0
51		13±3.0
52		15±9.5

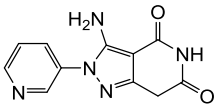
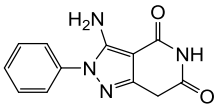
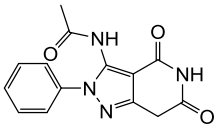
53		26±5.7
54		14±9.2
55		10±2.0
56		11±4.5
57		19±4.3
58		>37
59		42±2.0
60		>111

61		36±3.0
62		18±8.0
63		4.5±2.8
64		1.9±0.90
65		10±7.3
66		11±3.0
67		>111
68		30±5.0

69		21±8.0
70		16±5.8
71		5.5±2.0
72		5.6±2.6
73		1.7±0.30
74		6.4±4.2
75		8.0±5.1
76		7.1±5.3

77		>37
78		16±6.9
79		3.0±2.3
80		4.1±1.9
81		4.5±1.5
82		3.2±2.5
83		>111
84		5.1±2.0

85		9.3, 6.5[b]
86		>111
87		11±2.0
88		5.4±1.8
89		14±5.3
90		>111
91		>111
92		>111
93		>111

94		>111
95		>111
96		>111

[a] IC_{50}: concentration of a compound producing 50% inhibition, expressed as mean±standard deviation from at least three independent experiments.

[b] Value from a second independent experiment.

To confirm the observed potency with C6 or C7 analogues, selected compounds were also tested in a secondary assay using the whole cell extracts of the TDP2 expressing cells. As shown in Table 2, with the exception of **48** and **85**, all compounds tested retained activity in this assay with IC_{50} values consistent with those obtained from the assays using recombinant TDP2. These data strongly support a genuine and selective TDP2 inhibitory profile of the C6 or C7 substituted isoqunioline-1,3-dione chemotype. In addition, a few compounds (**36**, **40**, **43** and **84**) were also tested for sensitivity against DT40 knockout cells complemented with human hTDP2 using a previously reported assay.[6] Although synergy to Top2 inhibitor ETP was not observed with any of these compounds, three of them (**36**, **43** and **84**) exhibited potent cytotoxicity at low micromolar concentrations (CC_{90} = 2.5—12 μM). It is likely that further improved TDP2 inhibitors will produce the desired synergism.

Table 2. Inhibitory activity of selected compounds against hTDP2 whole cell extract (WCE).

Compd	*Rec. TDP2 IC_{50}* [a] *(μM)*	*WCE IC_{50}* [b] *(μM)*	*CC_{90}* [c] *(μM)*
36[d]	7.6±4.1	9.3	9.5
38	7.5±3.0	2.6	--
40[d]	3.0±1.0	5.2	>50
41	7.0±1.7	3.5	--
43[d]	8.5	25	2.4
44	13	50	--
48	23	>111	--
49	16±5.0	23	--
51	13±3.0	35	--
55	10±2.0	25	--
59	42±2.0	55	--
61	36±3.0	40	--

62	18±8.0	25	--
64	1.9±0.90	2.2	--
69	21±8.0	42	--
71	5.5±2.0	15	--
72	5.6±2.6	3.3	--
73	1.7±0.30	3.2	--
74	6.4±4.2	4.5	--
75	8.0±5.1	6.5	--
79	3.0±2.3	4.3	--
80	4.1±1.9	5.3	--
81	4.5±1.5	5.5	--
82	3.2±2.5	2.7	--
84[d]	5.1±2.0	11	12
85	9.3	>111	--

[a] IC_{50}: concentration of a compound producing 50% inhibition, expressed as mean±standard deviation from at least three independent experiments.

[b] IC_{50}: concentration of a compound producing 50% inhibition.

[c] CC_{90}: concentration of a compound killing 90% of the cells. Assay was done in the absence of EPT against hTDP2 DT40 cells.[6]

[d] Synergy was not observed when tested in combination with different concentrations of EPT.[6]

Molecular Modeling. With the lack of a TDP2 / inhibitor co-crystal structure, understanding on the inhibitor binding mode and mechanism of inhibition remains elusive. With reported inhibitors **6** and **7,** it is unclear exactly how they inhibit TDP2 as molecular modeling revealed little about their binding. To provide a predicted binding mode of our new inhibitors, we have constructed a homology model of hTDP2 based on the available crystal structure of mouse TDP2 (mTDP2; PDB code:4GZ1[15]) which shares a high degree of sequence identity (65%) with hTDP2. With this model, docking analysis was performed using Glide XP (v.6.4).[23, 24] The predicted binding mode of compound **56** in the active site of hTDP2 suggests an interaction between the carbonyl at position 1 of isoquinoline-1,3-dione core and the residues, H351 and the backbone NH of N264 of TDP2 (Fig. 4a). The NH at position 2 and carbonyl group at position 3 of **56** can coordinate to the magnesium ion (Mg^{2+}). The carbonyl group at position 7 of **56** forms a salt bridge with the R266 within the active site of hTDP2. Whereas in compound **40**, the carbonyl group at position 1 forms an H-bond interaction with the backbone NH of N264. The NH at position 2 and carbonyl group at position 3 within **40** coordinate to the E152 and magnesium ion (Mg^{2+}) respectively (Fig. 4b).

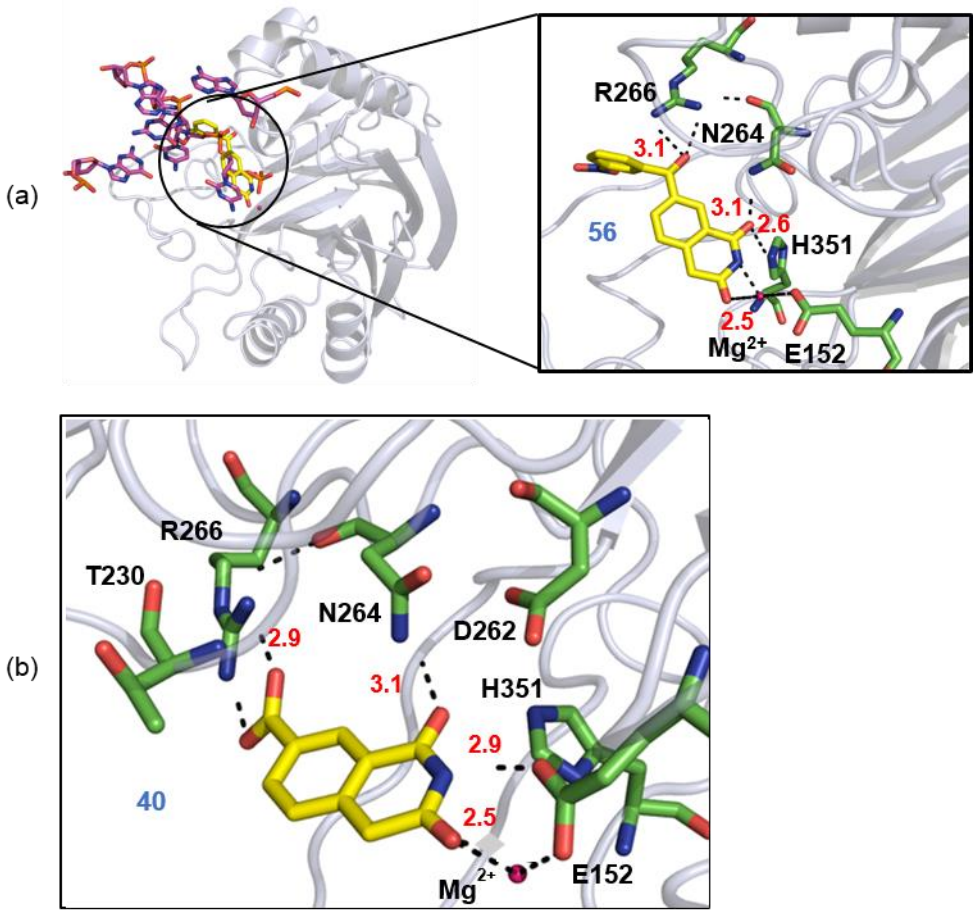


Fig. 4. Predicted binding mode of a) **56** and b) **40** within active site of hTDP2 (numbering was based on hTDP2 sequence). The active site residues are shown as green sticks and metal ions as magenta sphere. The H-bond interactions are depicted as black dotted lines and the distance between bonded atoms are represented in red. Homology model (hTDP2) was built using the crystal structure of mTDP2 (PDB code: 4GZ1[15]).

