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Synthesis, checkpoint kinase 1 inhibitory properties and in vitro antiproliferative activities of new pyrrolocarbazoles

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Abstract—In the course of structure–activity relationship studies on granulatimide analogues, new pyrrolo[3,4-*c*]carbazoles have been synthesized in which the imidazole heterocycle was replaced by a five-membered ring lactam system or a dimethylcyclopentanedione. Moreover, the synthesis of an original structure in which a sugar moiety is attached to the indole nitrogen and to a six-membered D ring via an oxygen is reported. The inhibitory activities of the newly synthesized compounds toward checkpoint kinase 1 and their in vitro antiproliferative activities toward three tumor cell lines: murine leukemia L1210, and human colon carcinoma HT29 and HCT116 are described.

1. Introduction

Over the past decade, a great deal of interest has been devoted to checkpoint kinase 1 (Chk1) as a therapeutic target for cancer treatment.[1–4] Chk1, a serine/threonine kinase, is a key signal transducer in DNA-damage response pathways. Normal cells respond to DNA damage by activating cell cycle checkpoints in G1/S and G2/M. When activated, these checkpoints prevent temporarily the progression of the cell cycle to allow time for DNA repair. In more than 50% of cancer cells, the G1 checkpoint is lacking due to loss of p53 or Rb proteins. Therefore, these cells are dependent upon the G2 checkpoint for DNA repair after damage. Chk1 is a major regulator of the G2 checkpoint. When active, it causes the inactivation of cyclin B/CDK1 and thereby cell cycle arrest. Accordingly, a combination of a DNA damaging agent and a G2 checkpoint abrogator should drive selectively G1 checkpoint-deficient cells into a mitotic catastrophe leading to cell death.[5–8] Thus, Chk1 inhibitors are expected to sensitize cancer cells, especially p-53 deficient cells, to DNA damaging agents. The combination of cisplatin, and topotecan, a well-known topoisomerase I inhibitor, with UCN-01, a potent Chk1 inhibitor, is currently being tested in clinical trials.[9,10]

Granulatimide and isogranulatimide (Fig. 1) are natural products isolated from the ascidian *Didemnum granulatum*[11,12] that have been previously identified as Chk1 inhibitors with IC_{50} values of 0.25 and 0.1 μM.[13] Like UCN-01, granulatimide, and isogranulatimide are ATP competitive Chk1 inhibitors. Their crystal structures in complex with Chk1 have been determined.[13,14] In the course of structure–activity relationship studies, granulatimide analogues, with and without substituents on the indole moiety, and in which the D and/or E rings were modified, have been synthesized.[15–30]

In our previous studies, we observed that the imide D ring of bis-imide **A** (Fig. 1) was sensitive to nucleophiles and that the upper carbonyl of the D ring was particularly important for the interaction with the ATP-binding site of Chk1. Therefore, our aim was to synthesize new bis-imide **A** analogues either with a lactam D ring in which the carbonyl group was oriented toward the imide E heterocycle, or with a five-membered D carbocycle containing two carbonyl functions instead of an imide D ring. Moreover, since in UCN-01, the sugar moiety stabilizes the drug in the ATP-binding site of Chk1 by additional hydrogen bonds, we wanted to attach a sugar part to compound **B** (Fig. 1) which proved to be a strong Chk1 inhibitor.[29] In this paper, we describe the synthesis

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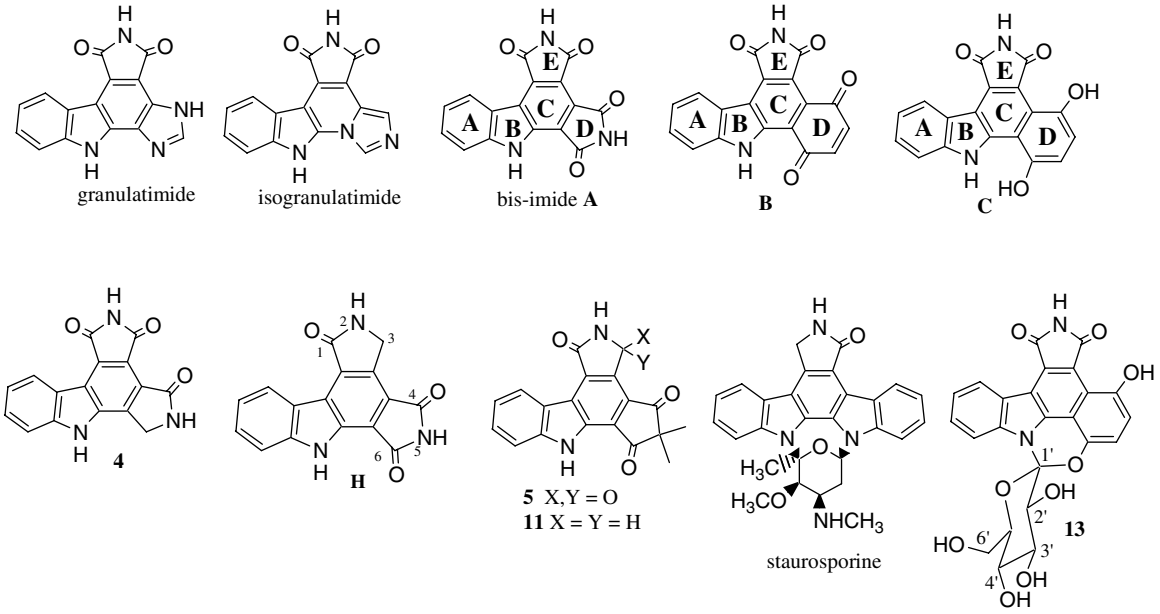


Figure 1. Chemical structures of granulatimide, isogranulatimide, staurosporine, compounds **A**, **B**, **C**, and **H** previously described and newly synthesized compounds **4**, **5**, **11**, and **13**.

of these new pyrrolo[3,4-*c*]carbazoles that can be considered as granulatimide analogues in which the imidazole heterocycle has been replaced by a five-membered ring system bearing one or two carbonyl functions, as well as the synthesis of a compound bearing a sugar unit linked to the indole nitrogen and to a diphenolic D ring. The Chk1 inhibitory activities and the cytotoxicities of the newly synthesized compounds toward three tumor cell lines (murine leukemia L1210, and human colon carcinoma HT29 and HCT116) are reported.

