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Synthesis and evaluation of 5-substituted 9-hydroxypyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-diones as check point 1 kinase inhibitors

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ABSTRACT

The structure–activity relationship (SAR) of 5-substituted pyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione derivatives **5** was investigated for their potential as Chk1 inhibitors for possible chemo- and radio-potentiators in anticancer chemotherapies. In silico virtual screening helped to optimize the substituent on the phenyl ring, and led to identification of the *m*-carbamoyl group among the 117 analogues tested. Further optimization studies focusing on the docking model of **15** in the active site of Chk1 revealed that **32b** (IC_{50} = 2.8 nM) was a more potent inhibitor than UNC-01.

1. Introduction

Many well-established cancer chemotherapies and radiotherapies exert their therapeutic effect through damage to DNA. However, the activation of cell cycle checkpoints provides an opportunity for cancer cells to repair DNA damage by reducing the antitumor effect of DNA-damaging treatments.[1] The G1 checkpoint mediated by p53 is a major barrier to genomic instability, but over half of all cancer cells are functionally defective for the p53 pathway.[2] These tumor cells are more reliant on the S and G2/M checkpoints to repair the damages. Premature mitotic entry of cells with unrepaired DNA leads to mitotic catastrophe and/or apoptosis. Therefore drugs that abrogate the DNA damage-induced S and G2/M checkpoints should selectively sensitize p53 deficient cancer cells to anticancer therapies.[1,3–5] The G2/M checkpoint is initiated by two sensor kinases, ataxia telangiectasia (ATM) and ATM- and Rad3-related (ATR), and two effector kinases, check point kinase 1 (Chk1) and check point kinase 2 (Chk2). Among them, Chk1 plays a crucial role in the G2/M checkpoint. Phosphorylation of Cdc25A phosphatase by Chk1 inactivates Cdc25A, which is responsible for activating the Cdc2 to regulate the cell-cycle upon removal of the inhibitory phosphate at Tyr15. Consequently, Chk1 inhibitors have been targeted as possible chemo- and radio-potentiators.[6–11]

Several Chk1 inhibitors have entered Phase clinical trials.[12–14] A pyrrolo[3,4-*c*]carbazole motif is one of the representative scaffolds for Chk1 inhibitors. UCN-01 (Fig. 1, **1**), which is an indolocarbazole natural product and currently in Phase II clinical trials[15] in the US, has been shown to enhance the therapeutic activity of DNA-damaging agents in animal models.[16] UCN-01, however, exhibited an extremely long half-life that resulted from avid binding to a human plasma protein, α_1-acid glycoprotein.[17] Although much effort has been expanded to discover chemotypes different from the indolocarbazole, most of these published Chk1 inhibitors originate from hits discovered by high throughput screening (HTS) of large conventional compound libraries,[18–20] except for a few examples.[21] The natural product staurosporine (**2**) did not show severe protein binding in spite of its close similarity in chemical structure to UCN-01, and isogranulatimide (**3**) is also known as a Chk1 inhibitor. Therefore, the pyrrolo[3,4-*c*]carbazole motif is still a good scaffold to develop novel Chk1 inhibitors.[22] It has been reported that the

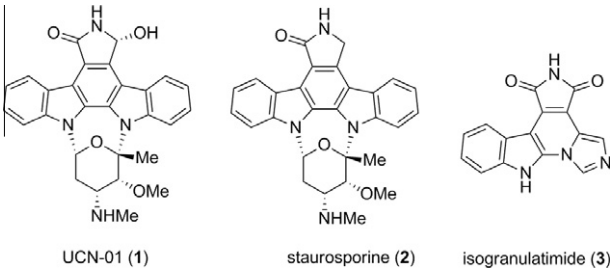

Figure 1. Structures of Chk1 inhibitors.

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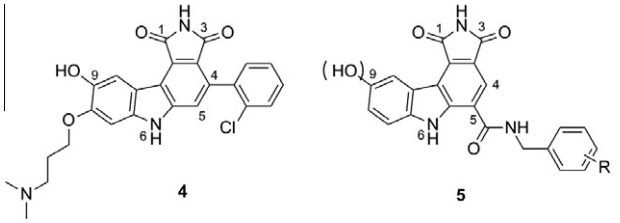


Figure 2. Structures of pyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione derivatives.

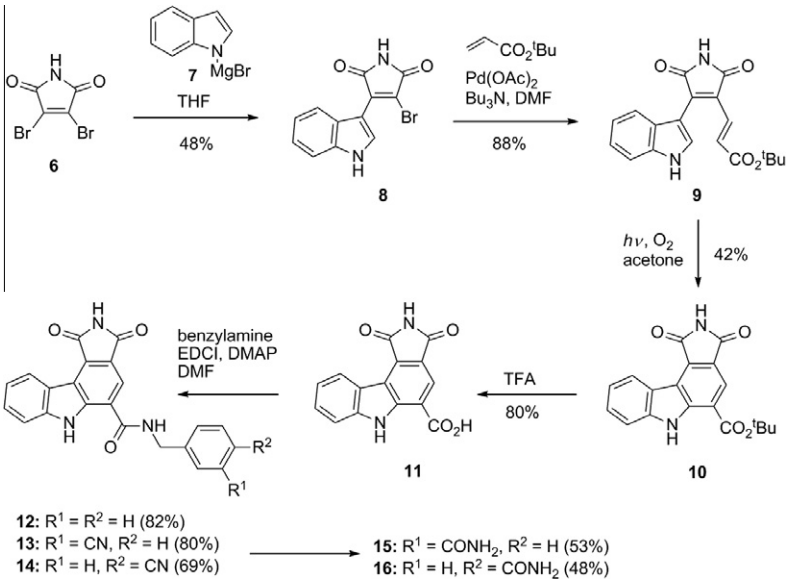
4-phenyl substituted pyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione derivative **4** (Fig. 2) exhibited potent Chk1 and Wee1 inhibitory activity.[23] X-ray crystal structure analysis of complex **4** and the kinase domain of Chk1 revealed that the phenyl group at the 4-position fit inside the hydrophobic pocket.[23a] This structure also indicated that the environment around the 5-position was close to a DFG (aspartic acid-phenylalanine-glycine) loop and a charged region, which binds to the phosphate group of ATP and catalyzes transfer of the phosphate to the substrate serine residue. It has been suggested that a relatively large substituent might be introduced, which could interact with amino acid residues around these regions. This observation prompted us to investigate the structure–activity relationship (SAR) of 5-substituted pyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione derivatives **5**. Here we report the synthesis and SAR of **5**.

2. Results and discussion

2.1. First generation inhibitors

We began our investigation by introducing an *N*-benzylcarbamoyl group as a substituent at the 5-position because of ease of modification as described later. First, compound **12** was synthesized to examine whether its introduction would still allow Chk1 inhibitory activity (Scheme 1). Bromoindolylmaleimide **8**, which was obtained from dibromomaleimide (**6**) and the magnesium salt of indole (**7**),[24] was cross-coupled with *t*-butyl acrylate by the Heck reaction to give **9** in 88% yield. Photochemical 6π-electron cyclization followed by oxidative aromatization gave the desired pyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione **10** in 42% yield. Deprotection of the *t*-Bu group afforded the carboxylic acid **11**. Condensation of **11** with benzylamine using EDCI and DMAP in DMF gave **12** in 82% yield.

The Chk1 inhibitory activity of **10**–**12** was then evaluated with a drug concentration of 10 μM (Table 1). The *t*-butyl ester **10**, the carboxylic acid **11**, and the benzyl amide **12** showed 1%, 46%, and 71% inhibition at 10 μM drug concentrations, respectively, and the IC_{50} value of **12** was 9000 nM. Although the activity was very weak, it was found that the *N*-benzylcarbamoyl group could be introduced at the 5-position. The catalytic activity of most of the protein kinases is regulated by the dynamics of the DFG-loop, the conformation of which is called 'DFG-in' for the active and 'DFG-out' for the inactive form. This aspect consequently makes it difficult to design an inhibitor to interact with this region.[25] However, the catalytic activity of Chk1 was regulated by the C-terminal domain, and the dynamics of the DFG-loop is quite limited among the DFG-in vs. DFG-out.[26] The properties of Chk1 allowed us to apply an in silico docking study in order to optimize the substituent on the phenyl group. A total 117 analogues with *ortho*-, *para*-, or *meta*-substituted *N*-phenyl-, -benzyl-, or -phenethylcarbamoyl groups (Fig. 3) were docked to the active site of the public domain crystal structure published for Chk1 (PDB accession code 1nvq)[26] using the Glide program,[27–29] and the docked structures were ranked by GlideScore. The *m*-carbamoylbenzyl derivative **15** (Scheme 1) showed the best score (GlideScore = −11.34). The docking structure indicated additional interactions of the carbamoyl moiety with Asp148 within the DFG-loop as shown in Figure 4a. In order to confirm the prediction, **15** was synthesized as shown in Scheme 1. As control compounds, the *m*-cyano derivative **13** and the *p*-carbamoyl derivative **16** were also prepared. These were predicted to show low affinities to Chk1 (GlideScore = −5.90 for **13**, −10.36 for **16**, respectively). Condensation of **11** with the corresponding amines gave the amides (80% for **13**, 69% for **14**). Hydration of the nitrile group of **13** and **14** provided the carbamoyl derivatives **15** and **16**, respectively. The Chk1 inhibitory activity of **15** was improved to 83% inhibition at 10 μM concentration and its IC_{50} value was 448 nM, which was a 20-fold increase in the activity compared to that of **12**. On the other hand, the *m*-cyano derivative **13** and the *p*-carbamoyl derivative **16** reduced the potency (48% for **13** and 27% for **16** at 10 μM drug concentrations). A further docking study using the global energy-minimized complex structure of **15** and the active site of Chk1 (PDB accession code 1nvq) indicated additional interactions



Scheme 1.

Table 1
Inhibitory activity of 5-substituted pyrrolo[3,4-*c*]carbazole-1,3-(2*H*,6*H*)-diones

Compound	Inhibitory activity at 10 μM (%)	IC_{50}[a] (nM)
10	1	ND
11	46	ND
12	71	9000
13	48	ND
14	46	ND
15	83	448
16	27	ND

Values are an average of three separate determinations; variation was generally ±15%.
[a] IC_{50}: concentration of drug (nM) to inhibit the phosphorylation of a Cdc25 substrate peptide by Chk1.

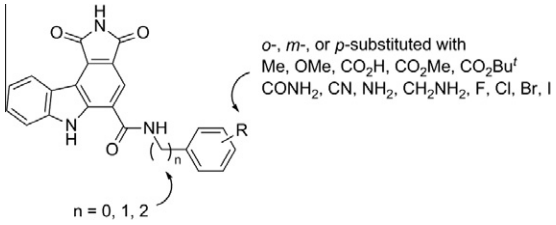


Figure 3. Chemical structures used for virtual screening by Glide®.

of the carbamoyl moiety with Lys132 in addition to that with Asp148 as shown in Figure 4b. These results clearly demonstrate the contribution of the *m*-carbamoyl group to the Chk1 binding. Compound **15** was further screened against a panel of 30 kinases at 0.1 μM concentration as shown in Figure 5. Compound **15** was relatively selective for the Calmodulin kinase (CaMK) family and was suggested to be a potential scaffold to design an inhibitor of the CaMK family including Chk1.