Conclusions

By screening our in-house collection of synthetic small molecules, isoquinoline-1,3-dione was identified as a novel inhibitor type of TDP2. The selective and potent inhibitory profile of this chemotype against TDP2 was further established through the chemical synthesis of 63 functional and scaffold analogues, and validated via a secondary assay

using WCE as well as a counter assay against TDP1, which shares some substrates with TDP2.[25] Analogue synthesis identified compound **64** as the best of the series with an IC_{50} of 1.9 μM against recombinant TDP2 and 2.2 μM against TDP2 in WCE assay. The main SAR observations also suggest that the C6 or C7 substitution is strongly preferred and that the OH substitution at the N2 position resulting in a CO-NOH-CO chelating triad is undesired. While efforts towards obtaining the TDP2 / inhibitor co-crystal structures are still underway, homology modeling did reveal a few specific interactions within the TDP2 active site that may contribute to understanding the molecular mechanism of TDP2 inhibition. Identifying this novel and selective TDP2 inhibitor type may provide an important path towards employing TDP2 as a drug target and developing TDP2 inhibitors as molecular probes.

Experimental

Chemistry

General Procedures. All commercial chemicals were used as supplied unless otherwise indicated. Flash chromatography was performed on a Teledyne Combiflash RF-200 with RediSep columns (silica) and indicated mobile phase. All moisture sensitive reactions were performed under an inert atmosphere of ultra-pure argon with oven-dried glassware. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 600 MHz spectrometer. Mass data were acquired on an Agilent TOF II TOS/MS spectrometer capable of ESI and APCI ion sources. Analysis of sample purity was performed on a Varian Prepstar SD-1 HPLC system with a Phenomenex Gemini, 5 micron C18 column (250mm x 4.6 mm). HPLC conditions**:** solvent A = H_2O, solvent B = MeCN; flow rate = 1.0 mL/min; compounds

were eluted with a gradient of 10% MeCN/H_2O for 0-5 min and to 80% MeCN/ H_2O from 5-30 min followed by 100% MeCN from 30-35 min and then 10% MeCN/H_2O from 35-40 min. Purity was determined by total absorbance at 254 nm. All tested compounds have a purity ≥ 98%.

General Procedure for Suzuki Coupling (21, 25). The mixture of compound **20 or 24** (1.0 eq), aryl boronic acid (1.6 eq), $Pd(PPh_3)_4$ (0.065 eq), K_2CO_3 (3.6 eq) in EtOH/H_2O (1:1) was irradiated at 150 °C for 30 min under microwave conditions. The black residue formed was filtered through celite and the solvent was concentrated in vacuo. The resulting aqueous solution was acidified (pH = 3) using 2N HCl. A white precipitate was obtained *via* filtration, which was washed with water and dried under vacuum to furnish desired compounds **(21, 25)** as colorless solid.

General Procedure for the S_NAr Reaction (23). To a suspension of NaH (32 mmol, 2.0 eq, 60% dispersion in oil) in DMSO (10 mL) at 0 °C was added methyl cyanoacetate (32 mmol, 2.0 eq) slowly and the mixture was stirred at r.t for 30 min before 2-fluoro-4-iodobenzonitrile (**22**, 16 mmol, 1.0 eq) in DMSO (16 mL) was added. The resulting solution was stirred at 90 °C for 8 h and quenched by adding 2N HCl (20 mL). After extraction with the EtOAc (2 x 30 mL), the combined organics were washed with $NaHCO_3$ (2 x 20 mL), brine (30 mL), dried over Na_2SO_4 and concentrated in vacuo to leave brown oil. The crude material was used for next step without further purification.

General Procedure for the Synthesis of Dinitriles (24). A solution of compound **23** in DMSO/H_2O (9:1) mixture was stirred at 120 °C for 16 h before being quenched with water. The aqueous solution was extracted using EtOAc (2 x 30 mL). The combined organics were washed with water (2 x 20 mL), brine (30 mL), dried over Na_2SO_4, and

concentrated in vacuo. The crude mixture was purified using CombiFlash with 0-20% EtOAc in hexane as an eluent to yield the desired products (**24**) as yellow solid. Yield: 70-85% over 2 steps.

General Procedure for the Synthesis of Arylketone (26). A mixture of compound **24** (1.0 eq), arylboronic acid (2.5 eq), $Pd(OAC)_2$ (0.1 eq), $Mo(CO)_6$ (1.5 eq), K_2CO_3 (3.0 eq) in anisole (0.2M, 5 mL) was irradiated at 140 °C for 30 min under microwave conditions. The black residue formed was filtered through celite and the solvent was concentrated in vacuo. The crude mixture was purified using CombiFlash with 0-50% EtOAc in hexane as an eluent to yield the desired product **26** as colorless solid. Yield: 50-62%.

General Procedure for Isoquinoline-1,3-dione (33-88). *Method A:* To a suspension of compound **21** (1.0 eq) in 1,2-dichlorobenzene was added urea (2.0 eq) and the resulting mixture was irradiated at 175 °C for 45 min under microwave conditions. The solvent was removed in vacuo to produce the crude product as brown solid which was purified using CombiFlash with 0-50% EtOAc in hexane as an eluent to furnish titled compounds (**33-88**) as yellow solid. *Method B:* A solution of compound **24 / 25 / 26** (0.45 mmol) in Conc. HCl (2 mL) was stirred at 70 °C for 4 h. After the solvent was removed in vacuo the residue was purified using CombiFlash with 0-30% EtOAc in hexane as an eluent to yield desired products (**33-88**) as colorless solid.

Isoquinoline-1,3(2*H*, 4*H*)-dione (33). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.28 (s, 1H), 8.01 (dd, J = 7.8, 1.4 Hz, 1H), 7.64 (td, J = 7.5, 1.4 Hz, 1H), 7.45 (t, J = 7.6 Hz, 1H), 7.38 (d, J = 7.6 Hz, 1H), 4.03 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.1, 165.5, 136.8, 133.6, 127.9, 127.7, 127.5, 125.1, 36.1. HRMS-ESI (−) *m/z* calculated for $C_9H_6NO_2$, 160.0404 [M-H]-; found: 160.0400.

5-Bromoisoquinoline-1,3(2*H*, 4*H*)-dione (34). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.48 (s, 1H), 8.06 (d, *J* = 7.7 Hz, 1H), 7.95 (d, *J* = 7.8 Hz, 1H), 7.43 (t, *J* = 7.8 Hz, 1H), 3.87 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.2, 164.1, 136.6, 135.7, 128.5, 126.9, 126.7, 122.2, 37.1. HRMS-ESI (−) *m/z* calculated for $C_9H_5BrNO_2$, 237.9509 [M-H]-; found: 237.9506.

6-Bromoisoquinoline-1,3(2*H*, 4*H*)-dione (35). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.37 (s, 1H), 7.92 (d, *J* = 8.1 Hz, 1H), 7.67-7.65 (m, 2H), 4.04 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.0, 162.4, 156.9, 137.1, 133.6, 129.9, 128.6, 127.6, 35.9. HRMS-ESI (−) *m/z* calculated for $C_9H_5BrNO_2$, 237.9509 [M-H]-; found: 237.9515.

6-Iodoisoquinoline-1,3(2*H*, 4*H*)-dione (36). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.35 (s, 1H), 7.83 (m, 2H), 7.74 (d, *J* = 8.7 Hz, 1H), 4.01 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.9, 165.5, 139.1, 136.9, 136.8, 136.4, 125.0, 102.5, 35.9. HRMS-ESI (−) *m/z* calculated for $C_9H_5INO_2$, 285.9370 [M-H]-; found: 285.9376.

7-Iodoisoquinoline-1,3(2*H*, 4*H*)-dione (37). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.38 (s, 1H), 8.25 (d, *J* = 1.8 Hz, 1H), 7.97 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.19 (d, *J* = 8.1 Hz, 1H), 3.96 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.4, 163.9, 141.5, 136.1, 135.3, 129.9, 126.8, 92.1, 35.5. HRMS-ESI (−) *m/z* calculated for $C_9H_5INO_2$, 285.9370 [M-H]-; found: 285.9367.

8-Iodoisoquinoline-1,3(2*H*, 4*H*)-dione (38). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.28 (s, 1H), 8.06 (d, *J* = 7.7 Hz, 1H), 7.40 (d, *J* = 7.7 Hz, 1H), 7.26 (t, *J* = 7.7 Hz, 1H), 4.05 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.7, 163.6, 141.8, 134.4, 133.4, 128.2, 124.6, 94.9, 37.5. HRMS-ESI (−) *m/z* calculated for $C_9H_5INO_2$, 285.9370 [M-H]-; found: 285.9372.

