Supporting Information for

# Synthesis and Characterization of Potent RIPK3 Inhibitors Based on a Tricyclic Scaffold

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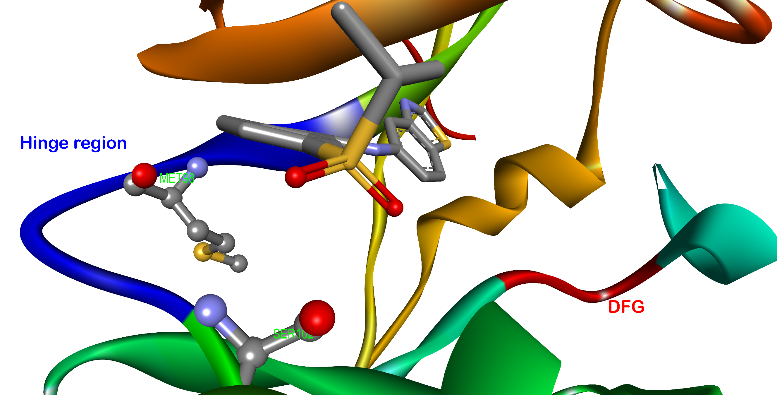
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1. Molecular docking study

The parent compound, GSK’872 was employed in Glide docking of Schrödinger 9.0 software package[1] based on RIPK3 crystal complex (PDB ID: 4M69[2]) from RCSB Protein Data Bank[3]. Detailed information of Glide docking pipeline can be seen in our previous studies.[4,5] The binding pose of GSK’872 from Glide docking and the interaction patterns between GSK’872 and RIPK3 were shown in Figure S1. The predicated results demonstrated that GSK’872 was a typical type Ⅰ inhibitor, targeting the active DFG-in conformation of RIPK3 (Figure S1a). Besides, GSK’872 can form favorable interactions (hydrogen bond) with two key residues of hinge region including Met98 and Ser102 located in the ATP binding site of RIPK3 (Figure S1b). Furthermore, we also observed that the tail group (6-(isopropylsulfonyl)quinoline) of GSK’872 was solvent-exposed and in close range of residues including Val28, Gly29, Lys30, Ala104, Ser147, Asn148 and Leu150 in the binding pocket of RIPK3. Based on these findings, we hypothesized that the structural modifications on the quinoline group of GSK’872 for engagement of favorable interaction patterns with these nearby residues may improve the inhibitory activity of rational-designed compounds.



(a)



(b)

Fig. S1. (a). The predicted binding pose of GSK’872 from Glide docking based on 4M69 as RIPK3 docking template, the DFG motif (Asp161-Phe162-Gly163) are colored in red and the residues of hinge region are colored in blue; (b). Key interaction patterns between GSK’872 and favorable residues in the binding pocket of RIPK3.

2. Chemistry

**N-(4-mercaptophenyl)acetamide (8).** To a solution of **7** (25.0 g, 200 mmol) in AcOH (120 mL) was added Ac2O (22.4 g, 220 mmol). The reaction was stirred at room temperature for 10 min. The reaction was poured into water (1.0 L) and the resulting solid was collected via filtration, washed with water and dried in vacuum to give the desired product **8** (31.0 g, 93 %) as a white solid. 1H NMR (400 MHz, DMSO*-d6*) δ 9.90 (s, 1H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 5.23 (s, 1H), 2.01 (s, 3H).

**N-(4-((2-chloroallyl)thio)phenyl)acetamide (9).** To a solution of **8** (31.0 g, 186 mmol) and K2CO3 (51.3 g, 372 mmol) in acetone (500 mL) was added 2,3-dichloroprop-1-ene (22.6 g, 205 mmol). The reaction was stirred at room temperature overnight. The precipitation was filtered off and the filtrate was concentrated. The residue was purified by silica gel column chromatography (DCM/ MeOH = 50/1) to give the desired product **9** (42 g, 94%) as a white solid. 1H NMR (400 MHz, DMSO*-d6*) δ 10.00 (s, 1H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 8.8 Hz, 2H), 5.32 (s, 1H), 5.21 (s, 1H), 3.83 (s, 2H), 2.03 (s, 3H). LCMS (ESI/APCI) m/z: 239.8 [M - H]-.

**8-hydroxy-2-methylthieno[2,3-g]quinoline 1,1-dioxide (10).** Intermediate **9** (20.0 g, 8.3 mmol) was added to *N*, *N*-diethylaniline (150 mL). The mixture was stirred at 220℃for 26 h under N2 atmosphere. The reaction was acidified with 6 N HCl. EA (200 mL) was added to dilute the solution and stirred for 30 min. The aqueous layer was extracted with EA (200 mL \* 2). The combined organic phase was washed with 6 N HCl (150 mL), dried over Na2SO4, filtered, and concentrated. The residue was purified by silica gel column chromatography (PE/EA=3/1) to give a crude product. The crude was rinsed with EtOH/H2O (100 mL/200 mL) to give the desired product **10** (5.0 g, 29%) as a yellow solid. 1H NMR (400 MHz, DMSO*-d6*) δ6 7.96 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.36 (s, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 6.91 (s, 1H), 2.56 (s, 3H), 2.19 (s, 3H). LC-MS (ESI/APCI) m/z: 205.9 [M + H] +.