2.2. Second generation inhibitors

The docking model of **15** with Chk1 indicated that there are negatively charged residues, which are associated with binding to the phosphate group of ATP, around the introduced benzyl group of **15**. This model suggested that only one of the hydrogen atoms of the *m*-carbamoyl group makes a contact with Asp148 while the other hydrogen atom is free from interaction with Chk1. Considering these observations, we introduced a positively charged substituent, which can interact with the negatively charged residues, on the nitrogen atom of the *m*-carbamoyl group in order to increase the inhibitory activity of **15**. This design simultaneously allowed us to improve the solubility of **15**. At the time our program began, it was reported that introducing a hydroxyl group at the 9-position increased the binding affinity for Chk1.[23] Therefore, the corresponding 9-hydroxy derivatives were also synthesized.

The synthesis of a series of analogues is shown in Scheme 2. Bromination of 3-methylbenzoic acid **17** by NBS and $(BzO)_2$ gave the bromide **18**, which was substituted by NaN_3 to afford the azide **19**. After the carboxylate group of **19** was protected with a *t*-Bu group, the azide group was reduced by catalytic hydrogenation, and the liberated amine was acylated with acryloyl chloride to give **21**, a precursor to the substituent introduced at the 5 position of the analogues. Heck reaction of **8** or **22**[30] with **21** cleanly provided **23a** or **23b** in 82% and 83% yield, respectively. Photocyclization followed by aromatization gave the pyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione **24a** in 73% and **24b** in 73% yield, respectively. Deprotection of the *t*-Bu group of **24** was effected by aqueous TFA (quant. for **25a**, 90% for **25b**). The *O*-methyl group at the 9-position of **25b** was removed at this stage by heating with pyridinium hydrochloride at 200 °C to give **25c**. The carboxylic acids **25a** and **25c** were then coupled with either mono-Boc protected ethylenediamine or propylenediamine to give **26a**–**27b**, respectively (45% for **26a**, 84% for **26b**, 57% for **27a**, 89% for **27b**). The Boc protecting groups were removed by 4 N HCl to afford the *N*-aminoalkyl analogues **28a**–**29b** (94% for **28a**, 86% for **28b**, 88% for **29a**, 75% for **29b**) as HCl salts. Compounds **28a**–**29b** were treated with *N,N′*-Boc_2-thiourea to give **30a**–**31b**, the Boc deprotection of which provided the *N*-guanidinoalkyl analogues **32a**–**33b** (75% for **32a**, 70% for **32b**, 70% for **33a**, 88% for **33b**) as HCl salts.

The Chk1 inhibitory activity was then evaluated, and the results are summarized in Table 2. Overall, introducing positively charged substituents at the *m*-carbamoyl group on the phenyl ring improved the Chk1 inhibitory activity up to 3.6-fold (**15** vs **32a**). The impact of the hydroxyl group at the 9-position was significant and all the 9-hydroxy analogues exhibited superior activity to the corresponding 9-unsubstituted analogues by a factor of 5–44. Compound **32b** was the most potent inhibitor with an IC_{50} value of 2.8 nM, which was twice as strong as that of UCN-01. Introduction of two substituents, namely the 9-hydroxyl and the *N*-guanidinoethyl groups increased the inhibitory activity by a magnitude of 160 compared to that of **15**. The length of the alkyl group linking the amino or guanidine group to the *m*-carbamoyl moiety did not significantly influence the inhibitory activity except in the case of **32b**. To rationalize the observed SAR from a 3D structural perspective, **32b** was docked in the active site of Chk1 in a manner similar to that of **15**. This model suggested that a very similar binding mode to that reported for **4** with Wee 1 (Fig. 6a,

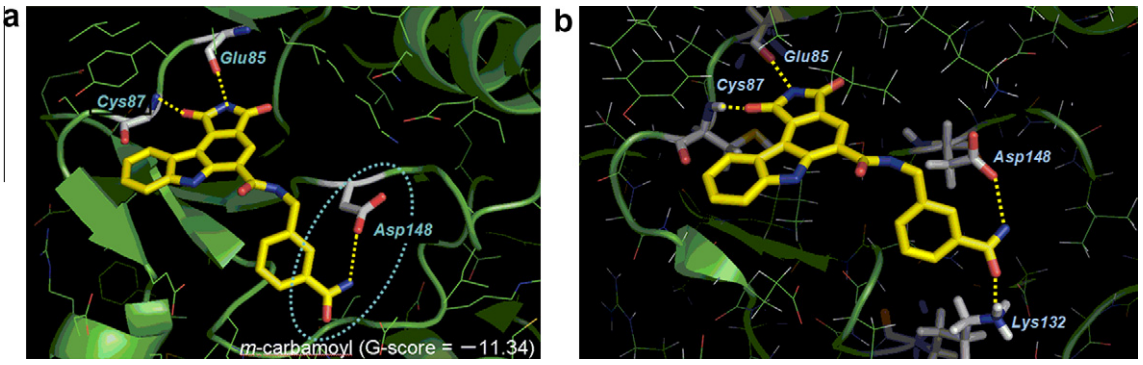


Figure 4. Docking model of **15** and Chk1. (a) Docking pose obtained by a virtual screening with Glide. (b) Proposed model for the global energy-minimum conformation of the complex **15** and Chk1 by conformation search using Macromodel.

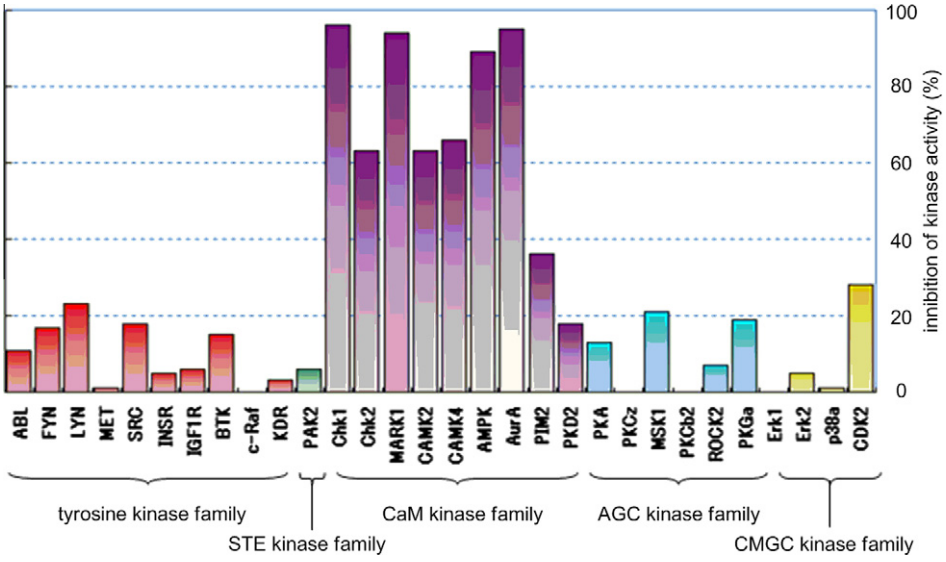


Figure 5. Kinase panel assay. Inhibitory activity of **15** at 0.1 μM was measured.

PDB code: 3biz), with matching occupancy of the binding pocket and key hydrogen bonds to the hinge domain (Glu85 and the hydrogen atom at the 2-position, and Cys87 and the carbonyl oxygen at the 3-position and the 9-hydroxyl group) as shown in Figure 6b. The substituent introduced at the 5-position made a contact with Asp148 in the DFG-loop; however, the mode of interaction was different from suggested for **15** (Fig. 4b). The orientation of the phenyl ring was distorted, and the *m*-carbamoyl group at the phenyl ring interacted only with Asp148. However, the guanidine group interacted with the two oxygen atoms of Asp148 through a hydrogen bond network.

An ideal Chk1 inhibitor would show no cytotoxicity or off-target effects in use as a single agent. None of the compounds listed in Table 2 showed any cytotoxicity against human acute lymphoma CCR-CEM and ML-1a cells up to 10 μM drug concentrations. Preliminary results to evaluate the potential of **28b** as a chemo-potentiator revealed that **28b** showed a very weak combination activity with the DNA-damaging agent, SN-38, at a drug concentration of 5 μM, resulting in up to fourfold sensitization of the IC_{50} value when **28b** was co-administered with SN-38 to HeLa cells.

3. Conclusion

Here we investigated the structure–activity relationship (SAR) of 5-substituted pyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione derivatives **5**. In silico virtual screening helped optimize the substituent on the phenyl ring, identifying the *m*-carbamoyl group among 117 analogues. Further optimization studies focusing on the docking model of **15** to the active site of Chk1 were pursued and it was found that the inhibitor **32b** (IC_{50} = 2.8 nM) was more potent than UNC-01.

4. Experimental

4.1. General experimental methods

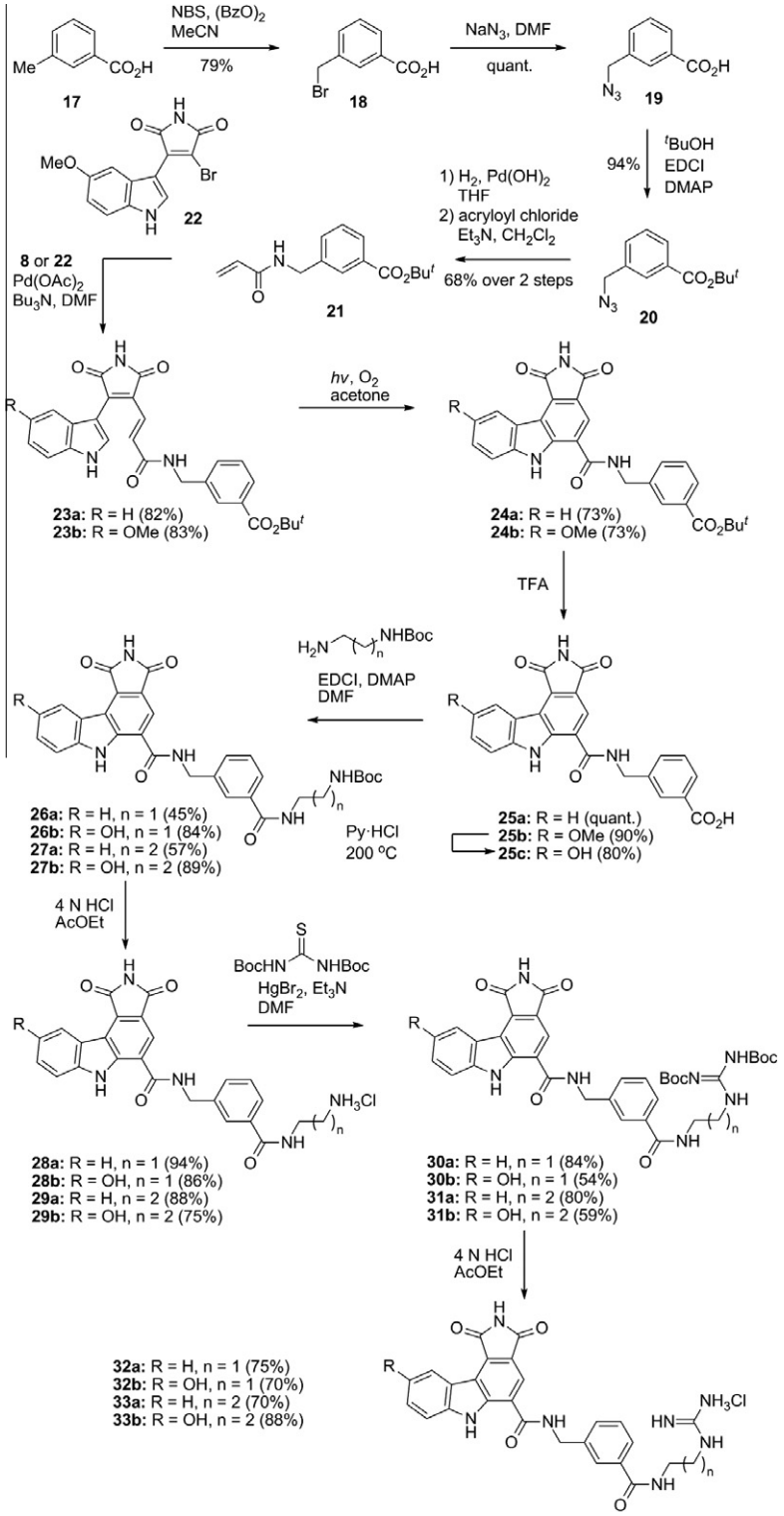
NMR spectra were obtained on a JEOL EX270, JEOL GX270, JEOL AL400 or JEOL ECA500, and were reported in parts per million (δ) relative to tetramethylsilane (0.00 ppm) as internal standard otherwise noted. Coupling constant (*J*) was reported in hertz (Hz). Abbreviations of multiplicity were as follows; s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, br: broad. Data were presented as follows; chemical shift (multiplicity, integration, coupling constant). Assignment was based on ^1H–^1H COSY, HMBC and HMQC NMR spectra. FABMS was obtained on a JEOL JMS-HX101 or JEOL JMS-700TZ. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60F254 plates. Normal-phase column chromatography was performed on Merck silica gel 5715 or Kanto Chemical silica gel 60 N (neutral). Flash column chromatography was performed on Merck silica gel 60.

