Research paper

Synthesis and structure-activity relationship of furoquin
inhibitors of Tyrosyl-DNA-phosphodiesterase 2 (TDP2)

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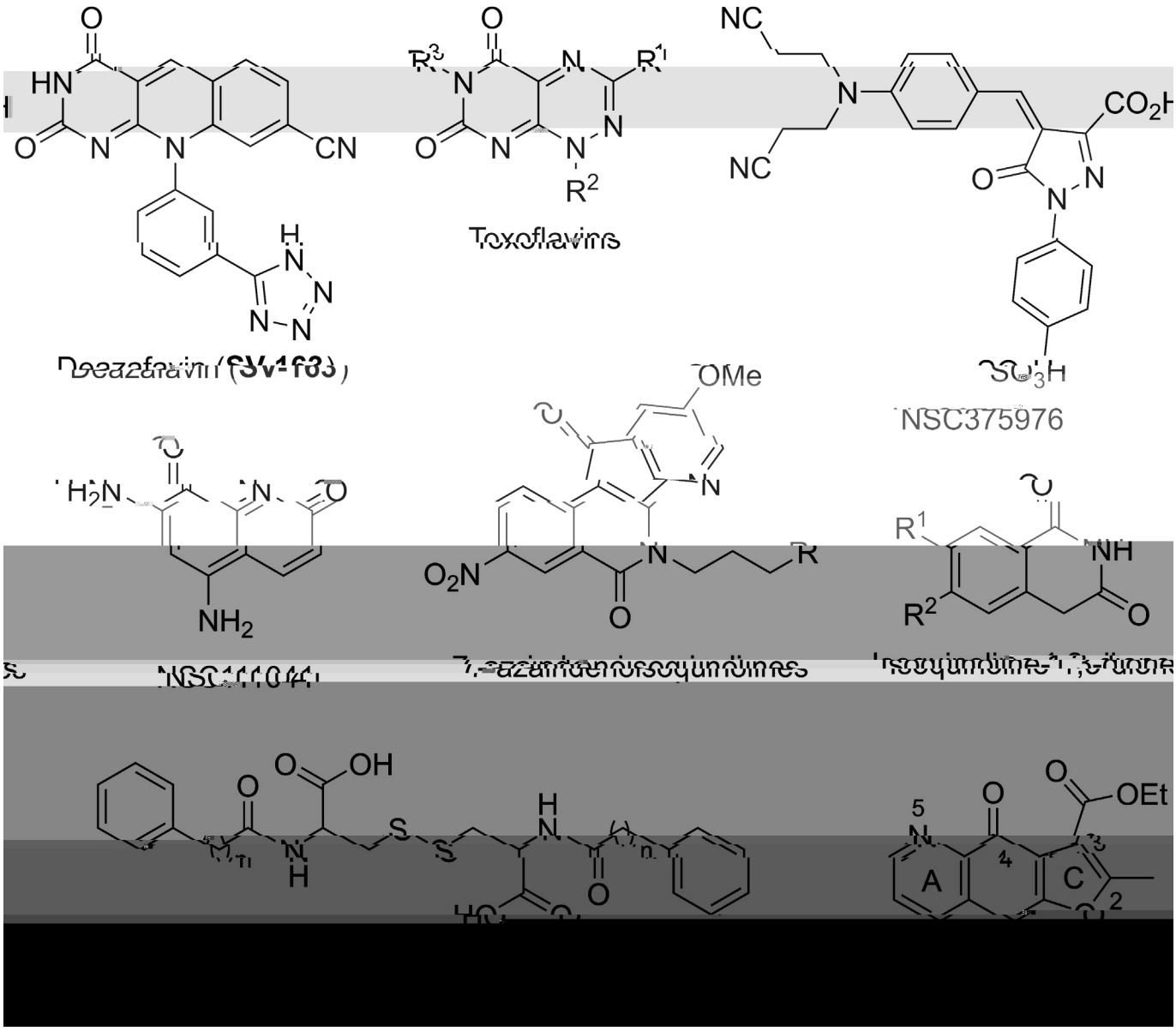


Fig. 1. The reported TDP2 inhibitors and our TDP2 hit **1**.

In this study, we report the discovery, synthesis and structure-activity relationship (SAR) of furoquinolinediones as an alternative scaffold of TDP2 inhibitor.

2. Results and discussion

2.1. Hit identification

In an effort to discover new chemotypes of TDP2 inhibitors, an in-house chemical library was screened against TDP2 using a single-stranded oligonucleotide TY19 substrate containing 5′-phosphotyrosyl group (Fig. 2A) [7]. A furoquinolinedione derivative **1** (Fig. 1) was found to inhibit recombinant human TDP2 with an IC_{50} (the concentration of compound that inhibits 50% of enzyme activity) value of 11 μM (Fig. 2B and E). Notably, compound **1** did not inhibit TDP1 (a counter enzyme characterized as hydrolyzing a DNA 3′-phosphotyrosyl bond) [2,10,29] at concentrations up to 111 μM (Fig. 2D), in which assay a single-stranded oligonucleotide N14Y substrate containing 3′-phosphotyrosyl group was used (Fig. 2A) [30]. The inhibitory activity of compound **1** against human TDP2 was also tested in whole cell extracts (TDP2 WCE) [28] and showed that **1** remained potent with IC_{50} value of 22 μM (Fig. 2C and E). The retention of the TDP2 inhibitory potency in the WCE context shows that **1** is not sequestered by binding to other cellular components, implying that **1** binds specifically to TDP2. To study the selectivity to TDP2, compound **1** was screened against TOP2 and topoisomerase IB (TOP1) by using relaxation assays, which indicated that **1** did not inhibit TOP1 and TOP2 relaxing activity at 25 μM concentration (Fig. 4). In addition, TOP1-mediated DNA cleavage assay indicated that compound **1** is unable to induce the formation of the covalent complex of TOP1 and DNA at up to 100 μM (Fig. 2S, Supporting Information), not a TOP1 poison [31]. These results indicate that furoquinolinediones represent a suitable chemotype for novel selective TDP2 inhibitors. Therefore, we undertook a systematic exploration of the SAR furoquinolinedione chemotype by probing analogues of **1**: 1) by modifying the ethoxy

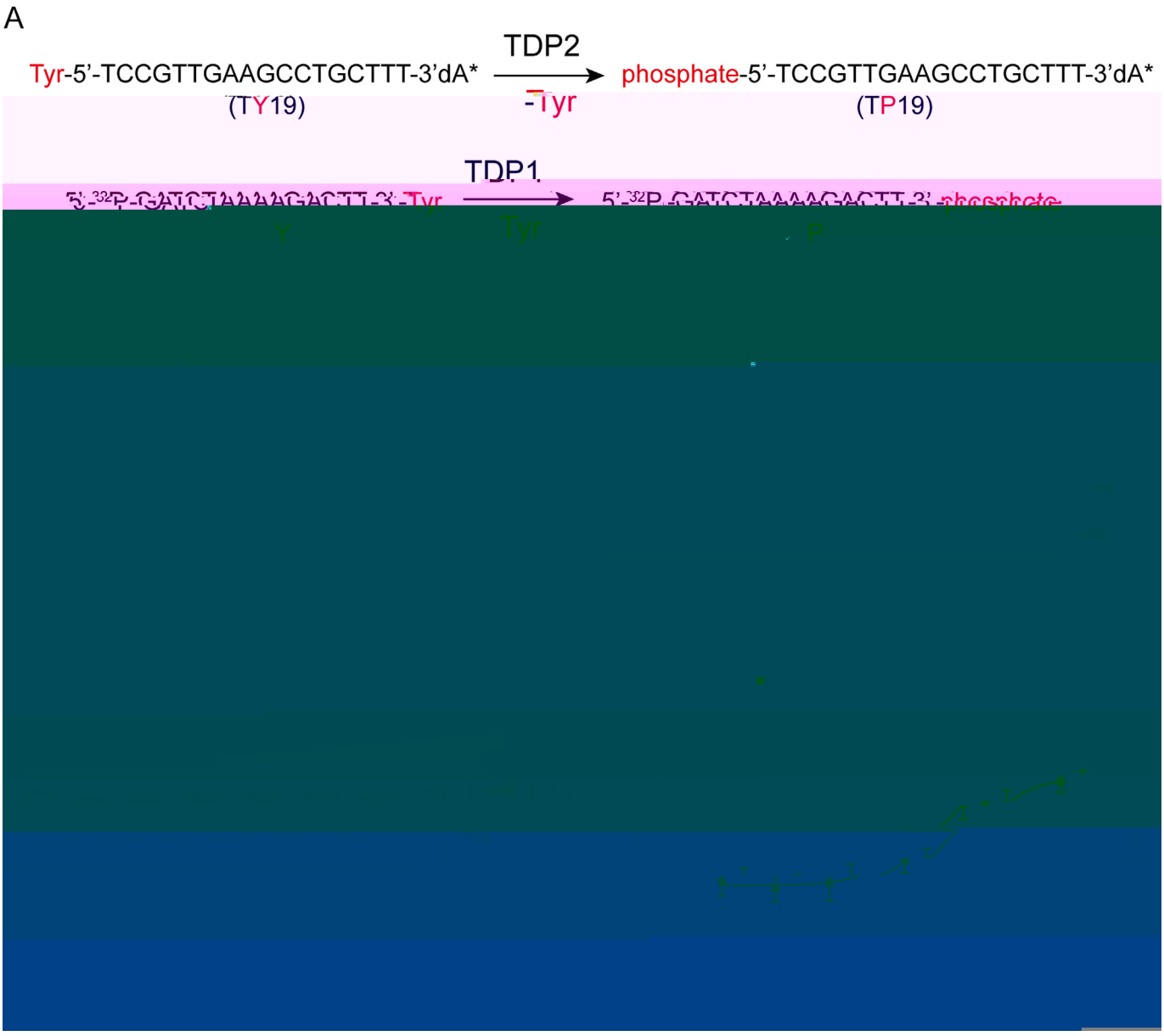


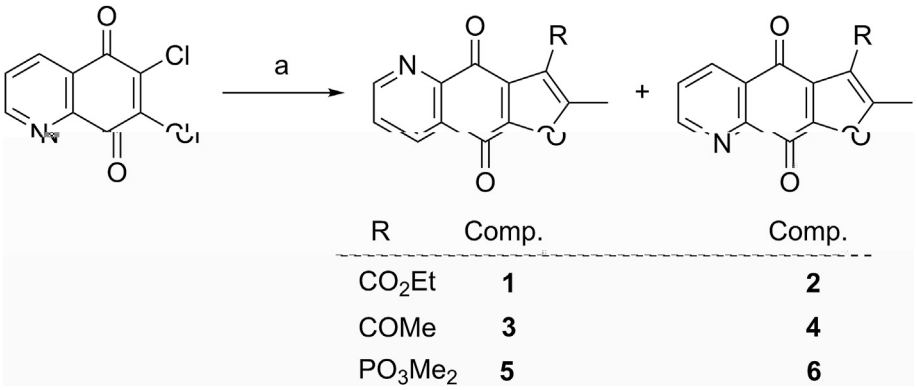
Fig. 2. (A) Schematic representation of TDP2 and TDP1-catalyzed phosphotyrosyl cleavage reaction. Representative inhibition gels of compound **1** against Rec TDP2 (B), TDP2 WCE (C) and TDP1 (D). (E) Dose-response curves of **1** against Rec TDP2 and TDP2 WCE expressed as mean ± SD. Testing concentrations: 0.46, 1.4, 4.1, 12.3, 37 and 111 μM.

carbonyl group at position 3; and 2) by converting the furan ring into diverse 5-member cycles including pyrrole, isoxazole and pyrazole.

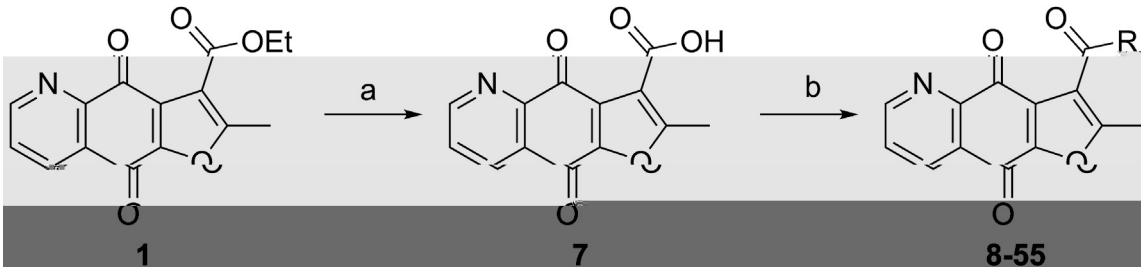
2.2. Chemistry

The synthesis of designed furoquinolinedione analogues is outlined in Schemes 1–8. Furoquinolinedione analogues **1**–**6** could be synthesized in a one-pot reaction as shown in Scheme 1 [32]. The reaction of 6,7-dichloroquinoline-5,8-dione with active methylene agent (AMR), such as ethyl acetoacetate, acetylacetone and dimethyl (2-oxopropyl)phosphonate, gave two furoquinolinedione products, N,O-anti isomer and N,O-syn isomer. The structures of isomers **1** and **2** were characterized with HRMS, 1D and 2D NMR spectroscopy. The structure of **1** was further confirmed with X-ray single crystal analysis (Supporting Information, Fig. 1S). The structural modification of the ester at position 3 of compounds **1** and **2** is shown in Schemes 2–6. Compounds **1** and **2** were hydrolyzed with aqueous sodium carbonate in isopropanol to give the corresponding carboxylic acids **7** and **66**, respectively. Treatment of acids **7** or **66** with thionyl chloride followed by amidation or esterification afforded the target amides **8**–**12** (Scheme 2, Table 2), **67** and **68** (Scheme 6), esters **13**–**55** (Scheme 2, Table 2), and **69** (Scheme 6). The reaction of bromide analogue **50** with pyridine derivatives gave the pyridinium analogues **56** and **57** (Scheme 3). Boc-protecting group of **51**–**53** was removed by treatment with trifluoroacetic acid leading to oxazole derivative **58** and anilines **59** and **60** (Scheme 4). The alkynes **54** and **55** were introduced into "click" reaction with various alkyl azides to prepare triazoles **61**–**64** (Scheme 5) [33]. In addition, 3-methyl analogue **77** (Fig. 4) was synthesized according to Cherkaoui method (Supporting Information, Scheme S1) [34].