**N-(2-methyl-1,1-dioxidobenzo[b]thiophen-5-yl)acetamide (11).** To a solution of **10** (5.0 g, 24.4 mmol) in MeOH/H2O (75 mL/ 75 mL) was added Oxone (22.5 g, 36.6 mmol). The mixture was stirred at room temperature overnight. The reaction was quenched with saturated Na2SO3 aqueous solution and extracted with DCM (100 mL \* 3). The combined organic phase was dried over Na2SO4, filtered and concentrated. The residue was rinsed with EA (70 mL) to give the desired product **11** (4.3 g, 76 %) as a white solid. 1H NMR (400 MHz, DMSO-*d6*) δ 10.40 (s, 1H), 7.82 (s, 1H), 7.76 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.24 (s, 1H), 2.09 (s, 6H). LC-MS (ESI/APCI) m/z: 237.8 [M + H] +.

**5-amino-2-methylbenzo[b]thiophene 1,1-dioxide (12).** To a solution of **11** (2.8 g, 11.8 mmol) in EtOH (50 mL) was added conc. HCl (10 mL). The reaction was stirred at 85℃ for 5 h. Saturated NaHCO3 aqueous solution was added with an ice bath to neutralize the conc. HCl. The aqueous was extracted with DCM (100 mL \* 2). The combined organic phase was dried over Na2SO4, filtered and concentrated to give the desired product **12** (2.3 g, 96%) as a white solid. 1H NMR (400 MHz, DMSO*-d6*) δ 7.39 (s, 1H), 7.01 (s, 1H), 6.54 (s, 2H), 6.15 (s, 2H), 2.04 (s, 3H). LC-MS (ESI/APCI) m/z: 195.8 [M + H] +.

**2,2-dimethyl-5-(((2-methyl-1,1-dioxidobenzo[b]thiophen-5-yl)amino)methylene)-1,3-dioxane-4,6-dione (13).** To a solution of **12** (2.3 g, 11.3 mmol) in EtOH (20 mL) was added 5-(methoxymethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (2.5 g, 13.6 mmol). The mixture was stirred at room temperature for 30 min. The resulting solid was collected via filtration, washed with EtOH (15 mL) and dried in vacuum to give the desired product **13** (3.8 g, 96%) as a yellowish solid. 1H NMR (400 MHz, DMSO*-d6*) δ 11.33 (s, 1H), 8.65 (s, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.79 (s, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.22 (s, 1H), 2.15 (s, 3H), 1.69 (s, 6H). LC-MS (ESI/APCI) m/z: 347.6 [M - H]-.

**8-hydroxy-2-methylthieno[2,3-g]quinoline 1,1-dioxide (14).** The diphenyl ether (90 mL) was added to a round-bottomed flask and the solvent was heated to 220 ℃ for 10 min. Intermediate **13** (3.7 g, 10.6 mmol) was added slowly to the solution. The mixture was stirred for 40 min. After cooling to room temperature, the resulting suspension was filtered, washed with ether (10 mL) and dried in vacuum to give the desired product **14** (1.6 g, 61%) as a grey solid. 1H NMR (400 MHz, CDCl3) δ 12.20 (s, 1H), 8.29 (s, 1H), 7.98 (s, 1H), 7.59 (s, 1H), 7.41 (s, 1H), 6.16 (s, 1H), 2.17 (s, 3H). LC-MS (ESI/APCI) m/z: 247.9 [M + H] +.

**8-chloro-2-methylthieno[2,3-g]quinoline 1,1-dioxide (15).** Intermediate **14** (1.6 g, 6.5 mmol) was added to POCl3 (30 mL) and the mixture was stirred at 110 ℃ for 2 h to afford a light brown solution. After cooling to room temperature, the excess POCl3 was removed in vacuum. The residue was dissolved in EA (25 mL) and neutralized with saturated NaHCO3 aqueous solution. The aqueous layer was extracted with EA (200 mL \* 2). The combined organic phase was dried over Na2SO4, filtered and concentrated. The residue was purified by silica gel column chromatography (PE/ EA= 1/1) to give the desired product **15** (1.3 g, 75%) as a yellow solid. 1H NMR (400 MHz, DMSO-*d6*) δ 8.98 (d, *J* = 4.8 Hz, 1H), 8.65 (s, 1H), 8.19 (s, 1H), 7.91 (d, *J* = 4.8 Hz, 1H), 7.53 (s, 1H), 2.24 (d, *J* = 1.2 Hz, 3H). LC-MS (ESI/APCI) m/z: 265.8 [M + H] +.

**General procedure for the synthesis of 16-20, 28-29.** To a solution of **15** (1 eq) in EtOH (1 mmol/ 4 mL) was added multiple aromatic amines (1.1 eq) and conc. HCl (0.1 eq). The mixture was stirred at reflux for 2 h. After cooling to room temperature. The resulting precipitation was collected via filtration, washed with EtOH (1 mmol/ 4 mL) and dried in vacuum to give the desired product **16**-**20**, **28**-**29**.