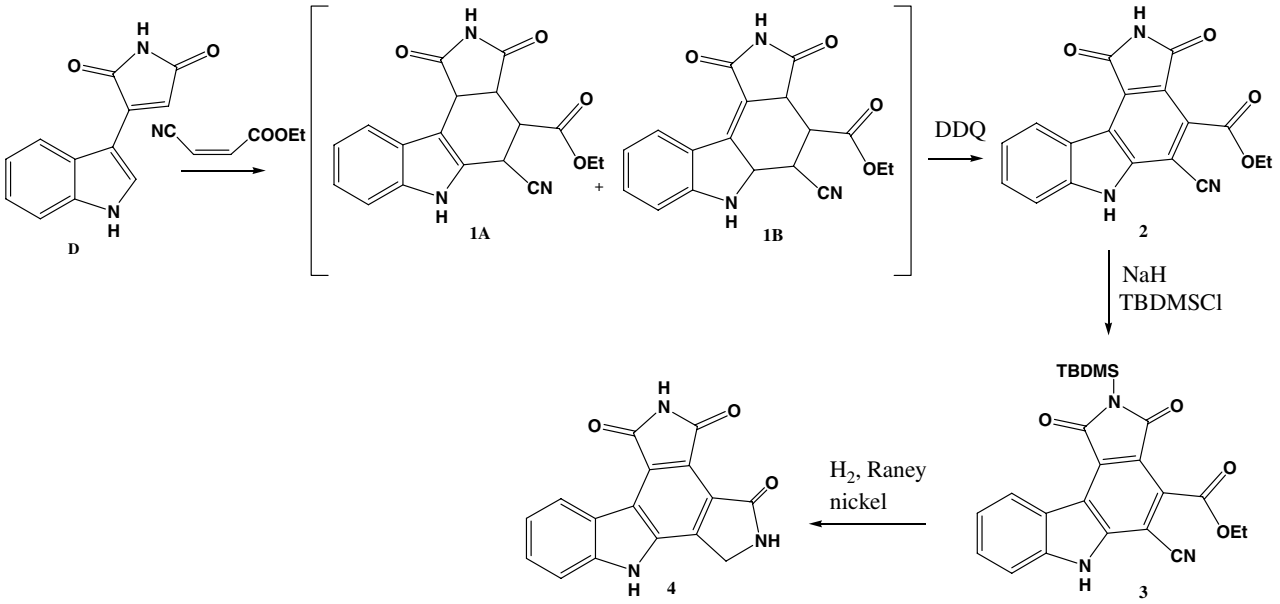
2. Results and discussion

2.1. Chemistry

Compound **4** with a lactam D heterocycle was prepared as outlined in Scheme 1. A Diels–Alder reaction between 3-(indol-3-yl)-maleimide and ethyl *cis*-β-cyanoacrylate, followed by the oxidation of the cycloadduct with DDQ gave compound **2**. 3-(Indol-3-yl)-maleimide was previously synthesized by Bergman et al.[31] and used as a dienophile for Diels–Alder reactions.[32,21] The structure of compound **2** was assigned from NMR experiments on indole intermediate **1A** before oxidation with DDQ. Actually, the Diels–Alder reaction led to two intermediate isomers indole **1A** and indoline **1B** that could be separated by chromatography. In Diels–Alder reactions with indolyl maleimide, we had previously observed the formation of indole and indoline isomers which could be identified from ^{1}H NMR data: an indole NH is shifted at about 11 ppm whereas an indoline NH is shifted at about 7.5 ppm.[23] All protons and carbons of compound **1A** were assigned from ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC, and HMBC correlations (Fig. 2). The C5 carbon (24.2 ppm) was much more shielded than the C4 carbon (39.7 ppm). Therefore, the cyano substituent was born by C5 (its small chemical shift value was due to the magnetic anisotropy of the triple bond) and the ethoxycarbonyl group was attached to C4.

Due to the insolubility of **2**, a direct hydrogenation did not work. Protection of the imide nitrogen with a *tert*-butyldimethylsilyl group led to the more soluble derivative **3**, which was hydrogenated in the presence of Raney Nickel to give lactam **4** with concomitant removal of the *tert*-butyldimethylsilyl substituent. Deprotection of the indole nitrogen could be due to the presence of aluminum hydroxide residues in Raney Nickel. The position of the *tert*-butyldimethylsilyl group was determined from a HMBC NMR experiment (Fig. 3). Two ^{2}J couplings between the exchangeable proton at 9.43 ppm and two quaternary carbons at 141.7 and 144.6 ppm as well as ^{3}J coupling between this proton and two quaternary carbons at 120.5 and 121.0 ppm showed that the TBDMS group was attached to the maleimide nitrogen. The quaternary carbon at 120.5 ppm could be assigned from its ^{3}J coupling with two protons at 7.62 and 7.47 ppm. The quaternary carbon at 144.6 ppm could be assigned from its unique ^{2}J coupling with the indolic NH at 9.43 ppm.

To obtain a five-membered D ring containing two carbonyl functions, a Diels–Alder reaction was first performed between indolyl maleimide and cyclopent-4-en-1,3-dione, but the cycloadduct was isolated as a mixture containing several isomers. In the literature, a large number of Diels–Alder cycloadditions are described using cyclopent-4-en-1,3-dione leading to cycloadducts in their enolic form.[33–37] Oxidation of the mixture of isomers either with DDQ or in TFA also led to a mixture of isomers, probably containing carbonyl and enol forms. To avoid the presence of enol isomers, the Diels–Alder reaction was carried out with 2,2-dimethyl-cyclopent-4-



Scheme 1. Synthesis of compound **4**.

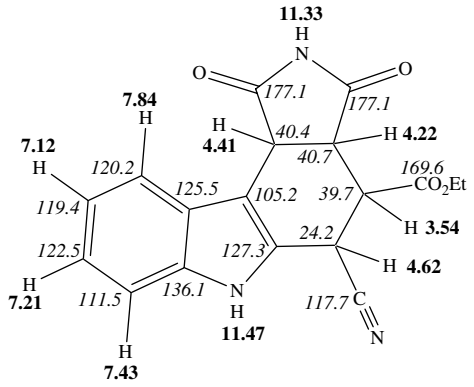


Figure 2. Chemical shifts of carbons (italics) and protons (bold) in **1A** assigned from ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC, and HMBC NMR correlations.

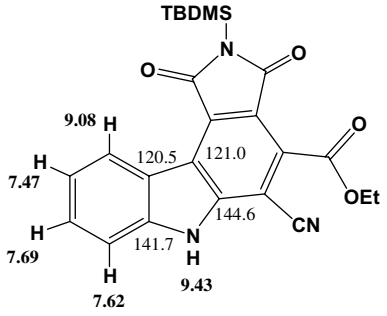


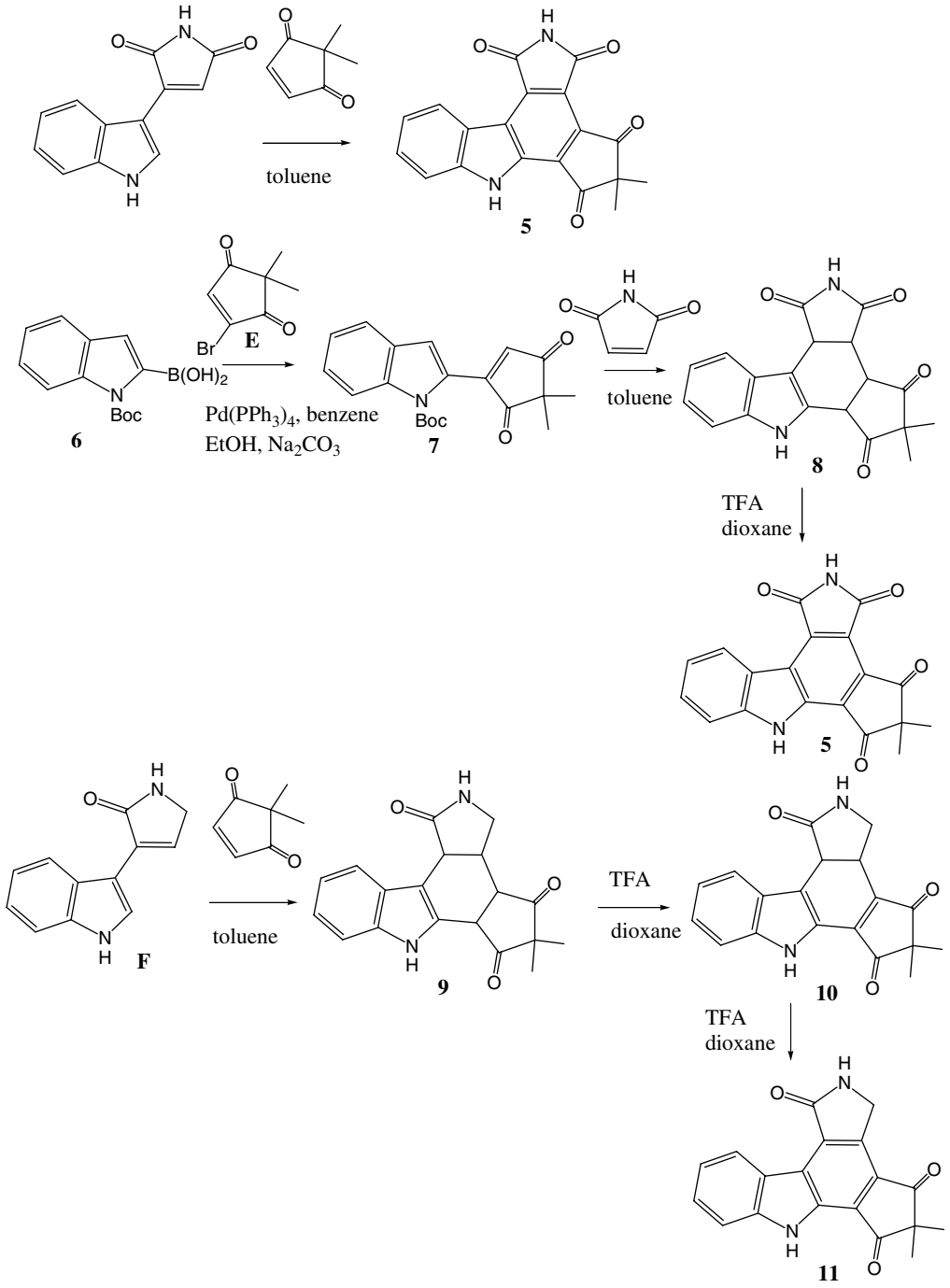
Figure 3. Assignment of the position of the TBDMS group in compound **3**. Chemical shifts of protons in bold.