4.2. 4-[(2-*tert*-Butoxycarbonyl)vinyl]-3,4-dihydro-3-(1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione (9)

A mixture of **8** (1.9 g, 6.4 mmol), *t*-butyl acrylate (1.9 mL, 12.7 mmol), $Pd(OAc)_2$ (143 mg, 0.6 mmol) and Bu_3N (1.7 mL, 7.1 mmol) in DMF (65 mL) was stirred at 60 °C for 2 h. The mixture was partitioned between AcOEt (150 mL) and H_2O (100 mL), and the organic layer was washed with brine (200 mL × 2), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, 5 × 15 cm, hexane/AcOEt = 3:1) to give **9** (1.9 g, 88%) as a red solid. ^1H NMR (500 MHz, DMSO-d_6) δ 12.20 (br s, 1H, indole-N*H*), 11.14 (br s, 1H, imide-N*H*), 7.94 (s, 1H, H-2′), 7.56 (d, 1H, H-4′, *J* = 8.1 Hz), 7.52 (d, 1H, CH=*CH*CO_2Bu^t, *J* = 16.1 Hz), 7.50 (d, 1H, H-7′, *J* = 8.1 Hz), 7.21 (t, 1H, H-5′, *J* = 6.9 Hz), 7.14 (t, 1H, H-6′, *J* = 6.9 Hz), 6.78 (d, 1H, *CH*=$CHCO_2Bu^t$, *J* = 16.1 Hz), 1.39 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.8, 171.1, 165.2, 138.0, 136.9, 132.5, 132.2, 125.1, 122.9, 122.6, 121.1, 120.6, 112.7, 104.9, 80.3, 27.7; ESIMS-LR *m/z* 338 $[M+H]^+$; ESIMS-HR calcd for $C_{19}H_{18}N_2O_4$ 338.3572, found 338.1249.

4.3. 5-*tert*-Butoxycarbonyl-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (10)

A solution of **9** (1.5 g, 4.3 mmol) in THF (170 mL) was irradiated with a medium-pressure mercury lamp (400 W) under oxygen atmosphere for 16 h. The solvent was removed, and the residue was triturated from hexane/AcOEt to give **10** (630 mg, 42%) as a yellow solid. ^1H NMR (500 MHz, DMSO-d_6) δ 12.13 (br s, 1H, indole-N*H*), 11.41 (br s, 1H, imide-N*H*), 8.82 (d, 1H, H-10, *J* = 6.9 Hz), 8.17 (s, 1H, H-4), 7.82 (d, 1H, H-7, *J* = 8.1 Hz), 7.57 (t, 1H, H-9, *J* = 6.9 Hz), 7.33 (t, 1H, H-8, *J* = 6.9 Hz), 1.65 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.7, 169.6, 164.5, 143.4, 142.3, 129.6, 128.8, 124.7, 122.7, 121.0, 120.7, 120.4, 119.4, 116.1, 113.0, 82.2, 27.9; ESIMS-LR *m/z* 336 $[M+H]^+$; ESIMS-HR calcd for $C_{19}H_{16}N_2O_4$ 336.3413, found 336.1101.

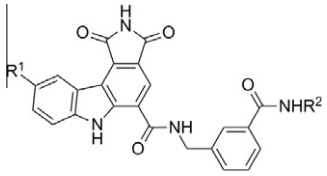


Scheme 2.

4.4. 5-Carboxy-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (11)

Compound **10** (322 mg, 0.91 mmol) was treated with TFA (25 mL) at room temperature for 3 h. The precipitate was filtered and washed with H_2O to give **11** (218 mg, 80%) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.85 (br s, 1H, CO_2H), 12.01 (s, 1H, indole-N*H*), 11.39 (s, 1H, imide-N*H*), 8.83 (d, 1H, H-10, J = 7.9 Hz), 8.21 (s, 1H, H-4), 7.82 (d, 1H, H-7, J = 8.0 Hz), 7.57 (t, 1H, H-9, J = 6.9 Hz), 7.31 (t, 1H, H-8, J = 6.9 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.7, 169.6, 166.8, 143.3, 142.2, 129.7, 128.7, 124.7, 122.9, 121.0, 120.8, 120.6, 119.5, 116.1, 113.0; ESIMS-LR *m/z* 280 $[M+H]^+$; ESIMS-HR calcd for $C_{15}H_8N_2O_4$ 280.2350, found 280.0476.

Table 2
Inhibitory activity of 5-substituted pyrrolo[3,4-*c*]carbazole-1,3-(2*H*,6*H*)-diones



Compound	R^1	R^2	IC_{50}[a] (nM)
15	H	H	448
28a	H	$CH_2CH_2NH_3Cl$	336
28b	OH	$CH_2CH_2NH_3Cl$	31
29a	H	$CH_2CH_2CH_2NH_3Cl$	251
29b	OH	$CH_2CH_2CH_2NH_3Cl$	46
32a	H	$CH_2CH_2NHC(NH)NH_3Cl$	123
32b	OH	$CH_2CH_2NHC(NH)NH_3Cl$	2.8
33a	H	$CH_2CH_2CH_2NHC(NH)NH_3Cl$	235
33b	OH	$CH_2CH_2CH_2NHC(NH)NH_3Cl$	22
UCN-01			5.6

Values are an average of three separate determinations; variation was generally ±15%.
[a] IC_{50}: concentration of drug (nM) to inhibit the phosphorylation of a Cdc25 substrate peptide by Chk1.

4.5. 5-(3-Cyanobenzylaminocarbonyl)-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (13)

A mixture of **11** (15 mg, 0.054 mmol), EDCI (15 mg, 0.081 mmol), DMAP (9.8 mg, 0.081 mmol) and *m*-cyanobenzylamine hydrochloride (13 mL, 0.081 mmol) in DMF (500 μL) was stirred at room temperature for 5 days. The mixture was partitioned between AcOEt (50 mL) and H_2O (20 mL × 2), and the organic layer was washed with 1 N aqueous HCl (50 mL), saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and concentrated. The residue was triturated from hexane/AcOEt to give **13** (17 mg, 0.43 mmol, 80%) as a yellow solid. ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.16 (s, 1H, indole-N*H*), 11.37 (s, 1H, imide-N*H*), 9.70 (t, 1H, amide-N*H*, J = 5.7 Hz), 8.84 (d, 1H, H-10, J = 8.0 Hz), 8.42 (s, 1H, H-4), 7.90–7.30 (m, 7H, H-7, H-8, H-9, H-2′, H-4′, H-5′, and H-6′), 4.63 (d, 2H, C*H*$_2$, J = 5.7 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.6, 166.5, 141.8, 132.9, 131.5, 131.3, 130.2, 129.0, 128.8, 125.0, 121.0, 120.8, 119.4, 119.4, 118.6, 113.7, 111.8, 42.7; ESIMS-LR *m/z* 417 [M+Na]$^+$; ESIMS-HR calcd for $C_{23}H_{14}N_4O_3Na$ 417.0946, found 417.0976.

4.6. 5-(3-Carbamoylbenzylaminocarbonyl)-1,3-dihydropyrrolo-[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (15)

A mixture of **13** (15 mg, 0.038 mmol), 35% aqueous H_2O_2 (17 μL, 0.18 mmol) and 6 N aqueous NaOH (2.2 μL, 0.013 mmol) in THF (2 mL) and EtOH (1 mL) was stirred at room temperature for 22 h. The mixture was partitioned between AcOEt (30 mL) and H_2O (20 mL × 2). The organic layer was washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated. The residue was triturated from hexane/AcOEt to give **6** (8 mg, 0.020 mmol, 53%) as a yellow solid. ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.10 (br s, 1H, indole-N*H*), 11.35 (br s, 1H, imide-N*H*), 9.66 (br s, 1H, amide-N*H*), 8.80 (d, 1H, H-10, J = 8.0 Hz), 8.42 (s, 1H, H-4), 7.89 (s, 1H, H-2′), 7.80 (d, 1H, H-7, J = 8.0 Hz), 7.73 (t, 1H, H-4′, J = 7.4 Hz), 7.56–7.53 (m, 3H, H-5′, H-6′, and N*H*$_2$), 7.41 (t, 1H, H-9, J = 7.6 Hz), 7.32 (t, 1H, H-8, J = 7.4 Hz), 4.61 (d, 2H, C*H*$_2$, J = 8.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.6, 166.5, 141.8, 132.9, 131.5, 131.3, 130.2, 128.8, 125.0, 121.0, 120.8, 119.4, 119.4, 118.6, 114.1, 113.7, 111.8, 42.7; FABMS-LR *m/z* 435 [M+Na]$^+$; FABMS-HR calcd for $C_{23}H_{16}N_4O_4Na$ 435.1091, found 435.1112.

4.7. 5-(4-Cyanobenzylaminocarbonyl)-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (14)

According to the procedure for the preparation of **13**, **14** (13 mg, 0.037 mmol, 69%) was obtained from **11** (15 mg, 0.054 mmol) and *p*-cyanobenzylamine hydrochloride (13 mg, 0.081 mmol) as a yellow solid. ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.16 (s, 1H, indole-N*H*), 11.37 (s, 1H, imide-N*H*), 9.70 (t, 1H, amide-N*H*, J = 5.7 Hz), 8.84 (d, 1H, H-10, J = 8.0 Hz), 8.42 (s, 1H, H-4), 7.90–7.30 (m, 7H, H-7, H-8, H-9, H-2′, H-3′, H-5′, and H-6′), 4.63 (d, 2H, CH_2, J = 5.7 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.9, 170.8, 166.6, 146.1, 132.9, 129.0, 128.7, 128.5, 125.0, 121.2, 120.4, 120.3, 119.5, 119.4, 118.5, 114.0, 113.9, 110.1, 43.1; ESIMS-LR *m/z* 417 [M+Na]$^+$; ESIMS-HR calcd for $C_{23}H_{14}N_4O_3Na$ 417.0944, found 417.0971.