To assess the role of furan ring for TDP2 inhibitory potency, analogues **70**–**76** were designed as shown in Schemes 7 and 8. The



Scheme 1. Synthesis of compounds **1**–**6**. Reagents and conditions: (a) CH_3COCH_2R, MeCN, K_2CO_3, reflux.



Scheme 2. Synthesis of compounds **8**–**55**. Reagents and conditions: (a) 2 N Na_2CO_3, i-PrOH, reflux. (b) i) chloroform, $SOCl_2$, reflux; ii) DMAP, TEA, amines for **8**–**12** or alcohols for **13**–**55**.

N,N-syn pyrroloquinolinedione **70** (Scheme 7) was obtained from the reaction of 7-bromoquinoline-5,8-dione with ethyl 3-aminocrotonate under $Mn(OAc)_3$ catalysis in a low yield (10%). Unfortunately, N,N-anti product **71** was not obtained in sufficient quantities to allow its evaluation as an inhibitor. The reaction of 6,7-dichloroquinoline-5,8-dione with ethyl acetoacetate and followed by treatment with methylamine mainly gave the N,N-syn pyrroloquinolinedione derivative **72**. As shown in Scheme 8, the reaction of 6,7-dichloroquinoline-5,8-dione with ethyl nitroacetate gave two isomers **73** and **74** with isoxazole at C ring. Two synthetic pathways (pathway a and b) were investigated, and unfortunately gave the target isomers in low yields (2–11%). The pyrazole analogues **75** and **76** were obtained from the reaction of quinoline-5,8-dione with ethyl diazoacetate (Scheme 8).

2.3. TDP2 and TDP1 inhibition

All prepared compounds were tested at six or eight three-fold dilution concentrations from 111 μM to 0.46 μM or 0.051 μM against recombinant TDP2 and TDP1. For the compounds with high TDP2 inhibitory activity, a further assay against TDP2 whole cell extracts (WCE) was conducted to determine their potency against native TDP2 enzyme in the presence of abundant cellular proteins. The inhibitory results are summarized in Tables 1–3 and expressed as IC_{50} value. Most compounds were selective against TDP2, as they did not show significant inhibition against TDP1 at the highest concentration tested of 111 μM. Only compounds **39** (TDP1 IC_{50} 48 μM) and **48** (TDP1 IC_{50} 38 μM) showed moderate TDP1 inhibitory potency.

Based on the TDP2 inhibition results, it could be observed that the ester functionality is preferred over amide or methyl groups at position 3 (**77**, >111 μM), either of which abolished TDP2 inhibitory activity in otherwise similarly substituted derivatives. For example, in ester/amid pairs **1** (IC_{50} = 11 μM)/**8** (inactive, i.e. IC_{50} > 111 μM), **2** (43 μM)/**67** (>111 μM), **27** (18 μM)/**9** (>111 μM), **29** (7.6 μM)/**10** (>111 μM) and **32** (14 μM)/**12** (>111 μM), the activity of ester analogues was abolished by changing to amide functionality (Tables 1–3). These findings point out the important role played by the neutral ester functionality in the activity of the furoquinolinedione class as TDP2 inhibitors. Furthermore, the furoquinolinedione core was esterified to a number of different groups both aliphatic and aromatic (Tables 2 and 3, **13**–**65**). The phosphorylethyl ester **24** (TDP2 IC_{50} = 9.0 μM) showed a slightly increased TDP2 inhibition. The phenyl esters **29** (TDP2 IC_{50} = 7.6 μM and WCE IC_{50} = 6.6 μM), **33** (11 and 7.5 μM), **63** (8.1 and 8.3 μM) and pyridinyl ester **41** (8.8 and 7.7 μM) showed increased inhibition against both recombinant TDP2 and TDP2 WCE. These results show that steric bulk is tolerated with respect to inhibitory properties. Despite that, a few analogues were found to be adversely affected by the introduction of larger conjugated aromatic groups, 2-naphthyl **40**, benzoylphenyl **46**, 3,4-methylenedioxyphenyl **49**, as these derivatives were determined to be inactive. Various functionality at the end of the conjugated groups electron-donating (e.g. **29**, **30**) and −withdrawing (e.g. **35**, **36**, **39**) substituents of the aryl analogues as well as amine (**44**) and acid (**64**) derivatives are tolerated.

The furan ring modified analogues revealed the importance of the oxygen present in the parent **1** and **2** for the TDP2 inhibitory activity. Conversion of furan to pyrrole **70**, N-methyl pyrrole **72** or diazoles **75** and **76** rendered compounds inactive. Isoxazoles **73** (TDP2 3.3 and WCE 3.1 μM) and **74** (1.9 and 2.1 μM) gained an improved inhibitory activity for both recombinant TDP2 and WCE, resulting in the two most potent analogues in the series to date. The representative gels of TDP2 inhibitions is shown in Fig. 3A. The deazaflavin SV-163 (Fig. 1) was used as the positive control [24]. The tested compounds show full inhibition at the highest

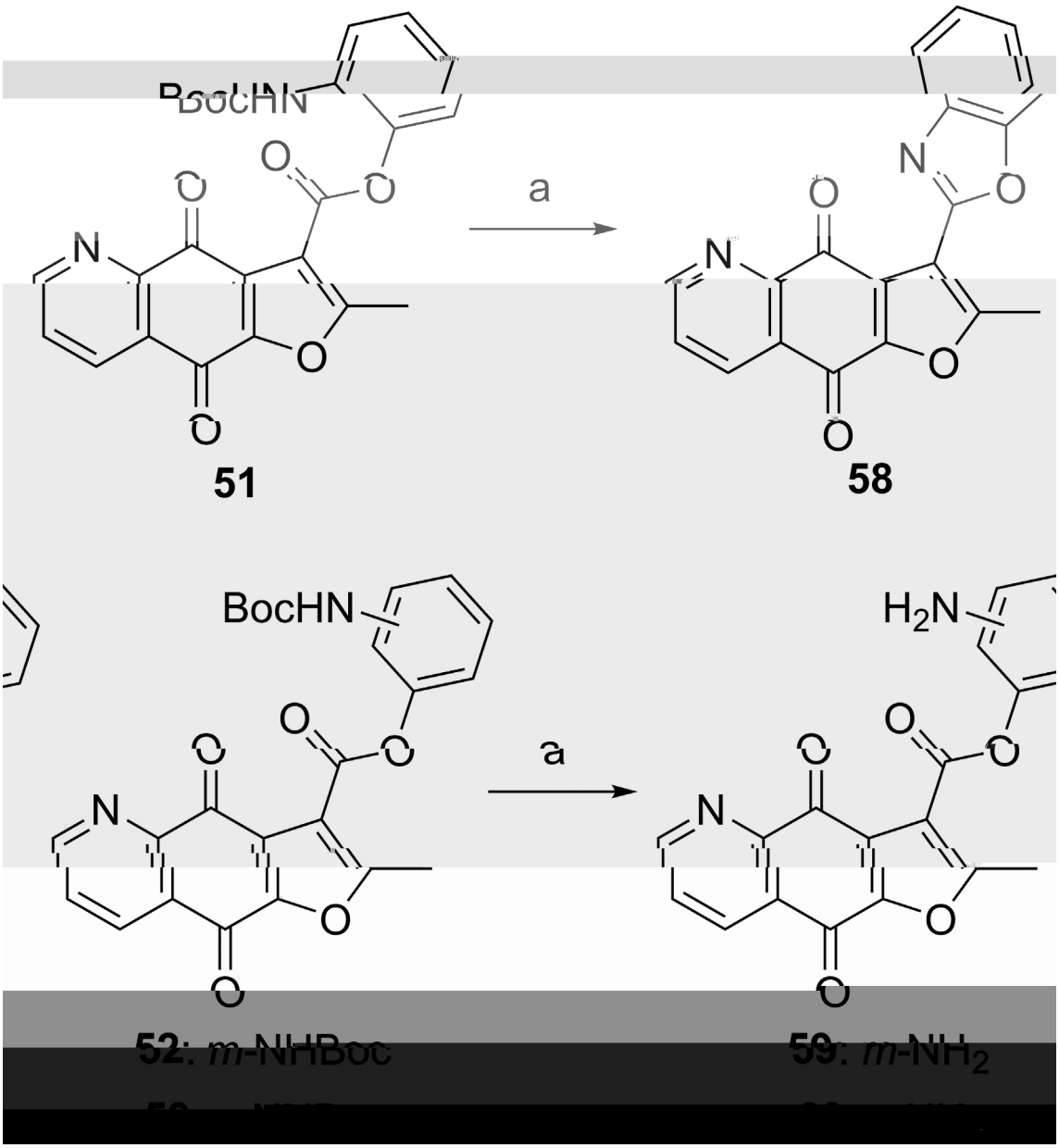
concentration (111 μM) and progressive dose-response (Fig. 3A). Conversely, they do not inhibit TDP1 up to 111 μM concentration (Fig. 3B), in which the indenoisoquinoline AM-8-3 (Fig. 4) was used as a positive control [35].

To study the selectivity of the two most potent compounds **73** and **74**, TOP1-mediated cleavage assay and relaxation assay and TOP2-medaited relaxation assay were performed and indicated that compounds **73** and **74** do not have inhibitory potency to TOP1 and TOP2 (Fig. 5A and Fig. 2S, Supporting Information). Compound **74** was also tested using TOP2-mediated in vivo complex of enzyme (ICE) assay (Fig. 5B), which indicated **74** is unable to induce the formation of TOP2cc at concentration up to 50 μM concentration. These results imply they are selective inhibitors of TDP2.

2.4. Molecular modeling

In order to obtain a molecular view of TDP2 inhibition by furoquinolinediones, we build a hypothetical binding model using in-silico docking. In the absence of the human TDP2 (hTDP2) molecular structure, we have employed homology modeling and constructed a hTDP2 structure using mouse TDP2 (mTDP2) as a template [4]. The assumption of mTDP2 suitability as a homology modeling template is born out of our observation that mTDP2 enzymatic performance and responsiveness to inhibitors can be made to mimic that of hTDP2 with few key mutations in the relative proximity of the catalytic site [28].

The docking of the furoquinolinediones **1** and **2** as well as their derivatives isoxazoles **73** and **74** into hTDP2 structure gave a theoretical insight into the binding mode of these inhibitors. According to the model of **73**-TDP2 (Fig. 6), the polycyclic core of **73** is



Scheme 4. Synthesis of compounds **58**–**60**. Reagents and conditions: (a) DCM, TFA, rt.

was stirred and heated under reflux for 3–6 h and cooled to room temperature. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give the N,O-anti and N,O-syn isomers, respectively.

4.2.1. Ethyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***1***)

Yellow solid, yield 9%, mp = 173.6–178.0 °C. ^{1}H NMR ($CDCl_3$) δ 9.06 (d, J = 4.0 Hz, 1H), 8.54 (d, J = 7.6 Hz, 1H), 7.70 (dd, J = 7.6, 4.7 Hz, 1H), 4.44 (q, J = 6.7 Hz, 2H), 2.76 (s, 3H), 1.48 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 175.4, 171.3, 164.4, 161.0, 153.4, 149.5, 148.1, 133.6, 127.6, 127.3, 126.3, 113.0, 60.7, 13.0. HRMS (ESI) m/z: 284.0551 $[M-H]^-$, calcd for $C_{15}H_{10}NO_5$ 284.0564. The structure of compound **1** was confirmed with 2D NMR spectra and X-ray single crystal analysis.

4.2.2. Ethyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[3,2-g]quinoline-3-carboxylate (***2***)

Yellow solid, yield 11%, mp = 173.5–178.2 °C. ^{1}H NMR ($CDCl_3$) δ 9.04 (d, J = 4.8 Hz, 1H), 8.54 (d, J = 8.0 Hz, 1H), 7.67 (dd, J = 7.6, 4.7 Hz, 1H), 4.46 (q, J = 7.1 Hz, 2H), 2.76 (s, 3H), 1.46 (t, J = 6.7 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 177.2, 171.5, 165.4, 161.6, 154.2, 151.6, 147.5, 135.4, 130.7, 128.0, 127.6, 113.7, 61.6, 14.3, 14.1. HRMS (ESI) m/z: 284.0574 $[M-H]^-$, calcd for $C_{15}H_{10}NO_5$ 284.0564. The structure of compound **2** was confirmed with 2D NMR spectra.

4.2.3. 3-Acetyl-2-methylfuro[2,3-g]quinoline-4,9-dione (***3***)

Yellow solid, yield 5%, mp = 219.5–220.4 °C. ^{1}H NMR ($CDCl_3$) δ 9.07 (d, J = 4.5 Hz, 1H), 8.55 (dd, J = 7.8, 1.2 Hz, 1H), 7.73 (dd, J = 7.8, 4.7 Hz, 1H), 2.81 (s, 3H), 2.70 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 195.3, 178.3, 172.3, 165.0, 154.4, 150.1, 148.9, 134.7, 128.5, 127.9, 127.6, 121.2, 31.9, 14.4. HRMS (ESI) m/z: 256.0613 $[M+H]^+$, calcd for $C_{14}H_{10}NO_4$ 256.0604.

Scheme 5. Synthesis of compounds **61**–**65**. Reagents and conditions: (a) i) 3-bromopropanol (for **61**, **63**) or 3-bromopropanic acid (for **62**, **64**), MeCN, NaN_3, reflux; ii) DMF, H_2O, 5 mol% $CuSO_4/5H_2O$, 10 mol% L-ascorbic acid sodium, 75C. (b) NaOH, EtOH, rt.

4.2.4. 3-Acetyl-2-methylfuro[3,2-g]quinoline-4,9-dione (***4***)

Yellow solid, yield 10%, mp = 219.0–220.1 °C. ^{1}H NMR ($CDCl_3$) δ 9.07 (dd, J = 4.7, 1.7 Hz, 1H), 8.54 (dd, J = 7.9, 1.7 Hz, 1H), 7.73 (dd, J = 7.9, 4.7 Hz, 1H), 2.79 (s, 3H), 2.70 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 195.1, 179.0, 171.5, 165.1, 154.6, 151.1, 147.6, 135.4, 130.4, 127.7, 127.1, 120.9, 31.9, 14.5. HRMS (ESI) m/z: 256.0593 $[M + H]^+$, calcd for $C_{14}H_{10}NO_4$ 256.0604.

