Synthesis and Biological Activities of New Checkpoint Kinase 1 Inhibitors Structurally Related to Granulatimide

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In the course of structure−activity relationship studies on granulatimide analogues, new pyrrolo[3,4-*c*]-carbazoles in which the imidazole heterocycle has been replaced by a five- or a six-membered ring bearing one or two carbonyl functions have been synthesized. Their checkpoint kinase 1 (Chk1) inhibitory properties and their in vitro antiproliferative activities toward three tumor cell lines—murine leukemia L1210 and human colon carcinoma HT29 and HCT116 have been determined. The results of molecular modeling in the ATP binding pocket of Chk1 are described. Among the newly synthesized compounds, compounds **13** and **16**, in which the imidazole was replaced by a quinone and a hydroquinone and which bear a hydroxy group on the indole moiety, are the most potent Chk1 inhibitors in this series with IC_{50} values of 27 and 23 nM, respectively.

Introduction

Granulatimide and isogranulatimide (Figure 1) are natural compounds isolated from the ascidian *Didemnum granulatum*.[1,2] These compounds were found to inhibit the cell cycle G2 checkpoint regulator checkpoint kinase 1 (Chk1[a]).[3−4] Normal cells respond to DNA damage by activating cell cycle checkpoints that stop temporarily the transitions from G1 to S phase and from G2 to M phase to allow time for DNA repair. In more than 50% of cancer cells, the G1 checkpoint is lacking, due to mutations of the *p53* gene.[5,6] In the *p53*-mutated cells, only the G2 checkpoint provides cancer cells with the opportunity to repair their DNA after damage. Accordingly, it has been proposed that treatment combining a DNA damaging agent with a G2 checkpoint inhibitor might promote cell death, by selective killing of *p53*-mutated tumor cells.[7−11]

The G2 checkpoint cascade of signal transduction leads to the inactivation of cyclin B/CDK1, the kinase responsible for the progression into M phase. In this cascade, ATM and ATR kinases phosphorylate (activate) Chk1 and Chk2, which in turn phosphorylate (inactivate) Cdc25 phosphatases.[12−14] In mammalian cell division, the Cdc25C phosphatase mediates cellular entry into mitosis. Cdc25C activates CDK1 by dephosphorylating its Tyr15 and Thr14 residues. In the presence of DNA damage, phosphorylation of Cdc25C by checkpoint kinase creates a binding site for 14-3-3 protein that can anchor Cdc25C in the cytoplasm, thus preventing CDK1 activation.[15]

Granulatimide and isogranulatimide are selective Chk1 inhibitors with IC_{50} values of 0.25 and 0.1 μM, respectively.[4] Like other carbazoles, such as staurosporine and UCN-01, granulatimide and isogranulatimide are ATP competitive Chk1 inhibitors. UCN-01 is currently in clinical trials in combination with DNA-damaging agents such as topotecan and fluorouracil.[16,17] The crystal structures of isogranulatimide, staurosporine, and UCN-01 in complex with Chk1 have been determined.[4,18] In the course of structure−activity relationship studies on granulatimide, structurally related compounds bearing modified heterocycles have been synthesized.[19−29] Among them, bis-imide granulatimide analogues (Figure 1) were potent Chk1 inhibitors. In this paper, we describe the synthesis of pyrrolo[3,4-*c*]-carbazoles that can be considered as granulatimide analogues in which the imidazole heterocycle has been replaced by a five- or six-membered ring carbocycle bearing one or two carbonyl functions. Compared with the previously synthesized bis-imides, the imide function in the D-ring has been replaced by one or two carbonyl functions, which could give an insight into the importance of each carbonyl and to the NH. 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) were evaluated.

Chemistry

To synthesize compounds **2** and **4** (Scheme 1), a Diels−Alder reaction was carried out between 3-indolylmaleimide[30] and cyclopent-2-enone and cyclohex-2-enone, providing the cycloadducts **1** and **3**, in only 31% and 40% yields, respectively. Unreacted starting material could be recovered. The orientation of the carbonyl group in compounds **1** and **3** was assigned from $^1H-^1H$ NMR COSY correlations (Figure 2). The position of the double bond in the cycloadducts (indoles and not indolines) was also assigned from the chemical shifts of the NH proton at 11.35 and 10.96 ppm, respectively (about 8 ppm in indolines and 11 ppm in indoles[31,32]).

Oxidation of compounds **1** and **3** using DDQ led to compounds **2** and **4** in 80% and 72% yields, respectively. To obtain compounds **8** and **10** (Scheme 2), analogues of compounds **2** and **4** with an opposite orientation of the carbonyl function, a Suzuki coupling using 2-*N*-Boc-indoleboronic acid[33] was considered. Various aryl substituents have been previously introduced in the 2-position of indoles via this method.[34−36] Suzuki coupling reactions between 3-bromocyclopent-2-enone[37] and 3-bromocyclohex-2-enone[38] and 2-*N*-Boc-indoleboronic acid provided compounds **6** and **9** in 80% and 69% yields, respectively. A Diels−Alder reaction between compound **6** and maleimide led to the indolyl cycloadduct **7** with concomitant removal of the Boc group in 67% yield. Compound **7** was

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[a] Abbreviations: Chk1, checkpoint kinase 1; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; Boc, *tert*-butoxycarbonyl; DMSO, dimethyl sulfoxide; EtOAc, ethyl acetate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; DTT, DL-dithiothreitol.

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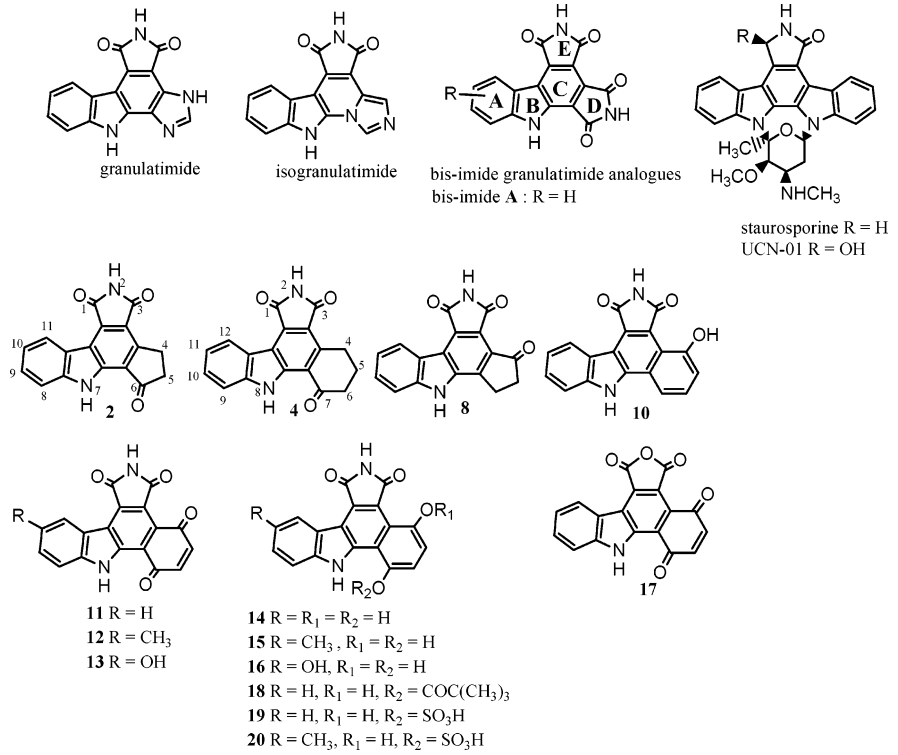
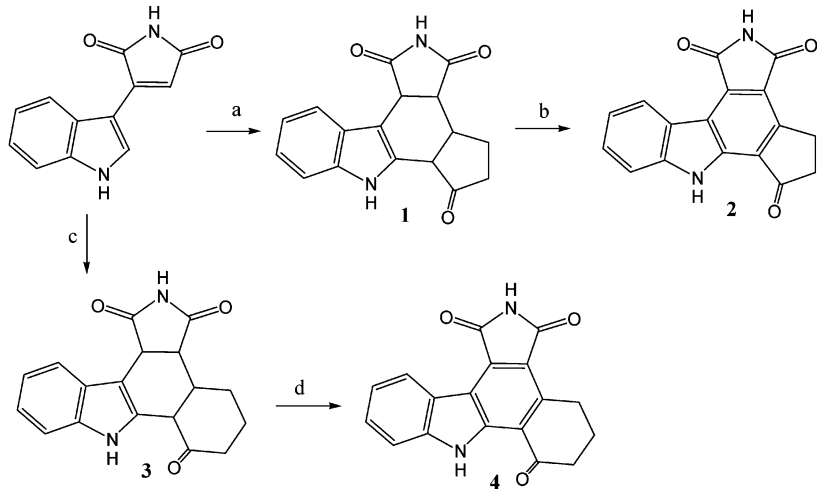