**N-(4-methyl-3-((2-methyl-1,1-dioxidothieno[2,3-g]quinolin-8-yl)amino)phenyl)acetamide (20).** Compound **20** was obtained as a white solid (65 mg, 49%). 1H NMR (400 MHz, DMSO*-d6*) δ 10.00 (s, 1H), 9.09 (s, 1H), 9.03 (s, 1H), 8.43 (d, *J* = 5.2 Hz, 1H), 7.89 (s, 1H), 7.64 (s, 1H), 7.44 (s, 1H), 7.40 (d, *J* = 8.0 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 6.22 (d, *J* = 5.2 Hz, 1H), 2.21 (s, 3H), 2.11 (s, 3H), 2.03 (s, 3H). LC-MS (ESI/APCI) m/z: 393.8 [M + H] +. Purity: 95.9%.

**8-amino-2-methylthieno[2,3-g]quinoline 1,1-dioxide (22).** To a solution of **15** (600 mg, 2.2 mmol) in NMP (20 mL) was added 4-methoxyaniline (466 mg, 3.4 mmol) and DIPEA (952 mg, 6.6 mmol). The mixture was stirred at 120 ℃ under microwave exposure for 2 h. The mixture was diluted with EA (40 mL) and the solvent was washed with saturated NaCl aqueous solution (20 mL \* 3). The organic phase was dried over Na2SO4, filtered and concentrated. The residue was dissolved in TFA (10 mL) and the mixture was stirred for at reflux 1 h. The solvent was removed in vacuum and saturated NaHCO3 aqueous solution was added to neutralize the remaining acid. Ether (20 mL) was added and the resulting solid was collected via filtration, washed with ether and dried in vacuum to give the desired product **22** (220 mg, 41 %) as a yellow solid. 1H NMR (400 MHz, DMSO-d6) δ 8.77 (s, 1H), 8.36 (d, J = 5.6 Hz, 1H), 7.76 (s, 1H), 7.38 (s, 1H), 7.17 (s, 2H), 6.62 (d, J = 5.2 Hz, 1H), 2.18 (s, 3H). LC-MS (ESI/APCI) m/z: 247.0 [M + H] +.

**General procedure for the synthesis of 23, 25, 27.** To a solution of **22** (1 eq) in 1,4-dioxane (1 mmol/ 8 mL) was added various aryl bromide (1.1 eq), XantPhos (0.05 eq), Pd2(dba)3 (0.05 eq) and Cs2CO3 (2 eq). The mixture was stirred at 100 °C overnight. The reaction was concentrated and purified by silica gel column chromatography (DCM/ MeOH = 20/1) to give the desired product **23**, **25**, **27**.

**N-(4-((2-methyl-1,1-dioxidothieno[2,3-g]quinolin-8-yl)amino)pyridin-2-yl)acetamide (23).** Compound **23** was obtained as a white solid (60 mg, 39%). 1H NMR (400 MHz, DMSO*-d6*) δ 10.45 (s, 1H), 9.68 (s, 1H), 8.91 (s, 1H), 8.73 (d, *J* = 4.4 Hz, 1H), 8.17 (d, *J* = 4.4 Hz, 1H), 8.14 (s, 1H), 7.99 (s, 1H), 7.46 (s, 2H), 7.05 (d, *J* = 4.4 Hz, 1H), 2.21 (s, 3H), 2.10 (s, 3H). LC-MS (ESI/APCI) m/z: 380.7 [M + H] +.

**N-(5-((2-methyl-1,1-dioxidothieno[2,3-g]quinolin-8-yl)amino)pyridin-3-yl)acetamide (25).** Compound **25** was obtained as a white solid (60 mg, 39%).1H NMR (400 MHz, DMSO*-d6*) δ 10.28 (s, 1H), 9.42 (s, 1H), 8.96 (s, 1H), 8.59 (d, *J* = 5.6 Hz, 1H), 8.45 (s, 1H), 8.32 (s, 1H), 8.20 (s, 1H), 7.94 (s, 1H), 7.46 (s, 1H), 7.10 (d, *J* = 5.2 Hz, 1H), 2.21 (s, 3H), 2.08 (s, 3H). LC-MS (ESI/APCI) m/z: 380.7 [M + H] +.

**General procedure for the synthesis of 21, 24, 26.** To a solution of **20**, **23**, **25** (1 eq) in EtOH (1 mmol/ 20 mL) was added conc. HCl (1 mmol/ 4 mL). The mixture was stirred at reflux for 1 h. The resulting solid was collected via filtration, washed with EtOH and dried in vacuum to give the desired product **21**, **24**, **26**.

**General procedure for the synthesis of 31a-31d.** To a solution of **30a-30d** (1 eq) in conc. H2SO4 (1 mmol/ 2 mL) was added conc. HNO3 (1 eq) dropwise with an ice bath. The mixture was stirred at 0 °C for 30 min. The reaction was poured into ice water and the aqueous layer was extracted with DCM (1 mmol/ 15 mL \* 2). The combined organic layer was dried over Na2SO4, filtered and concentrated. The residue was purified by silica gel column chromatography (PE/ EA = 50/1) to give the desired product **31a-31d**.