en-1,3-dione, prepared from commercially available 2-methyl-cyclopentan-1,3-dione as described by Agosta and Smith,[37] leading directly to oxidized compound **5** (Scheme 2). Compound **5** could also be obtained from 2,2-dimethyl-4-(2-*N*-Boc indolyl)-cyclopent-4-en-1,3-dione prepared via a Suzuki coupling between *N*-Boc indole boronic acid and 4-bromo-2,2-dimethyl-cyclopent-4-en-1,3-dione **E**[38] affording compound **7** (Scheme 2). A Diels–Alder reaction between **7** and maleimide gave cycloadduct **8** as the indole isomer, which was further oxidized into compound **5** in the presence of TFA.

Compound **11**, analogue of compound **5** with a lactam E heterocycle, was synthesized from 3-(1*H*-indol-3-yl)-1*H*-2,5-dihydro-pyrrol-2-one **F**[27] and 2,2-dimethylcyclopentanedione as outlined in Scheme 2. Cycloadduct **9** was obtained as the indole isomer. Oxidation of **9** into **11** was difficult to achieve. Using DDQ, the lactam function of the upper heterocycle was oxidized into imide. Intermediate **10** was obtained in the presence of TFA in the conditions indicated in the experimental section after heating at 80 °C for 48 h in 91% yield. When the mixture was heated at 80 °C for 3 days instead of 48 h, only the imide was isolated. The positions of the double bonds in compound **10** were assigned from the ^{1}H NMR spectrum showing a coupling between the two protons of the lactam heterocycle CH_2 and a vicinal proton. After isolation, a solution of compound **10** in THF was re-oxidized for 24 h at 80 °C to give the required compound **11** in 89% yield.

To enhance the solubility and/or modify the affinity for the ATP-binding site of Chk1, and since in staurosporine[15] (Fig. 1), a potent indolocarbazole Chk1 inhibitor, a sugar moiety is attached to the indole nitrogens, a glucosyl moiety was coupled to the indolyl maleimide before reaction with benzoquinone (Scheme 3).

A Diels–Alder reaction between *N*-BOM indolylmaleimide bearing a glucosyl moiety (compound **G**)[39] and benzoquinone gave compound **12** in which the sugar part was attached to the lower oxygen of the D cycle (Scheme 3). The mechanism involves probably a transfer of hydride from the sugar to the quinone oxygen, followed by the transfer of a proton to the phenolate

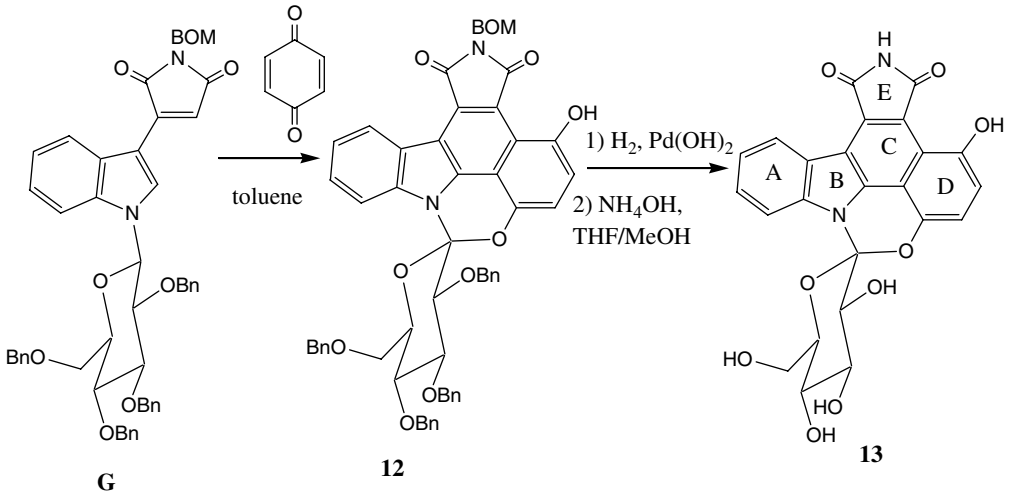


Scheme 2. Syntheses of compounds **5** and **11**.

ion.[40] Deprotection of the imide nitrogen and the hydroxy groups of the carbohydrate carried out by hydrogenolysis with $Pd(OH)_2$, as the catalyst, then aminolysis using NH_4OH in THF/MeOH led to compound **13**. In the ^1H NMR spectrum of compound **13**, the anomeric proton, a doublet, generally shifted about 7.5 ppm, was absent. Only two exchangeable protons were observed between 11 and 12 ppm (11.28 and 11.93 ppm). Thirteen quaternary carbons were observed in the ^{13}C NMR spectrum. The structure of spiro compound **13** was confirmed by ^1H–^1H COSY, HMBC, and HSQC NMR experiments. Moreover, only one exchangeable proton was observed in the ^1H NMR spectrum of intermediate **12**. As expected, compound **13** bearing a sugar moiety was much more soluble than quinone **B** and hydroquinone **C** (Fig. 1).

2.2. Chk1 inhibitory activities

The inhibitory activities toward Chk1 of compounds **4**, **5**, **11**, and **13** were evaluated and compared with those of granulatimide, isogranulatimide, bis-imide **A**, compound **H** (a regioisomer of **4** in which the D and E rings are inverted), quinone **B**, and hydroquinone **C** (IC_{50} values in μM, Table 1). The percentages of Chk1 inhibition at a compound concentration of 10 μM were determined



Scheme 3. Synthesis of compound **13**.

Table 1. Percentages of Chk1 inhibition at a drug concentration of 10 μM

Compound	% of Chk1 inhibition at 10 μM	IC_{50} Chk1 (μM)	L1210	HCT 116	HT29
Granulatimide	93.9	0.081	2.8	6.1	5.7
Isogranulatimide	89.7	0.44	10	13	13.7
A	94.4	0.020	32.7	nd	9.7
4	83	0.024	>50	8.54	0.58
H	85.4	0.051	nd	nd	nd
5	63	14.5	33	32.7	38.5
11	20	nd	2.2	1.7	2.5
13	73	1.92	21.2	46.4	57.8
B	86	0.269	6.8	5.3	17.4
C	96	0.311	1.1	3.1	5.6

IC_{50} values (μM) toward Chk1. In vitro antiproliferative activities against three tumor cell lines: murine leukemia L1210, and human HT29 and HCT116 colon carcinoma (IC_{50} μM).

and the IC_{50} values in micromolar were measured for the most efficient inhibitors. Compound **4** was a stronger Chk1 inhibitor than granulatimide, isogranulatimide, and its regioisomer **H**. The IC_{50} value of compound **4** toward Chk1 (24 nM) was in the same range as that of bis-imide **A**, suggesting that the lower carbonyl of the D ring is not essential for Chk1 inhibition. Imide **5** and lactam **11** with a 2,2-dimethylcyclopenten-4-dione as the D ring were weak Chk1 inhibitors, probably because of the two methyl groups that could induce steric hindrance in the ATP-binding site of Chk1.

The presence of the glycosyl moiety attached to both indole nitrogen and phenolic oxygen, though inducing a better solubility, is detrimental to Chk1 inhibition (compare compounds **13** and **C**).