4.8. 5-(4-Carbamoylbenzylaminocarbonyl)-1,3-dihydropyrrolo-[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (16)

According to the procedure for the preparation of **15**, **16** (7 mg, 0.018 mmol, 48%) was obtained from **14** (15 mg, 0.038 mmol) as a yellow solid. ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.16 (br s, 1H, indole-N*H*), 11.35 (br s, 1H, imide-N*H*), 9.69 (br s, 1H, amide-N*H*), 8.84 (d, 1H, H-10, J = 7.9 Hz), 8.45 (s, 1H, H-4), 7.92–7.77 (m, 4H, aromatic), 7.63–7.32 (m, 5H, aromatic, N*H*$_2$), 4.63 (d, 2H, C*H*$_2$, J = 5.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.8, 170.8, 166.6, 142.1, 132.9, 128.7, 128.5, 125.0, 121.2, 120.4, 120.3, 119.5, 119.4, 118.5, 112.4, 114.0, 113.9, 110.1, 43.1; ESIMS-LR *m/z* 435 [M+Na]$^+$; ESIMS-HR calcd for $C_{23}H_{16}N_4O_4$ 435.1076, found 435.10697.

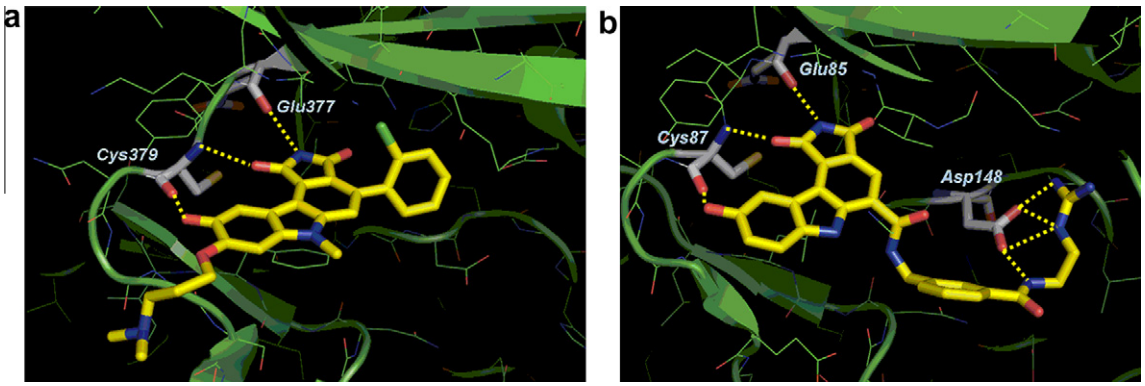


Figure 6. Structural comparison of **4** and **32b**. (a) X-ray crystal structure of the **4** and Wee1 active site (PDB code: 3biz). (b) Docking model of **32b** and Chk1 active site. Proposed model for the global energy-minimum conformation of the complex was calculated by conformation search using Macromodel.

4.9. 3-(Bromomethyl)benzoic acid (18)

A mixture of *m*-toluic acid (10.1 g, 73.5 mmol), *N*-bromosuccinimide (14.6 g, 80.8 mmol) and benzoyl peroxide (356 mg, 1.47 mmol) in MeCN (800 mL) was gently heated under reflux for 4 h. After concentrated, the residue was dissolved in Et_2O (300 mL) and cooled to −78 °C, and the precipitate was filtered off. The filtrate was concentrated in vacuo. Repeating this procedure twice gave **18** (12.2 g, 79%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.01 (s, 1H, H-2), 7.86 (d, 1H, H-6, J = 8.1 Hz), 7.67 (d, 1H, H-4, J = 7.5 Hz), 7.49 (t, 1H, H-5, J = 7.8 Hz), 4.77 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.9, 138.7, 133.7, 131.3, 130.1, 129.2, 129.1, 33.7; ESIMS-LR *m/z* 214, 216 $[M+H]^+$; ESIMS-HR calcd for $C_8H_7BrO_2$ 213.9629, found 213.9617.

4.10. 3-(Azidomethyl)benzoic acid (19)

A mixture of **18** (12.0 g, 55.7 mmol) and NaN_3 (5.4 g, 83.7 mmol) in DMF (600 mL) was stirred at room temperature for 2 h. The mixture was partitioned between AcOEt (500 mL) and H_2O (300 mL), and the organic layer was washed with brine (300 mL × 2), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, 3 × 12 cm, hexane/AcOEt = 3:1) to give **19** (9.8 g, quant.) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.94 (s, 1H, H-2), 7.91 (d, 1H, H-6, J = 8.1 Hz), 7.60 (d, 1H, H-4, J = 7.6 Hz), 7.51 (t, 1H, H-5, J = 7.9 Hz), 4.54 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.1, 136.3, 132.8, 131.3, 129.2, 129.1, 129.1, 53.1; ESIMS-LR *m/z* 177 $[M^+]$; ESIMS-HR calcd for $C_8H_7N_3O_2$ 177.0536, found 177.0336.

4.11. *tert*-Butyl 3-(Azidomethyl)benzoate (20)

A mixture of **19** (9.8 g, 55.3 mmol), EDCI (15.9 mg, 82.9 mmol) and DMAP (10.1 g, 82.9 mmol) in *t*-BuOH (300 mL) and DMF (200 mL) was stirred at room temperature for 4 h. The mixture was concentrated in vacuo to remove *t*-BuOH. The mixture was partitioned between AcOEt (400 mL) and H_2O (200 mL), and the organic layer was washed with 1 N aqueous HCl (300 mL), saturated aqueous $NaHCO_3$ (300 mL) and brine (300 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, 3 × 10 cm, hexane/AcOEt = 6:1) to give **20** (13.6 g, 94%) as a brown oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.89 (s, 1H, H-2), 7.86 (d, 1H, H-6, J = 8.1 Hz), 7.60 (d, 1H, H-4, J = 7.6 Hz), 7.52 (t, 1H, H-5, J = 7.8 Hz), 4.54 (s, 2H, CH_2), 1.52 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.6, 136.3, 132.8, 131.8, 129.0, 128.8, 128.7, 80.9, 53.1, 27.7; ESIMS-LR *m/z* 233 $[M+H]^+$; ESIMS-HR calcd for $C_{12}H_{15}N_3O_2$ 233.1158, found 233.0558.

4.12. *tert*-Butyl 3-Acrylamidomethylbenzoate (21)

A mixture of **20** (1.06 g, 4.55 mmol) and $Pd(OH)_2$ (130 mg) in THF (400 mL) was vigorously stirred under H_2 atmosphere for 1 h. The insoluble was filtered off through a Celite pad, and the filtrate was concentrated in vacuo. The residue in CH_2Cl_2 (50 mL) was treated with acryloyl chloride (481 μL, 5.92 mmol), Et_3N (824 μL, 5.92 mmol) at room temperature for 2 h. The mixture was partitioned between AcOEt (200 mL) and H_2O (200 mL). The organic layer was washed with 1 N aqueous HCl (200 mL), saturated aqueous $NaHCO_3$ (200 mL) and brine (200 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, 1.5 × 18 cm, hexane/AcOEt = 6:1) to give **21** (745 mg, 68% over 2 steps) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.95 (t, 1H, amide-N*H*, J = 6.3 Hz), 7.79 (s, 1H, H-2), 7.77 (s, 1H, H-6), 7.50 (d, 1H, H-4, J = 8.0 Hz), 7.44 (t, 1H, H-5, J = 7.8 Hz), 6.27 (dd, 1H, CH_2=C*H*, J = 9.7, 17.1 Hz), 6.12 (dd, 1H, CH_2=CH, J = 2.3, 17.2 Hz), 5.62 (dd, 1H, CH_2=CH, J = 2.3, 9.8 Hz), 4.77 (s, 2H, CH_2), 1.52 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.9, 164.7, 139.9, 132.0, 131.5, 131.4, 128.7, 127.8, 127.6, 125.6, 80.8, 41.9, 27.8; ESIMS-LR *m/z* 261 $[M+H]^+$; ESIMS-HR calcd for $C_{15}H_{19}NO_3$ 261.1364, found 261.1333.

4.13. *tert*-Butyl 3-{2*E*-3-[2,5-dihydro-4-(1*H*-indol-3-yl)-2,5-dioxo-1*H*-pyrrol-3-yl]acrylamidomethyl} benzoate (23a)

A mixture of **8** (389 mg, 1.3 mmol), **21** (701 mg, 2.7 mmol), $Pd(OAc)_2$ (31 mg, 0.13 mmol) and Bu_3N (479 μL, 2.0 mmol) in DMF (15 mL) was stirred at 60 °C for 5 h. The mixture was partitioned between AcOEt (80 mL) and H_2O (50 mL × 2), and the organic layer was washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, 3 × 12 cm, hexane/AcOEt = 3:1) to give **23a** (523 mg, 82%) as a red solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.11 (s, 1H, indole-N*H*), 11.08 (br s, 1H, imide-N*H*), 8.95 (br s, 1H, amide-N*H*), 7.87 (s, 1H, H-2), 7.77 (s, 1H, H-l), 7.55 (d, 1H, H-4′, J = 8.0 Hz), 7.50–7.39 (m, 4H, aromatic), 7.47 (d, 1H, C*H*=CHCONH, J = 16.4 Hz), 7.20 (d, 1H, CH=C*H*CONH, J = 16.3 Hz), 7.18 (t, 1H, H-5′, J = 7.5 Hz), 7.10 (t, 1H, H-6′, J = 7.5 Hz), 4.38 (d, 2H, CH_2, J = 5.8 Hz), 1.53 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.5, 171.9, 165.4, 165.3, 140.4, 137.3, 132.4, 131.9, 131.9, 129.2, 129.0, 128.4, 128.4, 128.1, 125.8, 124.8, 123.2, 121.4, 121.1, 113.0, 105.2, 81.3, 42.7, 28.3; ESIMS-LR *m/z* 494 $[M+Na]^+$; ESIMS-HR calcd for $C_{27}H_{25}N_3O_5Na$ 494.1682, found 494.1662.

4.14. *tert*-Butyl 3-{2*E*-3-[2,5-dihydro-4-(5-methoxy-1*H*-indol-3-yl)-2,5-dioxo-1*H*-pyrrol-3-yl]acrylamidomethyl} benzoate (23b)

According to the procedure for the preparation of **23a**, **23b** (128 mg, 83% as a red solid) was obtained from **22**[30] (100 mg, 0.31 mmol), **21** (123 mg, 0.47 mmol), $Pd(OAc)_2$ (7 mg, 0.31 mmol), Bu_3N (112 μL, 0.47 mmol). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.03 (s, 1H, indole-N*H*), 11.08 (br s, 1H, imide-N*H*), 8.98 (br s, 1H, amide-N*H*), 7.88–776 (m, 3H, aromatic), 7.58 (d, 1H, C*H*=CHCONH, J = 15.4 Hz), 7.50–7.39 (m, 4H, aromatic), 7.15 (d, 1H, CH=C*H*CONH, J = 15.5 Hz), 6.83 (dd, 1H, benzyl-5, J = 7.8 Hz), 4.39 (d, 2H, CH_2, J = 6.3 Hz), 3.76 (s, 3H, methyl), 1.50 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.0, 171.5, 164.9, 164.9, 154.6, 139.9, 136.7, 131.9, 131.6, 131.4, 128.9, 128.6, 127.8, 127.6, 127.2, 125.8, 122.9, 113.3, 113.1, 104.9, 102.1, 80.8, 54.8, 42.2, 27.8; FABMS-LR *m/z* 524 $[M+Na]^+$; FABMS-HR calcd for $C_{28}H_{27}N_3O_6Na$ 524.1809, found 524.1809.