**2-fluoro-5-nitrobenzaldehyde (31a).** Compound **31a** was obtained as a white solid (13.5 g, 65%). 1H NMR (400 MHz, CDCl3) δ 10.38 (s, 1H), 8.83 – 8.70 (m, 1H), 8.57 – 8.43 (m, 1H), 7.48 – 7.34 (m, 1H).

**1-(2-fluoro-5-nitrophenyl)ethan-1-one (31b).** Compound **31b** was obtained as a yellow solid (4.5 g, 86%). 1H NMR (400 MHz, CDCl3) δ 8.86 – 8.66 (m, 1H), 8.48 – 8.29 (m, 1H), 7.34 (t, *J* = 9.3 Hz, 1H), 2.70 (s, 3H).

**1-(2-fluoro-5-nitrophenyl)propan-1-one (31c).** Compound **31c** was obtained as a yellow oil (2.7 g, 35%). 1H NMR (400 MHz, CDCl3) δ 8.80-8.74 (m, 1H), 8.42-8.36 (m, 1H), 7.37-7.28 (m, 1H), 3.10-3.00 (m, 2H), 1.24 (t, *J* = 6.8 Hz, 3H). LC-MS (ESI/APCI) m/z: 197.8 [M + H] +.

**1-(2-fluoro-5-nitrophenyl)-2-methylpropan-1-one (31d).** Compound **31d** was obtained as a yellow oil (4.2 g, 70%). 1H NMR (400 MHz, CDCl3) δ 8.67 (s, 1H), 8.43 – 8.35 (m, 1H), 7.36 – 7.28 (m, 1H), 3.49 – 3.29 (m, 1H), 1.22 (d, *J* = 6.8 Hz, 6H). LC-MS (ESI/APCI) m/z: 211.9 [M + H] +.

**General procedure for the synthesis of 32a-32e.** To a solution of **31a-31e** (1 eq) in DMF(1 mmol/ 0.5 mL) was added sodium methanesulfinate or sodium ethyl sulfinate (1 eq). The mixture was stirred at room temperature for 1 h. K2CO3 (2 eq) was added and the mixture was stirred at 75 ℃ overnight. Water (1 mmol/ 3 mL) was added and the solution was extracted with EA (1 mmol/ 6 mL \* 2). The combined organic layer was dried over Na2SO4, filtered and concentrated. The residue was dissolved in DCM. Triethylamine (4 eq) and methanesulfonyl chloride (2 eq) were added dropwise. The mixture was stirred at room temperature overnight. The organic layer was removed and the residue was purified by silica gel column chromatography (PE/ EA = 2/1) to give the desired product **32a-32e**.

**5-nitrobenzo[b]thiophene 1,1-dioxide (32a).** Compound **32a** was obtained as a yellow solid (1.4 g, 8.3%).1H NMR (400 MHz, CDCl3) δ 8.43 (d, *J* = 8.0 Hz, 1H), 8.24 (s, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.33 (d, *J* = 7.2 Hz, 1H), 6.93 (d, *J* = 6.8 Hz, 1H).

**3-methyl-5-nitrobenzo[b]thiophene 1,1-dioxide (32b).** Compound **32b** was obtained as a yellow solid (4.8 g, 87%). 1H NMR (400 MHz, CDCl3) δ 8.44 (d, *J* = 8.0 Hz, 1H), 8.24 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 6.66 (s, 1H), 2.37 (s, 3H). LC-MS (ESI/APCI) m/z: 225.8 [M + H] +.

**2,3-dimethyl-5-nitrobenzo[b]thiophene 1,1-dioxide (32c).** Compound **32c** was obtained as a yellow solid (1.3 g, 78%). 1H NMR (400 MHz, CDCl3) δ 8.36 (d, *J* = 7.2 Hz, 1H), 8.19 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 2.24 (s, 3H), 2.21 (s, 3H). 1H NMR (400 MHz, CDCl3) δ 8.36 (d, *J* = 7.2 Hz, 1H), 8.19 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 2.24 (s, 3H), 2.21 (s, 3H).

**3-ethyl-5-nitrobenzo[b]thiophene 1,1-dioxide (32d).** Compound **32d** was obtained as a yellow solid (2.0 g, 62%). 1H NMR (400 MHz, CDCl3) δ 8.43 (d, *J* = 8.0 Hz, 1H), 8.25 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 6.63 (s, 1H), 2.73 (q, *J* = 7.2 Hz, 2H), 1.37 (t, *J* = 7.2 Hz, 3H).

**3-isopropyl-5-nitrobenzo[b]thiophene 1,1-dioxide (32e).**Compound **32e** was obtained as a yellow solid (1.3 g, 43%). 1H NMR (400 MHz, CDCl3) δ 8.42 (d, *J* = 8.0 Hz, 1H), 8.28 (s, 1H), 7.90 (d, *J* = 8.4 Hz, 1H),6.61 (s, 1H), 3.10 – 3.02 (m, 1H), 1.36 (d, *J* = 6.8 Hz, 6H).