2.3. Molecular modeling

To try to rationalize the above-described results, molecular modeling was carried out using as model the complex structure of Chk1/staurosporine[15] downloaded from the Protein Data Bank. Docking was performed with bis-imide **A** and compounds **4**, **5**, and **H** (Fig. 4). With bis-imide **A**, seven hydrogen bonds were observed. In addition to the two fundamental hydrogen bonds between the carbonyl on the left and the imide NH of the E heterocycle with Cys^{87} and Glu^{85}, respectively, hydrogen bonds were also observed between the indole NH and the carboxylate of the side chain of Glu^{91}, and between the upper carbonyl of the D heterocycle and the amine of the side chain of Lys^{38}. Moreover, three hydrogen bonds were formed via a water molecule: between the carbonyl on the right of the E heterocycle and the amine of Asp^{148}, between the imide NH of the D heterocycle and the carboxylate of the side chain of Asp^{148}, and between the lower carbonyl of the D heterocycle and the amine of the side chain of Lys^{132} and the carboxylate of the side chain of Glu^{91}. This important hydrogen bond net is in agreement with the strong inhibitory activity of bis-imide **A** toward Chk1 (IC_{50} value: 20 nM). Several water molecules in this area have been reported to make or not contacts with various families of Chk1 inhibitors.[41–47] The docking of compound **4** resulted in an orientation in the ATP-binding site of Chk1 that was similar to that of bis-imide **A**. Compared with bis-imide **A**, the same hydrogen bond net was found except for the carbonyl of the D heterocycle oriented toward the indole, which is missing in compound **4**. Compared with compound **4**, compound **H**, in which heterocycles D and E are inverted, made fewer hydrogen bonds with the enzyme, in particular, on the upper right side. The variations in the stabilization of the compounds in the ATP-binding site of Chk1 via hydrogen bonds appear to be partially responsible for the different Chk1 inhibitory potencies of bis-imide **A**, compound **4** and compound **H**.

Compared with bis-imide **A**, compound **5** was less stabilized by H-bonding inside the ATP-binding pocket, which could explain its weaker Chk1 inhibitory potencies (Fig. 4). Since the D ring is surrounded by a hydrophilic pocket, the replacement of the D maleimide heterocycle by a more hydrophobic cycle was unfavorable for the stabilization of the compound. It can be ob-

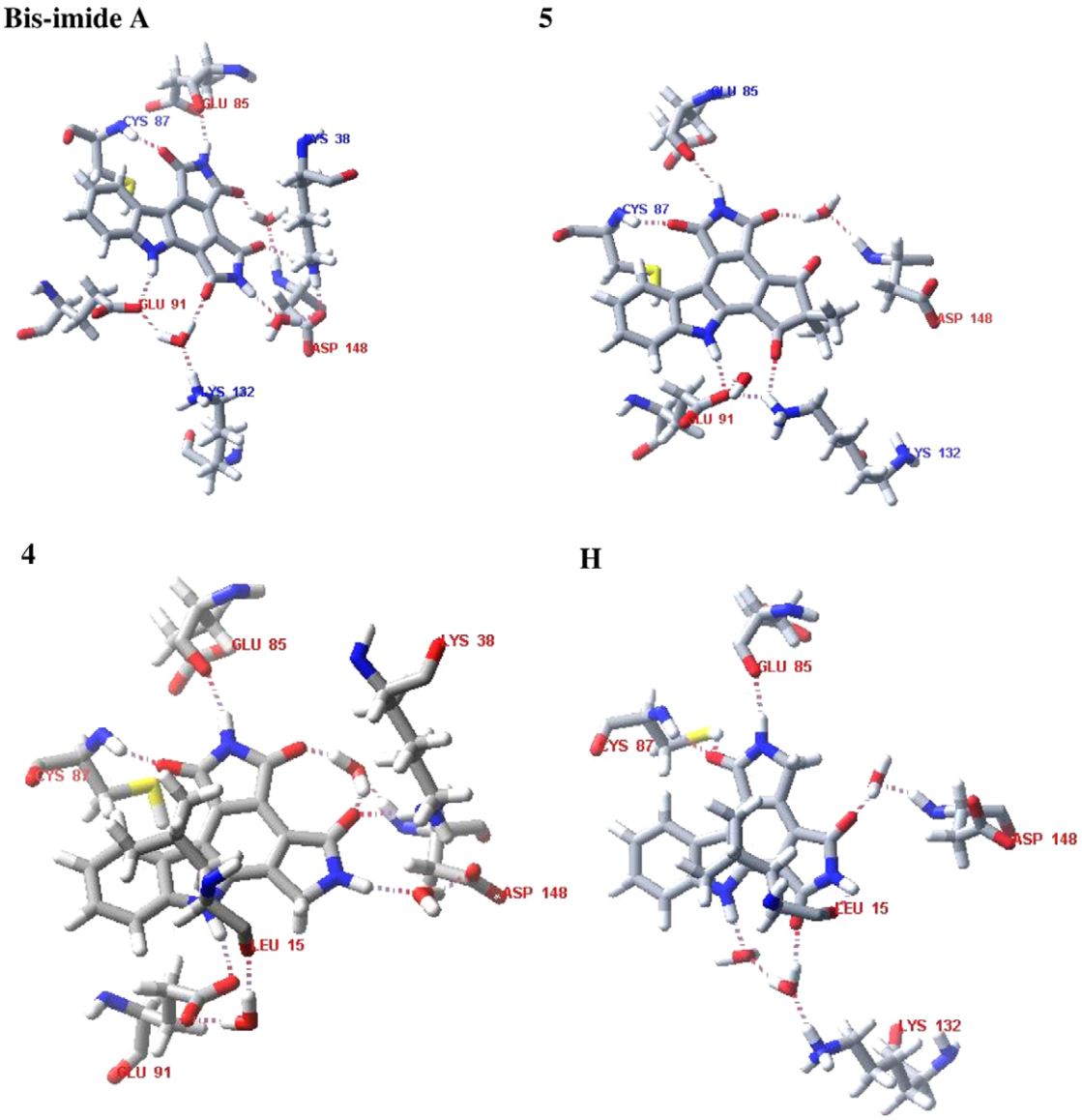


Figure 4. Docking of bis-imide **A** and compound **4**, **5**, and **H** displayed into the ATP-binding site of Chk1.

served that in the gem-dimethylated compound **5**, in contrast with bis-imide **A**, the upper carbonyl of the D ring did not participate in any hydrogen bonding. The superimposition of bis-imide **A** and compound **5** in the ATP-binding site of Chk1 (Fig. 5) shows a slight moving of Lys^{38} and Asp^{148} possibly due to the steric hindrance of the two methyl groups and also due to the presence of hydrophobic methyl groups in this hydrophilic pocket.

Docking was also performed with compound **13** and the results were compared with those obtained for the parent compound **C**[29] without the sugar moiety (Fig. 6). Compared with compound **C**, an identical hydrogen bond net was found in the upper region and, of course, a different hydrogen bond net was observed in the lower region. Besides two intramolecular hydrogen bonds between the OH in 2′ and the lower oxygen of the phenolic moiety, and between the OH in 4′ and the oxygen of the alcohol in 6′, the sugar part of compound **13** allowed two hydrogen bonds with Leu^{15} and Glu^{91}. But the pres-

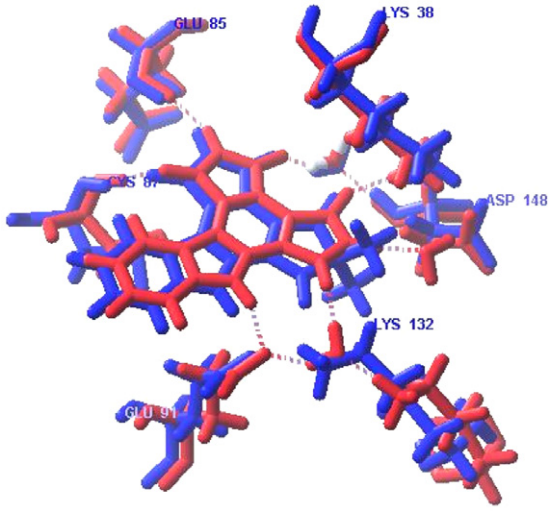


Figure 5. Superimposition of bis-imide **A** (in red) and compound **5** (in blue) in the ATP-binding site of Chk1.