1,3-Dioxo-1,2,3,4-tetrahydroisoquinoline-6-carboxylic acid (39). ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.37 (s, 1H), 11.40 (s, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.91 (s, 1H), 4.08 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.6, 166.3, 164.6, 136.8, 134.6, 128.6, 128.1, 127.6, 127.4, 35.8. HRMS-ESI (−) *m/z* calculated for $C_{10}H_6NO_4$, 204.0302 [M-H]-; found: 204.0301.

1,3-Dioxo-1,2,3,4-tetrahydroisoquinoline-7-carboxylic acid (40). ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.26 (s, 1H), 11.42 (s, 1H), 8.53 (d, J = 1.8 Hz, 1H), 8.14 (dd, J = 8.1, 1.8 Hz, 1H), 7.51 (d, J = 8.1 Hz, 1H), 4.11 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 175.0, 165.9, 162.8, 135.9, 135.2, 134.1, 130.2, 128.7, 127.2, 36.1. HRMS-ESI (−) *m/z* calculated for $C_{10}H_6NO_4$, 204.0302 [M-H]-; 204.0308.

6-(Trifluoromethyl)isoquinoline-1,3(2*H*, 4*H*)-dione (41). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.50 (s, 1H), 8.19 (d, J = 8.1 Hz, 1H), 7.82 (s, 1H), 7.80 (d, J = 8.3 Hz, 1H), 4.12 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.5, 164.4, 138.0, 132.7, 128.5, 124.9, 124.6, 123.7, 122.7, 36.0. HRMS-ESI (−) *m/z* calculated for $C_{10}H_5F_3NO_2$, 228.0278 [M-H]-; found: 228.0285.

6-Nitroisoquinoline-1,3(2*H*, 4*H*)-dione (42). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.59 (s, 1H), 8.29 (s, 1H), 8.25-8.25 (m, 2H), 4.17 (s, 2H). HRMS-ESI (−) *m/z* calculated for $C_9H_5N_2O_4$, 205.0255 [M-H]-; found: 205.0252.

6-(Furan-2-yl)isoquinoline-1,3 (2*H*, 4*H*)-dione (43). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.28 (s, 1H), 8.03 (d, J = 8.2 Hz, 1H), 7.86 (s, 1H), 7.77 (d, J = 8.2 Hz, 1H), 7.70 (s, 1H), 7.18 (d, J = 3.3 Hz, 1H), 6.67 (dd, J = 3.3, 1.7 Hz, 1H), 4.07 (s, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 175.4, 163.0, 150.9, 145.2, 135.0, 133.2, 129.3, 128.9, 128.0, 120.57,

112.9, 110.1, 39.2; HRMS-ESI (−) *m/z* calculated for $C_{13}H_8NO_3$, 226.0510 [M-H]-; found: 226.0506.

7-(Furan-2-yl)isoquinoline-1,3(2*H*, 4*H*)-dione (44). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.36 (s, 1H), 8.27 (s, 1H), 7.97 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.79 (s, 1H), 7.44 (d, *J* = 8.1 Hz, 1H), 7.09 (d, *J* = 3.3 Hz, 1H), 6.63 (dd, *J* = 3.3, 1.7 Hz, 1H), 4.04 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.0, 165.2, 151.9, 143.5, 135.6, 129.5, 128.8, 128.4, 125.6, 121.7, 112.4, 106.8, 35.9. HRMS-ESI (−) *m/z* calculated for $C_{13}H_8NO_3$, 226.0510 [M-H]-; found: 226.0504.

5-(Furan-2-yl)isoquinoline-1,3(2*H*, 4*H*)-dione (45). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.45 (s, 1H), 8.06 (d, *J* = 7.7 Hz, 1H), 8.00 (d, *J* = 7.7 Hz, 1H), 7.88 (d, *J* = 1.7 Hz, 1H), 7.56 (t, *J* = 7.8 Hz, 1H), 6.95 (d, *J* = 3.4 Hz, 1H), 6.69 (dd, *J* = 3.4, 1.7 Hz, 1H), 4.13 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.5, 165.3, 150.6, 143.6, 132.3, 131.6, 128.8, 127.5, 127.3, 126.1, 112.2, 110.6, 35.8. HRMS-ESI (−) *m/z* calculated for $C_{13}H_8NO_3$, 226.0510 [M-H]-; found: 226.0514.

6-(Furan-2-yl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (46). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.39 (s, 1H), 8.05 (d, *J* = 8.3 Hz, 1H), 7.87 (d, *J* = 0.7 Hz, 1H), 7.80 (d, *J* = 8.3 Hz, 1H), 7.71 (s, 1H), 7.19 (d, *J* = 3.4 Hz, 1H), 6.69-6.66 (m, 1H), 4.30 (s, 2H). HRMS-ESI (−) *m/z* calculated for $C_{13}H_8NO_4$, 242.0459[M-H]-; found: 242.0464.

7-(Furan-2-yl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (47). ^{1}H NMR (600 MHz, CD_3OD) δ 8.40 (d, *J* = 1.8 Hz, 1H), 7.95 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.61 (d, *J* = 1.3 Hz, 1H), 7.42 (d, *J* = 8.1 Hz, 1H), 6.89 (d, *J* = 3.3 Hz, 1H), 6.55 (dd, *J* = 3.3, 1.8 Hz, 1H). HRMS-ESI (−) *m/z* calculated for $C_{13}H_8NO_4$, 242.0459[M-H]-; found: 242.0466.

6-Phenylisoquinoline-1,3(2*H*, 4*H*)-dione (48). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.31 (s, 1H), 8.08 (d, *J* = 8.1 Hz, 1H), 7.76-7.74 (m, 3H), 7.71 (s, 1H), 7.52 (t, *J* = 7.6 Hz, 2H), 7.44 (t, *J* = 7.3 Hz, 1H), 4.10 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 175.7, 163.1, 157.6, 145.3, 137.8, 132.9, 132.9, 129.5, 129.2, 128.6, 127.2, 124.2, 39.1. HRMS-ESI (−) *m/z* calculated for $C_{15}H_{10}NO_2$, 236.0717 [M-H]-; found: 236.0712.

6-(Pyridin-3-yl)isoquinoline-1,3(2*H*, 4*H*)-dione (49). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.40 (s, 1H), 9.18 (s, 1H), 8.83 (d, *J* = 4.4 Hz, 1H), 8.62 (d, *J* = 7.8 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 7.96-7.84 (m, 3H), 4.11 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.6, 164.8, 144.7, 143.4, 139.6, 139.5, 137.4, 135.8, 128.2, 126.5, 125.8, 125.8, 125.2, 35.9. HRMS-ESI (−) *m/z* calculated for $C_{14}H_9N_2O_2$, 237.0670 [M-H]; found: 237.0672.

7-(Pyridin-3-yl)isoquinoline-1,3(2*H*, 4*H*)-dione (50). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.44 (s, 1H), 9.29 (s, 1H), 8.87 (d, *J* = 5.5 Hz, 1H), 8.82 (d, *J* = 8.1 Hz, 1H), 8.42 (d, *J* = 2.1 Hz, 1H), 8.15 (dd, *J* = 8.0, 2.1 Hz, 1H), 8.04 (dd, *J* = 8.0, 5.5 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 1H), 4.13 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.4, 165.6, 143.5, 142.8, 141.7, 138.5, 137.4, 134.3, 132.8, 129.7, 127.1, 126.7, 126.6, 36.6. HRMS-ESI (−) *m/z* calculated for $C_{14}H_9N_2O_2$, 237.0670 [M-H]-; found: 237.0673.

6-(3-Hydroxyphenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (51). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.30 (s, 1H), 9.64 (s, 1H), 8.07 (d, *J* = 8.2 Hz, 1H), 7.69 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.63 (d, *J* = 0.9 Hz, 1H), 7.31 (t, *J* = 7.9 Hz, 1H), 7.17-7.13 (m, 1H), 7.09 (t, *J* = 2.3 Hz, 1H), 6.85 (ddd, *J* = 8.2, 2.3, 0.9 Hz, 1H), 4.10 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.2, 165.3, 158.1, 145.1, 140.2, 137.4, 130.3, 128.2, 126.0, 125.6, 124.0, 117.9, 115.7, 113.9, 36.3. HRMS-ESI (−) *m/z* calculated for $C_{15}H_{10}NO_3$, 252.0666 [M-H]-; found: 252.0670.