4.2.5. Dimethyl-(2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g] quinolin-3-yl)phosphonate (***5***)

Yellow solid, yield 9%, mp = 183.4–183.9 °C. ^{1}H NMR ($CDCl_3$) δ 9.06 (d, J = 3.6 Hz, 1H), 8.55 (d, J = 7.8 Hz, 1H), 7.73 (dd, J = 7.7, 4.7 Hz, 1H), 3.95 (s, 3H), 3.92 (s, 3H), 2.83 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 177.0, 172.2, 169.3, 169.0, 154.4, 151.4, 151.3, 148.7, 134.8, 131.2, 131.1, 128.6, 127.5, 108.2, 106.1, 53.6, 53.6, 14.5. HRMS (ESI) m/z: 322.0473 $[M + H]^+$, calcd for $C_{14}H_{13}NO_6P$ 322.0475. The structure of compound **5** was confirmed with 2D NMR spectra.

4.2.6. Dimethyl-(2-methyl-4,9-dioxo-4,9-dihydrofuro[3,2-g] quinolin-3-yl)phosphonate (***6***)

Yellow solid, yield 10%, mp = 193.1–194.1 °C. ^{1}H NMR ($CDCl_3$) δ 9.06 (dd, J = 4.7, 1.7 Hz, 1H), 8.53 (dd, J = 7.9, 1.7 Hz, 1H), 7.73 (dd, J = 7.9, 4.7 Hz, 1H), 3.92 (s, 3H), 3.89 (s, 3H), 2.83 (d, J = 2.0 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 177.8, 171.3, 169.4, 169.1, 154.4, 152.4, 152.3, 147.7, 135.3, 130.3, 130.2, 130.2, 127.6, 107.5, 105.4, 53.4, 53.3, 14.5. HRMS (ESI) m/z: 322.0485 $[M + H]^+$, calcd for $C_{14}H_{13}NO_6P$ 322.0475. The structure of compound **6** was confirmed with 2D NMR spectra.

4.3. Hydrolysis of compounds ***1*** and ***2***

To a solution of compound **1** or **2** (4 mmol) in isopropanol (300 mL), a Na_2CO_3 aqueous solution (2 N, 30 mL) was added. The mixture solution was stirred and heated under reflux for 30 min and cooled to room temperature. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give the target compound **7** or **66**, respectively.

4.3.1. 2-Methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylic acid (***7***)

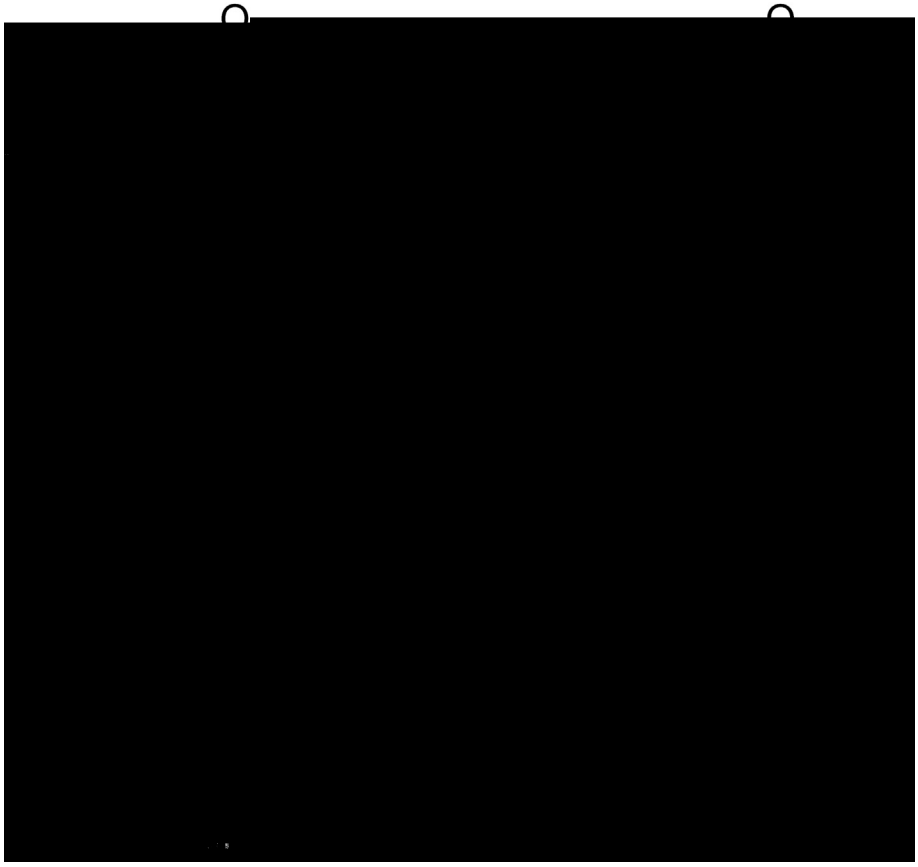
Yellow solid, yield 75%, ^{1}H NMR ($CDCl_3$) δ 9.12 (dd, J = 4.7, 1.7 Hz, 1H), 8.59 (dd, J = 7.9, 1.7 Hz, 1H), 7.82 (dd, J = 7.9, 4.7 Hz, 1H), 2.93 (s, 3H). ESI-MS m/z: 258.1 $[M + H]^+$.

4.3.2. 2-Methyl-4,9-dioxo-4,9-dihydrofuro[3,2-g]quinoline-3-carboxylic acid (***66***)

Yellow solid, yield 72%, mp = 176.9–177.5 °C. ^{1}H NMR (DMSO) δ 13.33 (s, 1H), 9.02 (d, J = 3.3 Hz, 1H), 8.43 (d, J = 7.6 Hz, 1H), 7.89–7.83 (m, 1H), 2.68 (s, 3H). ^{13}C NMR (DMSO) δ 178.6, 171.6, 164.4, 163.1, 154.3, 152.1, 148.0, 135.2, 130.9, 128.3, 127.4, 114.0, 14.2. HRMS (ESI) m/z: 258.0409 $[M + H]^+$, calcd for $C_{13}H_8NO_5$ 258.0397.

4.4. General procedures for the esterification or amidation of compounds ***7*** and ***66***

To a yellow solution of compound **7** or **66** (1 mmol) in new distilled chloroform (80 mL), a solution of thionyl chloride (1.5 mL) in new distilled chloroform (10 mL) was added dropwise. The mixture solution was stirred and heated under reflux for 5 h and cooled to room temperature. The solvent was evaporated under reduced pressure. The yellow residue was dissolved in new distilled chloroform (40 mL) and was added with a solution of DMAP (0.1 mmol), Et_3N (1.2 mmol) and corresponding amines or alcohols (1.2 mmol) in new distilled chloroform (20 mL). The reaction mixture was stirred and heated under reflux for 5 h and cooled to



Scheme 6. Reagents and conditions: (a) 2 N Na_2CO_3, i-PrOH, reflux. (b) i) chloroform, $SOCl_2$, reflux; ii) DMAP, TEA, amines for **67**–**68** or catechol for **69**.

room temperature. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give the amide or ester targets, respectively.

4.4.1. N-Ethyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxamide (***8***)

Yellow solid, yield 55%, mp = 237.6–238.5 °C. ^{1}H NMR ($CDCl_3$) δ 9.52 (s, 1H), 9.08 (d, J = 3.3 Hz, 1H), 8.56 (d, J = 7.4 Hz, 1H), 7.76 (dd, J = 7.7, 4.7 Hz, 1H), 3.50 (quint, J = 6.4 Hz, 2H), 2.93 (s, 3H), 1.34 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 181.0, 171.9, 167.3, 160.7, 154.5, 150.5, 148.6, 134.8, 128.6, 128.1, 126.6, 115.9, 34.5, 15.1, 14.3. HRMS (ESI) m/z: 307.0699 $[M + Na]^+$, calcd for $C_{15}H_{12}N_2O_4Na$ 307.0689.

4.4.2. N-Phenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g] quinoline-3-carboxamide (***9***)

Yellow solid, yield 41%, mp = 295.6–296.2 °C. ^{1}H NMR (DMSO) δ 11.10 (s, 1H), 9.06 (d, J = 3.2 Hz, 1H), 8.51 (dd, J = 7.8, 1.5 Hz, 1H), 7.91 (dd, J = 7.7, 4.7 Hz, 1H), 7.76 (d, J = 7.8 Hz, 2H), 7.42 (t, J = 7.1 Hz, 2H), 7.17 (d, J = 7.4 Hz, 1H), 2.78 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 180.6, 170.9, 167.2, 157.8, 153.6, 149.6, 147.5, 137.2, 133.9, 128.0, 127.6, 127.3, 125.1, 123.5, 119.2, 115.4, 14.4. HRMS (ESI) m/z: 331.0723 $[M - H]^-$, calcd for $C_{19}H_{11}N_2O_4$ 331.0724.

4.4.3. N-(2-hydroxyphenyl) 2-methyl-4,9-dioxo-4,9-dihydrofuro [2,3-g]quinoline-3-carboxamide (***10***)

Orange solid, yield 49%, mp = 290.1–292.3 °C. ^{1}H NMR (DMSO) δ 11.19 (s, 1H), 9.07 (d, J = 3.2 Hz, 1H), 8.51 (dd, J = 7.8, 1.5 Hz, 1H), 7.96 (s, 1H), 7.92 (dd, J = 7.7, 4.7 Hz, 1H), 7.59 (d, J = 8.2 Hz, 1H), 7.45 (t, J = 8.1 Hz, 1H), 7.23 (d, J = 7.8 Hz, 1H), 2.76 (s, 3H). ^{13}C NMR (DMSO) δ 180.2, 172.6, 168.8, 164.7, 159.8, 154.4, 150.5, 148.2, 140.3, 134.8, 133.8, 131.3, 129.1, 128.7, 127.5, 124.3, 119.5, 118.4, 14.4. HRMS (ESI) m/z: 347.0656 $[M - H]^-$, calcd for $C_{19}H_{11}N_2O_5$ 347.0673.

4.4.4. N-(4-Methoxyphenyl) 2-methyl-4,9-dioxo-4,9-dihydrofuro [2,3-g]quinoline-3-carboxamide (***11***)

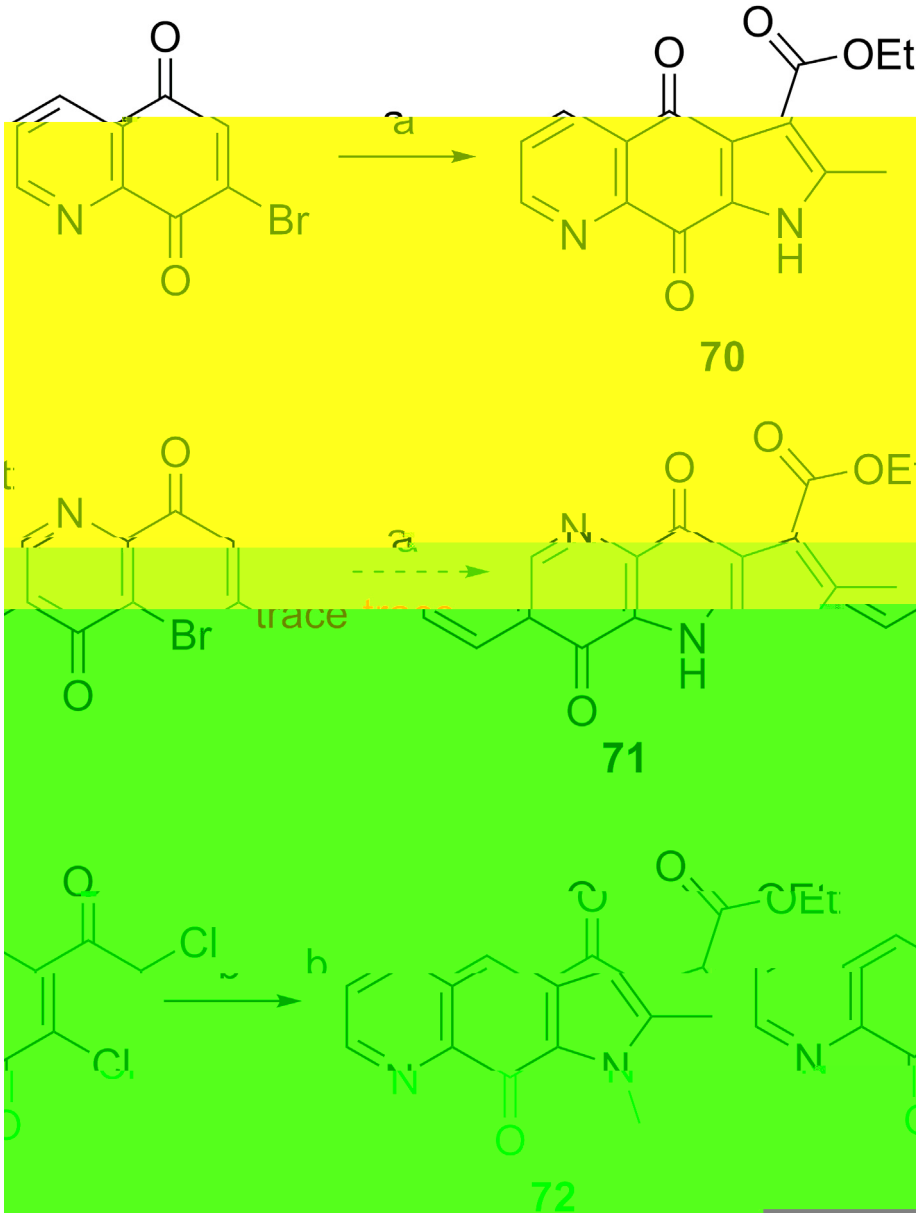
Red solid, yield 54%, mp = 332.7–333.2 °C. ^{1}H NMR (DMSO) δ 11.00 (s, 1H), 9.07 (d, J = 3.3 Hz, 1H), 8.50 (dd, J = 7.8, 1.6 Hz, 1H), 7.91 (dd, J = 7.7, 4.7 Hz, 1H), 7.67 (d, J = 9.0 Hz, 2H), 6.99 (d, J = 9.0 Hz, 2H), 3.77 (s, 3H), 2.77 (s, 3H). ^{13}C NMR (DMSO) δ 180.0, 172.1, 164.1, 158.5, 155.8, 153.8, 150.0, 148.6, 134.2, 133.4, 131.5, 128.6, 128.2, 126.9, 121.1, 114.2, 55.2, 14.0. HRMS (ESI) m/z: 361.0817 $[M - H]^-$, calcd for $C_{20}H_{13}N_2O_5$ 361.0830.

