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Design, synthesis and biological evaluation of thienopyridinones as Chk1 inhibitors

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ABSTRACT

A series of thienopyridinone derivatives was designed and synthesized as inhibitors of checkpoint kinase 1 (Chk1). Most of them exhibited moderate to good Chk1 inhibitory activities. Among them, compounds **8q**, **8t**, and **8w** with excellent Chk1 inhibitory activities (IC_{50} values of 4.05, 6.23, and 2.33 nM, respectively) displayed strong synergistic effects with melphalan, a DNA-damaging agent in the cell-based assay. Further kinase profiling indicated that compound **8t** was highly selective against CDK2/cyclinA, Aurora A, and PKC.

1. Introduction

Checkpoint kinase 1 (Chk1), as a serine/threonine protein kinase, plays an essential role in regulating cell cycle progression in response to DNA damage.[1] Predominantly activated by the upstream kinase, ataxia telangiectasia and rad3 related (ATR), Chk1 mediates the S and G2/M arrest through the proteolysis of Cdc25A, and leads to the inactivation of Cdk2, whose function is required in the cell cycle transition. Since most of human malignancies harbor mutations or defects in the DNA-binding domain of p53, they are more reliant on the later S or G2/M phase checkpoint for cell cycle arrest.[2] Activation of these checkpoints provides an opportunity for cancer cells to repair damaged DNA and thus reduces the effect of cancer chemotherapies and radiotherapy. While normal cells remaining at the G1 phase via p53, are less influenced by the abrogation of S and G2/M checkpoints. Therefore, inhibition of Chk1 by small molecules or siRNA against Chk1, in combination with traditional DNA damaging therapy could selectively target tumor cells, and has been recognized as a promising strategy for cancer therapy.[3]

Although clinical application of the first generation Chk1 inhibitors, exemplified by UCN-01, was hindered by its off-target effects and unfavorable pharmacokinetic profiles,[4] recent years have witnessed the development of many Chk1 inhibitors with various chemical scaffolds (Fig. 1).[5] Among these, most are single digit nM inhibitors of Chk1 with promising prospects for further optimization of cellular potency and selectivity profiles.[6] Particularly, nine candidates have entered into clinical evaluations for the treatment of cancer.[7]

At the beginning of our work, an initial survey of the literatures revealed that quinolin-2-one was a favorable core for exploring antitumor agents.[8] Besides, it is also incorporated in a well-known preclinical Chk1 inhibitor (Fig. 2, CHIR-124), which strongly inhibits Chk1 and potentiates the cytotoxicity of Topoisomerase I inhibitors.[9] For the pursuit of an improved potency and selectivity profile, we studied the structural characteristics of several Chk1 inhibitors and designed a series of novel thienopyridinone derivatives based on bioisosterism strategy incorporating the following concepts: (1) replacement of the quinolinone of CHIR-124 with thienopyridinone; (2) various alicyclic amine and aliphatic amine substituents were introduced at the 4-position of the thienopyridinone core; (3) small substituents such as methyl, chlorine, and bromine were introduced at the 6-position. All the synthesized compounds were evaluated for their Chk1 inhibitory activities, and representative compounds were performed in a combination

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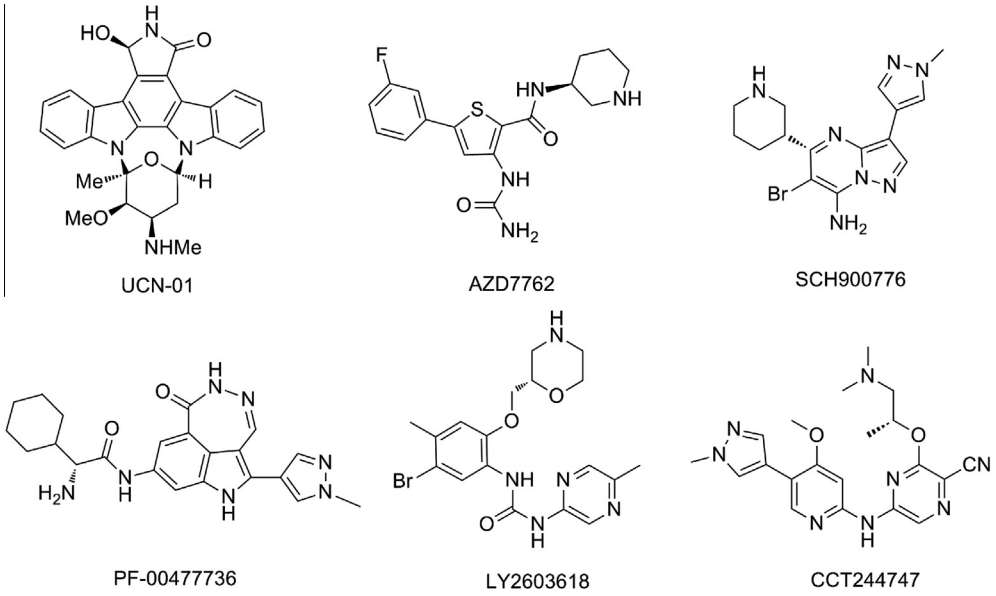


Figure 1. Structures of Chk1 inhibitors with various chemical scaffolds.

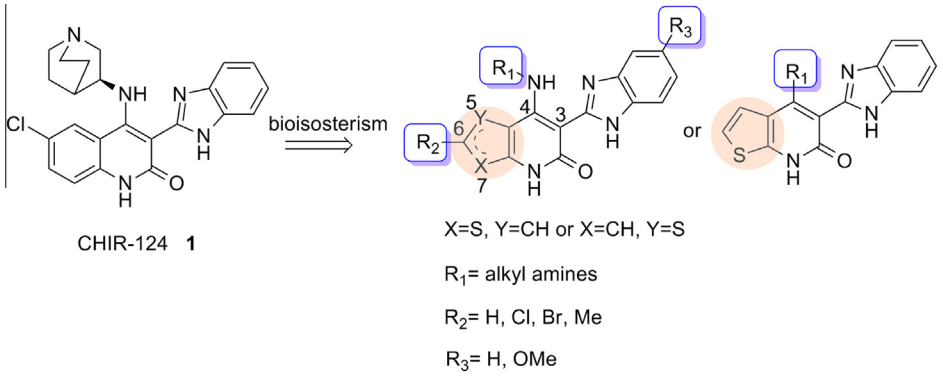


Figure 2. Design of novel thienopyridinone Chk1 inhibitors.

assay on human multiple myeloma cell line and further tested for their selectivities against other kinases.