4.15. 5-[(3-*tert*-Butoxycarbonyl)benzylaminocarboyl]-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (24a)

A solution of **23a** (554 mg, 1.1 mmol) in THF (170 mL) was irradiated for 1 day with a medium-pressure mercury lamp (400 W) under oxygen atmosphere. The solvent was removed, and the residue was triturated from hexane/AcOEt to give **14** (403 mg, 0.8 mmol, 73%) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.17 (s, 1H, indole-N*H*), 11.36 (s, 1H, imide-N*H*), 9.72 (t, 1H, amide-N*H*, J = 5.2 Hz), 8.84 (d, 1H, H-10, J = 8.0 Hz), 8.44 (s, 1H, H-4), 7.94 (s, 1H, H-6′), 7.82 (d, 1H, H-7, J = 8.0 Hz), 7.80 (d, 1H, H-4′, J = 8.0 Hz), 7.65 (d, 1H, H-2′, J = 8.0 Hz), 7.56 (t, 1H, H-9, J = 7.2 Hz), 7.48 (t, 1H, H-8, J = 7.4 Hz), 7.32 (t, 1H, H-3′, J = 8.0 Hz), 4.64 (d, 2H, CH_2, J = 5.7 Hz), 1.50 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.9, 169.9, 165.7, 164.9, 142.8, 142.2, 139.9, 132.0, 131.4, 128.7, 128.5, 128.0, 127.6, 124.6, 122.9, 120.7, 120.3, 119.4, 119.0, 117.9, 113.0, 80.8, 42.5, 27.8; ESIMS-LR *m/z* 469 $[M+H]^+$; ESIMS-HR calcd for $C_{27}H_{23}N_3O_5$ 469.1635, found 469.1633.

4.16. 5-[(3-*tert*-Butoxycarbonyl)benzylaminocarboyl]-9-methoxy-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (24b)

According to the procedure for the preparation of **24a**, **24b** (73 mg, 73% as a yellow solid) was obtained from **23b** (100 mg, 0.31 mmol). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.03 (s, 1H, indole-N*H*), 11.32 (s, 1H, imide-N*H*), 9.71(t, 1H, amide-N*H*, J = 5.7 Hz), 8.42 (s, 1H, H-4), 8.40 (d, 1H, H-9, J = 2.0 Hz), 7.99 (s, 1H, H-6′), 7.83 (d, 1H, H-7, J = 7.7 Hz), 7.73 (d, 1H, H-4′, J = 7.8 Hz), 7.66 (d, 1H, H-2′, J = 7.8 Hz), 7.48 (t, 1H, H-3′, J = 7.8 Hz), 7.21 (dd, 1H, H-8, J = 2.2, 7.7 Hz), 4.64 (d, 2H, C*H*$_2$, J = 5.7 Hz), 3.76 (s, 3H, methyl), 1.51 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.0, 165.7, 164.9, 154.0, 143.1, 140.0, 137.0, 132.0, 131.4, 128.7, 128.6, 128.0, 127.6, 128.1, 122.4, 120.3, 119.8, 119.0, 118.0, 117.7, 113.7, 106.5, 80.8, 55.5, 42.5, 27.8; EIMS-LR *m/z* 499 [M$^+$]; EIMS-HR calcd for $C_{28}H_{25}N_3O_6$ 499.1753, found 499.1771.

4.17. 5-[(3-Carboxy)benzylaminocarboyl]-1,3-dihydropyrrolo-[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (25a)

Compound **24a** (322 mg, 0.89 mmol) was treated with TFA (25 mL) at room temperature for 3 h. The precipitate was filtered and washed with H_2O to give **25a** (218 mg, quant.) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.56 (br s, 1H, CO_2H), 12.17 (s, 1H, indole-N*H*), 11.36 (s, 1H, imide-N*H*), 9.73 (t, 1H, amide-N*H*, J = 5.2 Hz), 8.84 (d, 1H, H-10, J = 8.0 Hz), 8.45 (s, 1H, H-4), 7.99 (s, 1H, H-6′), 7.84 (d, 1H, H-7, J = 8.0 Hz), 7.82 (d, 1H, H-4′, J = 7.9 Hz), 7.65 (d, 1H, H-2′, J = 7.9 Hz), 7.56 (t, 1H, H-9, J = 7.2 Hz), 7.49 (t, 1H, H-8, J = 7.4 Hz), 7.32 (t, 1H, H-3′, J = 7.9 Hz), 4.64 (d, 2H, C*H*$_2$, J = 5.7 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.4, 170.4, 167.8, 166.2, 143.4, 142.7, 140.5, 132.6, 131.4, 129.2, 129.0, 128.8, 128.5, 123.5, 121.2, 120.9, 119.9, 119.5, 118.4, 113.4; FABMS-LR *m/z* 412 [M+H]$^+$; FABMS-HR calcd for $C_{23}H_{15}N_3O_5$ 413.1012, found 412.0867.

4.18. 5-[(3-Carboxy)benzylaminocarboyl]-9-methoxy-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (25b)

According to the procedure for the preparation of **25a**, **25b** (40 mg, 90%) was obtained from **24b** (50 mg, 0.1 mmol) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.98 (br s, 1H, CO_2*H*), 12.04 (s, 1H, indole-N*H*), 11.33 (s, 1H, imide-N*H*), 9.70 (t, 1H, amide-N*H*, J = 5.7 Hz), 8.42 (s, 1H, H-4), 8.40 (d, 1H, H-10, J = 2.2 Hz), 7.98 (s, 1H, H-6′), 7.83 (d, 1H, H-7, J = 7.8 Hz), 7.73 (d, 1H, H-4′, J = 8.0 Hz), 7.66 (d, 1H, H-2′, J = 8.0 Hz), 7.48 (t, 1H, H-3′, J = 8.0 Hz), 7.21 (dd, 1H, H-8, J = 2.2, 7.8 Hz), 4.64 (d, 2H, C*H*$_2$, J = 5.7 Hz), 3.75 (s, 3H, methyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.0, 167.3, 165.7, 154.1, 143.2, 140.0, 137.0, 132.1, 130.9, 128.7, 128.6, 128.3, 127.9, 122.4, 120.3, 119.8, 118.9, 118.0, 117.7, 113.8, 106.5, 55.5, 42.6; FABMS-LR *m/z* 443 [M]$^+$; FABMS-HR calcd for $C_{24}H_{17}N_3O_6$ 443.1143, found 443.1169.

4.19. 5-[(3-Carboxy)benzylaminocarboyl]-9-hydroxy-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (25c)

A mixture of **25b** (100 mg, 0.22 mmol) and pyridinium hydrochloride (1.0 g) was heated at 200 °C under a $CaCl_2$ drying tube with stirring for 30 min. The mixture was partitioned between AcOEt (80 mL) and H_2O (60 mL × 2). The organic layer was washed with 1 N aqueous HCl (60 mL) and brine (40 mL), dried over Na_2SO_4, filtered and concentrated. The residue was triturated from hexane/AcOEt to give **32** (78 mg, 80%) as an orange solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.59 (br s, 1H, CO_2*H*), 11.89 (s, 1H, indole-N*H*), 11.30 (s, 1H, imide-N*H*), 9.69 (t, 1H, amide-N*H*, J = 5.7 Hz), 9.30 (s, 1H, O*H*), 8.42 (s, 1H, H-4), 8.27 (d, 1H, H-10, J = 2.3 Hz), 7.91 (s, 1H, H-6′), 7.81 (d, 1H, H-7, J = 8.0 Hz), 7.73 (d, 1H, H-4′, J = 7.9 Hz), 7.66 (d, 1H, H-2′, J = 7.9 Hz), 7.48 (t, 1H, H-3′, J = 7.9 Hz), 7.21 (dd, 1H, H-8, J = 2.2, 8.0 Hz), 4.64 (d, 2H, C*H*$_2$, J = 5.7 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 167.4, 165.4, 149.9, 143.2, 140.0, 137.0, 132.1, 130.9, 128.7, 128.6, 128.3, 127.9, 122.9, 120.3, 119. 8, 118.8, 118.1, 117.7, 113.8, 106.5, 42.6; FABMS-LR *m/z* 452 [M+Na]$^+$; FABMS-HR calcd for $C_{23}H_{15}N_3O_6Na$ 452.1243, found 452.1169.

4.20. 5-{3-[(2-*tert*-Butoxycarbonylamino)ethylcarbamoyl]-benzylaminocarboyl}-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (26a)

A mixture of **25a** (40 mg, 0.096 mmol), EDCI (28 mg, 0.15 mmol), DMAP (18 mg, 0.15 mmol) and *N*-Boc-ethylenediamine hydrochloride (23 mg, 0.15 mmol) in DMF (1.5 mL) was stirred at room temperature for 2 days. The mixture was partitioned between AcOEt (40 mL) and H_2O (20 mL × 2). The organic layer was washed with 1 N aqueous HCl (40 mL), saturated aqueous $NaHCO_3$ (40 mL) and brine (40 mL), dried over Na_2SO_4, filtered and concentrated. The residue was triturated from hexane/AcOEt to give **26a** (23 mg, 45%) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.16 (s, 1H, indole-NH), 11.35 (br s, 1H, imide-N*H*), 9.72 (br s, 1H, 5-CON*H*), 8.84 (d, 1H, H-10, J = 8.0 Hz), 8.46 (s, 1H, H-4), 8.46 (t, 1H, 5′-CON*H*, J = 5.7 Hz), 7.88 (s, 1H, H-6′), 7.82 (d, 1H, H-7, J = 8.0 Hz), 7.72 (d, 1H, H-4′, J = 8.1 Hz), 7.57 (d, 1H, H-2′, J = 8.1 Hz), 7.57 (t, 1H, H-9, J = 7.2 Hz), 7.43 (t, 1H, H-8, J = 7.4 Hz), 7.32 (t, 1H, H-3′, J = 8.1 Hz), 6.89 (t, 1H, N*H*Boc, J = 5.7 Hz), 4.64 (d, 2H, C*H*$_2$, J = 5.7 Hz), 3.27 (dd, 2H, NHCH$_2$C*H*$_2$NHBoc, J = 5.7, 6.3 Hz), 3.08 (dd, 2H, NHC*H*$_2$CH$_2$NHBoc, J = 5.7, 6.3 Hz), 1.34 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 169.9, 167.0, 166.4, 165.7, 155.8, 142.9, 142.2, 139.6, 134.6, 130.2, 128.5, 128.3, 126.7, 125.6, 124.6, 123.0, 120.7, 120.4, 119.4, 119.1, 118.0, 112.9, 77.7, 42.8, 28.2; FABMS-LR *m/z* 578 [M+Na]$^+$; FABMS-HR calcd for $C_{30}H_{29}N_5O_6Na$ 578.2045, found 578.2026.