4.4.5. N-(3-Chlorophenyl) 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxamide (***12***)

Orange solid, yield 50%, mp = 311.8–312.5 °C. ^{1}H NMR (DMSO) δ 11.11 (s, 1H), 10.02 (s, 1H), 9.05 (d, J = 3.3 Hz, 1H), 8.49 (d, J = 6.7 Hz, 1H), 8.18 (d, J = 7.8 Hz, 1H), 7.90 (dd, J = 7.7, 4.7 Hz, 1H), 6.99 (t, J = 7.2 Hz, 1H), 6.94 (d, J = 7.0 Hz, 1H), 6.82 (t, J = 7.3 Hz, 1H), 2.83 (s, 3H). ^{13}C NMR (DMSO) δ 180.0, 172.2, 165.7, 159.0, 153.8, 150.1, 148.6, 147.8, 134.1, 128.5, 128.1, 126.2, 124.7, 121.8, 118.9, 115.6, 115.1, 14.7. HRMS (ESI) m/z: 367.0480 $[M + H]^+$, calcd for $C_{19}H_{12}N_2O_4Cl$ 367.0501.

4.4.6. Methyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***13***)

Yellow solid, mp = 176.2–178.4 °C. ^{1}H NMR ($CDCl_3$) δ 9.06 (dd, J = 4.7, 1.7 Hz, 1H), 8.54 (dd, J = 7.9, 1.7 Hz, 1H), 7.71 (dd, J = 7.9, 4.7 Hz, 1H), 3.99 (s, 3H), 2.76 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.5, 172.3, 165.5, 162.3, 154.4, 150.6, 149.2, 134.6, 128.7, 128.4, 127.3, 113.6, 52.4,



Scheme 7. Synthesis of compounds **70**–**72**. Reagents and conditions: (a) ethyl 3-aminocrotonate, $Mn(OAc)_3$, MeCN, reflux. (b) i) T9F11Tf7397.41]

14.1. HRMS (ESI) m/z: 294.0386 $[M + Na]^+$, calcd for $C_{14}H_9NO_5Na$ 294.0373.

4.4.7. 2,2,2-Tri*fl*uoroethyl 4,9-dihydro-2-methyl-4,9-dioxofuro[2,3-g]quinoline-3-carboxylate (***14***)

Yellow solid, yield 61%, mp = 166.9–167.4 °C. ^1H NMR ($CDCl_3$) δ 9.06 (d, J = 3.5 Hz, 1H), 8.53 (dd, J = 7.8, 1.5 Hz, 1H), 7.71 (dd, J = 7.8, 4.7 Hz, 1H), 4.77 (q, J = 8.3 Hz, 2H), 2.78 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 175.1, 171.3, 165.6, 159.1, 153.6, 149.9, 148.0, 133.7, 127.4, 127.3, 126.4, 121.8 (q, $^1J_{CF}$ = 275.7 Hz), 111.1, 60.0 (q, $^2J_{CF}$ = 36.9 Hz), 13.3. HRMS (ESI) m/z: 362.0266 $[M + Na]^+$, calcd for $C_{15}H_8NO_5F_3Na$ 362.0247.

4.4.8. 2-((2-Methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carbonyl)oxy)acetic acid (***15***)

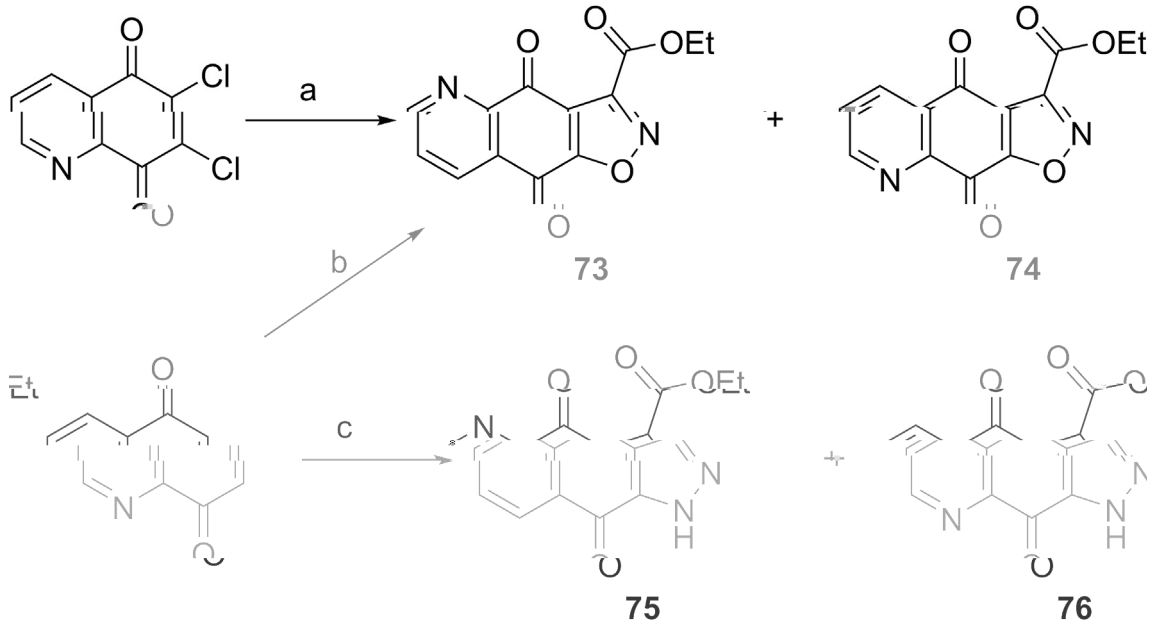
Yellow solid, yield 39%, mp = 205.0 °C (decomposing). ^1H NMR (CD_3OD) δ 8.95 (dd, J = 4.7, 1.6 Hz, 1H), 8.58 (dd, J = 7.9, 1.6 Hz, 1H), 7.84 (dd, J = 7.9, 4.8 Hz, 1H), 4.07 (s, 2H), 2.78 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.0, 171.8, 169.0, 166.0, 160.6, 153.7, 150.4, 148.4, 134.5, 128.2, 128.1, 127.3, 112.5, 60.9, 13.6. HRMS (ESI) m/z: 338.0283 $[M + Na]^+$, calcd for $C_{15}H_9NO_7Na$ 338.0271.

4.4.9. 2-Hydroxyethyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***16***)

Yellow solid, yield 24%, mp = 184.7–186.1 °C. ^1H NMR ($CDCl_3$) δ 9.08 (d, J = 3.2 Hz, 1H), 8.56 (d, J = 7.6 Hz, 1H), 7.73 (dd, J = 7.4, 4.4 Hz, 1H), 4.49 (t, J = 4.4 Hz, 2H), 4.00 (t, J = 4.4 Hz, 2H), 2.80 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.8, 172.3, 166.1, 162.3, 154.4, 150.6, 149.1, 134.7, 128.3, 128.3, 127.4, 113.6, 62.9, 59.5, 31.3, 14.1. HRMS (ESI) m/z: 300.0514 $[M - H]^-$, calcd for $C_{15}H_{10}NO_6$ 300.0499.

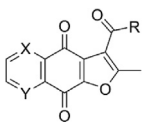
4.4.10. 3-Hydroxypropyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***17***)

Yellow solid, yield 25%, mp = 169.1–169.5 °C. ^1H NMR ($CDCl_3$)



Scheme 8. Synthesis of compounds **73**–**76**. Reagents and conditions: (a) MeCN, K_2CO_3, ethyl nitroacetate, reflux. (b) AcOH, $Mn(OAc)_3$, ethyl nitroacetate, 70 °C. (c) ethyl diazoacetate, toluene, reflux.

Table 1
The inhibitory activity of compounds **1**–**6** against TDP2.



Cpd.	X	Y	R	IC_{50} (μM)[a]	
				TDP2	TDP2 WCE
1	N	CH	CO_2Et	11 ± 1.0	22 ± 1.7
2	CH	N	CO_2Et	43 ± 5.5	54 ± 2.5
3	N	CH	COMe	35 ± 5.1	36 ± 2.4
4	CH	N	COMe	>111	ND[b]
5	N	CH	PO_3Me_2	>111	ND
6	CH	N	PO_3Me_2	99 ± 11	ND

[a] The IC_{50} was expressed as mean ± SD from at least three independent experiments unless otherwise indicated.
[b] "ND" means "not determined".

δ 9.06 (d, J = 3.3 Hz, 1H), 8.54 (d, J = 7.0 Hz, 1H), 7.71 (dd, J = 7.6, 4.7 Hz, 1H), 4.55 (t, J = 5.9 Hz, 2H), 3.91 (t, J = 5.9 Hz, 2H), 2.77 (s, 3H), 2.09 (quint, J = 5.8 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 176.8, 172.3, 166.1, 162.3, 154.4, 150.6, 149.1, 134.7, 128.3, 127.4, 113.6, 62.9, 59.5, 50.8, 31.3, 14.1. HRMS (ESI) m/z: 314.0653 $[M-H]^-$, calcd for $C_{16}H_{12}NO_6$ 314.0670.

4.4.11. 4-Hydroxybutyl 4,9-dihydro-2-methyl-4,9-dioxofuro[2,3-g]quinoline-3-carboxylate (***18***)

Yellow solid, yield 24%, mp = 128.0–128.5 °C. ^{1}H NMR ($CDCl_3$) δ 9.06 (d, J = 3.5 Hz, 1H), 8.54 (dd, J = 7.8, 1.1 Hz, 1H), 7.71 (dd, J = 7.8, 4.6 Hz, 1H), 4.43 (t, J = 6.4 Hz, 2H), 3.75 (t, J = 6.3 Hz, 2H), 2.76 (s, 3H), 1.95 (quint, J = 6.4 Hz, 2H), 1.81 (quint, J = 6.4 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 175.5, 171.3, 164.8, 161.1, 153.3, 149.6, 148.0, 133.7, 127.4, 127.3, 126.4, 112.8, 64.6, 61.3, 28.2, 24.0, 13.1. HRMS (ESI) m/z: 352.0810 $[M + Na]^+$, calcd for $C_{17}H_{15}NO_6Na$ 352.0792.

4.4.12. 5-Hydroxypentyl 4,9-dihydro-2-methyl-4,9-dioxofuro[2,3-g]quinoline-3-carboxylate (***19***)

Yellow solid, yield 42%, mp = 107.7–108.8 °C. ^{1}H NMR ($CDCl_3$) δ 9.05 (dd, J = 4.7, 1.7 Hz, 1H), 8.53 (dd, J = 7.9, 1.7 Hz, 1H), 7.70 (dd, J = 7.9, 4.7 Hz, 1H), 4.40 (t, J = 6.5 Hz,2H), 3.71 (t, J = 6.1 Hz, 2H), 2.76 (s, 3H), 1.93–1.85 (m, 2H), 1.73–1.55 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 176.5, 172.3, 165.6, 162.1, 154.3, 150.6, 149.1, 134.6, 128.6, 128.3, 127.3, 113.8, 65.6, 62.4, 32.2, 28.2, 22.2, 14.0. HRMS (ESI) m/z: 366.0966 $[M + Na]^+$, calcd for $C_{18}H_{17}NO_6Na$ 366.0948.

4.4.13. 2-(2-Hydroxyethoxy)ethyl 4,9-dihydro-2-methyl-4,9-dioxofuro[2,3-g]quinoline-3-carboxylate (***20***)

Brown solid, yield 31%, mp = 134.6–135.4 °C. ^{1}H NMR ($CDCl_3$) δ 9.05 (dd, J = 4.7, 1.7 Hz, 1H), 8.53 (dd, J = 7.9, 1.7 Hz, 1H), 7.71 (dd, J = 7.9, 4.7 Hz, 1H), 4.56–4.53 (m, 2H), 3.94–3.91 (m, 2H), 3.80–3.77 (m, 2H), 3.71–3.68 (m, 2H), 2.76 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.7, 172.3, 165.9, 161.9, 154.4, 150.6, 149.1, 134.6, 128.6, 128.3, 127.3, 113.5, 72.5, 68.6, 64.5, 61.6, 14.0. HRMS (ESI) m/z: 368.0752 $[M + Na]^+$, calcd for $C_{17}H_{15}NO_7Na$ 368.0741.

4.4.14. 2,3-Dihydroxypropyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***21***)

Yellow solid, yield 26%, mp = 193.7–195.3 °C. ^{1}H NMR ($CDCl_3$) δ 9.08 (dd, J = 4.7, 1.7 Hz, 1H), 8.55 (dd, J = 7.9, 1.7 Hz, 1H), 7.73 (dd, J = 7.9, 4.7 Hz, 1H), 4.47 (d, J = 5.2 Hz, 2H), 4.18 (quint, J = 5.2 Hz, 1H), 3.83–3.74 (m, 2H), 2.80 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 177.4, 172.2, 166.9, 161.9, 154.5, 150.9, 149.0, 134.8, 128.3, 127.8, 127.7, 112.9, 69.8, 66.9, 63.2, 14.1. HRMS (ESI) m/z: 330.0604 $[M-H]^-$, calcd for $C_{16}H_{12}NO_7$ 330.0619.

4.4.15. (2′S)-2,3-Dihydroxypropyl 4,9-dihydro-2-methyl-4,9-dioxofuro[2,3-g]quinoline-3-carboxylate (***22***)

Yellow solid, yield 24%, mp = 173.4–174.8 °C. ^{1}H NMR ($CDCl_3$) δ 9.09 (dd, J = 4.6, 1.6 Hz, 1H), 8.57 (dd, J = 7.8, 1.5 Hz, 1H), 7.75 (dd, J = 7.8, 4.7 Hz, 1H), 4.48 (d, J = 4.7 Hz, 2H), 4.19 (quint, J = 5.3 Hz, 1H), 3.85–3.76 (m, 2H), 2.81 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 177.4, 172.2, 167.0, 161.9, 154.5, 150.9, 149.0, 134.8, 128.3, 127.8, 127.7, 112.9, 69.8, 66.9, 63.2, 14.1. HRMS (ESI) m/z: 332.0750 $[M + H]^+$, calcd for

$C_{16}H_{14}NO_7$ 332.0765.