2. Results and discussion

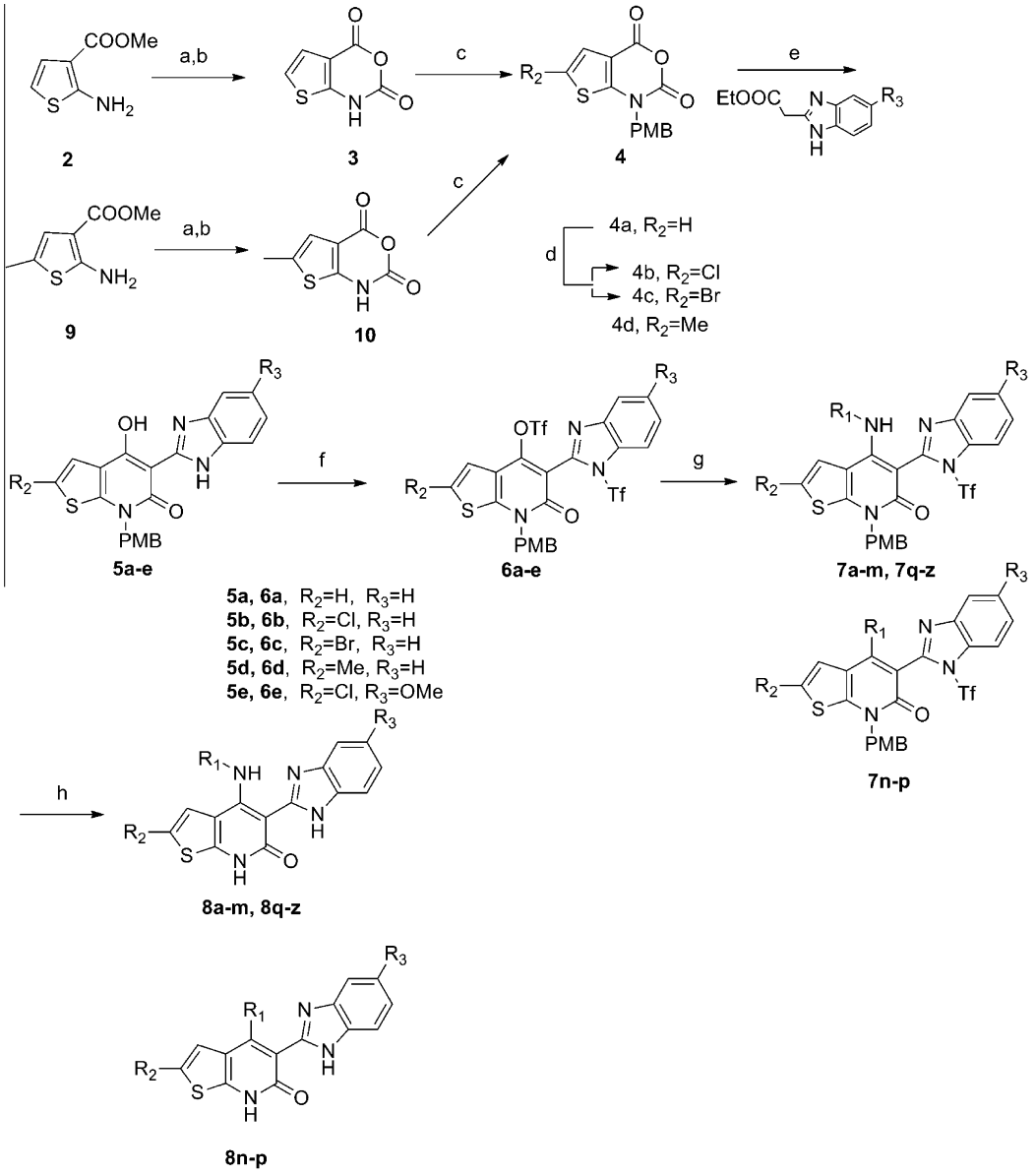
2.1. Chemistry

The synthetic routes for thienopyridinone derivatives were summarized in Schemes 1 and 2. Methyl 2-aminothiophene-3-carboxylates **2** and **9** were hydrolyzed with 1 N KOH aqueous solution under microwave radiation and then reacted with phosgene to give thiaisatoic anhydrides **3** and **10**, respectively.[10] Treatment of **3** and **10** with *p*-methoxybenzyl bromide (PMB) in the presence of K_2CO_3 in DMF afforded N-PMB thiaisatoic anhydrides **4a** and **4d**, respectively. Compound **4b** or **4c** was achieved by chlorination or bromination of **4a**. The obtained **4a–d** were reacted with 5-(un)substituted ethyl-2-benzimidazole-acetates in the presence of LHMDS in THF, which furnished the key intermediates **5a–e**. Compounds **5a–e** were then converted into corresponding bis-triflates **6a–e**. Treatment of **6a–e** with various alkyl amines under mild conditions provided **7a–z** in good yields. Finally, removal of the PMB and Tf groups under acidic conditions led to the target compounds **8a–z**.[11] The preparation of compounds **17a** and **17b** were conducted in a similar method starting from methyl 3-aminothiophene-2-carboxylate **11**.

2.2. Chk1 kinase assay

The obtained thienopyridinone derivatives were initially evaluated for their Chk1 kinase inhibitory activities by ADP-Glo luminescent assay, with AZD7762 employed as the positive control. The results are summarized in Table 1.

As shown in Table 1, most compounds exhibited Chk1 inhibitory potency in nanomolar level except for compounds **8f**, **8h**, **8i**, and **8k–p**. Incorporation of basic alicyclic amines at the 4-amino group of the thienopyridinone core (**8a–e**, **8q–z**, **17a**, and **17b)** resulted in desirable Chk1 inhibitory activity. Among them, (*S*)-3-piperidine was validated as the most preferred substituent (**8b**, **8r**, **8w**, Chk1 IC_{50} = 5.22, 3.53, 2.33 nM, respectively). In contrast, replacement of the alicyclic amines with cyclohexyl (**8f**) or aliphatic amines (**8h–j**) caused dramatic loss in potency. Compounds **8n–p**, of which, the nitrogen within piperidine or morpholine ring was directly linked to the thienopyridinone scaffold, only displayed a negligible inhibitory activity against Chk1 with an IC_{50} value >10 μM. Besides, compounds with small substituents, such as chlorine (**8q–t**), bromine (**8u**, **8v**), and methyl group at C-6 of thienopyridinone core (**8y**, **8z**) and incorporation of a methoxy group at the 5-position of benzimidazole ring (**8w**, **8x**) exhibited a slightly enhanced potency. Translocation of the sulfur atom in thienopyridinone from the 7-position to 5-position maintained potency as well,



Scheme 1. Synthesis of thieno[2,3-*b*]pyridin-6(7*H*)-ones **8a**–**z**. Reagents and conditions: (a) 1 N KOH, MeOH; (b) $COCl_2$, toluene, 62–65%; (c) PMB-Br, K_2CO_3, DMF, 80–92%; (d) for clorization/NCS, AcOH, 83%; for bromonization/NBS, DCM, 74%; (e) ethyl 2-(1*H*-benzo[*d*]imidazol-2-yl)acetate, LHMDS, THF, 36–43%; (f) $(TfO)_2O$, Py, DCM, 80–85%; (g) R_1-NH_2 (for **7a**–**m**, **7q**–**z**), or R_1 (for **7n**–**p**), ACN, 90–95%; (h) TFA/HCl (7:1), 40–62%.

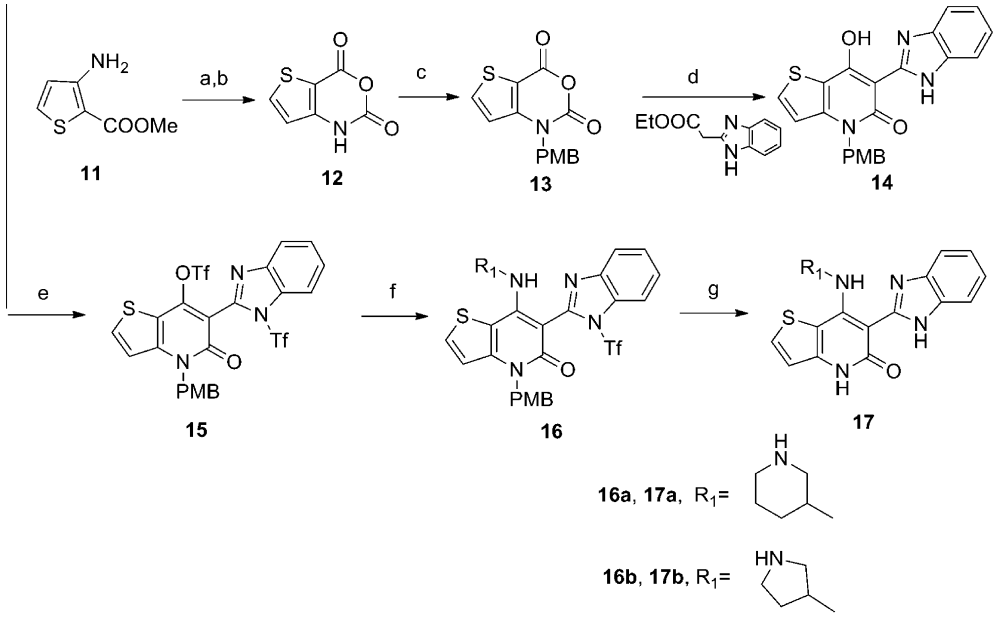
which indicated that the position of the sulfur atom had little impact on Chk1 binding affinity.

2.3. Cell synergy assay

It has been widely accepted that Chk1 inhibitors could enhance the antitumor effects of conventional DNA-damaging agents in combination assays.[12] Based on this consideration, potent Chk1 inhibitors **8q**, **8t**, and **8w** were further evaluated for their anti-proliferation activities against human multiple myeloma (MM) cell line RPMI-8266 that was p53-mutant.[13] As illustrated in Figure 3, when used as single agents, all the three compounds and the positive Chk1 inhibitor AZD7762 at 100 nM have little effect on the proliferation of RPMI-8266 cells. Whereas, they significantly potentiated the cytotoxicity of the DNA-damaging anti-MM agent melphalan (1–10 μM). In particular, compounds **8q** and **8t** showed no cytotoxicity in the absence of melphalan and thereby can be identified as ideal Chk1 inhibitors.[1,14]

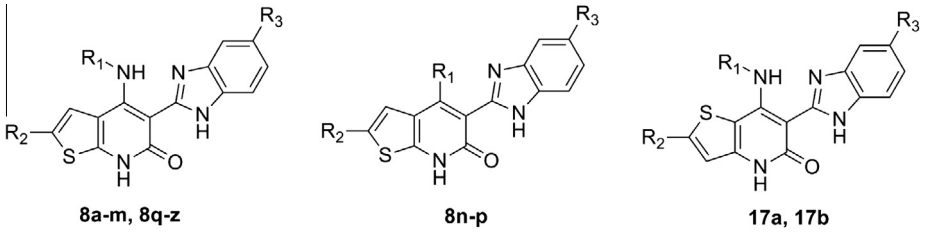
2.4. Kinase selectivity profiles

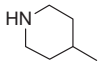
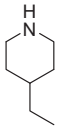
Inhibition of other kinases simultaneously may antagonize Chk1 inhibition phenotypes and even result in off-target effects, which highlights the benefit of monitoring kinase selectivity.[15] Therefore, nine compounds (**8a**, **8b**, **8e**, **8q**, **8t**, **8u**, **8w**, **8x**, and **17a**) were further evaluated for their selectivity profiles against Aurora A, CDK2/cyclinA, and PKC. As shown in Table 2, all the tested compounds demonstrated more than 20-fold selectivities over CDK2/cyclinA, Aurora A and PKC. Compounds **8e**, **8t**, and **8x** sharing the common 3-aminoquinuclidine fragment at the 4-position of thienopyridinone core exhibited nearly 100-fold selectivities over CDK2/cyclinA and PKC. Noticeably, compounds **8e** and **8t** showed almost no inhibition against Aurora A.