4.21. 5-{3-[(2-*tert*-Butoxycarbonylamino)ethylcarbamoyl]-benzylaminocarboyl}-9-hydroxy-1,3-dihydropyrrolo[3,4-*c*]-carbazole-1,3(2*H*,6*H*)-dione (26b)

According to the procedure for the preparation of **26a**, **26b** (67 mg, 84% as an orange solid) was obtained from **25c** (60 mg, 0.15 mmol) and *N*-Boc-ethylenediamine hydrochloride (34 mg, 0.22 mmol). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.96 (s, 1H, indole-N*H*), 11.29 (br s, 1H, imide-N*H*), 9.67 (br s, 1H, 5-CON*H*), 9.30 (s, 1H, O*H*), 8.89 (t, 1H, 5′-CON*H*, J = 5.3 Hz), 8.46 (s, 1H, H-4), 8.24 (d, 1H, H-10, J = 2.0 Hz), 7.88 (s, 1H, H-6′), 7.78 (d, 1H, H-7, J = 8.0 Hz), 7.62 (d, 1H, H-4′, J = 8.0 Hz), 7.57 (d, 1H, H-2′, J = 8.0 Hz), 7.45 (dd, 1H, H-8, J = 2.0, 8.0 Hz), 7.03 (t, 1H, H-3′, J = 8.0 Hz), 6.89 (t, 1H, N*H*Boc, J = 5.7 Hz), 4.64 (d, 2H, C*H*$_2$, J = 5.7 Hz), 3.49 (dd, 2H, NHCH$_2$C*H*$_2$NHBoc, J = 5.7, 6.3 Hz), 2.98 (dd, 2H, NHC*H*$_2$CH$_2$NHBoc, J = 5.3, 6.3 Hz), 1.34 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 167.9, 166.0, 165.8, 165.5, 155.8, 147.8, 145.3, 142.9, 142.2, 139.6, 134.6, 130.2, 128.5, 128.3, 126.7, 125.6, 124.6, 123.0, 120.7, 120.4, 119.4, 119.1, 118.0, 112.9, 77.70, 42.8, 28.2; FABMS-LR *m/z* 571 [M+H]$^+$; FABMS-HR calcd for $C_{30}H_{29}N_5O_7$ 571.2124, found 571.2169.

4.22. 5-{3-[(3-*tert*-Butoxycarbonylamino)propylcarbamoyl]-benzylaminocarboyl}-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (27a)

According to the procedure for the preparation of **26a**, **27a** (31 mg, 57% as a yellow solid) was obtained from **25a** (40 mg, 0.096 mmol) and *N*-Boc-propylenediamine hydrochloride (25 mg, 0.15 mmol). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.16 (s, 1H, indole-N*H*), 11.36 (s, 1H, imide-N*H*), 9.70 (t, 1H, 5-CON*H*, J = 5.7 Hz), 8.84 (d, 1H, H-10, J = 8.0 Hz), 8.46 (s, 1H, H-4), 8.44 (t, 1H, 5′-CON*H*,

J = 5.7 Hz), 7.88 (s, 1H, H-6′), 7.83 (d, 1H, H-7, J = 8.0 Hz), 7.72 (d, 1H, H-4′, J = 8.0 Hz), 7.57 (d, 1H, H-2′, J = 7.9 Hz), 7.56 (t, 1H, H-9, J = 7.2 Hz), 7.43 (t, 1H, H-8, J = 7.4 Hz), 7.33 (t, 1H, H-3′, J = 8.0 Hz), 6.80 (t, 1H, N*H*Boc, J = 5.8 Hz), 4.64 (d, 2H, CH_2, J = 5.7 Hz), 3.23 (dd, 2H, NHCH$_2$CH$_2$CH_2NHBoc, J = 5.8, 6.8 Hz), 2.94 (dd, 2H, NHCH_2CH$_2$CH$_2$NHBoc, J = 5.7, 6.8 Hz), 1.59 (m, 2H, NHCH$_2$CH_2CH$_2$-NHBoc), 1.34 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.8, 169.8, 166.1, 165.6, 155.5, 142.1, 139.6, 134.6, 130.1, 128.4, 128.2, 126.6, 125.4, 124.5, 122.9, 120.3, 119.3, 119.0, 118.5, 117.9, 112.8, 77.4, 42.7, 37.7, 36.6, 30.1, 28.2; FABMS-LR m/z 592 [M+Na]$^+$; FABMS-HR calcd for $C_{31}H_{31}N_5O_6Na$ 592.2201, found 592.2229.

4.23. 5-{3-[(3-*tert*-Butoxycarbonylamino)propylcarbamoyl]-benzylaminocarboyl}-9-hydroxy-1,3-dihydropyrrolo[3,4-*c*]-carbazole-1,3(2*H*,6*H*)-dione (27b)

According to the procedure for the preparation of **26a**, **27b** (73 mg, 89% as an orange solid) was obtained from **25c** (60 mg, 0.15 mmol) and *N*-Boc-propylenediamine hydrochloride (36 mg, 0.21 mmol). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.98 (s, 1H, indole-N*H*), 11.29 (br s, 1H, imide-N*H*), 9.70 (br s, 1H, 5-CON*H*), 9.29 (s, 1H, O*H*), 8.90 (t, 1H, 5′-CON*H*, J = 5.3 Hz), 8.46 (s, 1H, H-4), 8.24 (d, 1H, H-10, J = 2.2 Hz), 7.87 (s, 1H, H-6′), 7.78 (d, 1H, H-7, J = 8.0 Hz), 7.62 (d, 1H, H-4′, J = 8.0 Hz), 7.57 (d, 1H, H-2′, J = 8.0 Hz), 7.45 (t, 1H, H-8, J = 8.0 Hz), 7.03 (t, 1H, H-3′, J = 8.0 Hz), 6.89 (t, 1H, N*H*Boc, J = 5.7 Hz), 4.64 (d, 2H, CH_2, J = 5.3 Hz), 3.39 (dd, 2H, NHCH$_2$-CH$_2$CH_2NHBoc, J = 5.7, 6.3 Hz), 3.03 (dd, 2H, NHCH_2CH$_2$CH$_2$NHBoc, J = 5.3, 6.3 Hz), 1.78 (m, 2H, NHCH$_2$CH_2CH$_2$NHBoc), 1.34 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.8, 167.8, 166.1, 165.6, 155.5, 147.1, 143.1, 142.1, 139.6, 134.6, 130.1, 128.4, 128.2, 126.6, 125.4, 124.5, 122.9, 120.3, 119.3, 119.0, 118.5, 117.9, 112.8, 77.4, 42.7, 37.7, 36.6, 30.1, 28.2; FABMS-LR m/z = 585 [M+H]$^+$; FABMS-HR calcd for $C_{31}H_{31}N_5O_7$ 585.6123, found 585.6169.

4.24. 5-{3-[(2-Amino)ethylcarbamoyl]benzylaminocarboyl}-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione hydrochloride (28a)

A solution of **26a** (10 mg, 0.017 mmol) in THF (1 mL) was treated with 4 N HCl in AcOEt (1 mL) at room temperature for 4.5 h. The mixture was concentrated in vacuo, and the residue was triturated from hexane/AcOEt to give **28a** (8.1 mg, 0.016 mmol, 94%) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.16 (s, 1H, indole-N*H*), 11.37 (s, 1H, imide-N*H*), 9.74 (t, 1H, 5-CON*H*, J = 5.9 Hz), 8.84 (d, 1H, H-10, J = 8.0 Hz), 8.70 (br s, 1H, 5′-CON*H*), 8.46 (s, 1H, H-4), 7.91 (s, 1H, H-6′), 7.90 (br s, 3H, NH_3), 7.83 (d, 1H, H-7, J = 8.0 Hz), 7.78 (d, 1H, H-4′, J = 8.0 Hz), 7.56 (d, 1H, H-2′, J = 7.9 Hz), 7.56 (t, 1H, H-9, J = 7.2 Hz), 7.45 (t, 1H, H-8, J = 7.7 Hz), 7.33 (d, 1H, H-3′, J = 7.4 Hz), 4.65 (d, 2H, CH_2, J = 5.9 Hz), 3.48 (m, 2H, NHCH$_2$CH_2NH$_3$), 2.95 (m, 2H, NHCH_2CH$_2$NH$_3$); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 169.9, 166.8, 165.7, 142.9, 142.2, 139.7, 134.1, 130.5, 128.5, 128.5, 128.3, 126.8, 125.8, 124.6, 123.0, 120.7, 120.3, 119.4, 119.1, 118.0, 112.9, 42.7, 38.6, 37.1; FABMS-LR m/z 456 [M+H]$^+$; FABMS-HR calcd for $C_{25}H_{22}N_5O_4$ 456.1659, found 456.1646.

4.25. 5-[3-(2-Aminoethylcarbamoyl)benzylaminocarboyl]-9-hydroxy-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione hydrochloride (28b)

According to the procedure for the preparation of **28a**, **28b** (50 mg, 86% as an orange solid) was obtained from **25c** (66 mg, 0.11 mmol). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.89 (s, 1H, indole-N*H*), 11.30 (br s, 1H, imide-N*H*), 9.67 (br s, 1H, 5-CON*H*), 9.30 (s, 1H, O*H*), 8.69 (t, 1H, 5′-CON*H*, J = 5.3 Hz), 8.40 (s, 1H, H-4), 8.26 (d, 1H, H-10, J = 2.2 Hz), 7.92 (s, 1H, H-6′), 7.90 (br s, 3H, NH_3), 7.79 (d, 1H, H-7, J = 8.0 Hz), 7.62 (d, 1H, H-4′, J = 8.0 Hz), 7.58 (d, 1H, H-2′, J = 8.0 Hz), 7.45 (t, 1H, H-8, J = 8.0 Hz), 7.03 (t, 1H, H-3′, J = 8.0 Hz), 4.64 (d, 2H, CH_2, J = 5.3 Hz), 3.49 (m, 2H, NHCH$_2$CH_2NH$_3$), 2.98 (dd, 2H, NHCH_2CH$_2$NH$_3$, J = 5.3, 6.3 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 169.9, 166.7, 165.8, 151.8, 143.2, 139.7, 136.1, 134.1, 130.4, 128.5, 128.3, 126.7, 125.8, 122.1, 120.2, 118.7, 118.4, 117.6, 113.3, 108.9, 42.7, 38.6, 37.1, 34.1; ESIMS-LR m/z 472 [M+H]$^+$; EIMS-HR calcd for $C_{25}H_{22}N_5O_5$ 472.1621, found 472.1612.

4.26. 5-{3-[3-(Amino)propylcarbamoyl]benzylaminocarboyl}-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione hydrochloride (29a)

According to the procedure for the preparation of **28a**, **29a** (3.9 mg, 88% as a yellow solid) was obtained from **27a** (5.1 mg, 7.1 μmol). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.16 (s, 1H, indole-N*H*), 11.37 (s, 1H, imide-N*H*), 9.72 (t, 1H, 5-CON*H*, J = 5.7 Hz), 8.85 (d, 1H, H-10, J = 8.0 Hz), 8.70 (br s, 1H, 5′-CON*H*), 8.46 (s, 1H, H-4), 7.90 (s, 1H, H-6′), 7.90 (br s, 3H, NH_3), 7.83 (d, 1H, H-7, J = 7.9 Hz), 7.76 (d, 1H, H-4′, J = 8.0 Hz), 7.56 (d, 1H, H-2′, J = 8.0 Hz), 7.56 (t, 1H, H-9, J = 7.4 Hz), 7.45 (t, 1H, H-8, J = 7.5 Hz), 7.33 (d, 1H, H-3′, J = 8.0 Hz), 4.64 (d, 2H, CH_2, J = 5.7 Hz), 3.32 (dt, 2H, NHCH$_2$-CH$_2$CH_2NH$_3$, J = 6.3, 7.4 Hz), 2.80 (dd, 2H, NHCH_2CH$_2$CH$_2$NH$_3$, J = 5.7, 7.4 Hz), 1.79 (m, 2H, NHCH$_2$CH_2CH$_2$NH$_3$); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 169.9, 166.5, 165.8, 165.7, 142.9, 142.2, 134.3, 130.4, 128.5, 128.4, 126.6, 124.6, 123.0, 120.8, 120.7, 120.4, 119.4, 119.1, 118.0, 112.9, 42.7, 36.8, 36.3, 27.9; FABMS-LR m/z 470 [M+H]$^+$; FABMS-HR calcd for $C_{26}H_{24}N_5O_4$ 470.1834, found 470.1840.