4.4.16. (2′R)-2,3-Dihydroxypropyl 4,9-dihydro-2-methyl-4,9-dioxofuro[2,3-g]quinoline-3-carboxylate (***23***)

Yellow solid, yield 48%, mp = 159.0–162.7 °C. ^{1}H NMR ($CDCl_3$) δ 9.01 (dd, J = 4.7, 1.7 Hz, 1H), 8.48 (dd, J = 7.9, 1.7 Hz, 1H), 7.66 (dd, J = 7.9, 4.7 Hz, 1H), 4.40 (d, J = 5.2 Hz, 2H), 4.14–4.08 (m, 1H), 3.76–3.67 (m, 2H), 2.73 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 177.4, 172.2, 167.0, 161.9, 154.5, 150.9, 149.0, 134.8, 128.3, 127.8, 127.7, 112.9, 69.8, 66.9, 63.2, 14.1. HRMS (ESI) m/z: 354.0596 $[M + Na]^+$, calcd for $C_{16}H_{13}NO_7Na$ 354.0584.

4.4.17. 2-(Dimethoxyphosphoryl)ethyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***24***)

Yellow solid, yield 81%, mp = 182.5–183.7 °C. ^{1}H NMR ($CDCl_3$) δ 9.06 (dd, J = 4.6, 1.6 Hz, 1H), 8.54 (dd, J = 7.9, 1.6 Hz, 1H), 7.71 (dd, J = 7.9, 4.7 Hz, 1H), 4.64–4.58 (m, 2H), 3.78 (d, J = 11.2 Hz, 6H), 2.77

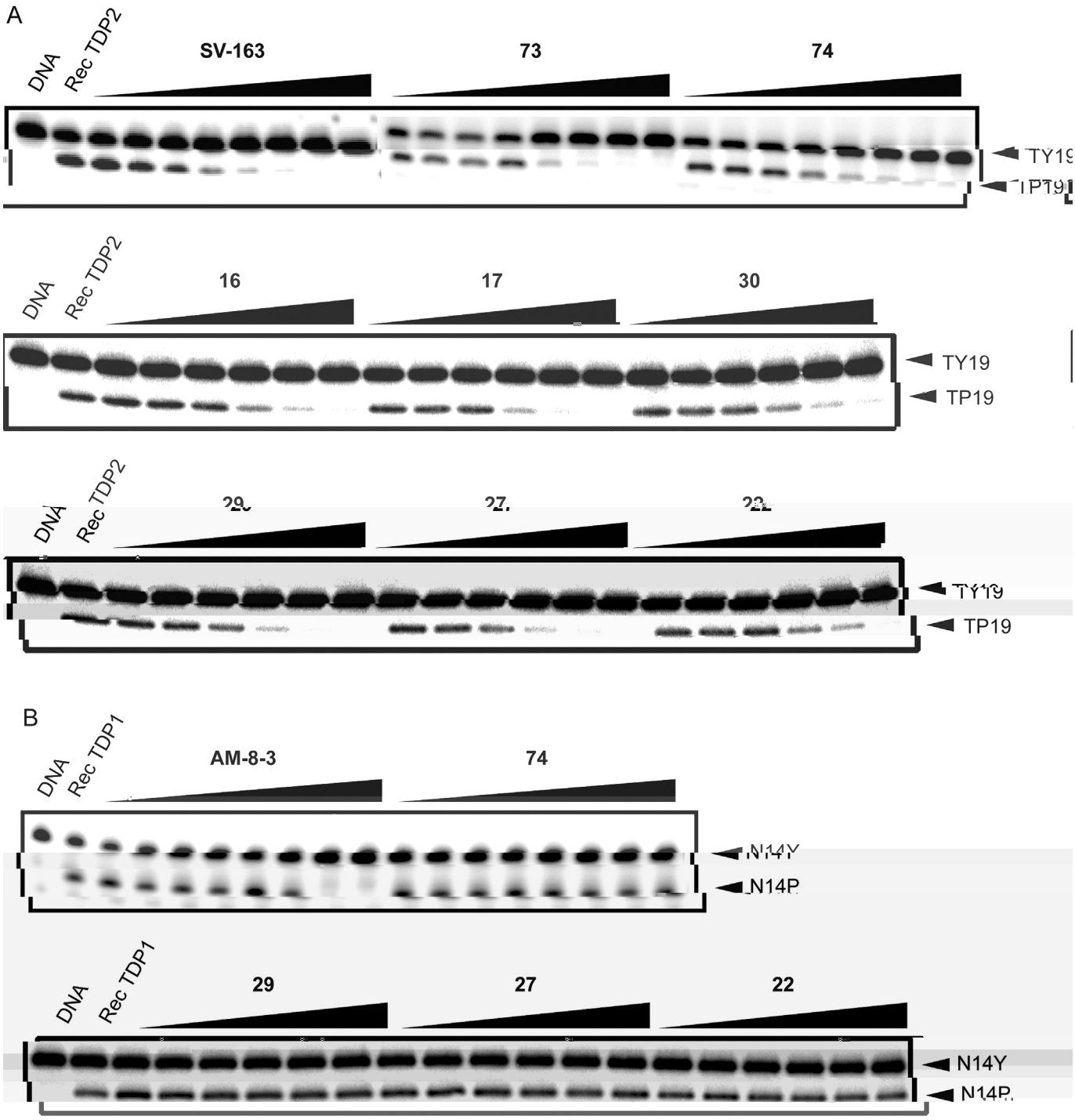


Fig. 3. Representative gels for the testing of compounds against Rec TDP2 (A) and TDP1 (B). The compounds were tested at concentrations increasing from 0.51 to 111 μM (for eight doses) or 0.46–111 μM (for six doses). The deazafavin SV-163 was tested as the positive control for TDP2 assay at concentrations increasing from 0.017 to 37 μM. The indenoisoquinoline AM-8-3 was tested as the positive control for TDP1 assay at concentrations increasing from 0.051 to 111 μM.

(s, 3H), 2.46 (dt, J = 19.2, 7.8 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 176.5, 172.2, 165.8, 161.5, 154.4, 150.6, 149.0, 134.7, 128.4, 128.3, 127.3, 113.7, 59.6, 52.5 (d, $^{2}J_{CP}$ = 6.3 Hz), 24.9 (d, $^{1}J_{CP}$ = 139.5 Hz), 14.0. HRMS (ESI) m/z: 392.0538 $[M-H]^-$, calcd for $C_{17}H_{15}NO_8P$ 392.0541.

4.4.18. 2-Cyanoethyl 4,9-dihydro-2-methyl-4,9-dioxofuro[2,3-g] quinoline-3-carboxylate (***25***)

Yellow solid, yield 49%, mp = 182.5–183.9 °C. ^{1}H NMR ($CDCl_3$) δ 9.06 (dd, J = 4.6, 1.6 Hz, 1H), 8.54 (dd, J = 7.9, 1.7 Hz, 1H), 7.72 (dd, J = 7.9, 4.7 Hz, 1H), 4.60 (t, J = 6.5 Hz, 2H), 2.99 (t, J = 6.5 Hz, 2H), 2.78 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.5, 172.2, 166.3, 161.2, 154.5, 150.8, 149.0, 134.7, 128.34, 128.32, 127.4, 116.8, 112.8, 59.7, 17.8, 14.2. HRMS (ESI) m/z: 311.0684 $[M+H]^+$, calcd for $C_{16}H_{11}N_2O_5$ 311.0662.

4.4.19. Phenethyl 4,9-dihydro-2-methyl-4,9-dioxofuro[2,3-g] quinoline-3-carboxylate (***26***)

Yellow solid, yield 53%, mp = 141.8–144.1 °C. ^{1}H NMR ($CDCl_3$) δ 9.07 (dd, J = 4.7, 1.7 Hz, 1H), 8.54 (dd, J = 7.9, 1.7 Hz, 1H), 7.70 (dd,

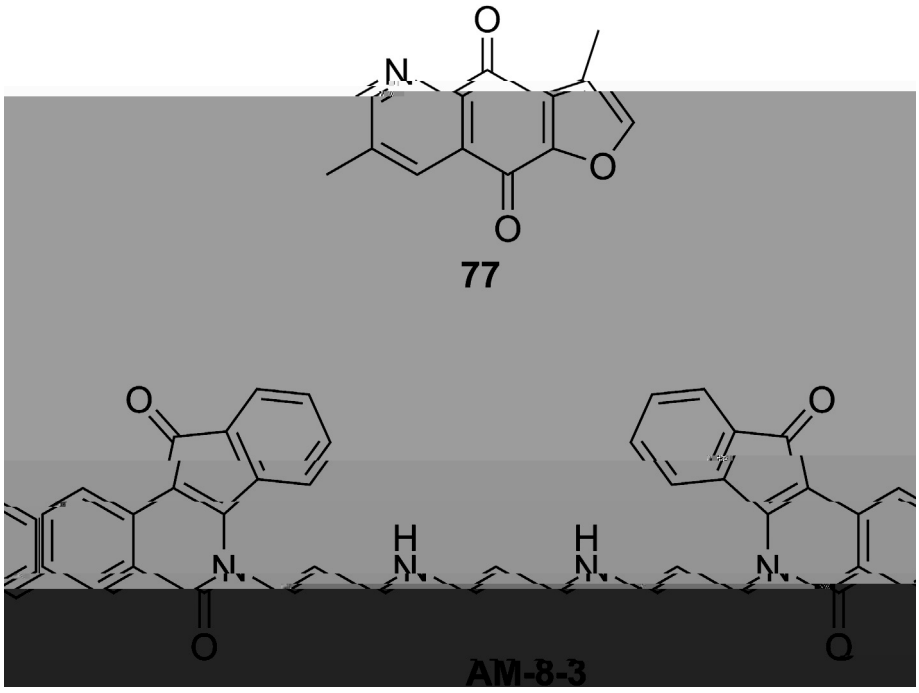


Fig. 4. The structures of compounds **77** and AM-8-3.

J = 7.9, 4.7 Hz, 1H), 7.33–7.28 (m, 4H), 7.25–7.20 (m, 1H), 4.60 (t, J = 7.3 Hz, 2H), 3.19 (t, J = 7.3 Hz, 2H), 2.65 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.5, 172.3, 165.5, 161.8, 154.4, 150.6, 149.1, 137.6, 134.6, 129.0, 128.6, 128.5, 128.4, 127.3, 126.6, 113.8, 66.1, 34.8, 14.0. HRMS (ESI) m/z: 384.0864 $[M + Na]^+$, calcd for $C_{21}H_{15}NO_5Na$ 384.0842.

4.4.20. Phenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g] quinoline-3-carboxylate (***27***)

Yellow solid, yield 36%, mp = 234.8–246.5 °C. ^{1}H NMR (DMSO) δ 9.03 (dd, J = 4.7, 1.7 Hz, 1H), 8.49 (dd, J = 7.9, 1.7 Hz, 1H), 7.87 (dd, J = 7.9, 4.7 Hz, 1H), 7.62–7.46 (m, 2H), 7.44–7.29 (m, 2H), 2.78 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.5, 172.3, 166.2, 160.4, 154.5, 150.7, 150.4, 149.1, 134.7, 129.4, 128.4, 127.4, 126.2, 121.6, 113.5, 14.1. HRMS (ESI) m/z: 332.0563 $[M - H]^-$, calcd for $C_{19}H_{10}NO_5$ 332.0564.

4.4.21. 4-((Dimethoxyphosphoryl)oxy)phenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***28***)

Yellow solid, yield 62%, mp = 118.7–120.3 °C. ^{1}H NMR ($CDCl_3$) δ 9.06 (dd, J = 4.7, 1.7 Hz, 1H), 8.56 (dd, J = 7.9, 1.7 Hz, 1H), 7.73 (dd, J = 7.9, 4.7 Hz, 1H), 7.44 (d, J = 8.9 Hz, 2H), 7.29 (d, J = 8.4 Hz, 2H), 3.89 (d, J = 11.2 Hz, 6H), 2.82 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.6, 172.3, 166.4, 160.3, 154.5, 150.7, 149.1, 148.3 (d, $^2J_{CP}$ = 6.8 Hz), 147.3, 134.7, 128.5, 128.4, 127.4, 122.9, 120.8 (d, $^3J_{CP}$ = 4.8 Hz), 113.2, 55.0 (d, $^2J_{CP}$ = 6.2 Hz), 14.1. HRMS (ESI) m/z: 458.0654 $[M + H]^+$, calcd for $C_{21}H_{17}NO_9P$ 458.0635.

4.4.22. 2-Hydroxyphenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***29***)

Yellow solid, yield 42%, mp = 252.1–253.2 °C. ^{1}H NMR (DMSO) δ 9.81 (s, 1H), 9.03 (d, J = 3.9 Hz, 1H), 8.49 (d, J = 7.7 Hz, 1H), 7.87 (dd, J = 7.6, 4.7 Hz, 1H), 7.23 (d, J = 7.8 Hz, 1H), 7.16 (t, J = 7.6 Hz, 1H), 7.00 (d, J = 7.9 Hz, 1H), 6.90 (t, J = 7.5 Hz, 1H), 2.80 (s, 3H). ^{13}C NMR (DMSO) δ 177.0, 172.8, 165.4, 159.7, 154.3, 151.4, 149.4, 149.3, 138.5, 134.6, 129.0, 128.5, 128.2, 127.6, 123.4, 119.7, 117.4, 116.1, 112.9, 14.5. HRMS (ESI) m/z: 348.0512 $[M - H]^-$, calcd for $C_{19}H_{10}NO_6$ 348.0514.

4.4.23. 3-Hydroxyphenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***30***)

Yellow solid, yield 20%, mp = 241.3–243.2 °C. ^{1}H NMR (DMSO) δ 9.81 (s, 1H), 9.03 (d, J = 3.1 Hz, 1H), 8.49 (d, J = 7.2 Hz, 1H), 7.87 (dd, J = 6.6, 4.9 Hz, 1H), 7.27 (d, J = 8.2 Hz, 1H), 6.88–6.68 (m, 4H), 2.76 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 180.5, 176.1, 170.2, 164.2, 162.0, 158.0, 155.1, 154.7, 152.7, 138.9, 133.7, 132.4, 131.7, 117.4, 117.2, 116.4, 112.7, 110.8, 17.8. HRMS (ESI) m/z: 348.0499 $[M - H]^-$, calcd for $C_{19}H_{10}NO_6$ 348.0514.