7-(3-Hydroxyphenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (52). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.35 (s, 1H), 9.58 (s, 1H), 8.17 (d, *J* = 1.9 Hz, 1H), 7.90 (dd, *J* = 7.9, 1.9 Hz, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.29 (t, *J* = 7.9 Hz, 1H), 7.12 (d, *J* = 7.7 Hz, 1H), 7.07 (s, 1H), 6.87-6.75 (m, 1H), 4.07 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.7, 165.1, 157.8, 139.9, 138.9, 135.5, 131.4, 129.9, 128.4, 125.2, 124.7, 117.1, 114.7, 113.1, 35.5. HRMS-ESI (−) *m/z* calculated for $C_{15}H_{10}NO_3$, 252.0666 [M-H]-; found: 252.0667.

8-(3-Hydroxyphenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (53). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.00 (s, 1H), 9.32 (s, 1H), 7.60 (t, *J* = 7.6 Hz, 1H), 7.37 (d, *J* = 7.3 Hz, 1H), 7.15 (d, *J* = 7.5 Hz, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 6.71 (dd, *J* = 8.2, 1.7 Hz, 1H), 6.61 (d, *J* = 7.5 Hz, 1H), 6.60-6.58 (m, 1H), 4.08 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.9, 164.6, 156.8, 144.3, 143.8, 137.9, 132.4, 130.7, 128.7, 127.6, 123.0, 119.44, 115.6, 113.8, 37.0. HRMS-ESI (−) *m/z* calculated for $C_{15}H_{10}NO_3$, 252.0666 [M-H]-; found: 252.0670.

7-(4-Hydroxyphenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (54). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.32 (s, 1H), 9.63 (s, 1H), 8.15 (d, *J* = 2.0 Hz, 1H), 7.87 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.54 (d, *J* = 8.6 Hz, 2H), 7.42 (d, *J* = 8.1 Hz, 1H), 6.87 (d, *J* = 8.6 Hz, 2H), 4.04 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.9, 165.2, 157.4, 139.0, 134.4, 130.9, 129.4, 128.4, 127.6, 125.2, 124.0, 115.8, 35.5. HRMS-ESI (−) *m/z* calculated for $C_{15}H_{10}NO_3$, 252.0666 [M-H]-; found: 252.0669.

6-(3-Nitrophenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (55). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.36 (s, 1H), 8.53 (t, *J* = 1.9 Hz, 1H), 8.29 (dd, *J* = 8.2, 1.9 Hz, 1H), 8.23 (d, *J* = 8.2 Hz, 1H), 8.12 (d, *J* = 8.1 Hz, 1H), 7.88 (d, *J* = 8.2 Hz, 1H), 7.86 (s, 1H), 7.81 (t, *J* = 8.1 Hz, 1H), 4.12 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.4, 165.5, 148.9, 142.7,

140.7, 138.0, 134.1, 131.1, 128.9, 126.9, 126.2, 125.3, 123.7, 122.0, 36.6. HRMS-ESI (−) *m/z* calculated for $C_{15}H_9N_2O_4$, 281.0568 [M-H]-; found: 281.0561.

7-(3-Nitrophenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (56). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.41 (s, 1H), 8.49 (s, 1H), 8.33 (s, 1H), 8.26 (d, *J* = 8.1 Hz, 1H), 8.22 (d, *J* = 8.1 Hz, 1H), 8.10 (d, *J* = 9.8 Hz, 1H), 7.79 (t, *J* = 7.9 Hz, 1H), 7.55 (d, *J* = 8.1 Hz, 1H), 4.11 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.3, 165.5, 148.9, 140.8, 137.4, 134.2, 133.74, 132.4, 131.2, 129.4, 125.9, 123.1, 122.3, 121.6, 36.3. HRMS-ESI (−) *m/z* calculated for $C_{15}H_9N_2O_4$, 281.0568 [M-H]-; found: 281.0570.

8-(3-Nitrophenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (57). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.12 (s, 1H), 8.23 – 8.19 (m, 1H), 8.08 (s, 1H), 7.72 (d, *J* = 7.6 Hz, 1H), 7.70-7.64 (m, 2H), 7.48 (d, *J* = 7.7 Hz, 1H), 7.26 (d, *J* = 7.5 Hz, 1H), 4.12 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.8, 165.0, 147.4, 144.1, 141.5, 138.5, 135.5, 132.8, 130.9, 130.7, 129.3, 123.6, 123.3, 121.8, 36.9. HRMS-ESI (−) *m/z* calculated for $C_{15}H_9N_2O_4$, 281.0568 [M-H]-; found: 281.0575

5-(3-Nitrophenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (58). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.42 (s, 1H), 8.30 (d, *J* = 7.8 Hz, 1H), 8.23 (s, 1H), 8.15 (d, *J* = 7.5 Hz, 1H), 7.89 (d, *J* = 7.5 Hz, 1H), 7.80 (t, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 7.1 Hz, 1H), 7.60 (t, *J* = 7.5 Hz, 1H), 3.85 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.1, 165.0, 147.7, 140.1, 138.0, 135.5, 134.4, 133.9, 129.9, 127.6, 127.2, 125.4, 123.5, 122.5, 35.0. HRMS-ESI (−) *m/z* calculated for $C_{15}H_9N_2O_4$, 281.0568 [M-H]-; found: 281.0572.

6-(3-Aminophenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (59). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.34 (s, 1H), 9.84 (s, 1H), 8.11 (d, *J* = 8.2 Hz, 1H), 7.71 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.65-7.63 (m, 2H), 7.61-7.52 (m, 2H), 7.32 (s, 1H), 4.11 (s, 2H). ^{13}C NMR (150 MHz,

DMSO-d_6) δ 171.4, 165.5, 144.1, 140.4, 137.9, 130.9, 128.8, 126.4, 125.9, 124.9, 122.2, 120.6, 117.5, 114.2, 36.6. HRMS-ESI (−) *m/z* calculated for $C_{15}H_{11}N_2O_2$, 251.0826 [M-H]-; found: 251.0829.

7-(3-Aminophenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (60). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.39 (s, 1H), 10.19 (s, 2H), 8.23 (d, *J* = 2.0 Hz, 1H), 7.95 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.69 (d, *J* = 7.9 Hz, 1H), 7.67 (s, 1H), 7.57 (t, *J* = 7.9 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.38-7.34 (m, 1H), 4.09 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.3, 165.6, 140.5, 138.3, 136.9, 134.9, 131.9, 130.9, 129.4, 126.1, 125.5, 122.3, 120.9, 36.3. HRMS-ESI (−) *m/z* calculated for $C_{15}H_{11}N_2O_2$, 251.0826 [M-H]-; found: 251.0829.

6-(4-Aminophenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (61). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.30 (s, 1H), 9.59 (s, 2H), 8.06 (d, *J* = 8.2 Hz, 1H), 7.81-7.77 (m, 2H), 7.74 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.69 (d, *J* = 1.7 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 2H), 4.08 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.4, 165.6, 145.7, 144.3, 137.8, 133.5, 128.6, 126.1, 125.7, 124.3, 122.4, 119.2, 36.6. HRMS-ESI (−) *m/z* calculated for $C_{15}H_{11}N_2O_2$, 251.0826 [M-H]-; found: 251.0830.

General Procedure for the Synthesis of Methanesulfonamide Derivative (62–64). To a solution of **59 / 60 / 61** (0.39 mmol, 1.0 eq) in dioxane (5 mL) was added pyridine (0.99 mmol, 2.5 eq), methanesulfonyl chloride (0.79 mmol, 2.0 eq) and the resulting solution was stirred at r.t for 1 h before being quenched with water. After extraction with EtOAc (2 x 20 mL), the combined organic layers were washed with 1N HCl (2 x 20 mL), brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude mixture was purified using CombiFlash with 0-5% methanol in DCM as an eluent to yield the desired product as colorless solid. Yield: 65-77%.

_N_-(3-(1,3-dioxo-1,2,3,4-tetrahydroisoquinolin-6-yl)phenyl)methanesulfonamide (62). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.32 (s, 1H), 9.89 (s, 1H), 8.10 (d, J = 8.2 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.64 (s, 1H), 7.53 (s, 1H), 7.50-7.45 (m, 2H), 7.28 (d, J = 7.0 Hz, 1H), 3.05 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.4, 165.6, 144.9, 140.4, 139.6, 137.9, 130.6, 128.7, 126.5, 126.0, 124.7, 123.1, 120.2, 118.6, 39.9, 36.6. HRMS-ESI (−) *m/z* calculated for $C_{16}H_{13}N_2O_4S$, 329.0602 [M-H]-; found: 329.0595.