Scheme 2. Synthesis of thieno[3,2-*b*]pyridin-5(4*H*)-ones **17a** and **17b**. Reagents and conditions: (a) 1 N KOH, MeOH; (b) $COCl_2$, toluene, 78%; (c) PMB-Br, K_2CO_3, DMF, 78%; (d) ethyl 2-(1*H*-benzo[*d*]imidazol-2-yl)acetate, LHMDS, THF, 26%; (e) $(TfO)_2O$, Py, DCM, 78%; (f) R_1-NH_2, ACN, 75–78%; (g) TFA/HCl (7:1), 47–48%.

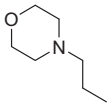
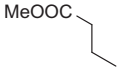
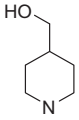
Table 1
Chk1 inhibitory profile for thienopyridinones **8a**–**z**, **17a**, and **17b**



Compd	R_1	R_2	R_3	Chk1[a] IC_{50} (nM)	Compd	R_1	R_2	R_3	Chk1[a] IC_{50} (nM)
8a		H	H	15.60	**8o**		H	H	>10 μM
8b		H	H	5.22	**8p**		H	H	>10 μM
8c		H	H	47.50	**8q**		Cl	H	4.05
8d		H	H	9.94	**8r**		Cl	H	3.53
8e		H	H	14.02	**8s**		Cl	H	40.98
8f		H	H	>10 μM	**8t**		Cl	H	6.23
8g		H	H	116.10	**8u**		Br	H	4.83
8h		H	H	>10 μM	**8v**		Br	H	15.34

(*continued on next page*)

Table 1 (*continued*)

Compd	R_1	R_2	R_3	Chk1[a] IC_{50} (nM)	Compd	R_1	R_2	R_3	Chk1[a] IC_{50} (nM)
8i		H	H	>10 μM	**8w**		Cl	OMe	2.33
8j		H	H	177.80	**8x**		Cl	OMe	5.36
8k		H	H	>10 μM	**8y**		Me	H	9.84
8l		H	H	>10 μM	**8z**		Me	H	23.20
8m		H	H	>10 μM	**17a**		H	H	7.54
8n		H	H	>10 μM	**17b**		H	H	24.73
AZD7762	—	—	—	7.89					

[a] Values are means of two experiments.

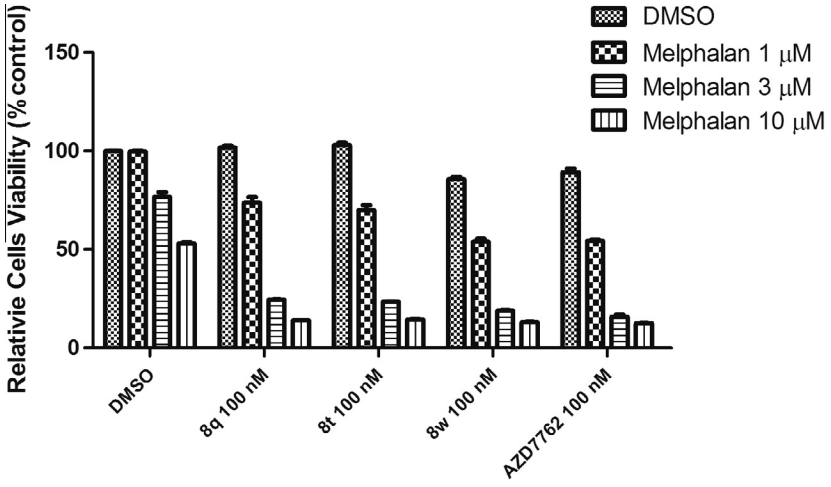


Figure 3. Synergistic effects of compounds **8q**, **8t**, and **8w** with melphalan.

Table 2
Kinase selectivity profiles of nine Chk1 inhibitors

Compd	IC_{50} (nM)			
	Chk1	CDK2/cyclinA	Aurora A	PKC
8a	15.60	848.82	790.81	695.85
8b	5.22	851.83	495.14	522.51
8e	14.02	1630.44	>10000	1001.81
8q	4.05	334.33	1051.51	168.89
8t	6.23	3187.14	>10000	2018.88
8u	4.83	246.20	1591.65	392.71
8w	2.33	393.33	328.20	204.48
8x	5.36	4304.83	1400.34	8186.03
17a	7.54	255.99	201.86	814.06

Compound **8w**, the most potent one in the Chk1 kinase assay, moderately inhibited other targets as well, which might partly explain its cytotoxic effect as a single agent in the cell-based assay.

2.5. Molecular docking study

Based on the biological evaluation results of Chk1 kinase inhibition, two representative compounds, the highly potent inhibitor **8t**, and the weak inhibitor **8o** were selected for further docking simulations to analyze their binding modes with Chk1 protein using the C-DOCKER protocol within Discovery Studio 2.1 software package. The published X-ray crystal structure of Chk1 (PDB ID: 2GDO) was used for the docking calculation. As shown in Figure 4, the critical donor-acceptor-donor motif has been retained in both **8t** and **8o**, which makes three hydrogen bonds with Chk1 hinge region residues Glu85 and Cys87. The presence of the 3-aminoquinuclidine substituent in **8t** fixed a basic tertiary-amine in the ribose binding pocket, which strongly contacted to acidic residues Glu134 and Glu91. Whereas, similar interactions cannot be observed between compound **8o** and Chk1 protein (Fig. 4B). It gave us a possible explanation that mere hydrogen bond interactions between

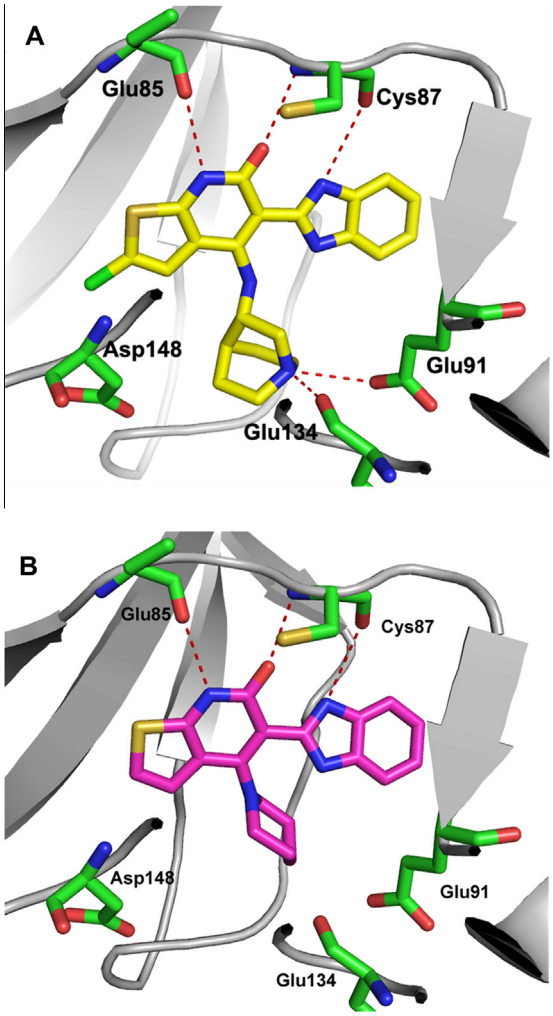


Figure 4. Docking mode comparison between compound **8t** and **8o** bound to Chk1. (A) Molecular docking analysis of **8t** (yellow backbone) with Chk1; (B) Molecular docking analysis of **8o** (pink backbone) with Chk1. Red dashed lines indicate hydrogen bonds, prepared using PyMOL, PDB ID: 2GDO.

ligands and Chk1 protein in this series were not sufficient for maintaining Chk1 inhibitory potency, and lack of the basic amine tail would cause dramatic drop of potency. These results predicted that compound **8t** would lead to potent biological activity, and this was confirmed by the experimental results.

3. Conclusion

In this study, a series of thienopyridinone derivatives was designed and synthesized as Chk1 inhibitors based on bioisosterism strategy. Preliminary structure–activity relationship (SAR) indicated that structural modification on the 4-position of thienopyridinone core greatly influenced the Chk1 inhibitory activities. Basic cyclic substituents at the 4-NH position were beneficial for maintaining potency. The introduction of 3-piperidine at the 4-amino group contributes to the improvement in both enzymatic and cellular synergistic efficacy, while the 3-quinuclidine fragment was confirmed as a priority for gaining selectivity over other Ser/Thr kinases. Based on the experimental results, it is noteworthy that compound **8t** not only demonstrated potent Chk1 inhibitory activity but also displayed synergistic effect with melphalan against RPMI-8266 tumor cell line. Additionally, it performed excellent selectivity profiles against some other kinases. With all these advantages, it can be recognized as a promising candidate for further investigation.

4. Experimental

4.1. Chemistry

Melting points were determined with a B-540 Büchi melting-point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a 500 MHz, ^{13}C NMR were recorded on a 125 MHz spectrometer at room temperature (chemical shifts are given in ppm (δ) relative to TMS as internal standard, coupling constants (J) are in hertz (Hz), and signals are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad singlet, etc.). Mass spectra (MS), ESI (positive) were recorded on an Esquire-LC-00075 spectrometer. Thin layer chromatography was carried out using plate silica gel F254 Merck. Reagents and solvents were purchased from common commercial suppliers and some unhydrous solvents were further purified before usage. All yields are unoptimized and generally represent the result of a single experiment.