4.4.24. 2-Chlorophenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g] quinoline-3-carboxylate (***31***)

Light yellow solid, yield 51%, mp = 212.4–214.0 °C. ^{1}H NMR ($CDCl_3$) δ 9.06 (dd, J = 4.6, 1.6 Hz, 1H), 8.56 (dd, J = 7.9, 1.7 Hz, 1H), 7.72 (dd, J = 7.9, 4.7 Hz, 1H), 7.51 (dd, J = 9.7, 8.1, 1.4 Hz, 2H), 7.38 (td, J = 7.8, 1.5 Hz, 1H), 7.31–7.23 (m, 1H), 2.85 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.4, 172.2, 166.2, 159.2, 154.4, 150.7, 149.0, 146.6, 134.7, 130.3, 128.7, 128.4, 128.0, 127.5, 127.4, 126.8, 123.9, 112.8, 14.2. HRMS (ESI) m/z: 368.0335 $[M + H]^+$, calcd for $C_{19}H_{11}NO_5Cl$ 368.0320.

4.4.25. 3-Chlorophenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g] quinoline-3-carboxylate (***32***)

Light yellow solid, yield 49%, mp = 212.9–214.2 °C. ^{1}H NMR ($CDCl_3$) δ 9.07 (dd, J = 4.6, 1.6 Hz, 1H), 8.56 (dd, J = 7.9, 1.6 Hz, 1H), 7.72 (dd, J = 7.9, 4.7 Hz, 1H), 7.52 (s, 1H), 7.37 (dd, J = 8.4, 4.7 Hz, 1H), 7.29 (dd, J = 4.4, 1.9 Hz, 1H), 2.83 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 176.5, 172.2, 166.6, 160.0, 154.5, 150.8, 150.7, 149.0, 134.7, 130.1, 128.4, 128.4, 127.4, 126.5, 122.2, 120.0, 112.9, 14.1. HRMS (ESI) m/z: 368.0341 $[M + H]^+$, calcd for $C_{19}H_{11}NO_5Cl$ 368.0320.

4.4.26. 4-Chlorophenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g] quinoline-3-carboxylate (***33***)

Light yellow solid, yield 71%, mp = 217.0–217.8 °C. ^{1}H NMR ($CDCl_3$) δ 9.07 (dd, J = 4.6, 1.6 Hz, 1H), 8.56 (dd, J = 7.9, 1.6 Hz, 1H), 7.72 (dd, J = 7.9, 4.7 Hz, 1H), 7.41 (br. s, 4H), 2.82 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.6, 172.3, 166.5, 160.2, 154.5, 150.8, 149.1, 148.9, 134.7,

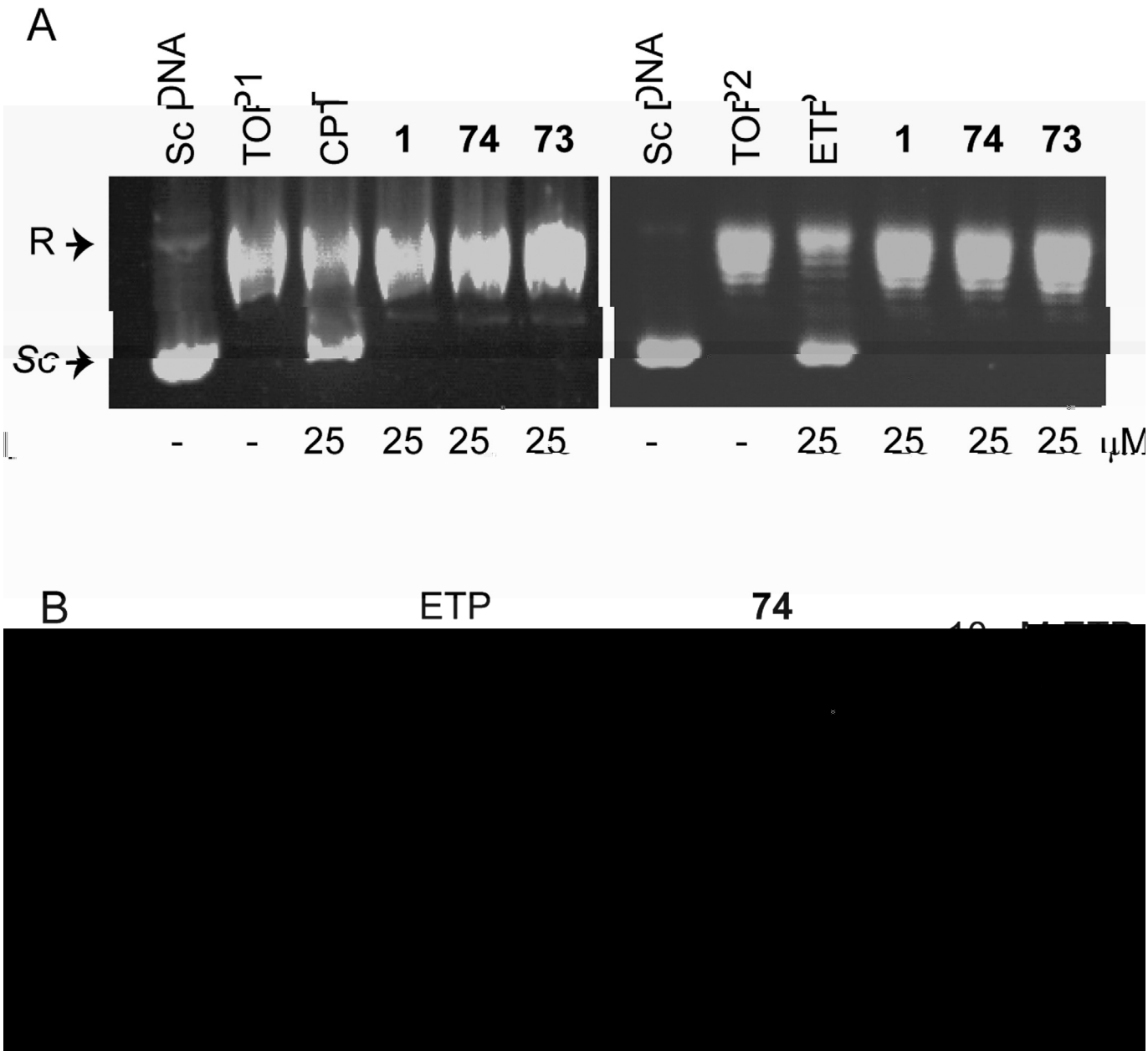


Fig. 5. The inhibition gels compounds against TOP1 and TOP2. (A) TOP1-mediated (left) and TOP2-mediated (right) relaxation assay gels. Lane 1, supercoiled pBR322 DNA alone; lane 2, DNA and enzyme; lanes 3–6, DNA, enzyme and the tested compound at 25 μM concentration, respectively. Camptothecin (CPT) and etoposide (ETP) were used as positive controls for TOP1 and TOP2, respectively. R, relaxed DNA; Sc, supercoiled DNA. (B) Detection of TOP2-DNA covalent cleavage complexes by in vivo complex of enzyme (ICE) assay using CCRF-CEM cells. Lane 1, untreated control; lanes 2 and 3, cells treated with ETP at 10 and 50 μM concentration, respectively; lanes 4 and 5, cells treated with **74** at 10 and 50 μM concentration, respectively; lane 6, cells co-treated with 10 μM ETP and 50 μM **74**.

131.6, 129.5, 128.4, 128.4, 127.5, 123.0, 113.1, 14.1. HRMS (ESI) m/z: 368.0334 $[M + H]^+$, calcd for $C_{19}H_{11}NO_5Cl$ 368.0320.

4.4.27. 2-Cyanophenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g] quinoline-3-carboxylate (***34***)

Light yellow solid, yield 53%, mp = 255.7–256.3 °C. ^1H NMR ($CDCl_3$) δ 9.06 (dd, J = 4.7, 1.7 Hz, 1H), 8.57 (dd, J = 7.9, 1.7 Hz, 1H), 7.92–7.61 (m, 4H), 7.42 (td, J = 7.5, 1.4 Hz, 1H), 2.87 (s, 3H). ^{13}C NMR (DMSO) δ 176.3, 172.3, 166.1, 159.1, 153.9, 151.9, 151.1, 148.8, 135.3, 134.1, 133.8, 128.5, 127.7, 127.4, 123.4, 119.5, 115.2, 111.0, 106.3, 14.2. HRMS (ESI) m/z: 381.0503 $[M + Na]^+$, calcd for $C_{20}H_{10}N_2O_5Na$ 381.0482.

4.4.28. 3-Cyanophenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g] quinoline-3-carboxylate (***35***)

Light yellow solid, yield 62%, mp = 221.0–221.8 °C. ^1H NMR ($CDCl_3$) δ 9.08 (dd, J = 4.7, 1.7 Hz, 1H), 8.57 (dd, J = 7.9, 1.7 Hz, 1H), 7.82 (d, J = 1.5 Hz, 1H), 7.79–7.73 (m, 2H), 7.63–7.53 (m, 2H), 2.84 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.6, 172.2, 166.9, 159.8, 154.6, 150.8, 150.5, 149.0, 134.8, 130.4, 129.9, 128.4, 128.3, 127.5, 126.6, 125.4, 117.8, 113.5, 112.6, 14.1. HRMS (ESI) m/z: 359.0679 $[M + H]^+$, calcd for $C_{20}H_{11}N_2O_5$ 359.0662.

4.4.29. 4-Cyanophenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g] quinoline-3-carboxylate (***36***)

Light yellow solid, yield 53%, mp = 244.5–244.7 °C. ^1H NMR

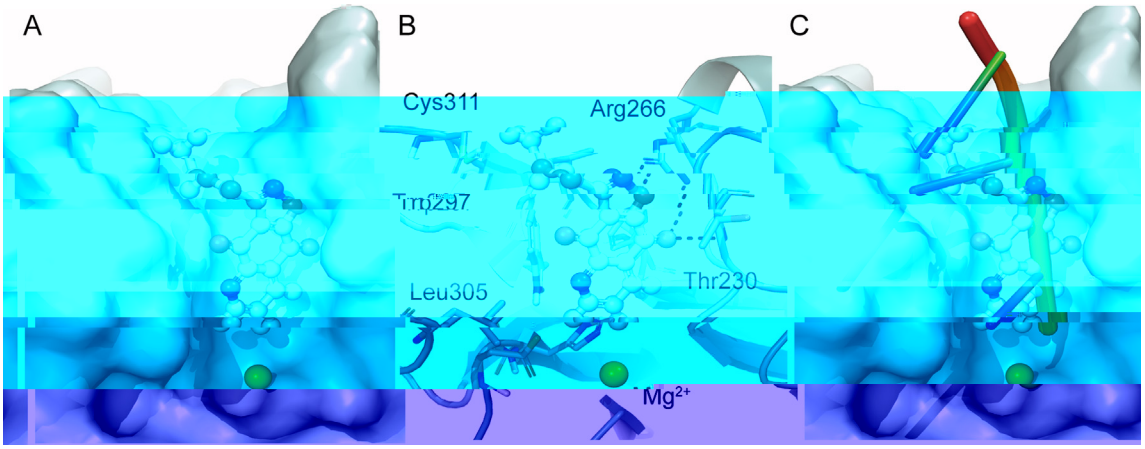


Fig. 6. The hypothetical binding mode of isoxazole derivative **73**. (A) An inhibitor steric fit in hypothetical binding mode of **73** (gray carbon atoms ball and stick riy298.2888007.9702348.661

($CDCl_3$) δ 9.08 (dd, J = 4.7, 1.7 Hz, 1H), 8.57 (dd, J = 7.9, 1.7 Hz, 1H), 7.76 (d, J = 8.8 Hz, 2H), 7.74 (dd, J = 7.8, 4.7 Hz, 1H), 7.63 (d, J = 8.8 Hz, 2H), 2.84 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.6, 172.2, 167.0, 159.6, 154.6, 153.6, 150.9, 149.0, 134.8, 133.7, 128.3, 128.2, 127.6, 122.7, 118.2, 112.6, 110.1, 14.1. HRMS (ESI) m/z: 381.0496 $[M + Na]^+$, calcd for $C_{20}H_{10}N_2O_5Na$ 381.0482.

4.4.30. 4-Bromophenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g] quinoline-3-carboxylate (***37***)

Light yellow solid, yield 69%, mp = 214.3–215.8 °C. ^{1}H NMR ($CDCl_3$) δ 9.07 (dd, J = 4.7, 1.7 Hz, 1H), 8.56 (dd, J = 7.9, 1.7 Hz, 1H), 7.72 (dd, J = 7.9, 4.7 Hz, 1H), 7.56 (d, J = 8.8 Hz, 2H), 7.36 (d, J = 8.8 Hz, 2H), 2.82 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.6, 172.3, 166.5, 160.1, 154.5, 150.8, 149.5, 149.1, 134.7, 132.5, 128.4, 127.4, 123.4, 119.3, 113.1, 14.1. HRMS (ESI) m/z: 411.9820 $[M + H]^+$, calcd for $C_{19}H_{11}NO_5$ Br 411.9815.

4.4.31. 4-(Methylsulfonyl)phenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***38***)

Light yellow solid, yield 57%, mp = 243.8–44.8 °C. ^{1}H NMR ($CDCl_3$) δ 9.10 (dd, J = 4.7, 1.7 Hz, 1H), 8.59 (dd, J = 7.9, 1.7 Hz, 1H), 8.07–8.03 (m, 2H), 7.76 (dd, J = 7.9, 4.7 Hz, 1H), 7.76–7.63 (m, 2H), 3.11 (s, 3H), 2.86 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.7, 172.3, 167.0, 159.8, 154.6, 154.4, 150.9, 149.1, 138.3, 134.8, 129.2, 128.4, 127.6, 122.7, 112.7, 44.7, 14.2. HRMS (ESI) m/z: 434.0334 $[M + Na]^+$, calcd for $C_{20}H_{13}NO_7SNa$ 434.0305.

4.4.32. 4-Nitrophenyl 4,9-dihydro-2-methyl-4,9-dioxofuro[2,3-g] quinoline-3-carboxylate (***39***)

Yellow solid, yield 44%, mp = 191.4–193.3 °C. ^{1}H NMR ($CDCl_3$) δ 9.11 (dd, J = 4.7, 1.7 Hz, 1H), 8.60 (dd, J = 7.9, 1.7 Hz, 1H), 8.39–8.34 (m, 2H), 7.77 (dd, J = 7.9, 4.7 Hz, 1H), 7.74–7.68 (m, 2H), 2.87 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.6, 172.2, 167.2, 159.5, 155.0, 154.6, 150.9, 149.0, 145.6, 134.8, 128.4, 128.2, 127.6, 125.2, 122.5, 112.5, 14.2. HRMS (ESI) m/z: 379.0583 $[M + H]^+$, calcd for $C_{19}H_{11}N_2O_7$ 379.0561.