**General procedure for the synthesis of 33a-33f.** To a solution of **32a-32f** (1 eq) in EtOH (1 mmol/ 3 mL) and water (1 mmol/ 1 mL) was added iron powder (5 eq) and ammonium chloride (5 eq). The mixture was stirred at 85 ℃ for 2 h. The solution was filtered via diatomite. The filtrate was extracted with DCM (1 mmol/ 10 mL \* 3). The combined organic phase was dried over Na2SO4, filtered and concentrated. The residue was purified by silica gel column chromatography (PE/ EA = 1/1) to give the desired product **33a-33f**.

**5-aminobenzo[b]thiophene 1,1-dioxide (33a).** Compound **33a** was obtained as a grey solid (0.8 g, 77%). 1H NMR (400 MHz, CDCl3) δ 8.42 (d, *J* = 8.4 Hz, 1H), 8.22 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.33 (d, *J* =6.8 Hz, 1H), 6.92 (d, *J* = 6.8 Hz, 1H). LC-MS (ESI/APCI) m/z: 181.9 [M + H] -.

**5-amino-3-methylbenzo[b]thiophene 1,1-dioxide (33b).** Compound **33b** was obtained as a grey solid (3.6 g, 88%). 1H NMR (400 MHz, CDCl3) δ 7.46 (d, *J* = 8.0 Hz, 1H), 6.66 (d, *J* = 8.0 Hz, 1H), 6.59 (s, 1H), 6.42 (s, 1H), 4.18 (s, 2H), 2.19 (s, 3H)。LC-MS (ESI/APCI) m/z: 195.9 [M + H] +.

**5-amino-2,3-dimethylbenzo[b]thiophene 1,1-dioxide (33c).** Compound **33c** was obtained as a yellow solid (540 mg, 78%). 1H NMR (400 MHz, CDCl3) δ 7.48 (d, *J* = 8.4 Hz, 1H), 6.60 (d, *J* = 8.0 Hz, 1H), 6.57 (s, 1H), 4.13 (br s, 2H), 2.11 (s, 3H), 2.07 (s, 3H). LC-MS (ESI/APCI) m/z: 209.9 [M + H] +.

**5-amino-3-ethylbenzo[b]thiophene 1,1-dioxide (33d).** Compound **33d** was obtained as a yellow solid (1.3 g, 74%). 1H NMR (400 MHz, CDCl3) δ 7.46 (d, *J* = 8.0 Hz, 1H), 6.65 (d, *J* = 8.0 Hz, 1H), 6.60 (s, 1H), 6.39 (s, 1H), 4.17 (br s, 2H), 2.55 (q, *J* = 6.8 Hz, 2H), 1.28 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI/APCI) m/z: 210.1 [M + H] +.

**5-amino-3-isopropylbenzo[b]thiophene 1,1-dioxide (33e).** Compound **33e** was obtained as a yellow solid (170 mg, 54%).1H NMR (400 MHz, CDCl3) δ 7.46 - 7.39 (m,1H), 6.67 – 6.59 (m, 2H), 6.35 (s, 1H), 2.92 – 2.81 (m, 1H), 1.26 (d, *J* = 6.8 Hz, 6H).

**General procedure for the synthesis of 34a-34f.** To a solution of **33a-33f** (1.0 eq) in EtOH (1 mmol/ 4 mL) was added 5-(methoxymethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (2.0 eq) slowly. The mixture was stirred at room temperature for 30 min. The resulting solid was collected via filtration, washed with EtOH (1 mmol/ mL) and dried in vacuum to give the desired product **34a-34f**.

**5-(((1,1-dioxidobenzo[b]thiophen-5-yl)amino)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (34a).** Compound **34a** was obtained as a yellow solid (1.4 g，90%). 1H NMR (400 MHz, CDCl3) δ 11.35 (d, *J* = 13.2 Hz, 1H), 8.66 (d, *J* = 13.6 Hz, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.26 (s, 1H), 7.23 (d, *J* = 7.2 Hz, 1H), 6.85 (d, *J* = 6.8 Hz, 1H), 1.77 (s, 6H). LC-MS (ESI/APCI) m/z: 333.7 [M - H] -.

**2,2-dimethyl-5-(((3-methyl-1,1-dioxidobenzo[b]thiophen-5-yl)amino)methylene)-1,3-dioxane-4,6-dione (34b).** Compound **34b** was obtained as a yellow solid (4.2 g, 65%). 1H NMR (400 MHz, DMSO*-d6*) δ 11.38 (d, *J* = 14.0 Hz, 1H), 8.77 (d, *J* = 14.0 Hz, 1H), 7.94 (s, 1H), 7.86 (d, *J* = 7.2 Hz, 1H), 7.77 (d, *J* = 6.8 Hz, 1H), 7.18 (s, 1H), 2.30 (s, 3H), 1.69 (s, 6H).