_N_-(3-(1,3-dioxo-1,2,3,4-tetrahydroisoquinolin-7-yl)phenyl)methanesulfonamide (63). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.37 (s, 1H), 9.84 (s, 1H), 8.20 (d, J = 2.0 Hz, 1H), 7.91 (dd, J = 8.1, 2.0 Hz, 1H), 7.52 (s, 1H), 7.49 (d, J = 8.1 Hz, 1H), 7.47-7.44 (m, 2H), 7.36-7.18 (m, 1H), 4.07 (s, 2H), 3.04 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.4, 165.7, 140.4, 139.6, 139.1, 136.5, 132.1, 130.6, 129.2, 126.0, 125.4, 122.6, 119.5, 118.2, 39.8, 36.2. HRMS-ESI (−) *m/z* calculated for $C_{16}H_{13}N_2O_4S$, 329.0602 [M-H]-; found: 329.0598.

_N_-(4-(1,3-dioxo-1,2,3,4-tetrahydroisoquinolin-6-yl)phenyl)methanesulfonamide (64). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.29 (s, 1H), 9.97 (s, 1H), 8.06 (d, J = 8.2 Hz, 1H), 7.76-7.72 (m, 3H), 7.67 (s, 1H), 7.33 (d, J = 8.6 Hz, 2H), 4.08 (s, 2H), 3.05 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 175.5, 163.1, 157.6, 144.7, 139.5, 132.9, 132.7, 132.2, 129.2, 128.3, 123.6, 119.6, 40.2, 36.5. HRMS-ESI (−) *m/z* calculated for $C_{16}H_{13}N_2O_4S$, 329.0602 [M-H]-; found: 329.0602.

General Procedure for the Synthesis of Acrylamide Derivative (65-66). To a solution of **60** / **61** (0.39 mmol, 1.0 eq) in dioxane (5 mL) was added pyridine (0.99 mmol, 2.5 eq), acryloyl chloride (0.79 mmol, 2.0 eq) and the resulting solution was stirred at r.t for 1 h before being quenched with water. After extraction with EtOAc (2 x 20 mL), the

combined organic layers were washed with 1N HCl (2 x 20 mL), brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude mixture was purified using CombiFlash with 0-10% methanol in DCM as an eluent to yield the desired product as colorless solid. Yield: 70-79%.

***N*-(4-(1,3-dioxo-1,2,3,4-tetrahydroisoquinolin-6-yl)phenyl) acrylamide (65).** ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.28 (s, 1H), 10.30 (s, 1H), 8.05 (d, *J* = 8.2 Hz, 1H), 7.81 (d, *J* = 8.7 Hz, 2H), 7.75 (dd, *J* = 8.7, 2.2 Hz, 3H), 7.69 (s, 1H), 6.46 (dd, *J* = 17.0, 10.2 Hz, 1H), 6.28 (dd, *J* = 17.0, 1.8 Hz, 1H), 5.78 (dd, *J* = 10.2, 1.8 Hz, 1H), 4.08 (s, 2H). HRMS-ESI (−) *m/z* calculated for $C_{18}H_{13}N_2O_3$, 305.0932 [M-H]-; found: 305.0928.

***N*-(3-(1,3-dioxo-1,2,3,4-tetrahydroisoquinolin-7-yl)phenyl)acrylamide (66).** ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.38 (s, 1H), 10.29 (s, 1H), 8.24 (s, 1H), 8.07 (s, 1H), 7.94 (d, *J* = 7.9 Hz, 1H), 7.72 (d, *J* = 6.6 Hz, 1H), 7.50 (d, *J* = 7.9 Hz, 1H), 7.45 (d, *J* = 7.4 Hz, 2H), 6.46 (dd, *J* = 16.8, 10.2 Hz, 1H), 6.29 (d, *J* = 16.8 Hz, 1H), 5.79 (d, *J* = 10.2 Hz, 1H), 4.08 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.1, 165.4, 163.5, 139.9, 139.41, 138.9, 136.1, 131.9, 131.7, 129.8, 128.9, 127.3, 125.7, 125.0, 121.9, 118.9, 117.5, 35.9. HRMS-ESI (−) *m/z* calculated for $C_{18}H_{13}N_2O_3$, 305.0932 [M-H]-; found: 305.0936.

2-(1,3-Dioxo-1,2,3,4-tetrahydroisoquinolin-6-yl)benzoic acid (67). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.90 (s, 1H), 11.31 (s, 1H), 8.02 (d, *J* = 7.8 Hz, 1H), 7.82 (d, *J* = 7.5 Hz, 1H), 7.63 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.43-7.36 (m, 3H), 4.06 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.1, 169.1, 165.3, 146.3, 140.2, 136.6, 131.9, 131.4, 130.6, 129.7, 128.3, 127.8, 127.6, 127.2, 123.9, 36.2. HRMS-ESI (−) *m/z* calculated for $C_{16}H_{10}NO_4$, 280.0615 [M-H]-; found: 280.0608.

4-(1,3-Dioxo-1,2,3,4-tetrahydroisoquinolin-6-yl)benzoic acid (68). ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.05 (s, 1H), 11.34 (s, 1H), 8.11 (d, J = 8.2 Hz, 1H), 8.06 (d, J = 8.2 Hz, 2H), 7.88 (d, J = 8.2 Hz, 2H), 7.83 (d, J = 8.3 Hz, 1H), 7.78 (s, 1H), 4.11 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.8, 166.8, 164.9, 143.4, 142.6, 137.3, 130.4, 129.9, 128.1, 127.1, 126.2, 125.6, 124.5, 35.9. HRMS-ESI (−) *m/z* calculated for $C_{16}H_{10}NO_4$, 280.0615 [M-H]-; found: 280.0620.

3-(1,3-Dioxo-1,2,3,4-tetrahydroisoquinolin-6-yl)benzoic acid (69). ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.16 (s, 1H), 11.32 (s, 1H), 8.26 (t, J = 1.6 Hz, 1H), 8.10 (d, J = 8.2 Hz, 1H), 8.00 (dd, J = 7.8, 1.8 Hz, 2H), 7.80 (dd, J = 8.2, 1.6 Hz, 1H), 7.76 (s, 1H), 7.65 (t, J = 7.8 Hz, 1H), 4.12 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.1, 167.2, 165.2, 143.9, 139.1, 137.7, 131.8, 131.5, 129.7, 129.3, 128.4, 127.7, 126.3, 125.8, 124.5, 36.2. HRMS-ESI (−) *m/z* calculated for $C_{16}H_{10}NO_4$, 280.0615 [M-H]-; found: 280.0616.

3-(1,3-Dioxo-1,2,3,4-tetrahydroisoquinolin-6-yl)benzamide (70). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.33 (s, 1H), 8.25 (s, 1H), 8.17 (s, 1H), 8.10 (d, J = 8.2 Hz, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 8.2 Hz, 1H), 7.79 (s, 1H), 7.59 (t, J = 7.7 Hz, 1H), 7.48 (s, 1H), 4.11 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 175.7, 167.7, 163.3, 157.8, 144.9, 137.9, 135.5, 133.2, 130.1, 129.7, 129.3, 129.1, 128.5, 126.2, 124.7, 37.8. HRMS-ESI (−) *m/z* calculated for $C_{16}H_{11}N_2O_3$, 279.0775 [M-H]-; found: 279.0778.

6-(3-Acetylphenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (71). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.16 (s, 1H), 8.09 (s, 1H), 7.94 (d, J = 8.1 Hz, 1H), 7.87-7.83 (m, 2H), 7.67 (d, J = 8.1 Hz, 1H), 7.63 (s, 1H), 7.50 (t, J = 7.7 Hz, 1H), 3.95 (s, 2H), 2.33 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 198.3, 171.4, 165.6, 144.3, 139.5, 138.0, 137.9, 132.0, 130.1,

128.7, 127.0, 126.7, 126.2, 125.6, 124.8, 36.6, 27.4. HRMS-ESI (−) *m/z* calculated for $C_{17}H_{12}NO_3$, 278.0823 [M-H]-; found: 278.0816.

6-(3-Chlorophenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (72). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.33 (s, 1H), 8.08 (d, *J* = 8.2 Hz, 1H), 7.82 (t, *J* = 1.8 Hz, 1H), 7.80 (d, *J* = 8.2 Hz, 1H), 7.76 (s, 1H), 7.73 (d, *J* = 7.7 Hz, 1H), 7.54 (t, *J* = 7.8 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 1H), 4.09 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.1, 165.2, 143.2, 140.9, 137.5, 134.1, 131.1, 128.5, 128.3, 126.9, 126.4, 125.8, 125.8, 124.6, 36.2. HRMS-ESI (−) *m/z* calculated for $C_{15}H_9ClNO_2$, 270.0327 [M-H]-; found: 270.0330.