4.4.33. 2-Naphthalenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g] quinoline-3-carboxylate (***40***)

Yellow solid, yield 78%, mp = 235.0–236.8 °C. ^{1}H NMR ($CDCl_3$) δ 9.08 (d, J = 4.4 Hz, 1H), 8.56 (d, J = 8.0 Hz, 1H), 7.95–7.86 (m, 4H), 7.72 (dd, J = 7.8, 4.8 Hz, 1H), 7.59 (dd, J = 8.8, 2.4 Hz, 1H), 7.54–7.47 (m, 2H), 2.86 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.6, 172.3, 166.3, 160.5, 154.4, 150.7, 149.1, 148.1, 134.7, 133.7, 131.6, 129.4, 128.5, 128.4, 127.8, 127.8, 127.4, 126.6, 125.8, 121.0, 118.6, 113.4, 14.1. HRMS (ESI) m/z: 382.0706 $[M - H]^-$, calcd for $C_{23}H_{12}NO_5$ 382.0721.

4.4.34. Pyridin-3-yl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g] quinoline-3-carboxylate (***41***)

Light yellow solid, yield 62%, mp = 218.2–220.1 °C. ^{1}H NMR ($CDCl_3$) δ 9.08 (dd, J = 4.7, 1.7 Hz, 1H), 8.77 (d, J = 2.5 Hz, 1H), 8.58 (dd, J = 3.6, 1.5 Hz, 1H), 8.56 (d, J = 1.7 Hz, 1H), 7.87 (dd, J = 8.3, 2.7, 1.4 Hz, 1H), 7.73 (dd, J = 7.9, 4.7 Hz, 1H), 7.43 (dd, J = 8.3, 4.8 Hz, 1H), 2.85 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.6, 172.3, 166.9, 160.0, 154.6, 150.9, 149.1, 147.4, 147.3, 143.5, 134.8, 129.2, 128.4, 127.5, 124.0, 112.7, 14.2. HRMS (ESI) m/z: 335.0673 $[M + H]^+$, calcd for $C_{18}H_{11}N_2O_5$ 335.0662.

4.4.35. 3-Acrylamidophenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro [2,3-g]quinoline-3-carboxylate (***42***)

Yellow solid, yield 51%, mp = 202.3–205.1 °C. ^{1}H NMR (DMSO) δ 10.40 (s, 1H), 9.03 (dd, J = 4.7, 1.6 Hz, 1H), 8.49 (dd, J = 7.9, 1.6 Hz, 1H), 7.87 (dd, J = 7.9, 4.7 Hz, 1H), 7.85 (t, J = 2.0 Hz, 1H), 7.59–7.54 (m, 1H), 7.46 (t, J = 8.1 Hz, 1H), 7.11–7.07 (m, 1H), 6.46 (dd, J = 17.0, 10.1 Hz, 1H), 6.29 (dd, J = 17.0, 1.9 Hz, 1H), 5.80 (dd, J = 10.1, 1.9 Hz, 1H), 2.77 (s, 3H). ^{13}C NMR (DMSO) δ 177.1, 172.8, 165.7, 163.8, 160.5, 154.3, 151.2, 150.7, 149.4, 140.7, 134.6, 132.1, 130.2, 129.0, 128.4, 128.2, 127.9, 117.4, 117.1, 113.0, 112.6, 14.3. HRMS (ESI) m/z: 403.0924 $[M + H]^+$, calcd for $C_{22}H_{15}N_2O_6$ 403.0925.

4.4.36. 4-Acrylamidophenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro [2,3-g]quinoline-3-carboxylate (***43***)

Yellow solid, yield 44%, mp = 206.5–208.8 °C. ^{1}H NMR (DMSO) δ 10.28 (s, 1H), 9.03 (dd, J = 4.5, 1.3 Hz, 1H), 8.47 (dd, J = 7.8, 1.3 Hz, 1H), 7.87 (dd, J = 7.8, 4.7 Hz, 1H), 7.79 (d, J = 8.8 Hz, 2H), 7.33 (d, J = 8.9 Hz, 2H), 6.46 (dd, J = 17.0, 10.1 Hz, 1H), 6.29 (dd, J = 17.0, 1.7 Hz, 1H), 5.78 (dd, J = 10.1, 1.7 Hz, 1H), 2.76 (s, 3H). ^{13}C NMR (DMSO) δ 176.5, 172.3, 165.1, 163.1, 160.2, 153.8, 150.7, 148.8, 145.6, 137.0, 134.1, 131.7, 128.5, 127.9, 127.7, 127.0, 121.9, 120.3, 112.2, 13.8. HRMS (ESI) m/z: 403.0919 $[M + H]^+$, calcd for $C_{22}H_{15}N_2O_6$ 403.0925.

4.4.37. 4-(3-Morpholinopropyl)phenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***44***)

Yellow solid, yield 35%, mp > 330 °C. ^{1}H NMR ($CDCl_3$) δ 9.06 (dd, J = 4.7, 1.7 Hz, 1H), 8.56 (dd, J = 7.9, 1.7 Hz, 1H), 7.72 (dd, J = 7.9, 4.7 Hz, 1H), 7.38–7.31 (m, 2H), 6.99–6.91 (m, 2H), 4.05 (t, J = 6.3 Hz,

2H), 3.74 (t, J = 4.6 Hz, 4H), 2.82 (s, 3H), 2.54 (t, J = 6.3 Hz, 2H), 2.50 (t, J = 4.6 Hz, 4H), 1.95 (t, J = 6.3 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 176.6, 172.3, 166.1, 160.8, 157.0, 154.5, 150.7, 149.1, 143.9, 134.7, 128.6, 128.4, 127.4, 122.4, 115.05, 113.5, 67.0, 66.5, 55.5, 53.7, 26.4, 14.1. HRMS (ESI) m/z: 477.1650 $[M + H]^+$, calcd for $C_{26}H_{24}N_2O_7$ 477.1656.

4.4.38. 4-(4-Acetylpiperazin-1-yl)phenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***45***)

Brown solid, yield 61%, mp = 209.1–210.0 °C. ^{1}H NMR ($CDCl_3$) δ 9.06 (d, J = 4.6 Hz, 1H), 8.56 (d, J = 7.8 Hz, 1H), 7.72 (dd, J = 7.8, 4.7 Hz, 1H), 7.35 (d, J = 8.8 Hz, 2H), 6.98 (d, J = 8.8 Hz, 2H), 3.81–3.74 (m, 2H), 3.67–3.62 (m, 2H), 3.21–3.18 (m, 2H), 3.17–3.14 (m, 2H), 2.82 (s, 3H), 2.16 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.6, 172.4, 169.0, 166.2, 160.8, 154.5, 150.7, 149.2, 149.1, 144.0, 134.8, 128.6, 128.4, 127.4, 122.2, 117.5, 113.5, 50.1, 49.8, 46.2, 41.3, 21.4, 14.2. HRMS (ESI) m/z: 460.1503 $[M + H]^+$, calcd for $C_{25}H_{22}N_3O_6$ 460.1503.

4.4.39. 4-Benzoylphenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***46***)

Yellow solid, yield 71%, mp = 187.8–190.2 °C. ^{1}H NMR ($CDCl_3$) δ 9.07 (d, J = 1.8 Hz, 1H), 8.56 (d, J = 7.7 Hz, 1H), 7.92 (d, J = 7.8 Hz, 2H), 7.82 (d, J = 7.4 Hz, 2H), 7.76–7.70 (m, 1H), 7.60 (m, 3H), 7.51 (t, J = 7.3 Hz, 2H), 2.85 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 195.6, 176.6, 172.3, 166.8, 160.0, 154.6, 153.5, 150.8, 149.0, 137.4, 135.4, 134.8, 132.6, 131.7, 130.0, 128.39, 128.36, 127.5, 121.6, 113.0, 14.2. HRMS (ESI) m/z: 438.0970 $[M + H]^+$, calcd for $C_{26}H_{16}NO_6$ 438.0972.

4.4.40. (S)-4-(2-Acetamido-3-methoxy-3-oxopropyl)phenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***47***)

Yellow solid, yield 81%, mp = 201.4–202.2 °C. ^{1}H NMR ($CDCl_3$) δ 9.04 (d, J = 4.7 Hz, 1H), 8.56 (d, J = 7.

4.4.50. N-3-Chlorophenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[3,2-g]quinoline-3-carboxamide (***68***)

Yellow solid, yield 83%, mp = 262.0–265.2 °C. ^{1}H NMR (DMSO) δ 11.13 (s, 1H), 9.07 (d, J = 3.9 Hz, 1H), 8.55 (d, J = 7.8 Hz, 1H), 7.93 (s, 1H), 7.90 (dd, J = 7.8, 4.8 Hz, 1H), 7.57 (d, J = 8.0 Hz, 1H), 7.44 (t, J = 8.0 Hz, 1H), 7.22 (d, J = 8.0 Hz, 1H), 2.76 (s, 3H). ^{13}C NMR (DMSO) δ 181.5, 171.4, 164.7, 159.7, 154.8, 151.6, 148.0, 140.2, 135.5, 133.8, 131.3, 130.6, 128.4, 126.4, 124.3, 119.4, 118.4, 116.0, 14.4. HRMS (ESI) m/z: 367.0494 $[M + H]^+$, calcd for $C_{19}H_{12}N_2O_4Cl$ 367.0480.

4.4.51. 2-Hydroxyphenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[3,2-g]quinoline-3-carboxylate (***69***)

Yellow solid, yield 48%, mp = 266.7–268.3 °C. ^{1}H NMR ($CDCl_3$) δ 9.10 (dd, J = 4.7, 1.7 Hz, 1H), 8.61 (dd, J = 7.9, 1.7 Hz, 1H), 8.52 (s, 1H), 7.76 (dd, J = 7.9, 4.7 Hz, 1H), 7.38 (dd, J = 8.1, 1.4 Hz, 1H), 7.23–7.15 (m, 1H), 7.12 (dd, J = 8.2, 1.6 Hz, 1H), 6.96–6.85 (m, 1H), 2.87 (s, 3H). ^{13}C NMR (DMSO) δ 178.1, 171.7, 165.5, 159.7, 154.3, 152.6, 149.4, 148.0, 138.5, 135.3, 131.0, 128.4, 127.6, 127.3, 123.4, 119.7, 117.4, 112.5, 14.5. HRMS (ESI) m/z: 350.0657 $[M + H]^+$, calcd for $C_{19}H_{12}NO_6$ 350.0659.

4.5. Synthesis of compounds ***56*** and ***57***

The reaction solution of compound **50** (40 mg, 0.1 mmol) and pyridines (0.5 mmol) in THF (20 mL) was stirred and heated under reflux for 8 h and cooled to room temperature. The reaction solution was added with ether (40 mL), and yellow precipitate appeared. The resulting precipitate was filtrated and purified by silica gel column chromatography to give target compound.

4.5.1. 1-(3-((2-Methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carbonyl)oxy)propyl)pyridin-1-ium bromide (***56***)

Yellow solid, yield 57%, mp > 332 °C. ^{1}H NMR (CD_3OD) δ 9.20 (dd, J = 5.8, 6.1 Hz, 2H), 9.02 (dd, J = 4.7, 1.6 Hz, 1H), 8.74–8.53 (m, 2H), 8.29–8.08 (m, 2H), 7.89 (dd, J = 7.9, 4.8 Hz, 1H), 5.08 (t, J = 7.4 Hz, 2H), 4.46 (t, J = 5.6 Hz, 2H), 2.74 (s, 3H), 2.64–2.48 (m, 2H). ^{13}C NMR (CD_3OD) δ 178.9, 173.3, 167.8, 163.4, 154.9, 152.6, 150.1, 147.1, 146.5, 146.5, 136.2, 130.1, 129.7, 129.7, 129.4, 128.9, 113.7, 62.4, 60.3, 31.5, 14.0. HRMS (ESI) m/z: 377.1149 $[M - Br]^+$, calcd for $C_{21}H_{17}N_2O_5Br$ 377.1132.

4.5.2. 4-(Dimethylamino)-1-(3-((2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carbonyl)oxy)propyl)pyridin-1-ium bromide (***57***)

Yellow solid, yield 69%, mp = 136.9–138.7 °C. ^{1}H NMR (CD_3OD) δ 9.01 (dd, J = 4.7, 1.5 Hz, 1H), 8.61 (dd, J = 7.9, 1.5 Hz, 1H), 8.35 (d, J = 7.7 Hz, 2H), 7.89 (dd, J = 7.9, 4.8 Hz, 1H), 7.01 (d, J = 7.7 Hz, 2H), 4.57 (t, J = 7.0 Hz, 2H), 4.40 (t, J = 5.6 Hz, 2H), 3.21 (s, 6H), 2.74 (s, 3H), 2.45–2.31 (m, 2H). ^{13}C NMR (CD_3OD) δ 178.6, 173.3, 167.7, 163.4, 158.0, 154.9, 152.5, 150.1, 143.5, 136.2, 130.0, 129.4, 128.9, 113.8, 109.1, 62.7, 56.0, 40.3, 30.9, 14.0. HRMS(ESI) m/z: 420.1555 $[M - Br]^+$, calcd for $C_{23}H_{22}N_3O_5Br$ 420.1554.

4.6. Deprotection of Boc protective group of compounds ***51–53***

To a solution of Boc-protected compound (90 mg, 0.2 mmol) in dichloromethane (6 mL), trifluoroacetic acid (1.3 mL) was added. The reaction solution was stirred for 1 h at room temperature. The solvent was evaporated under reduced pressure. The resulting solid was washed with ether to give the target product **59** and **60**. The compound **58** was obtained through the purification by silica gel column chromatography.

4.6.1. 3-(Benzo[d]oxazol-2-yl)-2-methylfuro[2,3-g]quinoline-4,9-dione (***58***)

Yellow solid, yield 84%, mp = 266.0 °C (decomposing). ^{1}H NMR ($CDCl_3$) δ 9.10 (dd, J = 4.7, 1.7 Hz, 1H), 8.60 (dd, J = 7.9, 1.7 Hz, 1H), 7.87–7.83 (m, 1H), 7.74 (dd, J = 7.9, 4.7 Hz, 1H), 7.69 (dd, J = 6.2, 2.5 Hz, 1H), 7.48–7.40 (m, 2H), 2.96 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.8, 172.2, 163.8, 155.7, 154.4, 151.0, 150.7, 149.1, 141.5, 134.7, 128.6, 127.4, 125.8, 124.8, 120.2, 111.1, 109.9, 14.3. HRMS (ESI) m/z: 331.0710 $[M + H]^+$, calcd for $C_{19}H_{11}N_2O_4$ 331.0713.