**5-(((2,3-dimethyl-1,1-dioxidobenzo[b]thiophen-5-yl)amino)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (34c).** Compound **34c** was obtained as a yellow solid (840 mg, 90%).1H NMR (400 MHz, DMSO-*d6*) δ 11.37 (d, *J* = 14.4 Hz, 1H), 8.78 (d, *J* = 14.0 Hz, 1H), 7.92 – 7.86 (m, 2H), 7.71 (d, *J* = 8.0 Hz, 1H), 2.21 (s, 3H), 2.07 (s, 3H), 1.69 (s, 6H).

**5-(((3-ethyl-1,1-dioxidobenzo[b]thiophen-5-yl)amino)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (34d).** Compound **34d** was obtained as a yellow solid (120 mg, 69%). 1H NMR (400 MHz, DMSO-*d6*) δ 12.19 (s, 1H), 8.26 (s, 1H), 8.04 – 7.98 (m, 1H), 7.67 (s, 1H), 7.33 (s, 1H), 6.17 (d, *J* = 7.6 Hz, 1H), 2.71 (q, *J* = 6.8 Hz, 2H), 1.25 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI/APCI) m/z: 362.1 [M - H] -.

**5-(((3-isopropyl-1,1-dioxidobenzo[b]thiophen-5-yl)amino)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (34e).** Compound **34e** was obtained as a white solid (300 mg, 100%).1H NMR (400 MHz, DMSO-*d6*) δ 11.38 (d, *J* = 14.4 Hz, 1H), 8.75 (d, *J* = 14.0 Hz, 1H), 8.01 (s, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.20 (s, 1H), 3.20 – 3.10 (m, 1H), 1.69 (s, 6H), 1.25 (d, *J* = 6.8 Hz, 6H).

**General procedure for the synthesis of 35a-35f.** The diphenyl ether (1 mmol/ 10 mL) was added to a round-bottomed flask and the solvent was heated to 240 ℃ for 5 min. Intermediate **34a-34f** (1 eq) was added dropwise to the solution. The mixture was stirred for 5 min. After cooling to room temperature, the resulting solid was collected via filtration, washed with ether (1 mmol/ 2 mL) and dried in vacuum to give the desired product **35a-35f**.

**8-hydroxythieno[2,3-g]quinoline 1,1-dioxide (35a).**Compound **35a** was obtained as a grey solid (450 mg, 77%). 1H NMR (400 MHz, DMSO-*d6*) δ 12.28 (s, 1H), 8.29 (s, 1H), 8.00 (d, *J* = 7.2 Hz, 1H), 7.80 (d, *J* = 6.8 Hz, 1H), 7.69 (s, 1H), 7.56 (d, *J* = 6.8 Hz, 1H), 6.18 (d, *J* = 7.2 Hz, 1H); LCMS (ESI/APCI) m/z 233.8 [M + H] +.

**8-hydroxy-3-methylthieno[2,3-g]quinoline 1,1-dioxide (35b).** Compound **35b** was obtained as a grey solid (765 mg, 54%).1H NMR (400 MHz, DMSO*-d6*) δ 12.19 (s, 1H), 8.26 (s, 1H), 8.02 (d, *J* = 6.8 Hz, 1H), 7.65 (s, 1H), 7.34 (s, 1H), 6.18 (d, *J* = 7.6 Hz, 1H), 2.32 (s, 3H). LC-MS (ESI/APCI) m/z: 247.6 [M + H] +.

**8-hydroxy-2,3-dimethylthieno[2,3-g]quinoline 1,1-dioxide (35c).** Compound **35c** was obtained as a yellow solid (270 mg, 45%). 1H NMR (400 MHz, DMSO-*d6*) δ 8.99 (d, *J* = 4.8 Hz, 1H), 8.62 (s, 1H), 8.23 (s, 1H), 7.92 (d, *J* = 4.8 Hz, 1H), 2.33 (s, 3H), 2.17 (s, 3H). LC-MS (ESI/APCI) m/z: 261.7 [M + H] +.

**3-ethyl-8-hydroxythieno[2,3-g]quinoline 1,1-dioxide (35d).** Compound **35d** was obtained as a brown solid (40 mg, 47%). 1H NMR (400 MHz, DMSO-*d6*) δ 12.19 (s, 1H), 8.26 (s, 1H), 8.04 – 7.98 (m, 1H), 7.67 (s, 1H), 7.33 (s, 1H), 6.17 (d, *J* = 7.6 Hz, 1H), 2.71 (q, *J* = 6.8 Hz, 2H), 1.25 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI/APCI) m/z: 262.1 [M + H] +.

**8-hydroxy-3-isopropylthieno[2,3-g]quinoline 1,1-dioxide (35e).** Compound **35e** was obtained as a yellow solid (70 mg). 1H NMR (400 MHz, DMSO-*d6*) δ 12.13 (s,1H), 8.27 (s, 1H), 8.03 (d, *J* = 6.8 Hz, 1H), 7.75 (s,1H), 7.36 (s,1H), 6.18 (d, *J* = 7.2 Hz, 1H), 3.14 – 3.05 (m, 1H), 1.27 (d, *J* = 6.8 Hz, 6H).