4.1.1. Synthesis of thiaisatoic anhydrides (3, 10, 12)

2-Aminothiophene-3-carboxylate **2** (4 g, 25 mmol) was suspended in an aqueous solution of potassium hydroxide 50 mL (1 N, 50 mmol). The mixture was then heated under microwave radiation (500 W) for 15 min. After cooling the solution at 0 °C, phosgene (1.6 equiv, 21 mL of 20% $COC1_2$ solution in toluene) was added dropwise with stirring. The mixture was allowed to stand at room temperature overnight. The resulting precipitate was filtered and washed successively with water and petroleum ether.

4.1.1.1. 3-Thiaisatoic anhydride (3). White solid (65%), mp: 230–231 °C (lit. 232 °C).

4.1.1.2. 6-Methyl-3-thiaisatoic anhydride (10). White solid (62%), mp: 195–197 °C (lit. 193–196 °C).

4.1.1.3. 2-Thiaisatoic anhydride (12). White solid (78%), mp: 230–232 °C (lit. 230 °C).

4.1.2. General procedure for the PMB protection of thiaisatoic anhydride derivatives (4a, 4d, 13)

To a precooled (0 °C) solution of thiaisatoic anhydride derivative (**3**) (10 mmol) in DMF (10 mL) was added K_2CO_3 (12 mmol). A solution of 4-methoxybenzylbromide (12 mmol) in DMF (3 mL) was added dropwise. After stirring at room temperature for 40 min, the reaction mixture was poured into ice and water (50 mL). The precipitate was filtered, washed with water and dried in vacuo and the crude product was used directly in the next step without further purification.

4.1.2.1. 1-(4-Methoxybenzyl)-1*H*-thieno[2,3-*d*][1,3]oxazine-2,4-dione (4a). White solid (92%), mp: 112–114 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.38 (d, J = 18 Hz, Ar-H, 2H), 7.28 (d, J = 12 Hz, Ar-H, 1H), 7. 24 (d, J = 12 Hz, Ar-H, 1H), 6.94 (d, J = 18 Hz, Ar-H, 2H), 5.06 (s, CH_2, 2H), 3.74 (s, CH_3, 3H). ESI-MS: m/z = 290 $[M+H]^+$.

4.1.2.2. 1-(4-Methoxybenzyl)-6-methyl-1*H*-thieno[2,3-*d*][1,3]oxazine-2,4-dione (4d). White solid (80%), mp: 130–132 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.37 (d, J = 18 Hz, Ar-H, 2H), 6.99 (s, Ar-H, 1H), 6.94 (d, J = 18 Hz, Ar-H, 2H), 5.01 (s, CH_2, 2H), 3.74 (s, CH_3, 3H), 2.37 (s, CH_3, 3H). ESI-MS: m/z = 304 $[M+H]^+$.

4.1.2.3. 1-(4-Methoxybenzyl)-1*H*-thieno[3,2-*d*][1,3]oxazine-2,4-dione (13). White solid (78%), mp: 179–181 °C (lit. 181–182 °C).

4.1.3. Synthesis of 6-chloro-1-(4-Methoxybenzyl)-1*H*-thieno[2,3-*d*][1,3]oxazine-2,4-dione (4b)

To a solution of compound **4a** (1.95 g, 6.74 mmol) in toluene (8 mL) and glacial acetic acid (8 mL), N-chlorosuccinimide (1.08 g, 8.09 mmol) was added in one portion. Then the mixture was stirred at 70 °C for 2 h. Upon cooling, the reaction mixture was extracted with CH_2Cl_2 and water. And then concentrated in vacuo, the resulting crude material was purified by column chromatography (petroleum ether/CH_2Cl_2 = 2:3, v/v) on silica gel to afford the product as a white solid. Yield: 83%, mp: 140–142 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.44 (s, Ar-H, 1H), 7.40 (d, J = 17 Hz, Ar-H, 2H), 6.95 (d, J = 17 Hz, Ar-H, 2H), 5.02 (s, CH_2, 2H), 3.75 (s, CH_3, 3H). ESI-MS: m/z = 324 $[M+H]^+$.

4.1.4. Synthesis of 6-bromo-1-(4-methoxybenzyl)-1*H*-thieno[2,3-*d*][1,3]oxazine-2,4-dione (4c)

N-bromosuccinimide (1.8 g, 10.11 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a solution of **4a** (1.95 g, 6.74 mmol) in CH_2Cl_2 (100 mL) under N_2 protection. After the reaction was stirred at room temperature for 2 h, water (100 mL) was added. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resulting crude material was purified by column chromatography (petroleum ether/ethyl acetate = 4:1, v/v) on silica gel to give a white solid. Yield: 74%, mp: 136–138 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.51 (s, Ar-H, 1H), 7.40 (d, J = 17 Hz, Ar-H, 2H), 6.95 (d, J = 17 Hz, Ar-H, 2H), 5.02 (s, CH_2, 2H), 3.75 (s, CH_3, 3H). ESI-MS: m/z = 368 $[M+H]^+$.

4.1.5. General procedure for LHMDS cyclization

LHMDS (1.65 mL, 1 M in THF, 1.65 mmol, 5.0 equiv) was added dropwise to a solution of ethyl 2-(1*H*-benzo[*d*]imidazol-2-yl)acetate (67.6 mg, 0.33 mmol, 1.0 equiv) and 1-(4-methoxybenzy-l)-1*H*-thieno[2,3-*d*][1,3]oxazine-2,4-dione (0.33 mmol, 1.0 equiv) in anhydrous THF (15 mL) at −78 °C. The resulting solution was slowly warmed to room temperature and then stirred overnight at 80 °C. After the reaction completed, quenched with a small amount of NH_4Cl (aq, satd). And then concentrated in vacuo, the resulting crude material was purified by column chromatography (petroleum ether/ethyl acetate/acetic acid = 500:100:20, v/v/v) on silica gel to afford the product as a white solid.

4.1.5.1. 3-(1*H*-Benzimidazole-2yl)-4-hydroxyl-1-(4-methoxybenzyl)-thieno[2,3-*b*]pyridin-6(7*H*)-one (5a). White solid (43%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.64 (br, NH, 1H), 7.81–7.77 (m, Ar-H, 2H), 7.35–7.31 (m, Ar-H, 4H), 7.30 (d, J = 11 Hz, Ar-H, 1H), 7.07 (d, J = 11 Hz, Ar-H, 1H), 6.91 (d, J = 17 Hz, Ar-H, 2H), 5.23 (s, CH_2, 2H), 3.71 (s, CH_3, 3H). ESI-MS: m/z = 404 $[M+H]^+$.

4.1.5.2. 3-(1*H*-Benzimidazole-2yl)-6-chloro-4-hydroxyl-1-(4-methoxybenzyl)-thieno[2,3-*b*]pyridin-6(7*H*)-one (5b). White solid (42%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.57 (br, NH, 1H), 7.80–7.78 (m, Ar-H, 2H), 7.35–7.33 (m, Ar-H, 2H), 7.32 (d, J = 18 Hz, Ar-H, 2H), 7.28 (s, Ar-H, 1H), 6.93 (d, J = 18 Hz, Ar-H, 2H), 5.17 (s, CH_2, 2H), 3.72 (s, CH_3, 3H). ESI-MS: m/z = 438 $[M+H]^+$.

4.1.5.3. 3-(1*H*-Benzimidazole-2yl)-6-bromo-4-hydroxyl-1-(4-methoxybenzyl)-thieno[2,3-*b*]pyridin-6-one (5c). White solid (36%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.58 (br, NH, 1H), 7.81–7.78 (m, Ar-H, 2H), 7.39 (s, Ar-H, 1H), 7.35–7.33 (m, Ar-H, 2H), 7.32 (d, J = 18 Hz, Ar-H, 2H), 6.93 (d, J = 18 Hz, Ar-H, 2H), 5.18 (s, CH_2, 2H), 3.72 (s, CH_3, 3H). ESI-MS: m/z = 482 $[M+H]^+$.

4.1.5.4. 3-(1*H*-Benzimidazole-2yl)-6-methyl-4-hydroxyl-1-(4-methoxybenzyl)-thieno[2,3-*b*]pyridin-6(7*H*)-one (5d). White solid (36%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.64 (br, NH, 1H), 7.79–7.77 (m, Ar-H, 2H), 7.33–7.31 (m, Ar-H, 2H), 7.31 (d, J = 18 Hz, Ar-H, 2H), 7.00 (s, Ar-H, 1H), 6.91 (d, J = 18 Hz, Ar-H, 2H), 5.18 (s, CH_2, 2H), 3.72 (s, CH_3, 3H), 2.39 (s, CH_3, 3H). ESI-MS: m/z = 418 $[M+H]^+$.

4.1.5.5. 6-Chloro-4-hydroxy-3-(5-methoxy-1*H*-benzo[*d*]imidazol-2-yl)-1-(4-methoxybenzyl)thieno[2,3-*b*]pyridin-2-one (5e). White solid (41%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.47 (br, NH, 1H), 7.68 (d, J = 17 Hz, Ar-H, 1H), 7.38 (s, Ar-H, 1H), 7.32 (d, J = 18 Hz, Ar-H, 2H), 7.27 (s, Ar-H, 1H), 6.97 (d, J = 17 Hz, Ar-H, 1H), 6.92 (d, J = 18 Hz, Ar-H, 2H), 5.17 (s, CH_2, 2H), 3.80 (s, CH_3, 3H), 3.72 (s, CH_3, 3H). ESI-MS: m/z = 468 $[M+H]^+$.