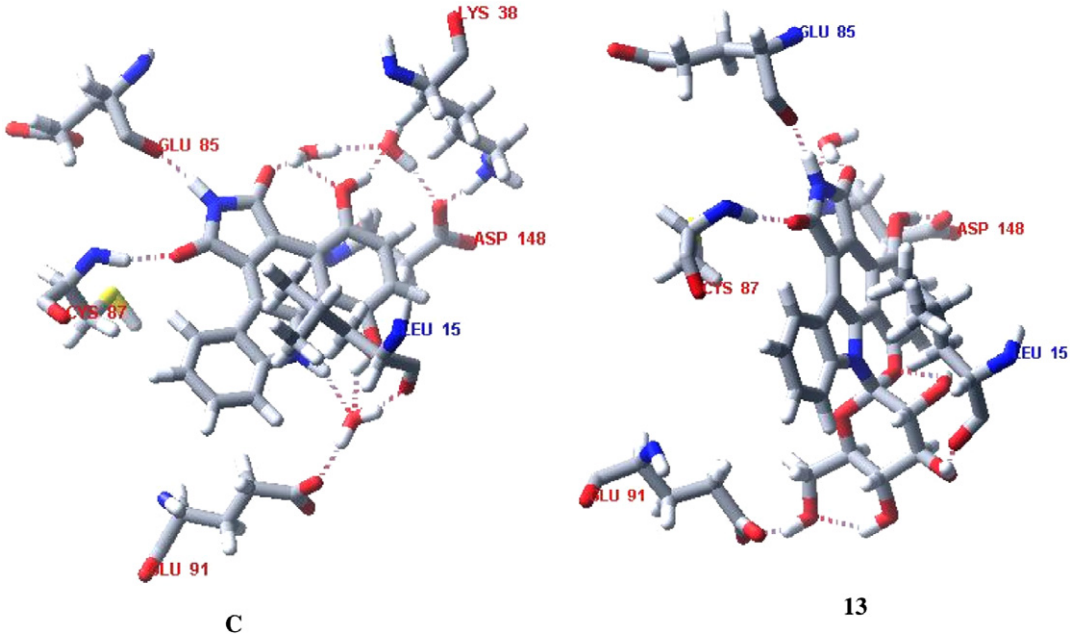


Figure 6. Docking of compounds **C** and **13** displayed into the ATP-binding site of Chk1.

ence of the sugar moiety eliminated the hydrogen bond net observed with the indolic NH and the lower phenolic OH in compound **C**. So, it is not possible to conclude from the molecular modeling results about the weaker Chk1 inhibitory potency of compound **13** with respect to compound **C**.

2.4. In vitro antiproliferative activities

The cytotoxicities of the newly synthesized compounds were evaluated toward three tumor cell lines: murine leukemia L1210, and human colon carcinoma HT29 and HCT116 and compared with those of granulatimide and isogranulatimide, bis-imide **A**, and compounds **H**, **B**, and **C**.

No parallelism was observed between the cytotoxicities and the Chk1 inhibitory activities which is not surprising because a Chk1 inhibitor is not expected to be cytotoxic by itself. However, the cytotoxicities of the new compounds were examined to get an insight into possible other targets than Chk1. The absence of cytotoxicity could also be due to a poor cell penetration. Compound **4**, the strongest Chk1 inhibitor of the new compounds described in this study, was not cytotoxic toward L1210 cells but strongly cytotoxic toward HT29 cells. Lactam **11**, that could not be considered as a Chk1 inhibitor, exhibited a similar significant cytotoxicity toward the three cell lines tested, suggesting other targets than Chk1 for this compound. Compound **13**, that inhibited Chk1 significantly, was not really cytotoxic. Compounds exhibiting significant cytotoxicities could inhibit other kinases than Chk1 which could account for their cytotoxic effects.

2.5. Src kinase inhibition

Staurosporine is a non-selective kinase inhibitor, UCN-01 is more selective, in contrast, compound **C** (Fig. 1), which have been tested toward 19 other kinases proved to be a selective Chk1 inhibitor.[29] To get an insight into the kinase selectivity, Src tyrosine kinase inhibitory activities were evaluated for compounds **11** and **13**. The percentages of Src inhibition at a drug concentration of 10 μM were 19% for compound **11** and 9% for compound **13**. Both were poor Src inhibitors, but the percentages of Chk1 and Src inhibition for compound **11** were in the same range, suggesting that compound **11** is not selective. Its capacity to inhibit other kinases than Chk1 could be responsible for the cytotoxicity of this compound.

3. Conclusion

In conclusion, this work reports the synthesis of new maleimide or maleamide carbazoles possessing various D rings with one or two carbonyl groups. Two methods have been developed for the synthesis of compound **5**, either by a Diels–Alder cycloaddition on indolylmaleimide or via a Suzuki coupling followed by a Diels–Alder reaction with maleimide to introduce the E ring. By analogy with staurosporine, carbazole **13**, in which the sugar moiety is attached to the indole nitrogen and to an oxygen born by the D ring, was also synthesized. Among the newly synthesized compounds, compound **4** with a lactam D ring was the strongest Chk1 inhibitor with an IC_{50} value of 24 nM. In spite of its weaker inhibitory potency toward Chk1 compared to the parent compound **C**, compound **13** represents an interesting structure providing a new avenue to the design of soluble Chk1 inhibitors. Actually, as it has been shown in our previous works,[29] it is easy to introduce various groups which could highly enhance the Chk1 inhibitory potency, for example, by forming additional hydrogen bonds with Cys^{87} or hydrophobic interactions with Gly^{90}.

4. Experimental

4.1. Chemistry

IR spectra were recorded on a Perkin-Elmer 881 spectrometer (ν in cm^{-1}). NMR spectra were performed on a Bruker AVANCE 400 and AVANCE 500 (chemical shifts δ in ppm) the following abbreviations are used: singlet (s), broad singlet (br s), doublet (d), doubled doublet (dd), triplet (t), doubled triplet (dt), multiplet (m), pseudo quadruplet (pq), quintuplet (quint), tertiary carbons (C tert), and quaternary carbons (C quat). The signals were assigned from ^1H–^1H COSY, HSQC, and HMBC NMR correlations. Low resolution mass spectra (ESI+) and HRMS were determined on a MS Hewlett–Packard engine. Chromatographic purifications were performed by flash silicagel Geduran SI 60 (Merck) 0.040–0.063 mm column chromatography.

4.1.1. 1,3,3a,4,5,10c-Hexahydro-5-cyano-4-ethyloxycarbonyl-2*H*,6*H*-pyrrolo[3,4-*c*]carbazole-1,3-dione (1A), 1,3,3a,4,5,5a-hexahydro-5-cyano-4-ethyloxycarbonyl-2*H*,6*H*-pyrrolo[3,4-*c*]carbazole-1,3-dione (1B) and 1,3-dihydro-5-cyano-4-ethyloxycarbonyl-2*H*,6*H*-pyrrolo[3,4-*c*]carbazole-1,3-dione (2). To a suspension of 3-(indol-3-yl)-maleimide **D** (500 mg, 2.35 mmol) in toluene (30 mL), ethyl *cis*-β-cyanoacrylate (24.0 mmol, 3.0 mL) was added. The mixture was refluxed for 5 days. After filtration, the solid residue was washed with CH_2Cl_2 to give a mixture of the Diels–Alder adducts **1A** and **1B** (200 mg, 0.61 mmol, 26% yield). A solution of the Diels–Alder adduct (200 mg) in dioxane (14 mL) was refluxed for 20 h in the presence of DDQ (295 mg, 1.29 mmol). The solvent was removed. Water was added to the residue. After filtration, the solid residue was washed with water and EtOAc to give **2** (177 mg, 0.53 mmol, 87% yield).