Figure 1. Structures of various well-known checkpoint kinase 1 inhibitors and structures of the newly synthesized compounds reported in this study.

Scheme 1[a]



[a] Reagents and conditions: (a) cyclopent-2-enone, toluene, sealed tube 120 °C, 5 days; (b) DDQ, dioxane, rt, 6 h; (c) cyclohex-2-enone, toluene, sealed tube 140 °C, 5 days; (d) DDQ, dioxane, rt, 24 h.

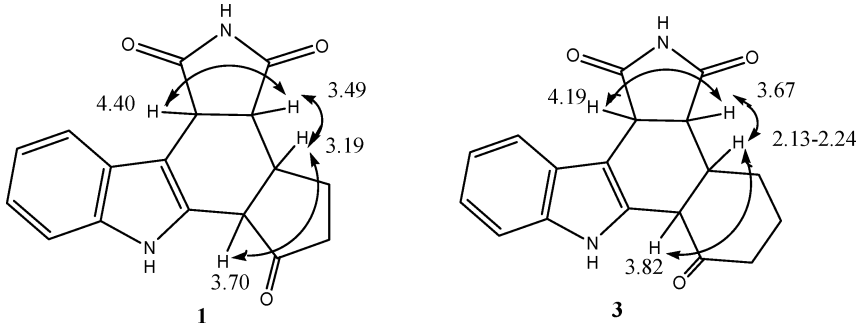
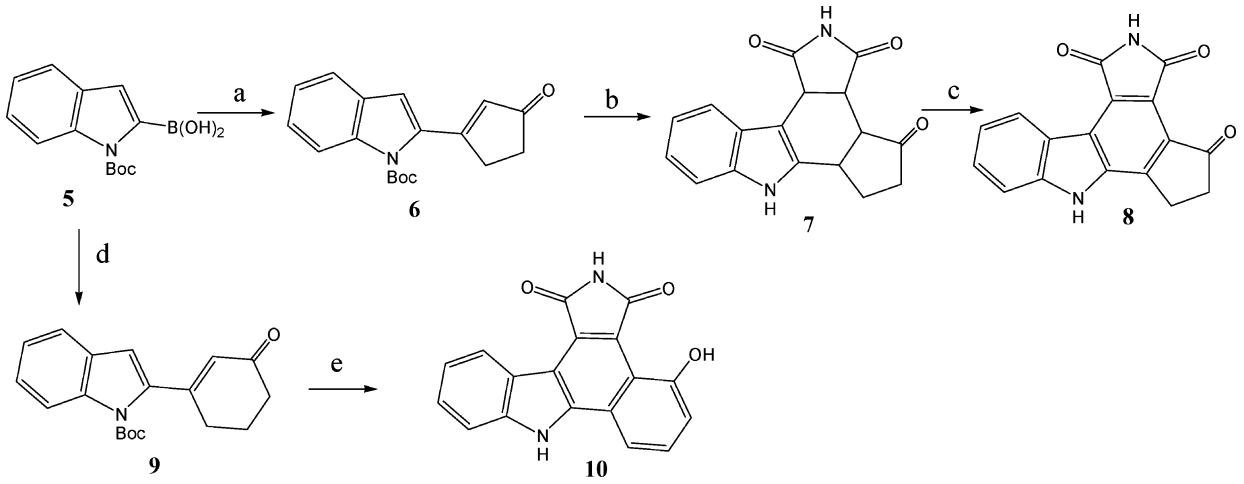


Figure 2. $^{1}H-^{1}H$ NMR COSY correlations in compounds **1** and **3** (chemical shifts in ppm).

further oxidized using DDQ to give ketone **8** in 98% yield. An identical sequence of reactions was performed from compound **9**, leading to phenol **10** in 53% yield for the two steps.

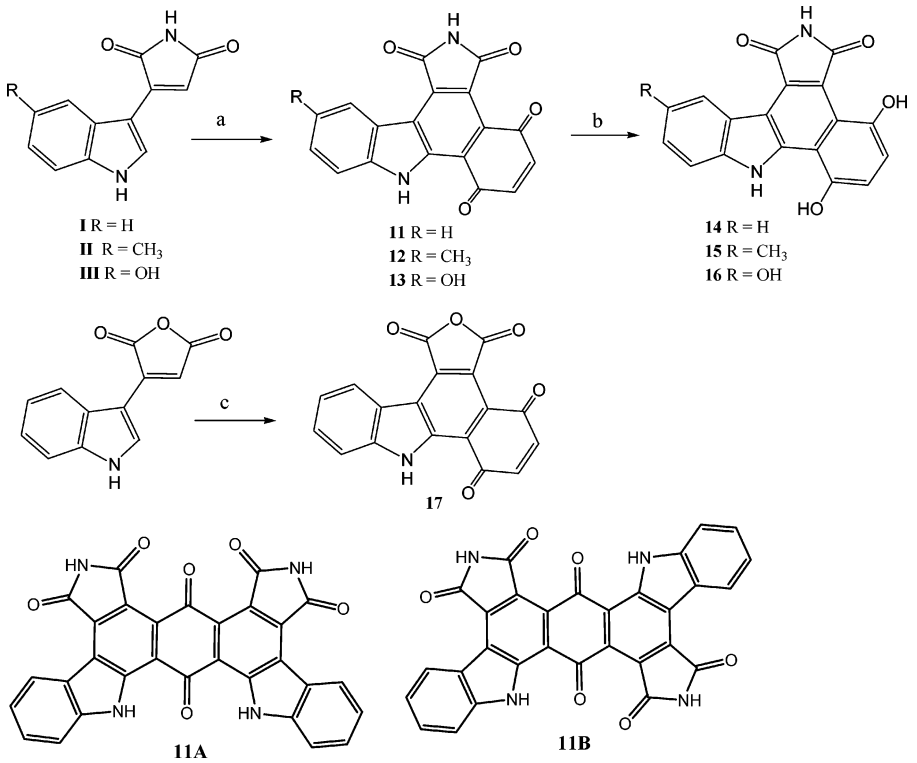
To obtain a six-membered D-ring containing two carbonyl functions, Diels−Alder reactions between indolylmaleimides **I**−**III**[23−26,28] and benzoquinone (40 equiv) were carried out and

Scheme 2[a]



[a] Reagents and conditions: (a) 3-bromocyclopent-2-enone, $Pd(PPh_3)_4$, C_6H_6, 2-*N*-Boc-indoleboronic acid in EtOH and then aqueous Na_2CO_3, reflux, 2 h; (b) maleimide, toluene, sealed tube, 140 °C, 3 days; (c) DDQ, dioxane, rt, 48 h; (d) 3-bromocyclohex-2-enone, $Pd(PPh_3)_4$, C_6H_6, 2*N*-Boc-indoleboronic acid in EtOH and then aqueous Na_2CO_3, reflux, 1.5 h; (e) (i) maleimide, toluene, sealed tube, 140 °C, 3 days; (ii) DDQ, dioxane, rt, 4 days.