4.6.2. 3-Aminophenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxamide (***59***)

Brown solid, yield 84%, mp > 300.0 °C. ^{1}H NMR (CD_3OD) δ 8.95 (d, J = 3.7 Hz, 1H), 8.57 (d, J = 7.7 Hz, 1H), 7.85 (dd, J = 7.8, 4.8 Hz, 1H), 7.54 (t, J = 8.1 Hz, 1H), 7.36–7.30 (m, 2H), 7.22 (d, J = 7.8 Hz, 1H), 2.76 (s, 3H). ^{13}C NMR (CD_3OD) δ 176.7, 171.8, 166.5, 160.1, 153.4, 151.4, 151.2, 148.6, 135.6, 134.7, 130.6, 128.6, 127.9, 127.7, 119.6, 118.8, 114.8, 112.1, 12.7. HRMS (ESI) m/z: 349.0813 $[M + H]^+$, calcd for $C_{19}H_{13}N_2O_5$ 349.0819.

4.6.3. 4-Aminophenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxamide (***60***)

Red solid, yield 62%, mp = 170.5 °C (decomposing). ^{1}H NMR (DMSO) δ 9.03 (d, J = 3.7 Hz, 1H), 8.49 (d, J = 7.3 Hz, 1H), 7.88 (dd, J = 7.7, 4.7 Hz, 1H), 7.23 (d, J = 8.6 Hz, 2H), 7.02 (d, J = 8.5 Hz, 2H), 2.76 (s, 3H). ^{13}C NMR (CD_3OD) δ 176.8, 171.8, 166.6, 160.2, 153.4, 151.2, 150.0, 148.6, 134.8, 130.0, 128.6, 127.9, 127.7, 123.4, 123.2, 112.1, 12.7. HRMS (ESI) m/z: 349.0807 $[M + H]^+$, calcd for $C_{19}H_{13}N_2O_5$ 349.0819.

4.7. General procedures for the synthesis of compounds ***61–64***

According to the reported "click chemistry" method with slight modification [33], A solution of 3-bromopropanol or 3-bromopropanic acid (1 mmol) and sodium azide (195 mg, 2 mmol) in acetonitrile (20 mL) was stirred and heated under reflux for 8 h, and cooled to room temperature. The solvent was evaporated under reduced pressure to give white solid. The resulting white solid was dissolved in water (5 mL) and was added with DMF (5 mL) and acetylene analogue (**54** or **55**, 0.5 mmol). The mixture solution was added with sodium ascorbate (20 mg, 0.1 mmol) and $CuSO_4/5H_2O$ (12 mg, 0.05 mmol). The reaction solution was stirred and heated at 75 °C for 3 h. The resulting suspension was diluted with water (50 mL). The aqueous solution was extracted with dichloromethane (20 mL x 2). The combined organic layer was washed with water (10 mL x 2) and saturated aqueous saline (10 mL) and dried with anhydride $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give the target compound.

4.7.1. 2-(1-(3-Hydroxypropyl)-1H-1,2,3-triazol-4-yl)ethyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***61***)

Brown solid, yield 24%, mp = 172.7–173.5 °C. ^{1}H NMR ($CDCl_3$) δ 9.02 (d, J = 4.5 Hz, 1H), 8.57 (dd, J = 7.9, 1.6 Hz, 1H), 7.98 (s, 1H), 7.75 (dd, J = 7.9, 4.7 Hz, 1H), 4.70 (t, J = 5.6 Hz, 2H), 4.58 (t, J = 5.6 Hz, 2H), 3.68 (t, J = 5.8 Hz, 2H), 3.27 (t, J = 5.6 Hz, 2H), 2.76 (s, 3H), 2.15 (quint, J = 5.9 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 176.5, 172.0, 166.5, 162.3, 154.0, 150.6, 148.7, 144.3, 135.2, 128.4, 128.1, 127.7,

4.7.2. 3-(4-(2-((2-Methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g] quinoline-3-carbonyl)oxy)ethyl)-1H-1,2,3-triazol-1-yl)propanoic acid (***62***)

Brown solid, yield 19%, mp = 216.6–217.4 °C. ^{1}H NMR ($CDCl_3$) δ 8.99 (dd, J = 4.8, 1.4 Hz, 1H), 8.65 (dd, J = 7.9, 1.5 Hz, 1H), 8.19 (s, 1H), 7.83 (dd, J = 7.9, 4.8 Hz, 1H), 4.78 (t, J = 5.6, 2H), 4.70 (t, J = 5.0, 2H), 3.24 (t, J = 5.0, 2H), 2.92 (t, J = 5.6, 2H), 2.79 (s, 3H). ^{13}C NMR (CD_3OD) δ 178.1, 173.3, 166.9, 163.2, 154.8, 152.4, 150.1, 145.3, 136.0, 130.0, 129.3, 129.2, 124.9, 114.2, 65.4, 47.3, 35.9, 26.1, 14.1. HRMS (ESI) m/z: 423.0923 $[M-H]^-$, calcd for $C_{20}H_{15}N_4O_7$ 423.0946.

4.7.3. 3-(1-(3-Hydroxypropyl)-1H-1,2,3-triazol-4-yl)phenyl 2-methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g]quinoline-3-carboxylate (***63***)

Light yellow solid, yield 55%, mp = 181.6–182.4 °C. ^{1}H NMR ($CDCl_3$) δ 9.08 (dd, J = 4.7, 1.7 Hz, 1H), 8.58 (dd, J = 7.9, 1.7 Hz, 1H), 7.93 (s, 1H), 7.85 (d, J = 1.8 Hz, 1H), 7.82 (s, 1H), 7.74 (dd, J = 7.9, 4.7 Hz, 1H), 7.52 (t, J = 7.9 Hz, 1H), 7.44–7.37 (m, 1H), 4.61 (t, J = 6.7 Hz, 2H), 3.72 (t, J = 5.8 Hz, 2H), 2.85 (s, 3H), 2.27–2.16 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 175.6, 171.3, 165.3, 159.3, 153.5, 149.7, 149.7, 148.0, 145.8, 133.8, 131.2, 128.9, 127.5, 127.4, 126.5, 122.4, 120.3, 119.7, 117.8, 112.3, 57.7, 45.9, 31.5, 13.2. HRMS (ESI) m/z: 457.1138 $[M-H]^-$, calcd for $C_{24}H_{17}N_4O_6$ 457.1154.

4.7.4. 3-(4-(3-((2-Methyl-4,9-dioxo-4,9-dihydrofuro[2,3-g] quinoline-3-carbonyl)oxy)phenyl)-1H-1,2,3-triazol-1-yl)propanoic acid (***64***)

Yellow solid, yield 80%, mp = 218.6–220.6 °C. ^{1}H NMR (DMSO) δ 12.54 (s, 1H), 9.04 (dd, J = 4.6, 1.6 Hz, 1H), 8.68 (s, 1H), 8.50 (dd, J = 7.9, 1.6 Hz, 1H), 7.89 (dd, J = 7.9, 4.7 Hz, 1H), 7.85 (d, J = 1.9 Hz, 1H), 7.82 (d, J = 7.8 Hz, 1H), 7.60 (t, J = 7.9 Hz, 1H), 7.35 (dd, J = 8.1, 1.4 Hz, 1H), 4.63 (t, J = 6.7 Hz, 2H), 2.97 (t, J = 6.7 Hz, 2H), 2.81 (s, 3H). ^{13}C NMR (DMSO) δ 176.6, 172.3, 171.7, 165.3, 160.0, 153.8, 150.8, 150.6, 148.8, 145.2, 134.1, 132.4, 130.3, 128.5, 127.9, 127.7, 122.9, 122.2, 121.0, 118.2, 112.1, 45.6, 33.9, 13.9. HRMS (ESI) m/z: 471.0920 $[M-H]^-$, calcd for $C_{24}H_{15}N_4O_7$ 471.0946.

4.8. Synthesis of sodium salt ***65***

To a solution of acid **64** (0.2 mmol) in ethanol (10 mL), a solution of NaOH in ethanol (20%, 0.2 mmol) was added dropwise at room temperature. The reaction solution was stirred at room temperature for 30 min. The resulting precipitate was filtered, washed with ethanol and dried to give the yellow solid **66** (85%). HRMS (ESI) m/z: 495.0938 $[M+H]^+$, calcd for $C_{24}H_{16}N_4{}_7Na$ 495.0911.

4.9. Synthesis of ethyl 2-methyl-4,9-dioxo-4,9-dihydro-1H-pyrrolo [3,2-g]quinoline-3-carboxylate (***70***)

The reaction solution of 7-bromoquinoline-5,8-dione (0.48 g, 2 mmol), $Mn(OAc)_3$ (0.80 g, 3 mmol) and ethyl 3-aminocrotonate (0.31 g, 2.4 mmol) in acetonitrile (40 mL) was stirred and heated under reflux for 3 h and cooled to room temperature. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give a yellow solid (**70**, 57 mg), yield 10%, mp = 215.3–217.6 °C. ^{1}H NMR ($CDCl_3$) δ 11.41 (s, 1H), 8.91 (d, J = 3.7 Hz, 1H), 8.51 (d, J = 7.6 Hz, 1H), 7.63 (dd, J = 7.7, 4.7 Hz, 1H), 4.43 (q, J = 7.2 Hz, 2H), 2.64 (s, 3H), 1.44 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 177.8, 173.9, 163.8, 153.2, 148.2, 143.7, 135.5, 132.3, 131.6, 127.4, 125.5, 113.8, 61.0, 14.3, 13.6. HRMS (ESI) m/z: 285.0870 $[M+H]^+$, calcd for $C_{15}H_{13}N_2O_4$ 285.0870. The structure of compound **70** was confirmed with 2D NMR spectra.

4.10. Synthesis of ethyl 1,2-dimethyl-4,9-dioxo-4,9-dihydro-1H-pyrrolo[3,2-g]quinoline-3-carboxylate (***72***)

The solution of 6,7-dichloroquinoline-5,8-dinoe (230 mg, 1 mmol), sodium acetate (160 mg, 2 mmol) and ethyl acetoacetate (0.13 mL, 1.1 mmol) in THF (10 mL) was stirred and heated under reflux for 3 h and cooled to room temperature. The solvent was evaporated under reduced pressure. The resulting residue was dissolved in dichloromethane (100 mL). The organic solution was washed with water (20 mL x 2), saturated aqueous saline (20 mL) and concentrated under reduced pressure to give yellow oil. The crude yellow oil was dissolved in ethanol (10 mL), and added with methylamine aqueous solution (40%, 0.5 mL, 4 mmol). The reaction solution was stirred and heated under reflux for 5 h and cooled to room temperature. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give a yellow solid (**72**, 27 mg), yield 9%, mp = 209.3–210.8 °C. ^{1}H NMR ($CDCl_3$) δ 8.97 (dd, J = 4.6, 1.6 Hz, 1H), 8.48 (dd, J = 7.8, 1.6 Hz, 1H), 7.62 (dd, J = 7.8, 4.7 Hz, 1H), 4.44 (q, J = 7.1 Hz, 2H), 4.10 (s, 3H), 2.52 (s, 3H), 1.44 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 197.6, 177.9, 174.3, 164.1, 153.5, 148.7, 143.1, 134.8, 130.9, 130.5, 127.0, 113.9, 61.2, 33.2, 14.1, 11.0. HRMS (ESI) m/z: 229.1017 $[M+H]^+$, calcd for C16H15N2O4 229.1026.

4.11. The synthesis of isoxazole analogues ***73*** and ***74***

Procedure A. Following "General Procedures for the synthesis of furoquinolinediones", the reaction of 6,7-dichloroquinoline-5,8-dione with ethyl nitroacetate gave the target products **73** (5%) and **74** (2%), respectively.

Procedure B. The isoxazole analogues were synthesized according Chuang method [38]. Briefly, a solution of quinoline-5,8-dione (1 mmol), ethyl nitroacetate (540 mg, 4 mmol) and manganese (III) acetate (1.61 g, 6 mmol) in acetic acid (15 mL) was stirred and heated at 70 °C overnight, followed by the addition of ethyl nitroacetate (540 mg, 4 mmol) and manganese (III) acetate (1.61 g, 6 mmol). The reaction solution was stirred and heated at 70 °C overnight again. The reaction mixture was diluted with dichloromethane (100 mL) and washed with saturated aqueous sodium bisulfite (50 mL), water (50 mL x 3) and aqueous saturated sodium bicarbonate (50 mL x 3). The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give the target compound **73** (11%) and **74** (8%), respectively.

4.11.1. Ethyl 4,9-dioxo-4,9-dihydroisoxazolo[5,4-g]quinoline-3-carboxylate (***73***)

Yellow solid, mp = 122.7–124.3 °C. ^{1}H NMR ($CDCl_3$ δ 9.14 (s, 1H), 8.62 (d, J = 7.9 Hz, 1H), 7.86–7.81 (m, 1H), 4.59 (q, J = 6.9 Hz, 2H), 1.50 (t, J = 6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 175.1, 170.6, 165.8, 157.6, 155.2, 153.4, 147.5, 135.9, 130.7, 128.6, 120.0, 63.6, 14.0. HRMS (ESI) m/z: 273.0494 $[M+H]^+$, calcd for $C_{13}H_9N_2O_5$ 273.0506. The structure of compound **73** was confirmed with 2D NMR spectra.

4.11.2. Ethyl 4,9-dioxo-4,9-dihydroisoxazolo[4,5-g]quinoline-3-carboxylate (***74***)

Yellow solid, yield 8%, mp = 119.5–120.6 °C. ^{1}H NMR ($CDCl_3$) δ 9.17 (dd, J = 4.7, 1.7 Hz, 1H), 8.64 (dd, J = 7.9, 1.7 Hz, 1H), 7.82 (dd, J = 7.9, 4.7 Hz, 1H), 4.59 (q, J = 7.1 Hz, 2H), 1.51 (t, J = 7.1 Hz, 3H).13C NMR ($CDCl_3$) δ 174.3, 171.6, 165.1, 157.6, 155.8, 153.8, 148.9, 135.6, 128.8, 128.0, 120.4, 63.7, 14.0. HRMS (ESI) m/z: 273.0496 $[M+H]^+$, calcd for $C_{13}H_9N_2O_5$ 273.0506. The structure of compound **74** was confirmed with 2D NMR spectra.

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