4.1.1.1. NMR data for compound 1A. ^1H NMR (500 MHz, DMSO-d_6): 1.34 (3H, t, J = 7.0 Hz), 3.54 (1H, t, J = 5.0 Hz), 4.22 (1H, dd, J_1 = 8.5 Hz, J_2 = 5.0 Hz), 4.29–4.34 (2H, m), 4.41 (1H, d, J = 8.5 Hz), 4.62 (1H, J = 5.0 Hz), 7.12 (1H, t, J = 8.0 Hz), 7.21 (1H, t, J = 7.5 Hz), 7.43 (1H, d, J = 8.0 Hz), 7.84 (1H, d, J = 8.0 Hz), 11.33 (1H, br s, NH), 11.47 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 14.0 (CH_3), 60.9 (CH_2), 24.2, 39.7, 40.4, 40.7 (CH), 111.5, 119.4, 120.2, 122.5 (CH arom), 117.7 (C≡N), 105.2, 125.5, 127.3, 136.1 (C quat arom), 169.6, 177.1 (2C) (C=O).

4.1.1.2. NMR data for compound 1B. ^1H NMR (400 MHz, DMSO-d_6): 1.34 (3H, t, J = 7.0 Hz), 3.09–3.14 (2H, m), 3.88 (1H, d, J = 6.0 Hz), 4.23–4.37 (2H, m), 4.54–4.60 (1H, m), 6.84 (1H, t, J = 7.5 Hz), 6.88 (1H, d, J = 8.5 Hz), 7.35 (1H, t, J = 7.5 Hz), 7.56 (1H, d, J = 3.5 Hz, NH), 8.50 (1H, d, J = 8.0 Hz), 11.36 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 14.0 (CH_3), 61.5 (CH_2), 35.0, 35.7, 43.8, 61.5 (CH), 111.1, 118.5, 127.4, 133.5 (CH arom.), 114.3, 118.8, 121.6, 147.3, 156.8 (C quat + C≡N), 167.9, 172.0, 176.0 (C=O).

Data for compound 2: mp >300 °C. IR (KBr) $\upsilon_{C=O}$ 1662, 1702 cm^{-1}, υ_{NH} 3356 cm^{-1}. HRMS (ESI+) $[M+Na]^+$ calcd for $C_{18}H_{11}N_3O_4Na$ 356.0647; found: 356.0661. ^1H NMR (400 MHz, DMSO-d_6): 1.40 (3H, t, J = 7.0 Hz), 4.50 (2H, d, J = 7.0 Hz), 7.40–7.47 (1H, m), 7.66–7.71 (2H, m), 8.84 (1H, d, J = 8.0 Hz), 11.80 (1H, br s, NH), 13.09 (1H, br s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 13.8 (CH_3), 62.6 (CH_2), 94.0, 114.1, 119.4, 119.5, 120.4, 129.0, 130.0, 142.8, 144.5 (C quat), 112.6, 121.8, 125.4, 129.9 (C tert arom), 164.1, 167.3, 168.3 (C=O).

4.1.2. 5-Cyano-1,3-dihydro-4-ethyloxycarbonyl-6-*tert*-butyldimethylsilyl-2*H*-pyrrolo[3,4-*c*]carbazole-1,3-dione (3). To a suspension of **2** (70 mg, 0.21 mmol) in THF (4 mL) was added NaH (60% dispersion in oil, 52 mg, 0.31 mmol) at 0 °C. After stirring for 10 min at 0 °C, TBDMSCl (0.52 mmol, 79 mg) was added. The mixture was stirred at room temperature for 30 min then water was added. After extraction with EtOAc, the organic phase was dried over $MgSO_4$ and the solvent was removed. The residue was purified by flash chromatography (eluent cyclohexane/EtOAc 7:3) to give **3** (90 mg, 0.20 mmol, 96% yield) as a yellow solid.

Mp 278 °C. IR (KBr) $\upsilon_{C=O}$ 1706, 1737, 1761 cm^{-1}, υ_{CN} 2231 cm^{-1}, υ_{NH} 3273 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{24}H_{26}N_3O_4Si$ 448.1693; found: 448.1674. ^1H NMR (400 MHz, $CDCl_3$): 0.60 (6H, s), 1.04 (9H, s), 1.50 (3H, dt, J_1 = 7.0 Hz, J_2 = 1.0 Hz), 4.63 (2H, dq, J_1 = 7.0 Hz, J_2 = 1.0 Hz), 7.47 (1H, dt, J_1 = 8.0 Hz, J_2 = 1.0 Hz), 7.62 (1H, d, J = 8.0 Hz), 7.69 (1H, t, J_1 = 8.0 Hz, J_2 = 1.0 Hz), 9.08 (1H, d, J = 8.0 Hz), 9.43 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): −4.0, 14.1, 26.5 (CH_3), 19.1 (C quat), 63.3 (CH_2), 111.6, 122.7, 126.8, 130.3 (C tert arom), 95.8, 114.1, 120.5, 120.9, 122.5, 129.4, 131.3, 141.6, 144.5 (C quat), 164.0, 171.0, 172.3 (C=O).

4.1.3. 2*H*,5*H*,7*H*-1,3,4,6-Tetrahydrodipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4-trione (4). A solution of **3** (69 mg, 0.15 mmol) in DMF (6 mL) was hydrogenated for 7 days (50 psi) in the presence of Raney Ni (150 mg). After filtration over Celite, the solvent was removed. EtOH was added to the residue. After filtration, compound **4** (10 mg, 0.034 mmol, 23% yield) was isolated as a pale yellow solid.

Mp >280 °C. IR (KBr) $\upsilon_{C=O}$ 1674, 1710, 1761 cm^{-1}, υ_{NH} 2997–3643 cm^{-1}. HRMS (ESI+) $[M+Na]^+$ calcd for $C_{16}H_9N_3O_3Na$ 314.0542; found: 314.0529. ^1H NMR (400 MHz, DMSO-d_6): 4.78 (2H, s), 7.40 (1H, dt, J_1 = 7.0 Hz, J_2 = 1.0 Hz), 7.64 (1H, dt, J_1 = 8.0 Hz, J_2 = 1.0 Hz), 7.73 (1H, d, J = 8.0 Hz), 8.78 (1H, s, NH), 9.03 (1H, d, J = 8.0 Hz), 11.17 (1H, br s, NH), 12.66 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 43.9 (CH_2), 111.9, 120.6, 125.3, 128.5 (C tert), 119.3, 120.2, 120.3, 126.8, 128.3, 133.2, 138.1, 142.0 (C quat), 167.4, 167.7, 170.2 (CO).

4.1.4. 2*H*,7*H*-1,3-Dihydro-5,5-dimethylcyclopenta[4,5-*a*]pyrrolo[3,4-*c*]carbazole-1,3,4,6-tetraone (5). A mixture of 3-(indol-3-yl)-maleimide (100 mg, 0.47 mmol) and 2,2-dimethylcyclopentenedione (350 mg, 2.83 mmol) in toluene (12 mL) was stirred at 140 °C in a sealed tube for 7 days. After cooling, the mixture was filtered off and the residue was washed with dichloromethane and

EtOAc to give **5** (30 mg, 0.09 mmol, 20% yield) as an orange solid.

Mp >280 °C. IR (KBr) $\upsilon_{C=O}$ 1700, 1726, 1742, 1767 cm^{-1}, υ_{NH} 3257, 3340 cm^{-1}. HRMS (ESI)+ $[M+H]^+$ calcd for $C_{19}H_{13}N_2O_4$ 333.0875; found: 333.0890. ^1H NMR (400 MHz, DMSO-d_6): 1.35 (6H, s), 7.52 (1H, t, J = 7.5 Hz), 7.75 (1H, t, J = 7.5 Hz), 7.91 (1H, d, J = 8.0 Hz), 9.06 (1H, d, J = 8.0 Hz), 11.68 (1H, s, NH), 13.07 (1H, s, NH).

4.1.5. 2,2-Dimethyl-4-(2-*N*-Boc-indolyl)-cyclopent-4-en-1,3-dione (7). A solution of 2-*N*-Boc-indole-boronic acid (670 mg, 2.57 mmol) in EtOH (18 mL) was added to a mixture of 2,2-dimethyl-5-bromo-cyclopentanedione **E** (437 mg, 2.15 mmol) and $Pd(PPh_3)_4$ (208 mg, 0.078 mmol) in benzene (36 mL). A solution of Na_2CO_3 (1.32 g) in water (49 mL) was added and the mixture was refluxed for 1.5 h. After cooling, water was added. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc from 95:5 to 8:2) to give **7** (157 mg, 0.46 mmol, 22% yield) as a yellow solid.