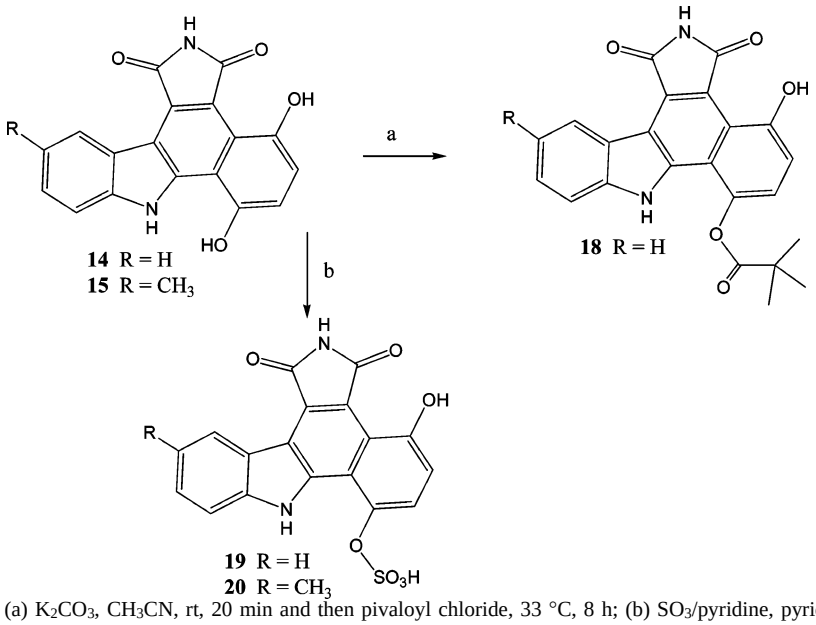
Scheme 3[a]



[a] Reagents and conditions: (a) benzoquinone, toluene, sealed tube, 140 °C, 12 h; (b) aqueous $Na_2S_2O_4$, THF, rt, 2−3 h; (c) benzoquinone, toluene, sealed tube, 140 °C, 48 h.

yielded directly oxidized compounds **11**−**13** (Scheme 3). When smaller amounts of benzoquinone were used, compounds **11A** and **11B** could be obtained from indolylmaleimide **I**. According to the proportions of indolylmaleimide and benzoquinone used, a single Diels−Alder reaction leading to compound **11** or a double Diels−Alder reaction leading to a mixture of **11A** and **11B** occurred. With a 40:1 ratio of benzoquinone/indolylmaleimide, only compound **11** was formed, whereas a 6:1 ratio of benzoquinone/indolylmaleimide led only to the formation of compounds **11A** and **11B**. The isomeric ratio **11A:11B** of 1.4:1 was determined from the ^{1}H NMR spectrum on the aromatic signals. Indeed, the signals of the aromatic protons are two pseudotriplets (both integrating for 2H) and four doublets (each integrating for 1H), at 7.96 and 9.00 ppm for the major isomer and at 8.04 and 9.08 ppm for the minor isomer. With 20 equiv of benzoquinone, a mixture of **11**, **11A**, and **11B** was obtained. Quinones **11**−**13** were reduced to hydroquinones **14**−**16** by reaction with aqueous sodium hydrosulfite according to a method described in naphthalene-1,4-diol series.[39] In hydroquinones **14**−**16**, a hydrogen bond could be formed between the upper hydroxy group of the D-cycle and the carbonyl group at the right side of the E imide heterocycle. The replacement of a ketone function by a phenol function was also tested to determine if it could modify the affinity of the molecules toward the ATP binding site of Chk1. Moreover, the aqueous solubility of hydroquinones is better than that of their parent quinones. Compound **17**, an analogue of compound **11** with an anhydride function in the E-ring, was prepared from indolyl maleic

Scheme 4[a]



[a] Reagents and conditions: (a) K_2CO_3, CH_3CN, rt, 20 min and then pivaloyl chloride, 33 °C, 8 h; (b) SO_3/pyridine, pyridine, 33 °C, 24 h.

anhydride and benzoquinone (Scheme 3). Since hydroquinones could be reoxidized into quinones, substitution of the phenolic function was carried out to prevent the oxidation. Pivalate **18** and sulfates **19** and **20** were prepared from diphenols **14** and **15** by reaction with pivaloyl chloride in the presence of potassium carbonate[40] and by reaction with sulfur trioxide/pyridine complex,[41] respectively (Scheme 4). Moreover, the pivalate could improve membrane crossing, whereas the sulfate could improve water solubility.

The orientation of the substitution in the pivalate and the sulfates was assigned from NMR experiments. The substitution of the imide nitrogen could be ruled out by the chemical shifts of the exchangeable protons. In DMSO, the chemical shift of the imidic NH was always higher than 11.5 ppm. In compounds **14** and **15**, the chemical shifts of the phenolic OH oriented toward the indolic NH were 10.84 and 10.75 ppm, respectively. In sulfates **19** and **20**, the chemical shifts of the exchangeable protons were all higher than 11.5 ppm, which confirmed that the substituent is located on the 7 position.

Table 1. Percentage of Chk1 Inhibition at a Drug Concentration of 10 μM, IC_{50} Values (μM) toward Chk1, and In Vitro Antiproliferative Activities against Three Tumor Cell Lines: Murine Leukemia L1210 and Human HT29 and HCT116 Colon Carcinoma (IC_{50} in μM)

compound	% of Chk1 inhibn at 10 μM	IC_{50} (μM)			
		Chk1	L1210	HCT116	HT29
granulatimide	93.9	0.081	2.8	6.1	5.7
isogranulatimide	89.7	0.44	10	13	13.7
bis-imide A	94.4	0.020	32.7	nd	9.7
4	15.3	nd	3.7	1.1	15.5
8	72	0.043	58.4	9.7	16.1
10	93	0.904	19.2	17.5	46.4
11	86	0.269	6.8	5.3	17.4
12	86	0.107	0.75	0.70	20.6
13	95	0.027	8.4	15.4	50.6
14	96	0.311	1.1	3.1	5.6
15	99	0.161	0.6	1.6	3.8
16	94	0.023	1.4	2.7	26.4
18	66	3.7	1.95	8.76	7.28
19	62	1.79	27.5	>50	46
20	70	0.893	25.6	>40	50

Checkpoint 1 Inhibitory Activities

The Chk1 inhibitory activities were evaluated only for the soluble compounds and were compared with those of granulatimide and isogranulatimide (Table 1). The percentages of Chk1 inhibition at a compound concentration of 10 μM were determined and the IC_{50} values in μM were measured for the most efficient inhibitors. Compound **4** with a six-membered D-cycle containing a carbonyl function oriented toward the indole NH did not inhibit Chk1 significantly. Unfortunately, the insolubility of its analogue **2** with a five-membered D-ring prevented its evaluation in biological tests. Compound **8**, an analogue of compound **2** with the carbonyl group oriented toward the E-heterocycle, and compound **10**, with a phenol D-ring in which the oxygen has the same orientation toward the imide as in **8**, were stronger Chk1 inhibitors than compound **4**, suggesting that the orientation of the carbonyl or the phenolic OH is important for Chk1 inhibition. Quinones **11**−**13** were moderate to potent Chk1 inhibitors. Of compounds **11**−**13**, the most potent compound was compound **13**, bearing a hydroxy group on the indole. Compound **13** was a stronger Chk1 inhibitor than granulatimide and inhibits Chk1 with an IC_{50} value in the same range as that of bis-imide **A**. In the bis-imide series, similar Chk1 inhibitory potencies were observed with substitutions in the 5 position of the indole moiety (OH > CH_3 > H).[29] Diphenols **14**−**16** were also potent Chk1 inhibitors, exhibiting Chk1 inhibitory properties very close to their quinone analogues **11**−**13**. The hydroxyl group at the 11-position seems to be important for potent Chk1 inhibition. A recent study on 4-phenylpyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-diones also shows the importance of the hydroxy group at this position on the indole for Chk1 inhibition.[42] The introduction of a pivalate (compound **18**) or a sulfate (compounds **19** and **20**) was detrimental to Chk1 inhibition (compare compound **14** with **18** and **19**, and compound **15** with **20**).