7-(3-Chlorophenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (73). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.38 (s, 1H), 8.24 (d, *J* = 1.8 Hz, 1H), 8.00 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.78 (d, *J* = 1.8 Hz, 1H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 8.1 Hz, 1H), 7.47 (d, *J* = 8.8 Hz, 1H), 4.08 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.1, 165.4, 141.3, 137.8, 136.8, 134.2, 132.1, 131.2, 129.1, 128.1, 126.7, 125.9, 125.7, 125.6, 36.1. HRMS-ESI (−) *m/z* calculated for $C_{15}H_9ClNO_2$, 270.0327 [M-H]-; found: 270.0330.

6-(4-Chlorophenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (74). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.33 (s, 1H), 8.08 (d, *J* = 8.2 Hz, 1H), 7.79-7.75 (m, 3H), 7.72 (s, 1H), 7.58 (d, *J* = 8.5 Hz, 2H), 4.09 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.2, 165.4, 143.7, 137.7, 133.8, 129.4, 129.1, 128.5, 126.2, 125.7, 124.5, 123.3, 36.4. HRMS-ESI (−) *m/z* calculated for $C_{15}H_9BrNO_2$, 313.9822 [M-H]-; found: 313.9826.

6-(3-Bromophenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (75). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.32 (s, 1H), 8.09-8.01 (m, 2H), 7.91-7.78 (m, 3H), 7.62-7.47 (m, 2H), 4.12 (s, 2H). HRMS-ESI (−) *m/z* calculated for $C_{15}H_9ClNO_2$, 270.0327 [M-H]-; found: 270.0329.

7-(4-Bromophenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (76). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.38 (s, 1H), 8.23 (s, 1H), 7.96-7.82 (m, 2H), 7.69-7.64 (m, 4H), 4.07 (s, 2H). HRMS-ESI (−) *m/z* calculated for $C_{15}H_9ClNO_2$, 270.0327 [M-H]-; found: 270.0326.

6-(2,6-Dimethoxyphenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (77). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.23 (s, 1H), 7.99 (d, *J* = 8.0 Hz, 1H), 7.35 (t, *J* = 8.4 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.21 (s, 1H), 6.77 (d, *J* = 8.4 Hz, 2H), 4.02 (s, 2H), 3.66 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.7, 165.9, 157.6, 140.6, 136.5, 130.6, 130.4, 127.3, 124.0, 118.1, 117.8, 105.0, 56.4, 36.6. HRMS-ESI (−) *m/z* calculated for $C_{17}H_{14}NO_4$, 296.0928 [M-H]-; found: 296.0931.

6-(2,5-Dimethoxyphenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (78). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.27 (s, 1H), 8.01 (d, *J* = 8.1 Hz, 1H), 7.57 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.48 (d, *J* = 1.6 Hz, 1H), 7.08 (d, *J* = 9.0 Hz, 1H), 6.96 (dd, *J* = 9.0, 3.1 Hz, 1H), 6.90 (d, *J* = 3.1 Hz, 1H), 4.05 (s, 2H), 3.75 (s, 3H), 3.71 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.2, 165.3, 153.5, 150.4, 143.3, 136.5, 129.3, 128.6, 128.4, 127.2, 123.7, 116.1, 114.6, 113.4, 56.3, 55.7, 36.2. HRMS-ESI (−) *m/z* calculated for $C_{17}H_{14}NO_4$, 296.0928 [M-H]-; found: 296.0931.

6-(3-Fluoro-4-methoxyphenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (79). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.29 (s, 1H), 8.03 (d, *J* = 8.2 Hz, 1H), 7.75 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.70 (s, 1H), 7.66 (dd, *J* = 12.9, 2.2 Hz, 1H), 7.63-7.52 (m, 1H), 7.29 (t, *J* = 8.8 Hz, 1H), 4.06 (s, 2H), 3.89 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.4, 165.6, 152.9, 151.4, 148.0, 147.9, 143.6, 137.7, 131.7, 128.5, 125.9, 124.1, 123.7, 114.9, 56.6, 36.6. HRMS-ESI (−) *m/z* calculated for $C_{16}H_{11}FNO_3$, 284.0728 [M-H]-; found: 284.0726.

6-(3-Chloro-4-fluorophenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (80). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.35 (s, 1H), 8.14-7.88 (m, 2H), 7.87-7.64 (m, 3H), 7.58 (s, 1H), 4.10 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.4, 165.5, 142.7, 137.8, 136.9, 133.9, 129.5, 128.6, 128.2, 128.2, 126.6, 126.0, 124.8, 117.8, 36.5. HRMS-ESI (−) *m/z* calculated for $C_{15}H_8ClFNO_2$, 288.0233 [M-H]-; found: 288.0237.

6-(2,4-Difluorophenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (81). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.34 (s, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 7.65 (dd, *J* = 12.1, 5.4 Hz, 1H), 7.63-7.60 (m, 1H), 7.56 (s, 1H), 7.47-7.39 (m, 1H), 7.25 (td, *J* = 8.4, 2.3 Hz, 1H), 4.09 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.7, 164.8, 158.1, 138.9, 136.9, 131.8, 127.9, 127.6, 127.4, 124.2, 112.2, 112.1, 104.7, 104.5, 35.8. HRMS-ESI (−) *m/z* calculated for $C_{15}H_8F_2NO_2$, 272.0529 [M-H]-; found: 272.0528.

6-(4-(Trifluoromethyl)phenyl)isoquinoline-1,3(2*H*, 4*H*)-dione (82). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.36 (s, 1H), 8.12 (d, *J* = 8.2 Hz, 1H), 7.97 (d, *J* = 8.2 Hz, 2H), 7.87 (d, *J* = 8.2 Hz, 2H), 7.83 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.79 (s, 1H), 4.11 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.2, 165.3, 143.4, 142.9, 137.8, 128.5, 128.4, 128.2, 126.8, 126.2, 126.2, 125.1, 124.9, 36.4. HRMS-ESI (−) *m/z* calculated for $C_{16}H_9F_3NO_2$, 304.0591 [M-H]-; found: 304.0586.

6-([1,1'-Biphenyl]-3-yl)isoquinoline-1,3(2*H*, 4*H*)-dione (83): ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.32 (s, 1H), 8.10 (d, *J* = 8.3 Hz, 1H), 7.99 (s, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.84 (s, 1H), 7.78 (d, *J* = 7.6 Hz, 2H), 7.74 (t, *J* = 7.2 Hz, 2H), 7.61 (t, *J* = 7.8 Hz, 1H), 7.51 (t, *J* = 7.6 Hz, 2H), 7.42 (d, *J* = 7.2 Hz, 1H), 4.11 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 175.8, 163.6, 158.1, 145.7, 141.8, 140.1, 139.0, 133.3, 130.4, 129.3, 129.1,

127.6, 127.4, 126.1, 125.9, 124.8, 124.6, 110.0, 35.8. HRMS-ESI (−) *m/z* calculated for $C_{21}H_{14}NO_2$, 312.1030 [M-H]-; found: 312.1020.

Synthesis of Benzothiazole (84). To the mixture of *o*-phenylenediamine (1 mmol) was added carboxylic acid **39** (1.0 mmol), *N*,*N*-diisopropylethylamine (1.5 mmol) and propylphosphonic anhydride (1.0 mmol, 50% w/w in EtOAc) and the resulting solution was irradiated at 100 °C for 30 min under microwave conditions before it was diluted with H_2O. The precipitate was collected *via* filtration and washed thoroughly with H_2O. Recrystallization of the crude sample in a mixture of EtOH/H_2O furnished the title compound as pale brown solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.43 (s, 1H), 8.22 (d, *J* = 7.9 Hz, 1H), 8.18 (d, *J* = 8.1 Hz, 1H), 8.17-8.10 (m, 3H), 7.60 (t, *J* = 7.6 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 1H), 4.18 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.6, 165.6, 164.5, 153.2, 137.7, 136.5, 134.6, 128.4, 126.8, 126.8, 126.1, 125.9, 125.5, 123.0, 122.4, 35.8. HRMS-ESI (−) *m/z* calculated for $C_{16}H_9N_2O_2S$, 293.0390 [M-H]-; found: 293.0384.

6-(Quinolin-3-yl)isoquinoline-1,3(2*H*, 4*H*)-dione (85). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.37 (s, 1H), 9.32 (d, *J* = 2.2 Hz, 1H), 8.78 (d, *J* = 2.2 Hz, 1H), 8.16 (d, *J* = 8.3 Hz, 1H), 8.11-8.06 (m, 2H), 8.00 (d, *J* = 8.3 Hz, 1H), 7.97 (s, 1H), 7.82 (t, *J* = 7.6 Hz, 1H), 7.69 (t, *J* = 7.6 Hz, 1H), 4.14 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 175.3, 163.0, 157.6, 148.9, 146.9, 142.2, 134.8, 133.2, 133.1, 130.7, 130.6, 129.2, 129.2, 128.9, 128.36, 127.6, 124.8, 39.1. HRMS-ESI (−) *m/z* calculated for $C_{18}H_{11}N_2O_2$, 287.0826 [M-H]-; found: 287.0826.