Mp 77 °C. IR (KBr) $\upsilon_{C=O}$ 1705, 1730 cm^{-1}. Mass (ESI+) $[M-Boc+H]^+$ 240, $[M-Boc+Na]^+$ 262, $[M+Na]^+$ 362. HRMS (ESI+) $[M-Boc+H]^+$ calcd for $C_{15}H_{14}NO_2$ 240.1025; found: 240.1041. ^1H NMR (400 MHz, DMSO-d_6): 1.30 (6H, s), 1.67 (9H, s), 7.02 (1H, s), 7.09 (1H, s), 7.27 (1H, t, J = 7.5 Hz), 7.41 (1H, t, J = 8.0 Hz), 7.61 (1H, d, J = 8.0 Hz), 8.03 (1H, d, J = 8.0 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): 19.9, 28.1 (CH_3), 47.6, 85.2, 128.7, 129.1, 137.6, 150.0 (C quat), 114.7, 115.2, 121.8, 123.3, 126.4, 138.0 (C tert), 151.7, 204.7, 206.1 (C=O).

4.1.6. 2*H*,7*H*-5,5-Dimethyl-1,3,3a,3b,6a,11c-hexahydrocyclopenta[4,5-*a*]pyrrolo[3,4-*c*]carbazole-1,3,4,6-tetraone (8). A solution of compound **7** (157 mg, 0.46 mmol) and maleimide (223 mg, 2.3 mmol) in toluene (3 mL) was stirred at 140 °C for 48 h in a sealed tube. The solvent was removed and the residue was purified by flash chromatography (eluent: EtOAc/cyclohexane from 3:7 to 7:3) to give **8** (75 mg, 0.22 mmol, 48% yield) as a yellow solid.

M_p >280 °C. IR (KBr) $\upsilon_{C=C}$ 1616, 1639 cm^{-1}, $\upsilon_{C=O}$ 1704, 1720, 1768 cm^{-1}, υ_{NH} 3400–3600 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{19}H_{19}N_2O_3$ 323.1396; found: 323.1410. ^1H NMR (400 MHz, DMSO-d_6): 1.09 (6H, s), 3.46 (2H, d, J = 8.0 Hz), 3.86 (2H, d, J = 8.0 Hz), 7.03 (1H, d, J = 7.5 Hz), 7.13 (1H, dt, J_1 = 7.5 Hz, J_2 = 1.0 Hz), 7.30 (1H, dt, J_1 = 7.5 Hz, J_2 = 1.0 Hz), 7.79 (1H, d, J = 7.0 Hz), 11.21 (1H, s, NH), 11.69 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 22.2 (2 CH_3), 46.2 (2 CH), 46.3 (2 CH), 111.3, 121.9, 125.8, 128.4 (C tert arom), 53.7, 94.0, 128.7, 146.2, 152.0 (C quat), 174.2, 176.3, 195.6, 214.0 (C=O).

4.1.7. 2*H*,7*H*-1,3-Dihydro-5,5-dimethylcyclopenta[4,5-*a*]pyrrolo[3,4-*c*]carbazole-1,3,4,6-tetraone (5). A solution of compound **8** (28 mg, 0.08 mmol) in TFA (64 μL) and dioxane (3 mL) was refluxed for 48 h. After evaporation, water was added to the residue. The mixture was filtered off to give compound **5** (24 mg, 0.072 mmol, 90% yield).

4.1.8. 2*H*,7*H*-5,5-Dimethyl-1,3,3a,3b,6a,11c-hexahydrocyclopenta[4,5-*a*]pyrrolo[3,4-*c*]carbazole-1,4,6-trione (9). A mixture of 3-(1*H*-indol-3-yl)-1*H*-2,5-dihydro-pyrrol-2-one **F** (219 mg, 1.11 mmol) and 2,2-dimethylcyclopentanedione (789 mg, 6.36 mmol) in toluene (4 mL) was stirred at 140 °C in a sealed tube for 4 days. After cooling, the mixture was filtered off and the residue was washed with dichloromethane to give **9** (226 mg, 0.70 mmol, 63% yield) as a pale yellow solid.

Mp >280 °C. IR (KBr) $\upsilon_{C=O}$ 1689, 1723, 1763 cm^{-1}, υ_{NH} 3346, 3376 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{19}H_{19}N_2O_3$ 323.1396; found: 323.1409. ^1H NMR (400 MHz, DMSO-d_6): 0.70 (3H, s), 1.19 (3H, s), 3.19 (1H, t, J = 7.5 Hz), 3.35–3.49 (3H, m), 3.79 (1H, m), 4.48 (1H, d, J = 8.5 Hz), 6.96 (1H, t, J = 7.5 Hz), 7.08 (1H, t, J = 7.5 Hz), 7.35 (1H, d, J = 8.0 Hz), 7.64 (1H, s, NH), 7.86 (1H, d, J = 8.0 Hz), 11.00 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 20.9, 21.6 (CH_3), 32.2, 38.2, 44.8, 46.6 (CH), 43.5 (CH_2), 50.6, 105.3, 126.2, 126.3, 137.0 (C quat), 111.2, 118.5, 120.9, 121.6 (C tert arom), 175.1, 212.6, 215.9 (C=O).

4.1.9. 2*H*,7*H*-5,5-Dimethyl-1,3,3a,11c-tetrahydrocyclopenta[4,5-*a*]pyrrolo[3,4-*c*]carbazole-1,4,6-trione (10). A mixture of **9** (30 mg, 0.093 mmol), TFA (72 μL) and dioxane (3 mL) was stirred at 80 °C for 48 h. After evaporation, water was added to the residue, and the mixture was filtered off to give **10** (27 mg, 0.084 mmol, 91% yield) as an orange solid.

Mp >280 °C. IR (KBr) $\upsilon_{C=C}$ 1619, 1635 cm^{-1}, $\upsilon_{C=O}$ 1690, 1723, 1763 cm^{-1}, υ_{NH} 3370–3565 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{19}H_{17}N_2O_3$ 321.1239; found: 321.1241. ^1H NMR (400 MHz, DMSO-d_6): 1.16 (3H, s), 1.17 (3H, s), 3.30 (1H, pt, J = 8.0 Hz), 3.75 (1H, pt, J = 9.0 Hz), 4.06 (1H, m), 4.21 (1H, d, J = 11.0 Hz), 7.09 (1 H, t, J = 7.0 Hz), 7.25 (1H, t, J = 7.5 Hz), 7.56 (1H, d, J = 8.0 Hz), 8.07 (1H, d, J = 8.0 Hz), 8.11 (1H, s, NH), 11.64 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 19.9, 20.0 (CH_3), 31.8, 41.2 (CH), 45.4 (CH_2), 47.2, 114.1, 123.8, 125.7, 139.8, 140.7, 147.1 (C quat), 112.7, 120.0, 122.2, 124.4 (C tert arom), 175.4, 203.6, 203.8 (C=O).

4.1.10. 2*H*,7*H*-5,5-Dimethyl-1,3-dihydrocyclopenta[4,5-*a*]pyrrolo[3,4-*c*]carbazole-1,4,6-trione (11). A mixture of **10** (37 mg, 0.016 mmol) and TFA (50 μL) in dioxane (4 mL) was stirred at 80 °C for 24 h. After evaporation, water was added to the residue. The mixture was filtered off to give **11** (33 mg, 0.10 mmol, 89% yield) as a yellow solid.