Molecular Modeling

To try to rationalize the above-described results, molecular modeling was carried out using as model the complex structure of Chk1/staurosporine[18] downloaded from the Protein Data Bank (PDB ID:1NVR). The various compounds were superimposed to staurosporine in the ATP binding site of the enzyme so that the hydrogen bonds between the imide NH and Glu85 and

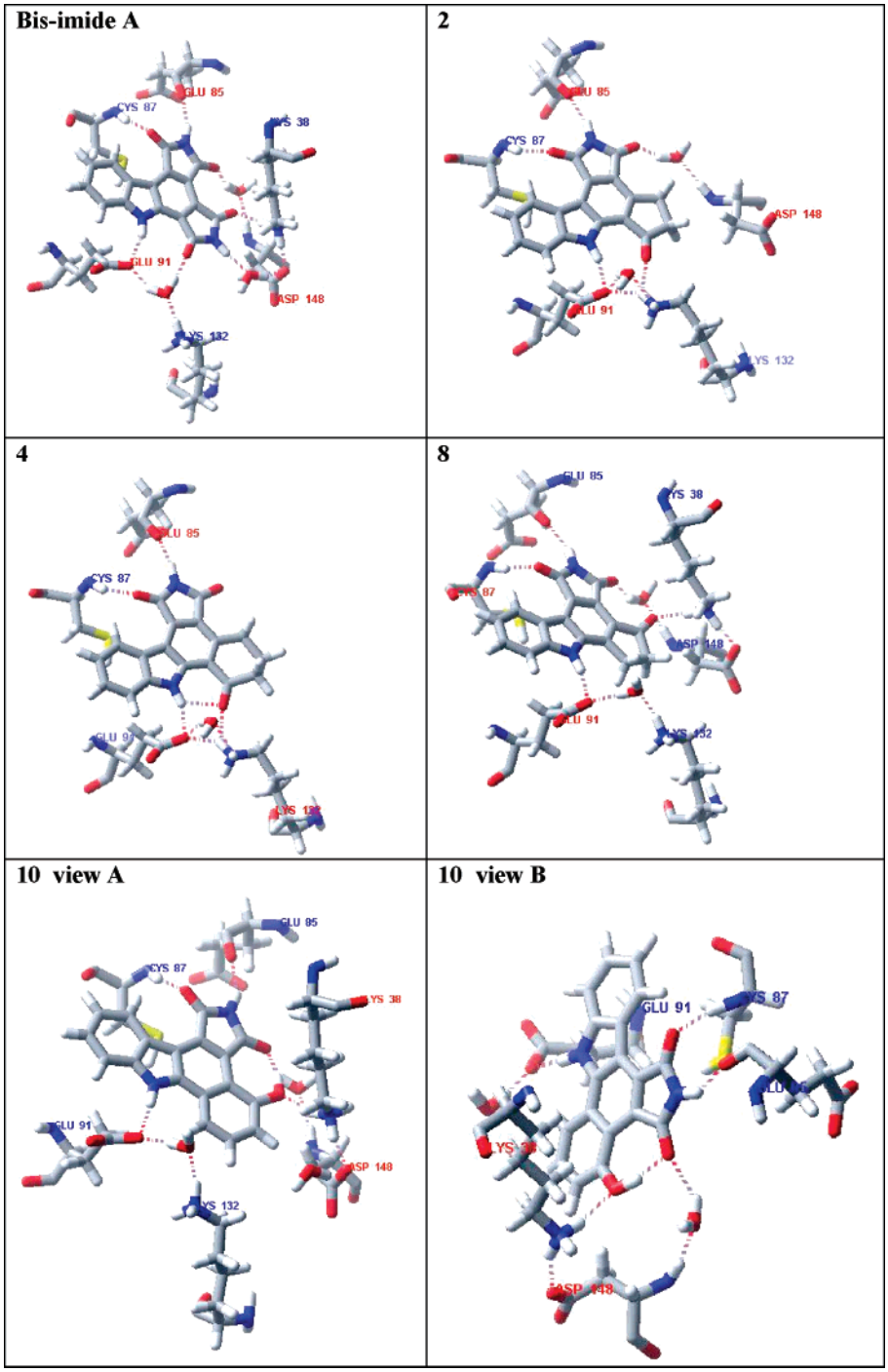


Figure 3. Bis-imide **A** and compounds **2**, **4**, **8**, **10** docked into the ATP binding site of Chk1.

between the carbonyl on the left of the E-heterocycle and Cys87 were maintained. Then, a complete mimization of the molecular system was carried out. Docking was performed with compounds **2**, **4**, **8**, **10**, and **11**−**16** and bis-imide **A** (Figures 3−5). 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 Cys87 and Glu85, respectively, hydrogen bonds were also observed between the indole NH and the carboxylate of the side chain of Glu91 and between the upper carbonyl of the D-heterocycle and the amine of the side chain of Lys38. Moreover, three hydrogen bonds were formed via a water molecule: between the carbonyl on the right of the E-heterocycle and the amine of Asp148, between the imide NH of the D-heterocycle and the carboxylate of the side chain of Asp148, and between the lower carbonyl of the D-heterocycle and the amine of the side chain of Lys132 and the carboxylate of the side chain of Glu91. This important hydrogen bond net is in agreement with the strong inhibitory activity of bis-imide **A** toward Chk1 (IC_{50} value 20 nM). Compared with bis-imide **A**, compounds **2**, **4**, **8**, and **10** were less stabilized by H-bonding inside the ATP binding pocket, which could explain their weaker Chk1 inhibitory potencies (Figure 3). Since the D-cycle is surrounded by a hydrophilic pocket, the replacement of the D maleimide heterocycle by more hydrophobic rings was unfavorable for the stabilization of the compounds. Intramolecular hydrogen bonds were formed for compound **4** between the indole NH and the carbonyl of the D-cycle and for compound **10** between the phenolic OH and the carbonyl on the right of the E-heterocycle. With compound **4**, only four hydrogen bonds