**General procedure for the synthesis of *36****-****40****.* Intermediate **35a-35e** (1 mmol/ 3 mL) was added to POCl3 (1 mmol/ 5 mL). The mixture was stirred at 110 ℃ for 2 h to afford a light brown solution. After cooling to room temperature, the excess POCl3 was removed in vacuum. The residue was dissolved in EtOH (1 mmol/ 10 mL) and benzo[d]thiazol-5-amine (1.1 eq) was added. The mixture was stirred at reflux for 2 h. The resulting was filtered, washed with EtOH and dried in vacuum to give the desired product **36-40**.

**methyl 5-nitrobenzo[b]thiophene-2-carboxylate (42).** To a solution of **41** (64 g, 346 mmol) in DMF (600 mL) was added K2CO3 (95.5 g, 692 mmol) and methyl 2-mercaptoacetate (34 mL, 381 mmol). The mixture was stirred at room temperature overnight. The reaction was poured into water (3.5 L) and the resulting solid was filtered, washed with water and dried in vacuum to give the desired product **42** (78.5 g, 96%) as a white solid. 1H NMR (400 MHz, CDCl3) δ 8.78 (s, 1H), 8.30 (d, *J* = 8.8 Hz, 1H), 8.19 (s, 1H), 8.00 (d, *J* = 8.8 Hz, 1H), 3.99 (s, 3H).

**5-nitrobenzo[b]thiophene-2-carboxylic acid (43).** To a solution of **42** (78.5 g, 331 mmol)in MeOH (400 mL) and H2O (400 mL) was added NaOH (53.0 g, 1.3 mol). The mixture was stirred at 70 ℃ for 4 h. After cooling to room temperature, the solution was poured into water (4 L) and acidified with concentrated hydrochloric acid. The resulting solid was filtered, washed with water, dried in vacuum to give the desired product **43** (72.6 g, 98%) as a white solid. 1H NMR (400 MHz, DMSO-*d6*) δ 8.97 (s, 1H), 8.49 – 8.19 (m, 3H).

**5-nitrobenzo[b]thiophene (44).** To a solution of **43** (65 g, 291 mmol) in quinoline (500 mL) was added copper powder (18.6 g, 291 mmol). The reaction was stirred at 170 ℃ under N2 atmosphere for 4 h. The solid was removed via filtration and washed with EA (400 mL). Another EA (2 L) was added to the filtrate to dilute the solution and the solution was acidified with conc. HCl with an ice bath. The organic layer was separated, washed with 2N HCl (400 mL), saturate NaHCO3 aqueous solution (400 mL), dried over Na2SO4, filtered and concentrated. The residue was rinsed with EA (200 mL) and dried in vacuum to give the desired product **44** (48 g, 78%) as a white solid. 1H NMR (400 MHz, DMSO-*d6*) δ8.84 (s, 1H), 8.29 (d, J = 9.2 Hz, 1H), 8.17 (d, *J* = 8.8 Hz, 1H), 8.05 (d, *J* = 5.2 Hz, 1H), 7.72 (d, *J* = 5.6 Hz, 1H).

**3-bromo-5-nitrobenzo[b]thiophene (45).** To a solution of **44** (19.4 g, 108 mmol) in DMF(250 mL) was added NBS (21.2 g, 119 mmol). The mixture was stirred at 60 ℃ under nitrogen atmosphere for 3 h. The solvent was removed in vacuum. The remaining residue was washed with EA (250 mL) and water (250 mL), dried in vacuum to give the desired product **45** (21.0 g, 75%) as a white solid. 1H NMR (400 MHz, DMSO-*d6*) δ8.49 (s, 1H), 8.40 (d, *J* = 8.8 Hz, 1H),8.32 – 8.25 (m, 2H).

**3-bromo-5-nitrobenzo[b]thiophene 1,1-dioxide (46).** To a solution of **45** (21.0 g, 81.4 mmol) in DCM (300 mL) with an ice bath was added 85% m-CPBA (42.0 g, 203.5 mmol). The mixture was stirred at room temperature overnight. The resulting solid was filtered off and the filtrate was quenched with Na2SO3, washed with saturated NaHCO3 aqueous solution and extracted with DCM (200 mL \* 2). The combined organic phase was dried over Na2SO4,filtered and concentrated. The residue was rinsed with EA and dried in vacuum to give the desired product **46** (20.8 g, 99%) as a white solid. 1H NMR (400 MHz, DMSO-*d6*) δ8.56 (dd, *J* = 8.4 Hz, *J* = 1.2 Hz, 1H), 8.34 (s, 1H), 8.30 (d, *J* = 8.4 Hz, 1H), 8.20 (d, *J* = 1.6 Hz, 1H).

**5-amino-3-bromobenzo[b]thiophene 1,1-dioxide (47).** To a solution of **46** (20.8 g, 71.7 mmol) in EtOH (300 mL) and H2O (100 mL) was added iron powder (16.1 g, 287 mmol) and NH4Cl(15.3 g, 287 mmol). The mixture was stirred at 85 ℃ for 2 h. The reaction was filtered through diatomaceous earth and the cake was washed with DCM. The resulting filtrate was extracted with DCM (500 mL \* 3). The combined organic phase was dried over Na2SO4, filtered and concentrated. The residue was rinsed with EA, dried in vacuum to give the desired product **47** (15.0 g, 81%) as a yellow solid. 1H NMR (400 MHz, DMSO-*d6*) δ 7.84 (s, 1H), 7.47 (s, 1H), 6.89 – 6.59 (m, 2H), 6.44 (s, 2H). LCMS (ESI/APCI) m/z: 257.7 [M - H] -.