6-Benzylisoquinoline-1,3(2*H*, 4*H*)-dione (86). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.20 (s, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.33-7.28 (m, 3H), 7.25 (d, *J* = 6.2 Hz, 3H), 7.19 (t, *J* = 7.2 Hz, 1H), 4.00 (s, 2H), 3.97 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.4, 165.6,

147.8, 135.6, 129.3, 129.3, 129.1, 129.0, 128.3, 128.2, 126.9, 126.7, 41.4, 36.4. HRMS-ESI (−) *m/z* calculated for $C_{16}H_{12}NO_2$, 250.0874 [M-H]-; found: 250.0868.

6-(3-Nitrobenzoyl)isoquinoline-1,3(2*H*, 4*H*)-dione (87). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.49 (s, 1H), 8.54 (dd, *J* = 7.9, 1.8 Hz, 1H), 8.47-8.44 (m, 1H), 8.20 (s, 1H), 8.18 (s, 1H), 7.89 (t, *J* = 8.0 Hz, 1H), 7.83-7.79 (m, 2H), 4.13 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 193.8, 171.1, 165.1, 148.2, 140.2, 138.1, 137.7, 136.2, 131.0, 129.5, 128.9, 128.4, 128.3, 127.8, 124.6, 36.5. HRMS-ESI (−) *m/z* calculated for $C_{16}H_9N_2O_5$, 309.0517 [M-H]-; found: 309.0510.

7-(3-Nitrobenzoyl)isoquinoline-1,3(2*H*, 4*H*)-dione (88). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.49 (s, 1H), 8.53 (d, *J* = 8.5 Hz, 1H), 8.45 (s, 1H), 8.32 (s, 1H), 8.17 (d, *J* = 7.6 Hz, 1H), 8.06 (d, *J* = 7.9 Hz, 1H), 7.89 (t, *J* = 8.0 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 4.17 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 192.7, 170.3, 164.5, 147.6, 141.8, 137.9, 135.4, 134.8, 133.8, 130.4, 128.7, 128.5, 126.9, 125.3, 123.8, 36.2. HRMS-ESI (−) *m/z* calculated for $C_{16}H_9N_2O_5$, 309.0517 [M-H]-; found: 309.0523.

Ethyl-6-bromo-1,3-dioxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylate (89). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.63 (s, 1H), 11.37 (s, 1H, enol isomer), 8.63 (s, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.91 (d, *J* = 8.4 Hz, 1H, enol isomer), 7.66-7.61 (m, 2H, enol isomer), 7.51 (d, *J* = 8.0 Hz, 1H), 4.47 (q, *J* = 6.9 Hz, 2H), 4.03 (s, 1H), 1.42 (t, *J* = 6.9 Hz, 3H). HRMS-ESI (−) *m/z* calculated for $C_{12}H_9BrNO_4$, 309.9720 [M-H]-; found: 309.9722.

Synthesis of Compound 90. The mixture of methyl-4-bromo-2-aminobenzoate (**27**,1.0 eq), phenylboronic acid (1.6 eq), $Pd(PPh_3)_4$ (0.065 eq), K_2CO_3 (3.6 eq) in DME/H_2O (4:1) was irradiated at 110 °C for 45 min under microwave conditions. The black residue formed was filtered through celite and the solvent was concentrated in vacuo. The crude

mixture was purified using CombiFlash with 0-50% EtOAc in hexane as an eluent to yield the desired product (**28**) as colorless solid. Yield: 50%.

To a solution of potassium isocyanate (1 mmol) in 1 mL of dry toluene was added compound **28** and the resulting mixture was stirred at r.t for 3 h before water was added. The aqueous solution was extracted with DCM (2 x 10 mL) and the combined organics were washed with $NaHCO_3$ (2 x 10 mL), brine (15 mL), dried over Na_2SO_4 and concentrated in vacuo to leave colorless oil. To the resulting oil in EtOH (2 mL) was added 1N NaOH (2 mL) and the reaction mixture was stirred under reflux for 1h before it was acidified (pH = 3) using 1N HCl. The precipitate was collected *via* filtration, washed with water, and then dried to obtain the titled compound (**90)** as colorless solid. Yield: 68%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.30 (s, 1H), 11.20 (s, 1H), 7.96 (d, J = 8.2 Hz, 1H), 7.67 (s, 1H), 7.66 (s, 1H), 7.53 (t, J = 7.6 Hz, 2H), 7.49-7.44 (m, 2H), 7.38 (d, J = 1.3 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 162.8, 150.5, 146.7, 141.5, 138.9, 129.3, 127.9, 127.8, 127.1, 113.5, 113.3, 113.0, 39.2. HRMS-ESI (−) *m/z* calculated for $C_{14}H_9N_2O_2$, 237.0670 [M-H]-; found: 237.0672.

Synthesis of Naphthalimides 91–92. A mixture of 4-bromo-1,8-naphthalic anhydride (**29**, 1.0 eq), 2-(tributylstannyl)furan (1.2 eq), $Pd(PPh_3)_4$ (0.03 eq) in toluene (5.0 mL) was stirred at 150 °C for 30 min under microwave conditions. The black residue formed was filtered through celite and the solvent was concentrated in vacuo. The crude mixture was purified using CombiFlash with 0-30% EtOAc in hexane as an eluent to yield 4-furylnaphthalic anhydride (**30**) as colorless solid. Yield: 70%.

A mixture of **29** / **30** (0.72 mmol) in ammonium hydroxide (15 mL) was stirred at 40 °C for 4 h. The resulting solution was extracted with EtOAc (2 x 20 mL) and the combined

organic extracts were washed with H_2O (2 x 20 mL), brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. Recrystallization of the crude sample from EtOH produced desired compounds (**91**–**92**) as colorless solid. Yield: 70-90%.

6-Bromo-1*H*-benzo[d]isoquinoline-1,3(2*H*)-dione (91). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.84 (s, 1H), 8.53-8.48 (m, 2H), 8.25 (d, *J* = 7.8 Hz, 1H), 8.18 (d, *J* = 7.8 Hz, 1H), 7.96 (dd, *J* = 8.3, 7.4 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 163.7, 163.6, 132.7, 131.4, 130.9, 130.4, 130.2, 129.7, 129.2, 128.8, 123.4, 122.6. HRMS-ESI (−) *m/z* calculated for $C_{12}H_5BrNO_2$, 273.9509 [M-H]-; found: 273.9504.

6-(Furan-2-yl)-1*H*-benzo[d]isoquinoline-1,3(2*H*)-dione (92). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.80 (s, 1H), 8.93 (d, *J* = 8.6 Hz, 1H), 8.52 (d, *J* = 7.1 Hz, 1H), 8.48 (d, *J* = 7.7 Hz, 1H), 8.12 (d, *J* = 7.7 Hz, 1H), 8.07 (d, *J* = 1.8 Hz, 1H), 7.98-7.87 (m, 1H), 7.30 (d, *J* = 3.4 Hz, 1H), 6.83 (dd, *J* = 3.4, 1.8 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.5, 164.1, 151.2, 145.5, 133.7, 132.3, 130.6, 130.1, 129.8, 128.1, 127.8, 125.9, 123.1, 113.3, 112.9, 39.1. HRMS-ESI (−) *m/z* calculated for $C_{16}H_8NO_3$, 262.0510 [M-H]-; found: 262.0512.

Synthesis of 93–95. To a suspension of 2-amino-1-propene-1,1,3-tricarbonitrile (**31**, 7.6 mmol, 1.0 eq) in EtOH (10 mL) was added arylhydrazine (8.4 mmol, 1.1 eq) slowly and the solution was heated at reflux for 30 min before it was cooled. The solvent was removed in vacuo to leave pale brown solid. Recrystallization of the crude mixture from EtOH furnished intermediates **32** as brown needles. Yield: 70-95%.

A solution of compound **32** (0.45 mmol) in Conc. HCl (2 mL) was stirred at 70 °C for 4 h. After the solvent was removed in vacuo the residue was purified using CombiFlash

with 0-100% EtOAc in hexane as an eluent to yield desired products (**93-95**) as colorless solid. Yield: 65-85%.