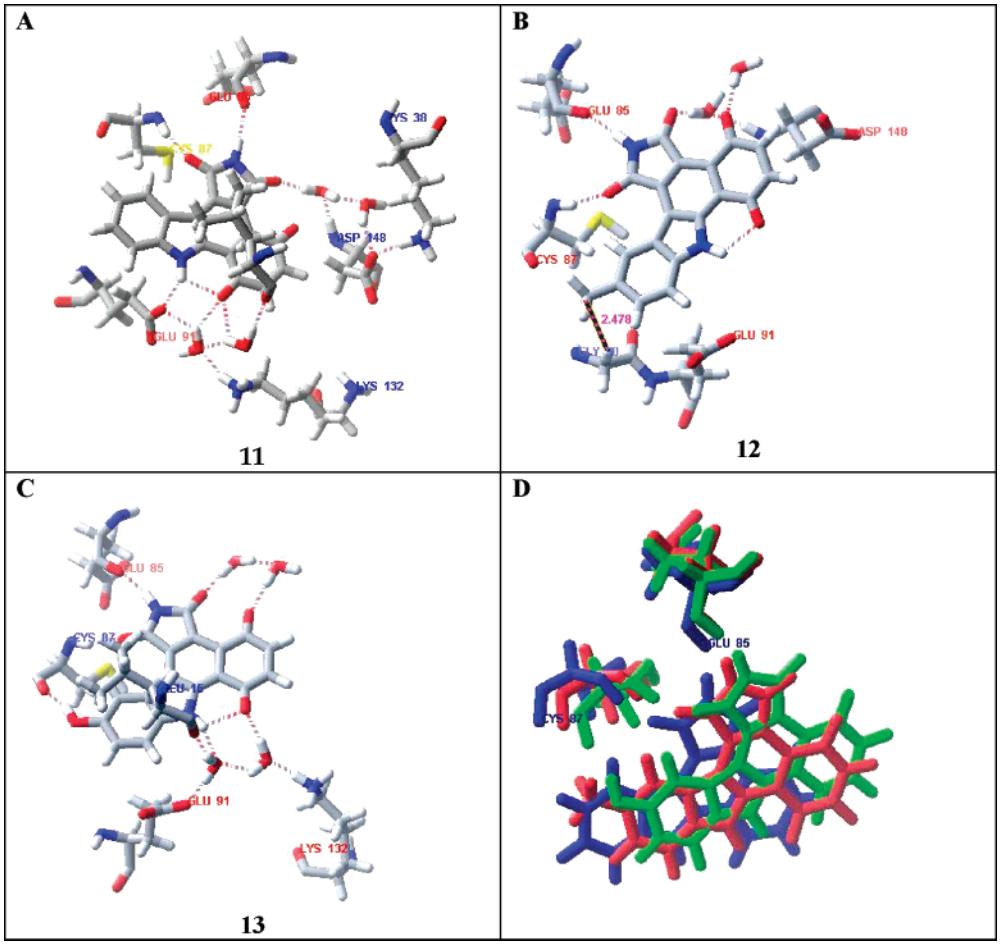


Figure 4. Views A, B, C: compounds **11**−**13** docked into the ATP binding site of Chk1. View D: Superimposition of compounds **11** (in blue), **12** (in red), and **13** (in green). A similar orientation is observed for compounds **12** and **13** due, for compound **12**, to the hydrophobic interactions with Gly90 and, for compound **13**, to the hydrogen bond between the hydroxy group and the carbonyl of Cys87. Compared with **12** and **13**, compound **11** is shifted to the left.

were observed with the surrounding residues. With compound **10**, four hydrogen bonds, which were also formed with bis-imide **A**, were observable in the two views A and B: the classical hydrogen bonds between the carbonyl on the left and the imide NH of the E-heterocycle with Cys87 and Glu85, a third one between the indole NH and the carboxylate of the side chain of Glu91, and the last one between the carbonyl on the right of the E-heterocycle and the amine of Asp148 via a water molecule. A fifth H-bond was formed between the oxygen of the phenol and the amine of the side chain of Lys38.

With compound **11** (Figure 4), the two fundamental hydrogen bonds with Glu85 and Cys87 were observed. Hydrogen-bonding also existed via a water molecule between the carbonyl on the right of the E imide heterocycle and Asp148. This side of the ligand seems to be hydrophilic with two water molecules and hydrophilic amino acid residues Lys38 and Asp148. At the bottom of compound **11**, an intramolecular hydrogen bond was observed between the indole NH and a carbonyl of the D-ring. Hydrogen bonds were also observed between the indole NH and the carboxylate of Glu91 and between a carbonyl of the D-cycle and Lys132 and Leu15 via water molecules. This important hydrogen bond net was in agreement with the inhibitory activity of compound **11** toward Chk1. Compared with compound **11**, compound **12**, which has a methyl group on the indole moiety, showed weaker hydrogen bonding. However, the Chk1 inhibitory activity of **12** was better than that of **11**. With compound **12**, hydrophobic interactions between the methyl group and Gly90 could be observed (Figure 4). Superimposition of compounds **11**−**13** showed a similar orientation for compounds **12** and **13** due, for compound **12**, to the hydrophobic interactions and, for compound **13**, to the hydrogen bond between the hydroxy group and the carbonyl of Cys87. Compared with **13**, compound **11** was shifted to the left. These different orientations and interactions are very probably responsible for the variations in the Chk1 inhibitory potencies of compounds **11**−**13**. Molecular modeling has also been carried out with hydroquinones **14**−**16** (Figure 5). Hydrophobic interactions were observed between the methyl group of **15** and Gly90. A hydrogen bond between the hydroxy group and the carbonyl of Cys87 stabilizes compound **16**. As for compounds **11**−**13**, superimposition of compounds **14**−**16** showed that the orientation of compound **14** is different from those of **15** and **16**. Compared with hydroquinones **15** and **16**, compound **14** was shifted to the right.

In Vitro Antiproliferative Activities

The cytotoxicities of the soluble compounds were evaluated toward three tumor cell lines—murine leukemia L1210 and human colon carcinoma HT29 and HCT116—and compared with those of granulatimide, isogranulatimide and bis-imide **A**. The most cytotoxic compounds in this series were compounds **4**, **12**, **14**, **15**, and **16**. Compounds **4**, **12**, and **16** were more cytotoxic against L1210 and HCT116 cells than against HT29 cells. Compounds **12** and **15** bearing a methyl group on the indole moiety were the most cytotoxic compounds against L1210 cells. There is no evident parallelism between the