4.1.5.6. 6-(1*H*-Benzo[*d*]imidazol-2-yl)-7-hydroxy-4-(4-methoxybenzyl)thieno[3,2-*b*]pyridin-5(4*H*)-one (14). This compound was obtained by two steps using the same method as Section 4.1.5. The firstly generated uncyclized intermediate was separated and then repeated the same operation again to get compound **14**. White solid (26% in two steps), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.59 (br, NH, 1H), 7.88 (d, J = 10 Hz, Ar-H, 1H), 7.79–7.77 (m, Ar-H, 2H), 7.34–7.32 (m, Ar-H, 2H), 7.31 (d, J = 18 Hz, Ar-H, 2H), 7.26 (d, J = 10 Hz, Ar-H, 1H), 6.88 (d, J = 18 Hz, Ar-H, 2H), 5.34 (s, CH_2, 2H), 3.69 (s, CH_3, 3H). ESI-MS: m/z = 404 $[M+H]^+$.

4.1.6. General procedure for the synthesis of bis-triflates 6a–e and 15

To a solution of **5a** (403 mg, 1 mmol) in anhydrous CH_2Cl_2 (80 mL) was added pyridine (20 mmol). The mixture was cooled to −10 °C, trifluoroacetic anhydride (8 mmol) in anhydrous CH_2Cl_2 (8 mL) was added dropwise. After the reaction was stirred at −5 °C for 4 h, saturated aqueous $NaHCO_3$ solution (20 mL) was added. The organic layer was separated, washed with 1 N aqueous HCl solution, 1 N aqueous $NaHCO_3$ solution, and brine, dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the residue obtained was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 4:1, v/v) to give **6a**.

4.1.6.1. 7-(4-Methoxybenzyl)-6-oxo-5-(1-((trifluoromethyl)sulfonyl)-1*H*-benzo[*d*]imidazol-2-yl)-6,7-dihydrothieno[2,3-*b*]pyridin-4-yl trifluoromethanesulfonate (6a). White solid (85%), mp: 185–187 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.04 (d, J = 15 Hz, Ar-H, 1H), 7.89 (d, J = 16 Hz, Ar-H, 1H), 7.72–7.64 (m, Ar-H, 2H), 7.64 (d, J = 12 Hz, Ar-H, 1H), 7.35 (d, J = 18 Hz, Ar-H, 2H), 7.27 (d, J = 12 Hz, Ar-H, 1H), 6.95 (d, J = 18 Hz, Ar-H, 2H), 5.67 (d, J = 31 Hz, CH_2, 1H), 5.18 (d, J = 31 Hz, CH_2, 1H), 3.74 (s, CH_3, 3H). ESI-MS: m/z = 668 $[M+H]^+$.

4.1.6.2. 2-Chloro-7-(4-methoxybenzyl)-6-oxo-5-(1-((trifluoromethyl)sulfonyl)-1*H*-benzo[*d*]imidazol-2-yl)-6,7-dihydrothieno[2,3-*b*]pyridin-4-yl trifluoromethanesufonate (6b). White solid (83%), mp: 157–158 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.04 (d, J = 16 Hz, Ar-H, 1H), 7.89 (d, J = 17 Hz, Ar-H, 1H), 7.72–7.64 (m, Ar-H, 2H), 7.37 (d, J = 18 Hz, Ar-H, 2H), 7.34 (s, Ar-H, 1H), 6.97 (d, J = 18 Hz, Ar-H, 2H), 5.65 (d, J = 31 Hz, CH_2, 1H), 5.10 (d, J = 31 Hz, CH_2, 1H), 3.75 (s, CH_3, 3H). ESI-MS: m/z = 702 $[M+H]^+$.

4.1.6.3. 2-Bromo-7-(4-methoxybenzyl)-6-oxo-5-(1-((trifluoromethyl)sulfonyl)-1*H*-benzo[*d*]imidazol-2-yl)-6,7-dihydrothieno[2,3-*b*]pyridin-4-yl trifluoromethanesulfonate (6c). Yellow solid (80%), mp: 153–155 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.05 (d, J = 16 Hz, Ar-H, 1H), 7.89 (d, J = 17 Hz, Ar-H, 1H), 7.72–7.64 (m, Ar-H, 2H), 7.42 (s, Ar-H, 1H), 7.37 (d, J = 18 Hz, Ar-H, 2H), 6.97 (d, J = 18 Hz, Ar-H, 2H), 5.65 (d, J = 31 Hz, CH_2, 1H), 5.10 (d, J = 31 Hz, CH_2, 1H), 3.75 (s, CH_3, 3H). ESI-MS: m/z = 746 $[M+H]^+$.

4.1.6.4. 7-(4-Methoxybenzyl)-2-methyl-6-oxo-5-(1-((trifluoromethyl)sulfonyl)-1*H*-benzo[*d*]imidazol-2-yl)-6,7-dihydrothieno[2,3-*b*]pyridin-4-yl trifluoromethanesulfonate (6d). White solid (82%), mp: 161–163 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.04 (d, J = 16 Hz, Ar-H, 1H), 7.89 (d, J = 17 Hz, Ar-H, 1H), 7.70–7.62 (m, Ar-H, 2H), 7.37 (d, J = 18 Hz, Ar-H, 2H), 7.03 (s, Ar-H, 1H), 6.98 (d, J = 18 Hz, Ar-H, 2H), 5.64 (d, J = 31 Hz, CH_2, 1H), 5.08 (d, J = 31 Hz, CH_2, 1H), 3.76 (s, CH_3, 3H), 2.43 (s, CH_3, 3H). ESI-MS: m/z = 682 $[M+H]^+$.

4.1.6.5. 2-Chloro-5-(5-methoxy-1-((trifluoromethyl)sulfonyl)-1*H*-benzo[*d*]imidaz-ol-2-yl)-7-(4-methoxybenzyl)-6-oxo-6,7-dihydrothieno[2,3-*b*]pyridin-4-yl trifluoromethanesulfonate (6e). White solid (85%), mp: 153–155 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.75 (d, J = 18 Hz, Ar-H, 1H), 7.57 (s, Ar-H, 1H), 7.37 (d, J = 18 Hz, Ar-H, 2H), 7.33 (s, Ar-H, 1H), 7.30 (d, J = 18 Hz, Ar-H, 1H), 6.97 (d, J = 18 Hz, Ar-H, 2H), 5.65 (d, J = 31 Hz, CH_2, 1H), 5.09 (d, J = 31 Hz, CH_2, 1H), 3.91 (s, CH_3, 3H), 3.75 (s, CH_3, 3H). ESI-MS: m/z = 732 $[M+H]^+$.

4.1.6.6. 4-(4-Methoxybenzyl)-5-oxo-6-(1-((trifluoromethyl)sulfonyl)-1*H*-benzo[*d*]imidazol-2-yl)-4,5-dihydrothieno[3,2-*b*]pyridin-7-yl trifluoromethanesulfonate (15). White solid (78%), mp: 157–159 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.42 (d, J = 11 Hz, Ar-H, 1H), 8.04 (d, J = 15 Hz, Ar-H, 1H), 7.89 (d, J = 16 Hz, Ar-H, 1H), 7.72–7.64 (m, Ar-H, 3H), 7.35 (d, J = 16 Hz, Ar-H, 2H), 6.92 (d, J = 17 Hz, Ar-H, 2H), 5.65 (d, J = 31 Hz, CH_2, 1H), 5.35 (d, J = 31 Hz, CH_2, 1H), 3.72 (s, CH_3, 3H). ESI-MS: m/z = 668 $[M+H]^+$.

4.1.7. General procedure for the synthesis of 4-substitued amino-3-benzimidazole-thiopyridinone intermediates (7a–z, 16a, and 16b)

To a solution of the bis-triflate (0.3 mmol) prepared above in acetonitrile (5 mL), the corresponding amine (0.8 mmol) dissolved in acetonitrile (3 mL) was added dropwise. The reaction mixture was stirred at room temperature for 2 h, and then evaporated under reduced pressure. The yellow oil obtained was used for the next step without further purification.

4.1.8. General procedure for the synthesis of thienopyridinones derivatives (8a–z, 17a, and 17b)

Compound **10a** (0.25 mmol) was dissolved in a mixture of trifluoroacetic acid and 12 N aqueous HCl solution (7:1, v/v), and heated at 90 °C for 18 h. The solution was cooled to room temperature, and saturated aqueous $NaHCO_3$ was added slowly to basify the solution to pH 9. Then the reaction mixture was extracted with ethyl acetate and brine, dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the residue obtained was purified by silica gel column chromatography (CH_2Cl_2/EtOH/TEA = 500:10:10–500:15:15, v/v/v), and then suspended in water and filtrated to get target compound **8a**.

4.1.8.1. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-4-(piperidin-3-ylamino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8a). White solid (56%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.10 (s, NH, 1H), 12.34 (d, J = 15 Hz, NH, 1H), 9.31 (br, NH, 1H), 7.72 (d, J = 9 Hz, Ar-H, 1H), 7.60 (d, J = 12 Hz, Ar-H, 2H), 7.28 (d, J = 12 Hz, Ar-H, 1H), 7.20–7.17 (m, Ar-H, 2H), 4.50–4.47 (m, CH, 1H), 3.57–3.54 (m, CH_2, 1H), 3.32–3.30 (m, CH_2, 1H), 3.09–3.02 (m, CH_2, 2H), 2.29–2.27 (m, CH_2, 1H), 2.08–2.06 (m, CH_2, 1H), 1.94–1.83 (m, CH_2, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.01, 152.44, 151.80, 148.87, 140.43, 131.72, 122.78, 121.73, 121.31, 116.87, 116.54, 111.79, 111.05, 90.05, 48.28, 47.41, 42.69, 29.82, 20.75. ESI-MS: m/z = 366 $[M+H]^+$.