**5-(((3-bromo-1,1-dioxidobenzo[b]thiophen-5-yl)amino)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (48).** To a solution of **47** (13.5 g, 51.9 mmol) in EtOH (120 mL) was added 5-(methoxymethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (14.3 g, 77.9 mmol). The mixture was stirred at room temperature for 30 min. The resulting solid was collected via filtration, washed with EtOH (20 mL) and dried in vacuum to give the desired product **48** (18.0 g, 86%) as a yellow solid.1H NMR (400 MHz, DMSO-*d6*) δ11.42 (d, *J* = 12.8 Hz, 1H), 8.68 (d, *J* = 13.6 Hz, 1H), 8.13 (s, 1H), 7.99 (s, 1H), 7.90 (s, 2H), 1.69 (s, 6H).

**3-bromo-8-hydroxythieno[2,3-g]quinoline 1,1-dioxide (49).**The diphenyl ether (720 mL) was added to a round-bottomed flask and the solvent was heated to 240 ℃ for 5 min. Intermediate **48** (18.0 g, 43.6 mmol) was added slowly to the solution. The mixture was stirred for 5 min. After cooling to room temperature, the resulting solid was collected via filtration, washed with ether (100 mL) and dried in vacuum to give the desired product **49** (5.5 g, 41%) as a grey solid. 1H NMR (400 MHz, DMSO-*d6*) δ12.31 (s, 1H), 8.35 (s, 1H), 8.25 (s, 1H), 8.08 (d, *J* = 7.2 Hz, 1H), 7.77 (s, 1H), 6.23 (d, *J* = 6.8 Hz, 1H). LCMS (ESI/APCI) m/z: 311.6 [M + H] +.

**8-(benzo[d]thiazol-5-ylamino)-3-bromothieno[2,3-g]quinoline 1,1-dioxide (50).** Intermediate **49** (5.5 g, 17.7 mmol) was added to POCl3 (50 mL) and then the mixture was stirred at 110 °C for 2 h to afford a light brown solution. After cooling to room temperature, the excess POCl3 was removed in vacuum. The residue was dissolved in EtOH (20 mL) and benzo[*d*]thiazol-5-amine (3.2 g, 21.3 mmol) was added subsequently. The mixture was stirred at reflux for 1 h. After cooling to room temperature. The resulting solid was collected via filtration, washed with EtOH and dried in vacuum to give the desired product **50** as a hydrochloride salt (6.0 g, 77%). 1H NMR (400 MHz, DMSO-*d6*) δ 11.37 (s, 1H), 9.55 (s, 1H), 9.43 (s, 1H), 8.63 (d, *J* = 6.8 Hz, 1H), 8.50 (s, 1H), 8.40 (d, *J* = 8.8 Hz, 1H), 8.27–8.21 (m, 2H), 7.62 (d, *J* = 8.8 Hz, 1H), 7.04 (d, *J* = 7.2 Hz, 1H). LC-MS (ESI/APCI) m/z: 443.5 [M + H] +.

**General procedure for the synthesis of 51-62.** To a solution of **50** (1eq) in 1,4-dioxane (1 mmol/ 20 mL) and water (1 mmol/ 2 mL) was added multiple aromatic borates or aromatic boric acid (1.5 eq), K2CO3 (3 eq) and Pd(dppf)Cl2 (0.1 eq). The mixture was stirred at 100 ℃ overnight. The solvent was removed in vacuum and the residue was purified by silica gel column chromatography (DCM/ MeOH = 1/1) to give the desired product **51-62**.

3. Preliminary in vitro safety and DMPK test

CYP inhibitory potency and human/mouse liver microsomes metabolic stability were evaluated as previously reported [6]. Caco-2 assays were reported before [7].

4. Kinase binding (Kd) assay [8]

The binding affinity of the test compounds for kinases was detected by a KINOMEscan assay. Kinase-tagged T7 phage strains were prepared in an *E. coli* host derived from the BL21 strain. *E. coli* were grown to log-phase and infected with T7 phage and incubated with shaking at 32°C until lysis. The lysates were centrifuged and filtered to remove cell debris. The remaining kinases were produced in HEK-293 cells and subsequently tagged with DNA for qPCR detection. Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 minutes at room temperature to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1% BSA, 0.05% Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific binding. Binding reactions were assembled by combining kinases, liganded affinity beads, and test compounds in 1x binding buffer (20% SeaBlock, 0.17x PBS, 0.05% Tween 20, 6 mM DTT). All reactions were performed in polypropylene 384-well plate. Each was a final volume of 0.02 ml. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05% Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05% Tween 20, 0.5 μM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The kinase concentration in the eluates was measured by qPCR.

5. Reference

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6. NMR spectra