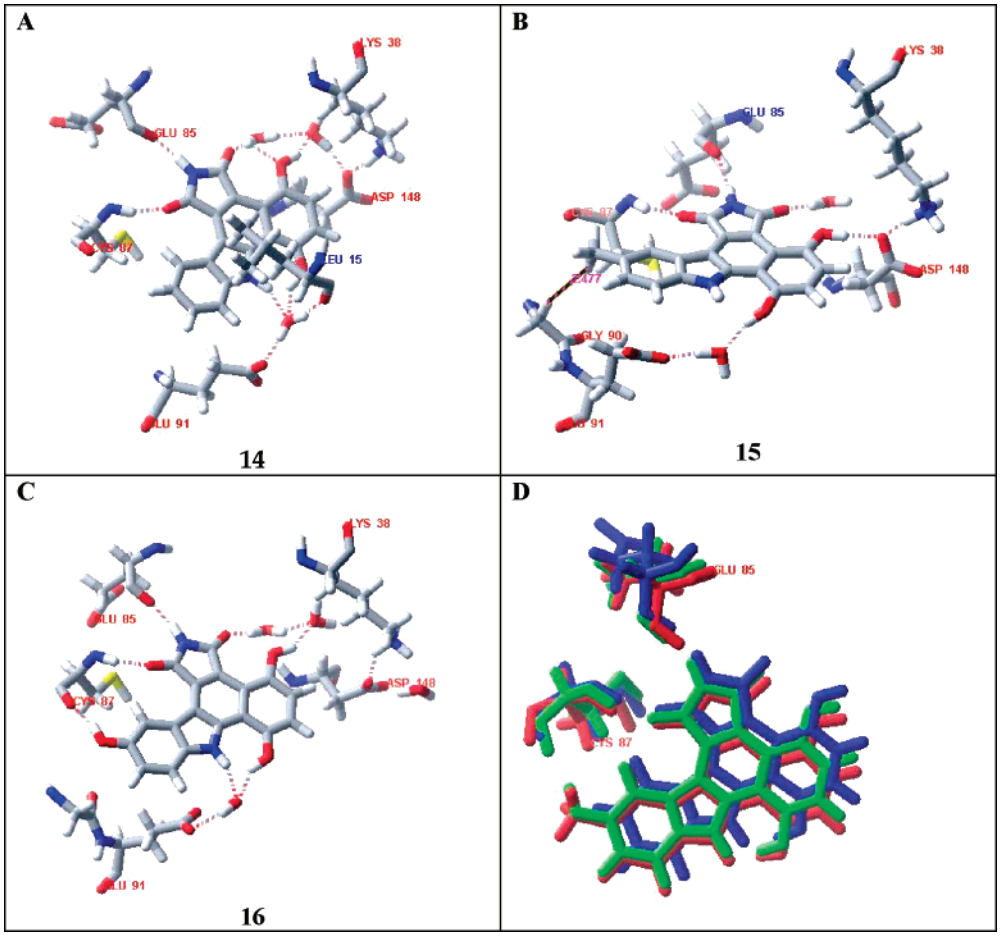


Figure 5. Views A, B, C: compounds **14**−**16** docked into the ATP binding site of Chk1. View D: Superimposition of **14** (in blue), **15** (in red), and **16** (in green). Hydrophobic interactions occur between the methyl group of **15** and hydrogens of Gly90. Compared with compounds **15** and **16**, compound **14** is shifted to the right.

Table 2. Percentage of Src Inhibition at a Drug Concentration of 10 μM (IC_{50} Values in μM)

compound	% of Src inhibn at 10 μM (IC_{50} in μM)	compound	% of Src inhibn at 10 μM (IC_{50} in μM)
granulatimide	58 (0.35)	**12**	52.5
bis-imide A	inactive	**13**	63
4	8.5	**14**	2.5
8	inactive	**16**	83.5 (5.4)
10	35		

cytotoxicities and the Chk1 inhibitory activities, since compound **4**, which was a poor Chk1 inhibitor, exhibited significant cytotoxicities, whereas compound **13**, which was a potent Chk1 inhibitor was not strongly cytotoxic. These results were not surprising because a Chk1 inhibitor is not expected to be strongly cytotoxic by itself. Nevertheless, it is possible that Chk1 inhibitors might also inhibit other kinases, which could account for some cytotoxic effects.

Kinase Selectivity

To get an insight into the kinase selectivity, Src tyrosine kinase inhibitory activities were evaluated for some compounds in this series (Table 2). Among the tested compounds, compound **16**, which was the strongest Chk1 inhibitor in this series, was also the strongest Src inhibitor. Moreover, quinone **13** and hydroquinone **15** were selected for analysis by kinase profiling at Upstate. The compounds were tested at 1 μM concentration in the presence of 10 μM ATP under standard conditions determined by Upstate for each of the 19 selected protein kinases. The data are presented as percent activity remaining (Table 3). Compound **15** was specific for Chk1 under the conditions used in this test, compared to 19 other protein kinases. Structural modifications in compound **13** led to a minor loss of activity toward Chk1, which was confirmed by analysis of IC_{50} values, and also a loss of enzyme specificity.

Table 3. Inhibition of 19 Protein Kinases by Compounds **13** and **15** at a Compound Concentration of 1 μM

kinase	**13**	**15**	kinase	**13**	**15**
CaMKII	40	34	GSK3α	3	32
CDK1/cyclin B	85	86	GSK3β	19	60
CDK2/cyclin A	62	69	MAPK1	77	95
CDK5/p25	33	28	MAPKAP-K2	73	57
CDK7/cyclin H	87	90	MEK1	98	96
CDK9/cyclin T1	61	117	p70S6K	27	42
Chk1	22	12	PKA	103	117
CK1δ	16	100	PKBα	87	87
CK2	92	108	PKCα	66	102
c-RAF	102	93			

[a] The data are presented as percent activity remaining.

Conclusion

In conclusion, this work reports the synthesis of new carbazole Chk1 inhibitors that possess various D-carbocycles bearing one or two carbonyl functions. Two synthetic methods have been developed for compounds with one carbonyl function in the D-cycle. For the synthesis of compounds with the carbonyl oriented toward the indole nitrogen, a Diels−Alder cycloaddition was

performed. A Suzuki coupling allowed the access to their regioisomers with the oxygen oriented toward the upper E-heterocycle. The Chk1 inhibitory activities were considerably stronger for compounds in which the oxygen was oriented toward the upper E-heterocycle. The inhibitory activity of quinones **11**−**13** and hydroquinones **14**−**16** toward Chk1 were in the same range. Among the newly synthesized compounds, the most potent Chk1 inhibitors were quinone **13** and hydroquinone **16**, bearing a hydroxyl group on the indole moiety, with IC_{50} values of 27 and 23 nM, respectively. Hydroquinone **15** was highly selective for Chk1. This study showed that the D-heterocycle can be replaced by a carbocycle without loss of Chk1 activity, providing a new avenue to the design of Chk1 inhibitors.

Experimental Section

Chemistry. IR spectra were recorded on a Perkin-Elmer Paragon 500 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 signal (br s), doublet (d), doubled doublet (dd), triplet (t), pseudotriplet (pt), doubled triplet (dt), multiplet (m), pseudoquadruplet (pq), quintuplet (quint), tertiary carbons (C tert), quaternary carbons (C quat). The signals were assigned from $^1H-^1H$ COSY, HSQC, and HMBC NMR correlations. Low-resolution mass spectra (ESI+ and APCI+) and HRMS were determined on a MS Hewlett-Packard engine. Chromatographic purifications were performed by flash silica gel Geduran SI 60 (Merck) 0.040−0.063 mm column chromatography. Purity evaluation was performed through analytical HPLC using a HP1090 liquid chromatograph. Detection wavelength is indicated for each compound. Solvents were (A) water, 0.1% TFA; (B) acetonitrile, 0.1% TFA; and (C) MeOH, 0.1% TFA. System I: Waters XTerra MS C18 column, 2.5 μm, 3.0 × 50 mm; from 95:5 A/B to 5:95 A/B in 15 min and then 5:95 A/B for 2 min; 0.45 mL/min. System II: Waters XTerra MS C18 column, 2.5 μM, 3.0 × 50 mm; from 95:5 A/C to 100% solvent (C) in 15 min and then 100% solvent (C) for 2 min; 0.40 mL/min.

3b,4,5,6a,7,11c-Hexahydro-1*H*-cyclopenta[*a*]pyrrolo[3,4-*c*]-carbazole-1,3,6(2*H*,3a*H*)-trione (1). Cyclopent-2-enone (400 μL, 4.8 mmol) was added to a suspension of 3-(indol-3-yl)maleimide **I**[30] (50 mg, 0.23 mmol) in toluene (6 mL). The mixture was stirred at 120 °C in a sealed tube for 5 days. The solvent was removed and the residue was purified by flash chromatography (eluent, cyclohexane/EtOAc from 7:3 to 5:5) to give compound **1** (21 mg, 0.07 mmol, 31% yield) as a yellow solid: mp >200 °C (dec); IR (KBr) $\upsilon_{C=O}$ 1710, 1767 cm^{-1}, υ_{NH} 3359 cm^{-1}; HRMS (ESI+) $[M + H]^+$ calcd for $C_{17}H_{15}N_2O_3$ 295.1083, found 295.1101; ^1H NMR (400 MHz, DMSO-d_6) 1.53−1.65 (1H, m), 1.86−1.94 (1H, m), 2.33 (1H, dd, J_1 = 19.0 Hz, J_2 = 8.0 Hz), 2.45 (1H, m), 3.19 (1H, m), 3.49 (1H, dd, J_1 = 8.0 Hz, J_2 = 7.0 Hz), 3.70 (1H, d, J = 7.0 Hz), 4.40 (1H, d, J = 8.0 Hz), 7.02 (1H, t, J = 8.0 Hz), 7.09 (1H, t, J = 8.0 Hz), 7.39 (1H, d, J = 8.0 Hz), 7.75 (1H, d, J = 8.0 Hz), 10.83 (1H, s, NH), 11.35 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6) 23.1, 37.1 (CH_2), 36.4, 38.7, 41.8, 46.4 (CH), 101.4, 118.7, 120.0, 121.3 (C tert arom), 102.9, 126.7, 127.0, 137.0 (C quat arom), 178.6, 179.2, 214.0 (C═O).