3-Amino-2-(4-fluorophenyl)-2*H*-pyrazolo[4,3-c]pyridine-4,6(5*H*,7*H*)dione (93). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.60 (s, 1H), 7.55 (dd, J = 8.9, 4.9 Hz, 2H), 7.36 (t, J = 8.8 Hz, 2H), 6.48 (s, 2H), 3.75 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.4, 162.5, 160.5, 152.9, 148.4, 148.2, 134.2, 126.6, 126.5, 116.7, 116.5, 92.9, 32.2. HRMS-ESI (−) *m/z* calculated for $C_{12}H_8FN_4O_2$, 259.0637 [M-H]-; found: 259.0636.

3-Amino-2-(pyridin-2-yl)-2*H*-pyrazolo[4,3-c]pyridine-4,6(5*H*,7*H*)dione (94). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.64 (s, 1H), 8.51-8.43 (m, 1H), 8.02 – 7.96 (m, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.77 (s, 2H), 7.35-7.29 (m, 1H), 3.80 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.1, 162.2, 153.8, 149.7, 149.6, 147.5, 140.0, 121.1, 113.2, 92.8, 32.2. HRMS-ESI (+) *m/z* calculated for $C_{11}H_{10}N_5O_2$, 244.0829 [M+H]-; found: 244.0832.

3-Amino-2-phenyl-2*H*-pyrazolo[4,3-c]pyridine-4,6(5*H*,7*H*)dione (95). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.60 (s, 1H), 7.76 – 7.45 (m, 4H), 7.41 (dd, J = 5.6, 3.0 Hz, 1H), 6.45 (s, 2H), 3.76 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.8, 161.9, 147.8, 147.4, 137.3, 129.2, 123.2, 92.5, 31.7. HRMS-ESI (+) *m/z* calculated for $C_{12}H_{11}N_4O_2$, 243.0882 [M+H]-; found: 243.0890.

***N*-(4,6-dioxo-2-phenyl-4,5,6,7-tetrahydro-2*H*-pyrazolo[4,3-c]pyridine-3-yl)acetamide (96).** To a solution of compound **95** (1.0 eq) in AcOH (5.0 mL) was added acetic anhydride (1.2 eq) and stirred at 70 °C for 5 h. The solvent was evaporated in vacuo to leave pale solid. Recrystallization of crude sample from MeOH produced the title compound (**96**) as colorless solid. Yield: 62%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.94

(s, 1H), 10.32 (s, 1H), 7.64 – 7.36 (m, 5H), 3.95 (s, 2H), 1.97 (s, 3H). HRMS-ESI (−) *m/z* calculated for $C_{14}H_{11}N_4O_3$, 283.0837 [M-H]-; found: 283.0831.

Biology

Recombinant TDP1 Assay

A 5′-[^{32}P]-labeled single-stranded DNA oligonucleotide containing a 3′-phosphotyrosine (N14Y)[26] was incubated at 1 nM with 10 pM recombinant TDP1 in the absence or presence of inhibitor for 15 min at room temperature in WCE buffer. When indicated, parallel reactions were performed in the LMP1 assay buffer containing 50 mM Tris HCl, pH 7.5, 80 mM KCl, 2 mM EDTA, 1 mM DTT, 40 μg/mL BSA, and 0.01% Tween-20.[27] Reactions were terminated by the addition of 1 volume of gel loading buffer [99.5% (v/v) formamide, 5 mM EDTA, 0.01% (w/v) xylene cyanol, and 0.01% (w/v) bromophenol blue]. Samples were subjected to a 16% denaturing PAGE with multiple loadings at 12-min intervals. Gels were dried and exposed to a PhosphorImager screen (GE Healthcare). Gel images were scanned using a Typhoon 8600 (GE Healthcare), and densitometry analyses were performed using the ImageQuant software (GE Healthcare).

Whole Cell Extract TDP1 Assay

DT40 knockout cells (1 x 10^7) for TDP1 (TDP1-/-) complemented with human TDP1 (hTDP1) were collected, washed, and centrifuged. Cell pellets were then resuspended in 100 μL of CellLytic M cell lysis reagent (SIGMA-Aldrich C2978). After 15 min on ice, lysates were centrifuged at 12,000 g for 10 min, and supernatants were transferred to a new tube. Protein concentrations were determined using a Nanodrop spectrophotometer (Invitrogen), and whole cell extracts were stored at −80 °C. The N14Y DNA substrate was incubated at 1 nM with 5 μg/mL of whole cell extracts in the absence or presence of

inhibitor for 15 min at room temperature in the LMP1 assay buffer. Samples were then analyzed similarly to the recombinant TDP1 assay (see above).

Recombinant TDP2 Assay

TDP2 reactions were carried out as described previously[14] with the following modifications. The 18-mer single-stranded oligonucleotide DNA substrate (TY18, α^{32}P-cordycepin-3'-labeled) was incubated at 1 nM with 25 pM recombinant human TDP2 in the absence or presence of inhibitor for 15 min at room temperature in the LMP2 assay buffer containing 50 mM Tris-HCl, pH 7.5, 80 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 40 μg/mL BSA, and 0.01% Tween 20. Reactions were terminated and treated similarly to WCE and recombinant TDP1 reactions (see above).

Whole Cell Extract TDP2 Assay

DT40 knockout cells (1 x 10^7) for TDP1 (TDP2-/-) complemented with human TDP2 (hTDP2) were collected, washed, and centrifuged. hTDP2 whole cell extracts were prepared and stored similarly to hTDP1 whole cell extracts. The TY18 DNA substrate was incubated at 1 nM with 5 μg/mL of whole cell extracts in the absence or presence of inhibitor for 15 min at room temperature in the LMP2 assay buffer. Reactions were terminated and treated similarly to WCE and recombinant TDP1 reactions (see above).

Molecular Modeling

Molecular modeling was performed using the Schrodinger small molecule drug discovery suite 2014-3. Homology models generated from templates having a higher degree of sequence identity with the target are found to be reliable and accurate for the utilization in structure based drug design. Due to the high sequence homology between hTDP2 and mTDP2 (65%), an available crystal structure of mTDP2 (PDB code: 4GZ1[15]) was used to

build a homology model of hTDP2. The sequence of hTDP2 was imported into the program Prime[28, 29] (Schrödinger Inc.) and the model was built using mTDP2 (PDB code: 4GZ1[15]) as a template. This model was subjected to the loop refinement and energy minimization to improve accuracy and quality of the model. This model was subjected to protein preparation wizard[30, 31] (Schrodinger Inc) in which missing hydrogens were added, zero-order bonds to metals were created followed by the generation of metal binding states. The structure of protein was minimized using OPLS 2005 force field[32] to optimize hydrogen bonding network and converge heavy atoms to the RMSD of 0.3 Å.

The receptor grid generation tool in Maestro (Schrodinger Inc) was used to define an active site around the metal **(Mg^{2+})** to cover all the residues within 12 Å from it. Compound **40** and **56** were drawn using Maestro and subjected to Lig Prep[33] to generate conformers, possible protonation at pH of 7±3 and metal binding states which serves as an input for docking process. All the dockings were performed using Glide XP[23, 24] (Glide, version 6.4) mode. The van der Waals radii of non-polar atoms for each of the ligands were scaled by a factor of 0.8. The predicted favourable binding mode of compounds **40** and **56** features the critical interaction between the ligands and E152, T230, D262, N264, R266, H351 (residues are labeled according to hTDP2 sequence) and the metal ion within the active site of hTDP2. All the ligands docked were further refined post docking by minimizing under implicit solvent to account for the local protein flexibility.

ASSOCIATED CONTENT

Supporting Information Available. Characterization data, including ^{1}H NMR data of intermediate chemotypes **21**, **24, 25** and **32**. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS USED

TDP2, tyrosyl DNA phosphodiesterase 2; TDP1, tyrosyl DNA phosphodiesterase 1; Top2, topoisomerase II; SAR, structure-activity-relationship; ETP, etoposide; HBV, hepatitis B virus; cccDNA, covalently closed circular DNA; RC-DNA, relaxed circular DNA; EEP, the exonuclease-endonuclease-phosphatase; hAPE1, human apurinic/apyrimidinic endonuclease 1; HTS, high-throughput screening; PK, pharmacokinetics; WCE, whole cell extract; HID, 2-hydroxyisoquinoline-1,3-dione; MW, microwave irradiation.

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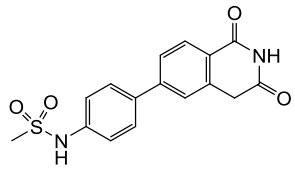
TOC Graphic



IC_{50} Rec. Tdp2 = 1.9 μM
IC_{50} WCE= 2.2 μM
IC_{50} Tdp1 > 111 μM