Mp >280 °C. IR (KBr) $\upsilon_{C=C}$ 1619, 1634 cm^{-1}, $\upsilon_{C=O}$ 1694, 1722 cm^{-1}, υ_{NH} 3400–3565 cm^{-1}. HRMS (ESI)+ $[M+H]^+$ calcd for $C_{19}H_{15}N_2O_3$ 319.1083; found: 319.1084. ^1H NMR (400 MHz, DMSO-d_6): 1.38 (6H, s), 5.10 (2H, s), 7.43 (1H, dt, J_1 = 7.5 Hz, J_2 = 0.5 Hz), 7.70 (1H, dt, J_1 = 8.0 Hz, J_2 = 1.0 Hz), 7.86 (1H, d, J = 8.0 Hz), 9.03 (1H, d, J = 8.0 Hz), 9.37 (1H, s, NH),

12.83 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 20.3 (CH_3), 45.7 (CH_2), 49.9, 119.6, 125.2, 126.3, 130.0, 130.6, 130.7, 133.2, 143.5 (C quat), 112.9, 121.1, 125.5, 129.4 (C tert arom), 168.2, 203.2, 203.6 (C=O).

4.1.11. 1,3-Dihydro-2-benzyloxymethyl-7,8-(1,1-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl))-4-hydroxybenzo[1,2-*a*]pyrrolo[3,4-*c*]carbazole-1,3-dione (12). A solution of compound **G** (450 mg, 0.53 mmol) in toluene (30 mL) was refluxed in the presence of *p*-benzoquinone (1.73 g, 16.0 mmol). The solvent was removed and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc/toluene 10:4:2) to give **12** (170 mg, 0.177 mmol, 33% yield) as a red solid.

Mp 55 °C. IR (KBr) $\upsilon_{C=O}$ 1685, 1758 cm^{-1}, υ_{NH} 3444 cm^{-1}. Mass (ESI+) $[M+Na]^+$ 981. ^{1}H NMR (400 MHz, $CDCl_3$): 2.96 (1H, d, J = 11.5 Hz), 3.92 (1H, dd, J_1 = 11.0 Hz, J_2 = 2.0 Hz), 4.02 (1H, dd, J_1 = 11.0 Hz, J_2 = 2.0 Hz), 4.06 (1H, d, J = 11.0 Hz), 4.11 (1H, d, J = 9.0 Hz), 4.19–4.29 (2H, m), 4.53 (1H, d, J = 10.0 Hz), 4.57 (1H, d, J = 12.0 Hz), 4.67 (1H, d, J = 12.0 Hz), 4.73–4.77 (4H, m), 4.85 (1H, d, J = 11.0 Hz), 4.97 (1H, d, J = 11.0 Hz), 5.25 (2H, d, J = 1.5 Hz), 5.91 (2H, d, J = 7.5 Hz), 6.71 (2H, t, J = 8.0 Hz), 6.86 (1H, t, J = 7.5 Hz), 7.07 (1H, d, J = 8.5 Hz), 7.10 (1H, d, J = 8.5 Hz), 7.16–7.45 (22 H, m), 7.85 (1H, d, J = 8.0 Hz), 8.76 (1H, d, J = 8.0 Hz), 10.80 (1H, s, exchangeable proton). ^{13}C NMR (100 MHz, $CDCl_3$): 66.9, 67.9, 71.7, 73.5, 74.5, 75.5, 75.8 (CH_2), 74.1, 76.9, 82.0, 82.8, 112.8, 113.1, 114.6, 122.9, 125.1, 126.0, 126.9, 127.3, 127.4, 127.6, 127.7, 127.8, 128.0, 128.4, 128.5, 128.6 (C tert), 107.2, 109.5, 111.4, 115.3, 118.8, 123.0, 136.1, 137.3, 137.9, 138.0, 138.1, 138.4, 142.2, 148.2 (C quat), 168.3, 173.4 (C=O).

4.1.12. 2*H*-1,3-Dihydro-7,8-(1,1-D-glucopyranosyl)-4-hydroxybenzo[1,2-*a*]pyrrolo[3,4-*c*]carbazole-1,3-dione (13). A solution of **12** (43 mg, 0.045 mmol) in a mixture THF/MeOH (2.0/0.5 mL) was hydrogenated (1 bar) in the presence of $Pd(OH)_2$ (22 mg) at room temperature for 12 h. The mixture was filtered over Celite and the solvents were removed. A solution of the residue in THF/NH_4OH (4.5/9.0 mL) was stirred at room temperature for 4.5 h then the solvents were removed. The residue was purified by flash chromatography (eluent EtOAc) to give **13** (18 mg, 0.038 mmol, 84% yield) as a red solid.

Mp 240–242 °C. IR (KBr) $\upsilon_{C=O}$ 1687, 1757 cm^{-1}, $\upsilon_{NH,OH}$ 3300–3600 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{24}H_{19}N_2O_9$ 479.1091; found: 479.1103. ^{1}H NMR (400 MHz, DMSO-d_6): 3.61–3.78 (3H, m), 3.88–3.91 (1H, m), 4.09–4.16 (2H, m), 4.79 (1H, t, J = 5.5 Hz, OH), 5.30 (1H, d, J = 5.5 Hz, OH), 5.41 (1H, d, J = 5.5 Hz, OH), 5.52 (1H, d, J = 5.5 Hz, OH), 7.10 (1H, d, J = 8.0 Hz), 7.27 (1H, d, J = 8.0 Hz), 7.55 (1H, dt, J_1 = 7.0 Hz, J_2 = 1.0 Hz), 7.67 (1H, dt, J_1 = 7.0 Hz, J_2 = 1.0 Hz), 7.98 (1H, d, J = 8.0 Hz), 8.80 (1H, d, J = 8.0 Hz), 11.28 (1H, s), 11.93 (1 H, s). ^{13}C NMR (100 MHz, acetone-d_6): 61.1 (CH_2), 70.0, 74.1, 74.6, 76.8, 112.8, 113.6, 114.2, 122.4, 124.6, 127.0 (C tert), 106.9, 108.3, 109.0, 111.6, 120.1, 123.2, 128.6, 138.4, 138.5, 141.7, 148.4 (C quat), 169.4, 175.1 (C=O).

4.2. Molecular modeling

For docking experiments, all molecular mechanics calculations were performed by the Macromodel[48] molecular modeling software. We used as model the complex structure of Chk1/staurosporine[15] downloaded from the Protein Data Bank (1NVR file).

Energy minimization was done with AMBER force field[49,50] using the Truncated Newton Conjugate Gradient method (TNCG).

4.3. Chk1 inhibitory assays

Human Chk1 full-length enzyme with an N-terminal GST sequence was either purchased from Upstate Biochemicals (No. 14-346) or purified from extracts of Sf9 cells infected with a baculovirus encoding GST-Chk1. Assays for compound testing were based upon the method described by Davies et al.[51]

4.4. Growth inhibition assays

Tumor cells were provided by American Type Culture Collection (Frederik, MD, USA). They were cultivated in RPMI 1640 medium (Life Science technologies, Cergy-Pontoise, France) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 10 mM Hepes buffer (pH 7.4). Cytotoxicity was measured by the microculture tetrazolium assay as described.[52] Cells were continuously exposed to graded concentrations of the compounds for four doubling times, then 15 μL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide were added to each well and the plates were incubated for 4 h at 37 °C. The medium was then aspirated and the formazan solubilized by 100 μL of DMSO. Results are expressed as IC_{50}, concentration which reduced by 50% the optical density of treated cells with respect to untreated controls.

4.5. Src inhibition assays

Inhibitors were diluted with a Tecan Evo150 robot. The kinase assay was performed with 4 μL of inhibitor (10% dimethylsulfoxide (DMSO)), 10 μL of kinase assay buffer 4× concentrated (80 mM $MgCl_2$, 200 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), 0.4 mM ethylenediamine-tetraacetic acid (EDTA), 2 mM DL-dithiothreitol (DTT)), 10 μL substrate peptide (KVEKIGEGYYGVVYK, 370 nM), and 6 μL Src kinase (stock GTP purified diluted with 1× kinase assay buffer to 200 nM). 10 μL co-substrate (40 μM ATP with 0.2 μCi P^{33}-γ-ATP) was added with a Precision 2000 (Biotek Robotic). The assay was incubated for 20 min at 30 °C, then stopped by adding 200 μL of 0.85% orthophosphoric acid, and transferred to a phosphocellulose filter microplate (Whatman-P81). The plate was washed three times with 200 μL of 0.85% orthophosphoric acid and dried with 200 μL acetone. The remaining activity was measured on a Topcount with 25 μL scintillation solution (Packard UltimaGold).

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