4,7-Dihydro-1*H*-cyclopenta[*a*]pyrrolo[3,4-*c*]carbazole-1,3,6-(2*H*,5*H*)-trione (2). A mixture of **1** (31 mg, 0.10 mmol) and DDQ (100 mg, 0.44 mmol) in dioxane (2.5 mL) was stirred for 6 h at room temperature. After filtration, the solid residue was washed with water to give **2** (22 mg, 0.08 mmol, 80% yield) as a yellow solid: mp >300 °C; IR (KBr) $\upsilon_{C=O}$ 1690, 1715, 1758 cm^{-1}, υ_{NH} 3216, 3333 cm^{-1}; HRMS (ESI+) $[M + Na]^+$ calcd for $C_{17}H_{10}N_2O_3Na$ 313.0589, found 313.0602; ^1H NMR (400 MHz, DMSO-d_6) 2.89 (2H, m), 3.52 (2H, m), 7.42 (1H, t, J = 8.0 Hz), 7.62 (1H, t, J = 8.0 Hz), 7.79 (1H, d, J = 8.0 Hz), 8.88 (1H, d, J = 8.0 Hz), 11.45 (1H, s, NH), 12.62 (1H, s, NH); due to the insolubility of the compound, the ^{13}C NMR spectrum could not be recorded; HPLC purity, system I, >99%, detection wavelength 254 nm, t_R = 15.0 min; system II, >99%, detection wavelength 309 nm, t_R = 18.4 min.

3b,5,6,7a,8,12c-Hexahydrobenzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3,7(2*H*,3a*H*,4*H*)-trione (3). Cyclohex-2-enone (460 μL, 4.8 mmol) was added to a suspension of 3-(indol-3-yl)maleimide **I**[30] (50 mg, 0.23 mmol) in toluene (6 mL). The mixture was stirred at 140 °C in a sealed tube for 5 days. The solvent was removed and the residue was purified by flash chromatography (eluent, cyclohexane/EtOAc from 7:3 to 5:5) to give **3** (28 mg, 0.09 mmol, 40% yield) as a yellow solid: mp 152 °C; IR (KBr) $\upsilon_{C=O}$ 1660, 1717, 1770 cm^{-1}, υ_{NH} 3113−3628 cm^{-1}; MS (ESI+) $[M + H]^+$ 309; ^1H NMR (400 MHz, DMSO-d_6) 1.66 (1H, m), 1.98 (1H, d, J = 13.0 Hz), 2.13−2.24 (2H, m), 2.37 (1H, d, J = 13.0 Hz), 2.68−2.85 (2H, m), 3.67 (1H, dd, J_1 = 7.0 Hz, J_2 = 4.0 Hz), 3.82 (1H, d, J = 13.0 Hz), 4.19 (1H, d, J = 8.0 Hz), 7.01 (1H, t, J = 8.0 Hz), 7.09 (1H, t, J = 8.0 Hz), 7.49 (1H, d, J = 8.0 Hz), 7.79 (1H, d, J = 8.0 Hz), 10.86 (1H, s, NH), 10.96 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6) 26.1, 28.1, 41.2 (CH_2), 40.8, 42.0, 45.0, 46.6 (CH), 111.6, 118.5, 119.7, 121.0 (C tert arom), 104.2, 125.3, 131.3, 136.2 (C quat arom), 177.7, 179.0, 207.8 (C═O).

5,6-Dihydrobenzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3,7(2*H*,4*H*,8*H*)-trione (4). A mixture of **3** (50 mg, 0.16 mmol) and DDQ (145 mg, 0.64 mmol) in dioxane (2.5 mL) was stirred for 24 h at room temperature. After filtration, the solid residue was washed with water then was dried to give **4** (35 mg, 0.11 mmol, 72% yield) as a yellow solid: mp >300 °C; IR (KBr) $\upsilon_{C=O}$ 1657, 1706 cm^{-1}, υ_{NH} 3200−3450 cm^{-1}; HRMS (ESI+) $[M + H]^+$ calcd for $C_{18}H_{13}N_2O_3$ 305.0926, found 305.0942; ^1H NMR (400 MHz, DMSO-d_6) 2.17−2.24 (2H, m), 2.79−2.84 (2H, m), 3.55−3.60 (2H, m), 7.35 (1H, t, J = 8.0 Hz), 7.63 (1H, t, J = 8.0 Hz), 7.88 (1H, d, J = 8.0 Hz), 8.86 (1H, d, J = 8.0 Hz), 11.44 (1H, s, NH), 12.40 (1H, s, NH); due to the insolubility of the compound, the ^{13}C NMR spectrum could not be recorded; HPLC purity, system I, 97.5%, detection wavelength 312 nm, t_R = 10.9 min; system II, 97.8%, detection wavelength 329 nm, t_R = 15.5 min.

1-*tert*-Butoxycarbonyl-2-(3-oxocyclopent-1-en-1-yl)-1*H*-indole (6). A solution of 2-*N*-Boc-indoleboronic acid[33] (100 mg, 0.38 mmol) in EtOH (3 mL) was added to a mixture of 3-bromocyclopent-2-enone (50 mg, 0.31 mmol), $Pd(PPh_3)_4$ (29 mg, 0.025 mmol), and benzene (6 mL). Then a solution of Na_2CO_3 (181 mg, 1.71 mmol) in water (7 mL) was added and the mixture was refluxed for 2 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 (eluant, cyclohexane/EtOAc 8:2) to give **6** (74 mg, 0.25 mmol, 80% yield) as a pale yellow solid: mp 102−104 °C; IR (KBr) $\upsilon_{C=O}$ 1680, 1744 cm^{-1}; MS (ESI+) $[M + Na]^+$ 320; ^1H NMR (400 MHz, DMSO-d_6) 1.52 (9H, s), 2.52−2.56 (2H, m), 3.06−3.08 (2H, m), 6.32 (1H, s), 7.23 (1H, s), 7.34 (1H, t, J = 8.0 Hz), 7.48 (1H, t, J = 8.0 Hz), 7.73 (1H, d, J = 8.0 Hz), 8.08 (1H, d, J = 8.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) 27.4 (CH_3), 30.2, 34.5 (CH_2), 84.8, 128.2, 135.2, 137.9, 149.3 (C quat), 112.9, 114.6, 121.8, 123.4, 126.2, 129.9 (C tert), 166.0, 207.8 (CO).

3b,5,6,6a,7,11c-Hexahydro-1*H*-cyclopenta[*a*]pyrrolo[3,4-*c*]-carbazole-1,3,4(2*H*,3a*H*)-trione (7). A mixture of **6** (300 mg, 1.01 mmol) and maleimide (686 mg, 7.07 mmol) in toluene (4 mL) was heated at 140 °C in a sealed tube for 3 days. After cooling, the mixture was filtered, and the solid residue was washed with dichloromethane to give **7** (200 mg, 0.68 mmol, 67% yield) as a white solid: mp > 280 °C; IR (KBr) $\upsilon_{C=O}$ 1714, 1768 cm^{-1}, υ_{NH} 3307, 3390 cm^{-1}; MS (ESI+) $[M + H]^+$ 295, $[M + Na]^+$ 317; ^1H NMR (400 MHz, DMSO-d_6) 2.00 (1H, m), 2.22−2.36 (3H, m), 3.13 (1H, t, J = 6.5 Hz), 3.62−3.66 (2H, m), 4.22 (1H, dd, J_1 = 8.0 Hz, J_2 = 1.0 Hz), 7.02 (1H, t, J = 8.0 Hz), 7.11 (1H, t, J = 8.0 Hz), 7.34 (1H, d, J = 8.0 Hz), 7.86 (1H, d, J = 8.0 Hz), 11.15 (1H, s, NH), 11.22 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6) 25.8, 35.6 (CH_2), 34.1, 40.3, 41.1, 47.7 (CH), 104.1, 126.4, 135.1, 136.5 (C quat arom), 110.8, 118.6, 120.5, 121.3 (C tert arom), 178.1, 179.2, 214.0 (CO).