4.1.8.2. (*S*)-5-(1*H*-Benzo[*d*]imidazol-2-yl)-4-(piperidin-3-ylamino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8b). White solid (55%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.11 (s, NH, 1H), 12.33 (d, J = 15 Hz, NH, 1H), 10.05 (br, NH, 1H), 7.70 (br, Ar-H, 1H), 7.60 (d, J = 12 Hz, Ar-H, 2H), 7.28 (d, J = 12 Hz, Ar-H, 1H), 7.20–7.16 (m, Ar-H, 2H), 4.50–4.45 (m, CH, 1H), 3.55 (d, J = 24 Hz, CH_2, 1H), 3.29 (d, J = 25 Hz, CH_2, 1H), 3.06–2.99 (m, CH_2, 2H), 2.28 (d, J = 19 Hz, CH_2, 1H), 2.06–2.03 (m, CH_2, 1H), 1.93–1.81 (m, CH_2, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.03, 152.47, 151.79, 148.87, 122.82, 121.71, 121.65, 121.37, 116.86, 116.51, 111.86, 111.79, 111.04, 89.96, 48.50, 47.76, 42.88, 29.98, 21.02. ESI-MS: m/z = 366 $[M+H]^+$.

4.1.8.3. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-4-(pyrrolidin-3-ylamino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8c). White solid (51%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.07 (s, NH, 1H), 12.51 (d, J = 13 Hz, NH, 1H), 10.02 (br, NH, 1H), 7.67–7.61 (m, Ar-H, 2H), 7.57 (d, J = 12 Hz, Ar-H, 1H), 7.26 (d, J = 12 Hz, Ar-H, 1H), 7.19–7.16 (m, Ar-H, 2H), 5.01–4.96 (m, CH, 1H), 3.62–3.59 (m, CH_2, 1H), 3.46–3.42 (m, CH_2, 1H), 3.38–3.33 (m, CH_2, 1H), 3.23–3.19 (m, CH_2, 1H), 2.46–2.39 (m, CH_2, 1H), 2.22–2.16 (m, CH_2, 1H). ESI-MS: m/z = 352 $[M+H]^+$.

4.1.8.4. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-4-(piperidin-4-ylamino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8d). White solid (56%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.12 (s, NH, 1H), 12.35 (d, J = 15 Hz, NH, 1H), 8.75 (br, NH, 1H), 7.72–7.70 (m, Ar-H, 1H), 7.58–7.56 (m, Ar-H, 2H), 7.26 (d, J = 12 Hz, Ar-H, 1H), 7.20–7.16 (m, Ar-H, 2H), 4.57–4.56 (m, CH, 1H), 3.41–3.36 (m, CH_2, 2H), 3.28–3.23 (m, CH_2, 2H), 2.29–2.26 (m, CH_2, 2H), 1.96–1.89 (m, CH_2, 2H). ESI-MS: m/z = 366 $[M+H]^+$.

4.1.8.5. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-4-(quinuclidin-3-ylamino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8e). White solid (46%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.09 (s, NH, 1H), 12.62 (d, J = 14 Hz, NH, 1H), 7.72–7.69 (m, Ar-H, 1H), 7.60–7.57 (m, Ar-H, 1H), 7.52 (d, J = 12 Hz, Ar-H, 1H), 7.25 (d, J = 12 Hz, Ar-H, 1H), 7.19–7.16 (m, Ar-H, 2H), 4.57–4.54 (m, CH, 1H), 3.71–3.67 (m, CH_2, 1H), 3.19–3.09 (m, CH_2, 2H), 3.05–3.03 (m, CH_2, 2H), 3.03–3.00 (m, CH_2, 1H), 2.39–2.36 (m, CH, 1H), 2.27–2.25 (m, CH_2, 1H), 1.94–1.88 (m, CH_2, 1H), 1.87–1.75 (m, CH_2, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.12, 152.80, 152.06, 148.63, 140.63, 131.69, 122.90, 121.53, 121.20, 116.59, 115.98, 111.62, 111.22, 89.13, 57.03, 51.15, 46.85, 45.94, 26.90, 24.98, 19.79. ESI-MS: m/z = 392 $[M+H]^+$.

4.1.8.6. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-4-(cyclohexylamino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8f). White solid (55%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.11 (s, NH, 1H), 12.29 (d, J = 16 Hz, NH, 1H), 7.70–7.67 (m, Ar-H, 1H), 7.58–7.55 (m, Ar-H, 1H), 7.45 (d, J = 12 Hz, Ar-H, 1H), 7.22 (d, J = 12 Hz, Ar-H, 1H), 7.18–7.14 (m, Ar-H, 2H), 4.23–4.19 (m, CH, 1H), 2.06–2.04 (m, CH_2, 2H), 1.85–1.82 (m, CH_2, 2H), 1.65–1.49 (m, CH_2, 5H), 1.45–1.39 (m, CH_2, 1H). ESI-MS: m/z = 365 $[M+H]^+$.

4.1.8.7. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-4-((piperidin-4-ylmethyl)amino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8g). White solid (55%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.05 (s, NH, 1H),

12.18 (t, J = 11 Hz, NH, 1H), 7.69–7.67 (m, Ar-H, 1H), 7.64 (d, J = 14 Hz, Ar-H, 1H), 7.56–7.54 (m, Ar-H, 1H), 7.19–7.15 (m, Ar-H, 3H), 3.76–3.73 (m, CH_2, 2H), 3.26–3.23 (m, CH_2, 2H), 2.86–2.80 (m, CH_2, 2H), 2.08–1.95 (m, CH_2, CH, 3H), 1.50–1.42 (m, CH_2, 2H). ESI-MS: m/z = 380 $[M+H]^+$.

4.1.8.8. 4-((2-Aminoethyl)amino)-5-(1*H*-benzo[*d*][*d*]imidazol-2-yl)thieno[2,3-*b*]pyridin-6(7*H*)-one (8h). White solid (59%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.04 (br, NH, 1H), 12.15 (t, J = 10 Hz, NH, 1H), 7.64–7.62 (m, Ar-H, 3H), 7.23 (d, J = 12 Hz, Ar-H, 1H), 7.18–7.15 (m, Ar-H, 2H), 4.01 (dd, J = 25 Hz, 13 Hz, CH_2, 2H), 3.16 (t, J = 14 Hz, CH_2, 2H). ESI-MS: m/z = 326 $[M+H]^+$.

4.1.8.9. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-4-((2-(methylamino)ethyl)amino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8i). White solid (57%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.08 (s, NH, 1H), 12.21 (t, J = 11 Hz, NH, 1H), 10.01 (br, NH, 1H), 7.66 (d, J = 12 Hz, Ar-H, 3H), 7.25 (d, J = 12 Hz, Ar-H, 1H), 7.19–7.16 (m, Ar-H, 2H), 4.16 (dd, J = 25 Hz, 13 Hz, CH_2, 2H), 3.34 (t, J = 14 Hz, CH_2, 2H), 2.66 (s, CH_3, 3H). ESI-MS: m/z = 340 $[M+H]^+$.

4.1.8.10. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-4-((2-(dimethylamino)ethyl)amino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8j). White solid (62%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.99 (br, NH, 1H), 12.23 (br, NH, 1H), 12.20 (t, J = 10 Hz, NH, 1H), 7.67 (d, J = 15 Hz, Ar-H, 2H), 7.53 (br, Ar-H, 1H), 7.17 (d, J = 15 Hz, Ar-H, 1H), 7.16–7.12 (m, Ar-H, 2H), 3.90 (dd, J = 27 Hz, 13 Hz, CH_2, 2H), 2.72 (t, J = 15 Hz, CH_2, 2H), 2.35 (s, $CH_3\times$ 2, 6H). ESI-MS: m/z = 354 $[M+H]^+$.

4.1.8.11. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-4-((2-morpholinoethyl)amino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8k). White solid (62%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.04 (br, NH, 1H), 12.27 (br, NH, 1H), 12.09 (t, J = 8 Hz, NH, 1H), 7.68 (d, J = 12 Hz, Ar-H, 2H), 7.52–7.50 (m, Ar-H, 1H), 7.17 (d, J = 12 Hz, Ar-H, 1H), 7.16–7.14 (m, Ar-H, 2H), 3.94 (dd, J = 21 Hz, 10 Hz, CH_2, 2H), 3.67 (t, J = 9 Hz, $CH_2\times$ 2, 4H), 2.78 (t, J = 12 Hz, CH_2, 2H), 2.58 (br, $CH_2\times$ 2, 4H). ESI-MS: m/z = 396 $[M+H]^+$.

4.1.8.12. Methyl-3-((5-(1*H*-benzo[*d*]imidazol-2-yl)-6-oxo-6,7-dihydrothieno[2,3-*b*]pyridin-4-yl)-amino)propanoate (8l). White solid (62%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.01 (br, NH, 1H), 12.36 (br, NH, 1H), 12.20 (t, J = 8 Hz, NH, 1H), 7.69–7.67 (m, Ar-H, 1H), 7.62 (d, J = 12 Hz, Ar-H, 1H), 7.53–7.52 (m, Ar-H, 1H), 7.23 (d, J = 11 Hz, Ar-H, 1H), 7.18–7.14 (m, Ar-H, 2H), 4.09 (dd, J = 12 Hz, CH_2, 2H), 3.66 (s, CH_3, 3H), 3.92 (t, J = 12 Hz, CH_2, 2H). ESI-MS: m/z = 369 $[M+H]^+$.