4.27. 5-[3-(3-Aminopropylcarbamoyl)benzylaminocarboyl]-9-hydroxy-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione hydrochloride (29b)

According to the procedure for the preparation of **28a**, **29b** (48 mg, 75% as an orange solid) was obtained from **25c** (72 mg, 0.12 mmol). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.88 (s, 1H, indole-N*H*), 11.30 (br s, 1H, imide-N*H*), 9.67 (br s, 1H, 5-CON*H*), 9.29 (s, 1H, O*H*), 8.70 (t, 1H, 5′-CON*H*, J = 5.3 Hz), 8.46 (s, 1H, H-4), 8.26 (d, 1H, H-10, J = 2.0 Hz), 7.92 (s, 1H, H-6′), 7.90 (br s, 3H, NH_3), 7.76 (d, 1H, H-7, J = 8.0 Hz), 7.63 (d, 1H, H-4′, J = 8.0 Hz), 7.56 (d, 1H, H-2′, J = 8.0 Hz), 7.44 (t, 1H, H-8, J = 8.1 Hz), 7.03 (t, 1H, H-3′, J = 8.1 Hz), 4.62 (d, 2H, CH_2, J = 5.3 Hz), 3.30 (dd, 2H, NHCH$_2$CH$_2$CH_2NH$_3$, J = 5.3, 6.3 Hz), 2.80 (dd, 2H, NHCH_2CH$_2$CH$_2$NH$_3$, J = 5.3, 6.3 Hz), 1.79 (m, 2H, NHCH$_2$CH_2CH$_2$NH$_3$); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 169.9, 166.5, 165.8, 151.8, 143.2, 139.7, 136.1, 134.4, 130.3, 128.5, 128.3, 126.6, 125.6, 122.1, 120.3, 120.1, 118.7, 118.4, 117.6, 113.4, 108.9, 42.7, 37.7, 36.2, 27.3; EIMS-LR m/z 486 [M+H]$^+$; FAB-MS-HR calcd for $C_{26}H_{24}N_5O_5$ 486.1768, found 486.1777.

4.28. 5-{3-[2-(Di-*tert*-butoxycarbonylguanidino)ethylcarbamoyl]benzylaminocarboyl}-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (30a)

A mixture of **28a** (10 mg, 0.018 mmol), di-Boc-thiourea (6.7 mg, 0.023 mmol), $HgBr_2$ (8.6 mg, 0.023 mmol) and Et_3N (14 μL, 0.092 mmol) in DMF (1 mL) was stirred at room temperature for 2 h. Insoluble was filtered off through a Celite pad, and filtrate was concentrated. The residue was partitioned between AcOEt (30 mL) and H_2O (20 mL × 2). The organic layer was washed with brine (40 mL), dried over Na_2SO_4, filtered and concentrated. The residue was triturated from hexane/AcOEt to give **30a** (12 mg, 84%) as an orange solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.16 (s, 1H, indole-N*H*), 11.47 (s, 1H, N*H*Boc), 11.36 (s, 1H, imide-N*H*), 9.70 (t, 1H, 5-CON*H*,

J = 6.0 Hz), 8.84 (d, 1H, H-10, *J* = 8.1 Hz), 8.56 (t, 1H, 5′-CONH, *J* = 6.0 Hz), 8.45 (s, 1H, H-4), 8.44 (t, 1H, guanidine-NH, *J* = 6.2 Hz), 7.87 (s, 1H, H-6′), 7.82 (d, 1H, H-7, *J* = 8.1 Hz), 7.70 (d, 1H, H-4′, *J* = 8.0 Hz), 7.56 (d, 1H, H-2′, *J* = 8.0 Hz), 7.55 (t, 1H, H-9, *J* = 8.1 Hz), 7.42 (t, 1H, H-8, *J* = 8.1 Hz), 7.32 (t, 1H, H-3′, *J* = 8.0 Hz), 4.63 (d, 2H, CH_2, *J* = 6.0 Hz), 3.47 (dt, 2H, $CONHCH_2CH_2NH$, *J* = 6.2, 6.8 Hz), 3.39 (dt, 2H, $CONHCH_2CH_2NH$, *J* = 6.1, 6.7 Hz), 1.41 (s, 9H, *t*-butyl), 1.39 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 169.9, 166.6, 165.7, 155.8, 151.9, 142.9, 142.2, 139.6, 134.6, 130.3, 128.5, 128.3, 126.7, 125.5, 124.6, 122.9, 120.7, 120.4, 119.4, 119.1, 118.0, 112.9, 82.8, 79.2, 78.21 42.8, 38.8, 28.0, 27.6; ESIMS-LR *m/z* 698 $[M+H]^+$; ESIMS-HR calcd for $C_{36}H_{40}N_7O_8Na$ 698.2938, found 698.2929.

4.29. 5-{3-[2-(Di-*tert*-butoxycarbonylguanidino)ethylcarbamoyl]benzylaminocarboyl}-9-hydroxy-1,3-dihydropyrrolo-[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (30b)

According to the procedure for the preparation of **30a**, **30b** (19 mg, 54% as an orange solid) was obtained from **28b** (25 mg, 0.051 mmol). ^1H NMR (500 MHz, DMSO-d_6) δ 11.88 (s, 1H, indole-NH), 11.29 (s, 1H, H-r), 11.31 (s, 1H, imide-NH), 9.70 (t, 1H, 5-CONH, *J* = 5.3 Hz), 9.29 (s, 1H, OH), 8.52 (t, 1H, 5′-CONH, *J* = 5.3 Hz), 8.48 (s, 1H, H-4), 8.44 (t, 1H, guanidine-NH, *J* = 5.0 Hz), 8.24 (d, 1H, H-10, *J* = 2.0 Hz), 7.88 (s, 1H, H-6′), 7.75 (d, 1H, H-7, *J* = 8.1 Hz), 7.61 (d, 1H, H-4′, *J* = 8.0 Hz), 7.60 (d, 1H, H-2′, *J* = 8.0 Hz), 7.45 (t, 1H, H-8, *J* = 8.1 Hz), 7.03 (t, 1H, H-3′, *J* = 8.0 Hz), 4.63 (d, 2H, CH_2, *J* = 5.3 Hz), 3.43 (m, 2H, $CHNHCH_2CH_2NH$), 3.39 (m, 2H, $CONHCH_2CH_2NH$), 1.45 (s, 9H, *t*-butyl), 1.31 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 169.9, 166.6, 165.8, 163.1, 155.7, 151.8, 143.2, 139.6, 136.2, 134.6, 139.2, 128.5, 128.6, 126.7, 125.5, 122.1, 120.3, 118.7 118.3, 117.6, 113.4, 108.9, 82.8, 78.2, 42.7, 38.8, 28.0, 27.6; FABMS-LR *m/z* 714 $[M+H]^+$; FABMS-HR calcd for $C_{36}H_{40}N_7O_9$ 714.2892, found 714.2897.

4.30. 5-{3-[3-(Di-*tert*-butoxycarbonylguanidino)propylcarbamoyl]benzylaminocarboyl}-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (31a)

According to the procedure for the preparation of **30a**, **31a** (17 mg, 80% as an orange solid) was obtained from **29a** (15 mg, 0.029 mmol). ^1H NMR (500 MHz, DMSO-d_6) δ 12.16 (s, 1H, indole-NH), 11.47 (s, 1H, H-r), 11.35 (s, 1H, imide-NH), 9.69 (t, 1H, 5-CONH, *J* = 6.0 Hz), 8.84 (d, 1H, H-10, *J* = 8.1 Hz), 8.54 (t, 1H, 5′-CONH, *J* = 6.0 Hz), 8.46 (s, 1H, H-4), 8.44 (t, 1H, guanidine-NH, *J* = 8.0 Hz), 7.88 (s, 1H, H-6′), 7.82 (d, 1H, H-7, *J* = 8.1 Hz), 7.72 (d, 1H, H-4′, *J* = 8.0 Hz), 7.56 (d, 1H, H-2′, *J* = 8.0 Hz), 7.55 (t, 1H, H-9, *J* = 8.1 Hz), 7.44 (t, 1H, H-8, *J* = 8.1 Hz), 7.32 (t, 1H, H-3′, *J* = 8.0 Hz), 4.64 (d, 2H, CH_2, *J* = 6.0 Hz), 3.34 (m, 2H, $CONHCH_2CH_2CH_2NH$), 3.25 (m, 2H, $CONHCH_2CH_2CH_2NH$), 1.69 (m, 2H, $CONHCH_2CH_2CH_2NH$), 1.42 (s, 9H, *t*-butyl), 1.33 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.9, 169.9, 166.3, 165.7, 163.1, 155.4, 152.0, 142.9, 142.2, 139.6, 134.7, 130.2, 128.5, 128.3, 126.6, 125.5, 124.6, 122.9, 120.7, 120.4, 119.4, 119.1, 117.9, 112.9, 82.8, 79.2, 78.2, 42.7, 37.9, 36.5, 31.3, 29.0, 28.3, 28.0, 27.6; ESIMS-LR *m/z* 712 $[M+H]^+$; ESIMS-HR calcd for $C_{37}H_{42}N_7O_8$ 712.3073, found 712.3095.

4.31. 5-{3-[3-(Di-*tert*-butoxycarbonylguanidino)propylcarbamoyl]benzylaminocarboyl}-9-hydroxy-1,3-dihydropyrrolo-[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione (31b)

According to the procedure for the preparation of **30a**, **31b** (19 mg, 59% as an orange solid) was obtained from **28b** (25 mg, 0.049 mmol). ^1H NMR (500 MHz, DMSO-d_6) δ 11.92 (s, 1H, indole-NH), 11.27 (s, 1H, H-r), 11.35 (s, 1H, imide-NH), 9.69 (t, 1H, 5-CONH, *J* = 5.5 Hz), 9.30 (s, 1H, OH), 8.54 (t, 1H, 5′-CONH, *J* = 5.3 Hz), 8.46 (s, 1H, H-4), 8.44 (t, 1H, OH, *J* = 5.0 Hz), 8.24 (d, 1H, H-10, *J* = 2.1 Hz), 7.88 (s, 1H, H-6′), 7.75 (d, 1H, H-7, *J* = 8.1 Hz), 7.63 (d, 1H, H-4′, *J* = 8.0 Hz), 7.59 (d, 1H, H-2′, *J* = 8.0 Hz), 7.45 (t, 1H, H-8, *J* = 8.1 Hz), 7.03 (t, 1H, H-3′, *J* = 8.0 Hz), 4.63 (d, 2H, CH_2, *J* = 5.5 Hz), 3.39 (m, 2H, $CONHCH_2CH_2CH_2NH$), 3.39 (m, 2H, $CONHCH_2CH_2CH_2NH$), 1.69 (m, 2H, $CONHCH_2CH_2CH_2NH$), 1.42 (s, 9H, *t*-butyl), 1.33 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 170.0, 166.4, 166.3, 165.8, 163.2, 162.4, 155.4, 152.0, 151.8, 143.2, 139.7, 136.1, 130.2, 128.5, 128.4, 126.7, 125.5, 122.1, 120.3, 120.1, 118.7, 118.4, 117.6, 113.4, 108.9, 82.9, 78.2, 42.7, 38.0, 36.6, 30.1, 28.2, 27.7; FABMS-LR *m/z* 728 $[M+H]^+$; FABMS-HR calcd for $C_{37}H_{42}N_7O_9$ 728.3063, found 728.3081.