4.1.8.13. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-4-((2-hydroxyethyl)amino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8m). White solid (41%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.03 (br, NH, 1H), 12.27 (br, NH, 1H), 12.19 (t, J = 9 Hz, NH, 1H), 7.67–7.65 (m, Ar-H, 1H), 7.62 (d, J = 12 Hz, Ar-H, 1H), 7.56–7.55 (m, Ar-H, 1H), 7.18 (d, J = 11 Hz, Ar-H, 1H), 7.16–7.12 (m, Ar-H, 2H), 3.90–3.87 (m, CH_2, 2H), 3.82–3.79 (m, CH_2, 2H). ESI-MS: m/z = 327 $[M+H]^+$.

4.1.8.14. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-4-(4-(hydroxymethyl)piperidin-1-yl)thieno[2,3-*b*]pyridin-6(7*H*)-one (8n). White solid (40%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.05 (br, NH, 1H), 12.40 (br, NH, 1H), 7.57 (br, Ar-H, 2H), 7.24 (d, J = 11 Hz, Ar-H, 1H), 7.17–7.15 (m, Ar-H, 2H), 7.12 (br, Ar-H, 1H), 3.28–3.25 (m, CH_2, 2H), 3.21 (d, J = 12 Hz, CH_2, 2H), 2.63–2.62 (m, CH_2, 2H), 1.54–1.52 (m, CH_2, 2H), 1.38 (br, CH, 1H), 1.22–1.16 (m, CH_2, 2H). ESI-MS: m/z = 381 $[M+H]^+$.

4.1.8.15. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-4-(piperidin-1-yl)thieno[2,3-*b*]pyridin-6(7*H*)-one (8o). White solid (45%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.36 (br, NH, 1H), 7.57 (br, Ar-H, 2H), 7.26 (d, J = 11 Hz, Ar-H, 1H), 7.17–7.15 (m, Ar-H, 2H), 7.14 (d, J = 12 Hz, Ar-H, 1H), 2.92 (br, $CH_2\times$ 2, 4H), 1.46 (br, $CH_2\times$ 3, 6H). ESI-MS: m/z = 351 $[M+H]^+$.

4.1.8.16. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-4-morpholinothieno[2,3-*b*]pyridin-6(7*H*)-one (8p). White solid (45%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.39 (br, NH, 1H), 7.58 (br, Ar-H, 2H), 7.28 (d, J = 11 Hz, Ar-H, 1H), 7.22 (d, J = 11 Hz, Ar-H, 1H), 7.20–7.16 (m, Ar-H, 2H), 3.57 (t, J = 9 Hz, $CH_2\times$ 2, 4H), 2.94 (t, J = 9 Hz, $CH_2\times$ 2, 4H). ESI-MS: m/z = 353 $[M+H]^+$.

4.1.8.17. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-2-chloro-4-(piperidin-3-ylamino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8q). White solid (53%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.03 (br, NH, 1H), 12.32 (d, J = 15 Hz, NH, 1H), 9.69 (br, NH, 1H), 7.63 (br, Ar-H, 3H), 7.19–7.16 (m, Ar-H, 2H), 4.36–4.34 (m, CH, 1H), 3.47–3.45 (m, CH_2, 1H), 3.23–3.20 (m, CH_2, 1H), 2.96–2.90 (m, CH_2, 2H), 2.25–2.23 (m, CH_2, 1H), 2.01–1.96 (m, CH_2, 1H), 1.88–1.79 (m, CH_2, 2H). ESI-MS: m/z = 400 $[M+H]^+$.

4.1.8.18. (*S*)-5-(1*H*-Benzo[*d*]imidazol-2-yl)-2-chloro-4-(piperidin-3-ylamino)thieno[2,3-*b*]pyridi-*n*-6(7*H*)-one (8r). White solid (52%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.05 (br, NH, 1H), 12.24 (d, J = 16 Hz, NH, 1H), 7.62 (br, Ar-H, 2H), 7.53 (s, Ar-H, 1H), 7.17–7.16 (m, Ar-H, 2H), 4.23–4.18 (m, CH, 1H), 3.35–3.33 (m, CH_2, 1H), 3.06–3.03 (m, CH_2, 1H), 2.82–2.76 (m, CH_2, 2H), 2.20–2.19 (m, CH_2, 1H), 1.91–1.89 (m, CH_2, 1H), 1.81–1.67 (m, CH_2, 2H). ESI-MS: m/z = 400 $[M+H]^+$.

4.1.8.19. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-2-chloro-4-(pyrrolidin-3-ylamino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8s). White solid (49%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.02 (br, NH, 1H), 12.54 (d, J = 13 Hz, NH, 1H), 10.13 (br, NH, 1H), 7.65 (br, Ar-H, 2H), 7.63 (s, Ar-H, 1H), 7.19–7.16 (m, Ar-H, 2H), 5.01–4.95 (m, CH, 1H), 3.66–3.62 (m, CH_2, 1H), 3.51–3.46 (m, CH_2, 1H), 3.43–3.39 (m, CH_2, 1H), 3.24 (dd, J = 24 Hz, 8 Hz, CH_2, 1H), 2.49–2.39 (m, CH_2, 1H), 2.23–2.17 (m, CH_2, 1H). ESI-MS: m/z = 386 $[M+H]^+$.

4.1.8.20. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-2-chloro-4-(quinuclidin-3-ylamino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8t). White solid (53%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.02 (br, NH, 1H), 12.63 (d, J = 15 Hz, NH, 1H), 11.08 (br, NH, 1H), 7.71–7.70 (m, Ar-H, 1H), 7.58–7.56 (m, Ar-H, 2H), 7.20–7.16 (m, Ar-H, 2H), 4.55 (br, CH, 1H), 3.73–3.69 (m, CH_2, 1H), 3.18 (t, J = 15 Hz, CH_2, 2H), 3.10 (t, J = 16 Hz, CH_2, 2H), 3.06–3.05 (m, CH_2, 1H), 2.43–2.42 (m, CH, 1H), 2.28 (br, CH_2, 1H), 1.99–1.93 (m, CH_2, 1H), 1.89–1.80 (m, CH_2, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.39, 157.73, 152.05, 151.04, 146.99, 140.35, 131.75, 121.85, 121.39, 119.42, 116.73, 111.90, 109.91, 90.75, 54.52, 48.81, 45.86, 45.28, 25.91, 22.33, 17.93. ESI-MS: m/z = 426 $[M+H]^+$.

4.1.8.21. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-2-bromo-4-(piperidin-3-ylamino)thieno[2,3-*b*]pyridin-6(7*H*)7*H*)-one (8u). White solid (41%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.03 (br, NH, 1H), 12.28 (d, J = 11 Hz, NH, 1H), 9.82 (br, NH, 1H), 7.67 (s, Ar-H, 1H), 7.63 (br, Ar-H, 2H), 7.18–7.16 (m, Ar-H, 2H), 4.32–4.30 (m, CH, 1H), 3.40–3.39 (m, CH_2, 1H), 3.17–3.15 (m, CH_2, 1H), 2.89–2.85 (m, CH_2, 2H), 2.23–2.21 (m, CH_2, 1H), 1.99–1.95 (m, CH_2, 1H), 1.85–1.76 (m, CH_2, 2H). ESI-MS: m/z = 444 $[M+H]^+$.

4.1.8.22. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-2-bromo-4-(pyrrolidin-3-ylamino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8v). White solid (43%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.00 (br, NH, 1H), 12.55 (d, J = 13 Hz, NH, 1H), 10.27 (br, NH, 1H), 7.71 (s, Ar-H, 1H), 7.66 (br, Ar-H, 2H), 7.20–7.16 (m, Ar-H, 2H), 5.03–4.98 (m, CH, 1H), 3.67–3.63 (m, CH_2, 1H), 3.53–3.47 (m, CH_2, 1H), 3.45–3.40 (m, CH_2, 1H), 3.25 (dd, J = 24 Hz, 7 Hz, CH_2, 1H), 2.48–2.40 (m, CH_2, 1H), 2.25–2.18 (m, CH_2, 1H). ESI-MS: m/z = 430 $[M+H]^+$.

4.1.8.23. (*S*)-2-Chloro-5-(5-methoxy-1*H*-benzo[*d*]imidazol-2-yl)-4-(piperidin-3-ylamino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8w). White solid (58%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.92 (br, NH, 1H), 12.24 (br, NH, 1H), 9.99 (br, NH, 1H), 7.61 (s, Ar-H, 1H), 7.51 (br, Ar-H, 1H), 7.27 (d, J = 18 Hz, Ar-H, 1H), 6.82 (dd, J = 18 Hz, 5 Hz, Ar-H, 1H), 4.36–4.35 (m, CH, 1H), 3.79 (s, CH_3, 3H), 3.50 (d, J = 22 Hz, CH_2, 1H), 3.28 (d, J = 25 Hz, CH_2, 1H), 3.00–2.93 (m, CH_2, 2H), 2.26–2.24 (m, CH_2, 1H), 2.02–2.00 (m, CH_2, 1H), 1.87–1.81 (m, CH_2, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.38, 155.49, 146.84, 138.40, 130.99, 128.76, 127.71, 125.58, 122.05, 119.42, 118.47, 111.61, 109.78, 91.01, 55.39, 48.38, 47.95, 42.93, 30.03, 21.21. ESI-MS: m/z = 430 $[M+H]^+$.

4.1.8.24. 2-Chloro-5-(5-methoxy-1*H*-benzo[*d*]imidazol-2-yl)-4-(quinuclidin-3-ylamino)thieno-[2,3-*b*]pyridin-6(7*H*)-one (8x). White solid (61%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.99 (br, NH, 1H), 12.39 (d, J = 14 Hz, NH, 1H), 7.51 (s, Ar-H, 1H), 7.43 (d, J = 18 Hz, Ar-H, 1H), 7.28 (s, Ar-H, 1H), 6.81 (d, J = 18 Hz, Ar-H, 1H), 4.30 (br, CH, 1H), 3.78 (s, CH_3, 3H), 3.44–3.39 (m, CH_2, 1H), 2.89–2.68 (m, CH_2, 5H), 2.12–2.10 (m, CH, 1H), 2.04 (br, CH_2, 1H), 1.77–1.75 (m, CH_2, 1H), 1.69–1.65 (m, CH_2, 1H), 1.52 (br, CH_2, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.74, 155.53, 152.64, 150.71, 141.42, 132.44, 122.18, 118.71, 117.07, 110.38, 109.88, 99.54, 95.41, 90.32, 55.44, 50.68, 46.83, 45.92, 45.68, 26.83, 24.84, 19.74. ESI-MS: m/z = 456 $[M+H]^+$.

4.1.8.25. 5-(1*H*-Benzo[*d*][*d*]imidazol-2-yl)-2-methyl-4-(piperidin-3-ylamino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8y). White solid (54%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.12 (br, NH, 1H), 12.21 (d, J = 16 Hz, NH, 1H), 9.75 (br, NH, 1H), 7.67–7.58 (m, Ar-H, 2H), 7.28 (s, Ar-H, 1H), 7.18–7.15 (m, Ar-H, 2H), 4.38–4.36 (m, CH, 1H), 3.46–3.42 (m, CH_2, 2H), 3.20–3.17 (m, CH_2, 1H), 2.94–2.89 (m, CH_2, 2H), 2.49 (s, CH_3, 3H), 2.25–2.23 (m, CH_2, 1H), 1.87–1.77 (m, CH_2, 2H). ESI-MS: m/z = 380 $[M+H]^+$.

4.1.8.26. 5-(1*H*-Benzo[*d*]imidazol-2-yl)-2-methyl-4-(pyrrolidin-3-ylamino)thieno[2,3-*b*]pyridin-6(7*H*)-one (8z). White solid(50%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.08 (br, NH, 1H), 12.40 (d, J = 14 Hz, NH, 1H), 9.96 (br, NH, 1H), 7.64 (br, Ar-H, 2H), 7.27 (s, Ar-H, 1H), 7.18–7.15 (m, Ar-H, 2H), 4.97–4.92 (m, CH, 1H), 3.62–3.59 (m, CH_2, 1H), 3.47–3.42 (m, CH_2, 1H), 3.38–3.34 (m, CH_2, 1H), 3.20 (dd, J = 23 Hz, 7 Hz, CH_2, 1H), 2.48 (s, CH_3, 3H), 2.44–2.38 (m, CH_2, 1H), 2.20–2.14 (m, CH_2, 1H). ESI-MS: m/z = 366 $[M+H]^+$.

4.1.8.27. 6-(1*H*-Benzo[*d*]imidazol-2-yl)-7-(piperidin-3-ylamino) thieno[3,2-*b*]pyridin-5(4H)-one (17a). White solid (48%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.18 (br, NH, 1H), 12.33 (d, J = 16 Hz, NH, 1H), 12.10 (br, NH, 1H), 9.15 (br, NH, 1H), 8.13 (d, J = 11 Hz, Ar-H, 1H), 7.73–7.71 (m, Ar-H, 1H), 7.60–7.59 (m, Ar-H, 1H), 7.20–7.18 (m, Ar-H, 2H), 7.11 (d, J = 11 Hz, Ar-H, 1H), 4.58–4.52 (m, CH, 1H), 3.65 (dd, J = 24 Hz, 6 Hz, CH_2, 1H), 3.29 (d, J = 25 Hz, CH_2, 1H), 3.18–3.14 (m, CH_2, 1H), 3.09–3.04 (m, CH_2, 1H), 2.33–2.31 (m, CH_2, 1H), 2.09–2.06 (m, CH_2, 1H), 1.93–1.81 (m, CH_2, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.30, 158.39, 152.39, 151.30, 143.70, 140.46, 132.80, 131.74, 121.76, 121.34, 117.16, 111.85, 106.44, 89.92, 48.15, 47.52, 42.66, 30.41, 20.56. ESI-MS: m/z = 366 $[M+H]^+$.

4.1.8.28. 6-(1*H*-Benzo[*d*]imidazol-2-yl)-7-(pyrrolidin-3-ylamino) thieno[3,2-*b*]pyridin-5(4H)-one (17b). White solid (47%), mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.17 (s, NH, 1H), 12.33 (d, J = 16 Hz, NH, 1H), 12.09 (br, NH, 1H), 8.97 (br, NH, 1H), 8.13 (d, J = 11 Hz, Ar-H, 1H), 7.73–7.71 (m, Ar-H, 1H), 7.59–7.58 (m, Ar-H, 1H), 7.19–7.18 (m, Ar-H, 2H), 7.10 (d, J = 10 Hz, Ar-H, 1H), 4.54–4.52 (m, CH, 1H), 3.64 (dd, J = 24 Hz, 5 Hz, CH_2, 1H), 3.28 (d, J = 25 Hz, CH_2, 1H), 3.18–3.14 (m, CH_2, 1H), 3.08–3.03 (m, CH_2, 1H), 2.32 (d, J = 19 Hz, CH_2, 1H), 2.09–2.07 (m, CH_2, 1H). ESI-MS: m/z = 352 $[M+H]^+$.

4.2. Biological evaluation

4.2.1. In vitro Chk1 kinase assay

The Chk1 inhibitory activities of compounds **8a**–**z**, **17a**, and **17b** were determined using the luminescent ADP-Glo assay kit (Promega Corporation), according to the manufacturer's instructions. White low-volume 384-well polystyrene plates (ProxiPlate-384 Plus, PerkinElmer, Waltham, MA) were used, in which containing 1 μL of each compound dilution and 2 μL of 4 ng/mL solution of Chk1 kinase. The reactions were started via the addition of 2 μL substrates to a final assay volume of 5 μL per well. After incubation for 60 min at room temperature, 5 μL ADP-Glo™ Reagent was added to terminate the kinase reaction and deplete the unconsumed ATP. After an additional incubation for 40 min at room temperature, 10 μL kinase detection reagent was added to convert ADP to ATP and luciferase or luciferin was introduced to detect ATP. After incubation for 40 min at room temperature, luminescence was measured using a plate-reading luminometer. IC_{50} values were calculated using GraphPad Prism 4 software. AZD7762 was used as the positive control. Assays were performed in duplicate and repeated on separate days.

4.2.2. Cell synergy assay

The inhibitory effect of Chk1 inhibitors on multiple myeloma cell growth was assessed with MTS assay. Briefly, in a volume of 100 μL, human myeloma RPMI 8226 cells were seeded in 96-well plates at a density of 5×10^3 cells per well. Cells were added with 100 nM Chk1 inhibitor or with melphalan (1, 3 and 10 μM) or both of them, cultured for 72 h, and incubated with MTS at a final concentration of 0.5 mg/mL for 2–4 h. Optical density was determined at 490 nm (background subtraction at 690 nm) with a SpectraMax 340 microplate reader (Molecular Devices, Sunnyvale, CA, USA).

4.2.3. Kinase selectivity assay

For the kinase selectivity assays, the recombinant Aurora A protein was expressed in *Escherichia coli* cells, CDK2/cyclin A was purchased from Carna company. Aurora A and PKC kinase assays were carried out by using the Invitrogen Z′-LYTETM Kinase Assay kits. CDK2/cyclin A kinase assay was carried out using the HTRF Kinase Assay Kit (Cisbio). Compounds were initially tested at a final concentration of 1 μg/mL. Compounds displaying more than 40% inhibition at 1 μg/mL were further tested for dose-response IC_{50} values. And results represented an average of at least two experiments.

4.3. Molecular docking

Docking analysis were carried out by using C-DOCKER module (Discovery Studio, version 2.1; Accelrys, San Diego, CA, USA, 2008) to compare the binding modes between compound **8t** bound to Chk1 and **8o** bound to Chk1. The X-ray crystal structure of Chk1 (PDB ID: 2GDO) was used for the docking calculation. After removing the ligand and water molecules, the CHARMm-force field was

applied to the protein. And the ATP binding pocket was chosen as the active site with a radius set as 9 Å. The ligands were generated random conformations using CHARMm-based molecular dynamics (1000 steps), and then docked into the defined Chk1 binding site. The other parameters were set as default. The final binding conformation of **8t** and **8o** was determined based on the calculated CDOCKING ENERAGE. The most stable binding modes among the top 10 docking poses of **8t** and **8o** were presented in Figure 4, respectively.

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