4.32. 5-{3-[2-(Guanidino)ethylcarbamoyl]benzylaminocarboyl}-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione hydrochloride (32a)

According to the procedure for the preparation of **28a**, **32a** (2.4 mg, 75% as an orange solid) was obtained from **30a** (4.5 mg, 6.5 μmol). ^1H NMR (500 MHz, DMSO-d_6) δ 12.16 (s, 1H, indole-NH), 11.37 (s, 1H, imide-NH), 9.71 (t, 1H, 5-CONH, *J* = 6.0 Hz), 8.84 (d, 1H, H-10, *J* = 8.1 Hz), 8.62 (t, 1H, 5′-CONH, *J* = 6.0 Hz), 8.46 (s, 1H, H-4), 7.90 (s, 1H, H-6′), 7.82 (d, 1H, H-7, *J* = 8.1 Hz), 7.74 (d, 1H, H-4′, *J* = 8.0 Hz), 7.58 (d, 1H, H-2′, *J* = 8.0 Hz), 7.55 (t, 1H, H-9, *J* = 8.1 Hz), 7.46 (t, 1H, H-8, *J* = 8.1 Hz), 7.33 (t, 1H, H-3′, *J* = 8.0 Hz), 6.81 (br s, 3H, NH_3), 4.64 (d, 2H, CH_2, *J* = 6.0 Hz), 3.36 (m, 2H, $CONHCH_2CH_2NH$), 3.28 (m, 2H, $CONHCH_2CH_2NH$); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.7, 168.9, 167.4, 165.5, 142.7, 141.1, 139.6, 134.6, 130.2, 128.5, 128.3 126.6, 125.6, 124.6, 122.7, 120.6, 120.3, 119.4, 119.1, 118.0, 112.9, 79.2, 42.7, 38.5, 36.6; ESIMS-LR *m/z* 498 $[M+H]^+$; ESIMS-HR calcd for $C_{26}H_{24}N_7O_4$ 498.1889, found 498.1887.

4.33. 5-{3-[2-(Guanidino)ethylcarbamoyl]benzylaminocarboyl}-9-hydroxy-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3-(2*H*,6*H*)-dione hydrochloride (32b)

According to the procedure for the preparation of **28a**, **32b** (8.1 mg, 70% as an orange solid) was obtained from **30b** (15 mg, 0.021 mmol). ^1H NMR (500 MHz, DMSO-d_6) δ 11.90 (s, 1H, indole-NH), 11.30 (br s, 1H, imide-NH), 9.67 (br s, 1H, 5-CONH), 9.30 (s, 1H, OH), 8.64 (t, 1H, 5′-CONH, *J* = 5.3 Hz), 8.40 (s, 1H, H-4), 8.26 (d, 1H, H-10, *J* = 2.0 Hz), 7.90 (s, 1H, H-6′), 7.75 (d, 1H, H-7, *J* = 8.0 Hz), 7.63 (d, 1H, H-4′, *J* = 8.0 Hz), 7.58 (d, 1H, H-2′, *J* = 8.0 Hz), 7.45 (t, 1H, H-8, *J* = 8.0 Hz), 7.41–6.77 (br s, 4H, guanidine), 4.62 (d, 2H, CH_2, *J* = 5.7 Hz), 3.39 (dd, 2H, $CONHCH_2CH_2NH$, *J* = 5.7, 6.3 Hz), 2.28 (dd, 2H, $CONHCH_2CH_2NH$, *J* = 5.7, 6.3 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 169.9, 166.8, 165.8, 157.0, 151.8, 143.2, 139.8, 136.1, 134.2, 130.5, 128.5, 128.4, 126.6, 125.6, 122.1, 120.3, 118.7, 118.4, 117.6, 113.4, 108.9, 42.7, 40.4, 38.6, 30.7; FABMS-LR *m/z* 514 $[M+H]^+$; FABMS-HR calcd for $C_{26}H_{24}N_7O_5$ 514.1821, found 514.1989.

4.34. 5-{3-[3-(Guanidino)propylcarbamoyl]benzylaminocarboyl}-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione hydrochloride (33a)

According to the procedure for the preparation of **28a**, **33a** (3.2 mg, 70% as an orange solid) was obtained from **31a** (7.1 mg, 9.8 μmol). ^1H NMR (500 MHz, DMSO-d_6) δ 12.16 (s, 1H, indole-NH), 11.37 (s, 1H, imide-NH), 9.72 (t, 1H, 5-CONH, *J* = 6.0 Hz), 8.84 (d, 1H, H-10, *J* = 8.1 Hz), 8.55 (t, 1H, 5′-CONH, *J* = 6.0 Hz), 8.46 (s, 1H, H-4), 7.89 (s, 1H, H-6′), 7.82 (d, 1H, H-7, *J* = 8.1 Hz), 7.74 (d, 1H, H-4′, *J* = 8.0 Hz), 7.58 (d, 1H, H-2′, *J* = 8.0 Hz), 7.54 (t,

1H, O*H*, *J* = 8.1 Hz), 7.44 (t, 1H, H-8, *J* = 8.1 Hz), 7.33 (t, 1H, H-3′, *J* = 8.0 Hz), 6.88 (br s, 3H, NH_3), 4.64 (d, 2H, CH_2, *J* = 6.0 Hz), 3.28 (dt, 2H, COHCH$_2$CH$_2$CH_2NH, *J* = 6.3, 6.8 Hz), 3.12 (s, 1H, guanidine-N*H*), 3.11 (dt, 2H, CONHCH_2CH$_2$CH$_2$NH, *J* = 6.0, 6.8 Hz), 1.69 (m, 2H, CONHCH$_2$CH_2CH$_2$NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.9, 169.9, 166.4, 165.7, 142.9, 142.1, 139.6, 134.6, 130.2, 128.5, 128.3 126.6, 125.6, 124.6, 122.9, 120.7, 120.3, 119.4, 119.1, 118.0, 112.9, 79.2, 42.7, 38.5, 36.6, 28.7; ESIMS-LR *m/z* 512 [M+H]$^+$; ESIMS-HR calcd for $C_{27}H_{26}N_7O_4$ 512.2079, found 512.2095.

4.35. 5-{3-[3-(Guanidino)propylcarbamoyl]benzylaminocarboyl}-9-hydroxy-1,3-dihydropyrrolo[3,4-*c*]carbazole-1,3-(2*H*,6*H*)-dione hydrochloride (33b)

According to the procedure for the preparation of **28a**, **33b** (10.1 mg, 88% as an orange solid) was obtained from **31b** (15 mg, 0.020 mmol). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.88 (s, 1H, indole-N*H*), 11.30 (br s, 1H, imide-N*H*), 9.67 (br s, 1H, 5-CON*H*), 9.29 (s, 1H, O*H*), 8.55 (t, 1H, 5′-CON*H*, *J* = 5.3 Hz), 8.40 (s, 1H, H-4), 8.26 (d, 1H, H-10, *J* = 2.0 Hz), 7.88 (s, 1H, H-6′), 7.76 (d, 1H, H-7, *J* = 8.0 Hz), 7.63 (d, 1H, H-4′, *J* = 8.0 Hz), 7.54 (d, 1H, H-2′, *J* = 8.0 Hz), 7.44 (t, 1H, H-8, *J* = 8.0 Hz), 7.05 (t, 1H, H-3′, *J* = 8.0 Hz), 7.41–6.77 (br s, 4H, guanidine-N*H*), 4.62 (d, 2H, CH_2, *J* = 5.7 Hz), 3.34 (dd, 2H, COHCH$_2$CH$_2$CH_2NH, *J* = 5.7, 6.3 Hz), 3.14 (dd, 2H, COHCH_2CH$_2$-CH$_2$NH, *J* = 5.3, 6.3 Hz), 1.70 (m, 2H, COHCH$_2$CH_2CH$_2$NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 169.9, 166.4, 165.8, 156.9, 151.8, 143.2, 139.7, 136.1, 134.6, 130.2, 128.5, 128.3, 126.6, 125.6, 122.1, 120.2, 120.0, 118.7, 118.3, 117.6, 113.3, 108.9, 42.7, 38.4, 36.5, 28.7; ESIMS-LR *m/z* 528 [M+H]$^+$; ESIMS-HR calcd for $C_{27}H_{26}N_7O_5$ 528.1983, found 528.2183.

5. Molecular docking

UCN-01/the active site of Chk1 complex structure (PDB code: 1nvq) was used for the docking. The docking calculations were performed using the Schrödinger software suite with default settings if not indicated otherwise. For protein preparation, all the crystallographic water molecules were removed, hydrogens were added, and bond orders were assigned using Maestro's Protein Preparation Wizard (Maestro ver. 8.6). The added hydrogen atoms were minimized with all heavy atoms fixed using the OPLS2001 force field. For each of the three structures, energy grids were built using the default value of protein atom scaling. This box was centered around the centroid of the UCN-01. Docking was performed using Schrödinger Glide.[26–28] Calculation was conducted using the standard-precision (SP) mode with default settings. As for global energy-minimized conformation search, key binding site side residues were refined in the presence of inhibitor by a 100,000-step MCMM conformational search in MacroModel 9 (Schrodinger, LLC, New York, NY).

6. Chk1 kinase assay

Recombinant human Chk1 and UCN-01 were obtained from Calbiochem. The test compounds and UCN-01 were dissolved in DMSO, and diluted in four different concentrations, respectively. The assay was performed using a Calbiochem K-LISA Checkpoint Activity Kit according to the manufacturer's instructions. Chk1 inhibitory activity was calculated based on Chk1 activity in the presence and absence of compounds. Kinase activity was determined by reading the absorbance at dual wave lengths 450/540 nm on a microplate reader from BIO-RAD, and data were analyzed using SOFTmax Pro.

7. Mobility sift assay for kinase panel assay

To test the enzyme selectivity of compounds, a kinase panel assay was carried out using ProfilerPro Kits (Caliper Life Sciences, Inc., Hopkinton, MA). The procedure was according to the instruction manual of ProfilerPro kit 1 and 2. In 384-well plates, 16 μL of 1.625× concentration compounds was first added to the enzyme plates, followed by a 15-min preincubation at room temperature. 10 μL of a 2.6× mixture of peptide substrate and ATP in 2.6× kinase buffer was added to initiate the reaction. Each assay was run at the apparent K_m ($K_{m,app}$) concentration of ATP and at a fixed low concentration (1.5 μM) of peptide for 90 min. Reactions were stopped by the addition of Termination buffer. For percent inhibition screening, compounds were tested in duplicate at both 0.1 μM and 10 μM. After the reactions were stopped, the plates were read on a Caliper EZReder using a four-sipper chip and separation conditions that were optimized for each kinase. Product-to-sum ratios, indicative of percent conversion from substrate to product, were taken as the assay signal.

8. Cell culture

CCRF-CEM and ML-1a cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and HeLa cells were grown in MEM supplemented with 10% FBS, 1% nonessential amino acids at 37 °C in a 5% CO_2 incubator.

9. Cell cytotoxicity assay

SN-38 was purchased from LKT Laboratories. All the data generated were the result of three separate experiments performed in triplicate. Exponential growing cells were seeded in 96-well plates. After 24 h, the cells were treated with SN-38 with or without the various doses of the test compounds for 48 h. After the treatment, Cell counting Kit-8 reagents (Dojindo Laboratories) that measured the amount of live cell were added to the incubated cells and allowed to develop for 2 h and the absorbance at 450 nm was measured using a microplate reader from BIO-RAD. The control value corresponding to untreated cells was taken as 100% and the viability of treated samples was expressed as a percentage of the control. IC_{50} values were determined as concentrations that reduced cell viability by 50%.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.09.042.

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