6,7-Dihydro-1*H*-cyclopenta[*a*]pyrrolo[3,4-*c*]carbazole-1,3,4-(2*H*,5*H*)-trione (8). A mixture of **7** (180 mg, 0.61 mmol) and DDQ (552 mg, 2.44 mmol) in dioxane (12 mL) was stirred at room temperature for 48 h. After filtration, the solid residue was washed

with water and EtOAc to give **8** (176 mg, 0.60 mmol, 98% yield) as a pale yellow solid: mp >280 °C; IR (KBr) $\upsilon_{C=O}$ 1715, 1753 cm^{-1}, υ_{NH} 3488, 3548 cm^{-1}; HRMS (ESI+) $[M + Na]^+$ calcd for $C_{17}H_{10}N_2O_3Na$ 313.0589, found 313.0602; ^1H NMR (400 MHz, DMSO-d_6) 2.85 (2H, m), 3.46 (2H, m), 7.40 (1H, t, $J = 7.5$ Hz), 7.65 (1H, t, $J = 7.5$ Hz), 7.73 (1H, d, $J = 8.0$ Hz), 8.99 (1H, d, $J = 8.0$ Hz), 11.14 (1H, s, NH), 12.67 (1H, s, NH); due to the insolubility of the compound, the ^{13}C NMR spectrum could not be recorded; HPLC purity, system I, >99%, detection wavelength 230 nm, $t_R = 10.1$ min; system II, >99%, detection wavelength 325 nm, $t_R = 14.8$ min.

1-*tert*-Butoxycarbonyl-2-(3-oxocyclohex-1-en-1-yl)-1*H*-indole (9). A solution of 2-*N*-Boc-indoleboronic acid[33] (180 mg, 0.69 mmol) in EtOH (5 mL) was added to a mixture of 3-bromocyclohex-2-enone (101 mg, 0.57 mmol) and $Pd(PPh_3)_4$ (56 mg, 0.048 mmol) in benzene (11 mL). A solution of Na_2CO_3 (345 mg, 3.26 mmol) in water (13 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 8:2) to give **9** (148 mg, 0.48 mmol, 69% yield) as a yellow-brown solid: mp 67−68 °C; IR (KBr) $\upsilon_{C=O}$ 1661, 1732 cm^{-1}; HRMS (ESI+) $[M + Na]^+$ calcd for $C_{19}H_{21}NO_3Na$ 334.1419, found 334.1427; ^1H NMR (400 MHz, DMSO-d_6) 1.54 (9H, s), 2.06 (2H, quint, $J = 6.0$ Hz), 2.40 (2H, t, $J = 6.5$ Hz), 2.52 (2H, t, $J = 6.0$ Hz), 6.09 (1H, d, $J = 1.0$ Hz), 6.52 (1H, s), 7.13 (1H, t, $J = 7.0$ Hz), 7.23 (1H, t, $J = 7.5$ Hz), 7.43 (1H, d, $J = 8.0$ Hz), 7,93 (1H, d, $J = 8.0$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6) 23.0, 30.1, 37.6 (CH_2), 28.1 (CH_3), 84.7, 128.9, 137.7, 139.7, 149.9 (C quat), 111.0, 115.4, 121.3, 123.3, 125.4, 126.8 (C tert), 155.2, 199.3 (C=O).

4-Hydroxybenzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (10). A mixture of **9** (150 mg, 0.48 mmol) and maleimide (470 mg, 4.19 mmol) in toluene (3 mL) was stirred at 140 °C in a sealed tube for 3 days. After cooling and then filtration, the solid residue was washed with dichloromethane to give the Diels−Alder cycloadduct. A mixture of the cycloadduct (128 mg), DDQ (377 mg, 1.66 mmol), and dioxane (10 mL) was stirred at room temperature for 4 days. After filtration, the residue was washed with water and then purified by flash chromatography (eluent, cyclohexane/EtOAc 5:5) to give **10** (80 mg, 0.256 mmol, 53% yield for the two steps) as a red solid: mp >300 °C; IR (KBr) $\upsilon_{C=O}$ 1739 cm^{-1}, $\upsilon_{NH,OH}$ 3229 cm^{-1}; HRMS (ESI+) $[M + H]^+$ calcd for $C_{18}H_{11}N_2O_3$ 303.0770, found 303.0777; ^1H NMR (400 MHz, DMSO-d_6) 7.17 (1 H, dd, $J_1 = 7.5$ Hz, $J_2 = 0.5$ Hz), 7.43 (1H, dt, $J_1 = 7.5$ Hz, $J_2 = 0.5$ Hz), 7.61 (1H, dt, $J_1 = 7.0$ Hz, $J_2 = 1.0$ Hz), 7.76 (1H, d, $J = 8.0$ Hz), 7.77 (1H, d, $J = 8.0$ Hz), 8.18 (1H, d, $J = 7.5$ Hz), 8.94 (1H, d, $J = 8.0$ Hz), 11.90 (1H, br s), 12.35 (1H, s), 12.94 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) 111.7, 117.2, 118.8, 121.1, 124.5, 127.9, 140.1, 141.5, 154.3 (C quat arom), 111.8, 113.4 (2C), 120.9, 124.1, 126.7, 129.7 (C tert arom), 170.2, 175.9 (C=O); HPLC purity, system I, 95.9%, detection wavelength 210 nm, $t_R = 8.4$ min; system II, 95.3%, detection wavelength 269 nm, $t_R = 12.0$ min.

Benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3,4,7(2*H*,8*H*)-tetraone (11). Benzoquinone (611 mg, 5.66 mmol) was added to a suspension of 3-(indol-3-yl)maleimide **I**[30] (30 mg, 0.14 mmol) in toluene (7 mL). The mixture was stirred at 140 °C in a sealed tube for 12 h. After cooling, the mixture was filtered. The solid residue was washed successively with water and EtOAc and then was dried to give **11** (28 mg, 0.09 mmol, 63% yield) as a brown solid: mp >300 °C; IR (KBr) $\upsilon_{C=O}$ 1721, 1766 cm^{-1}, υ_{NH} 3314 cm^{-1}; HRMS (ESI+) $[M + Na]^+$ calcd for $C_{18}H_8N_2O_4Na$ 339.0382, found 339.0385; ^1H NMR (400 MHz, DMSO-d_6) 7.13 (1H, d, $J = 10.0$ Hz), 7.18 (1H, d, $J = 10.0$ Hz), 7.43 (1H, t, $J = 7.5$ Hz), 7.68 (1H, t, $J = 8.0$ Hz), 7.93 (1H, d, $J = 8.0$ Hz), 9.03 (1H, d, $J = 8.0$ Hz), 11.57 (1H, s, NH), 12.64 (1H, s, NH); due to the insolubility of the compound, the ^{13}C NMR spectrum could not be recorded; HPLC purity, system I, 96.1%, detection wavelength 312 nm, $t_R = 10.9$ min; system II, >99%, detection wavelength 329 nm, $t_R = 15.4$ min.

Benzo[1,2-*a*:5,4-*a*′]dipyrrolo[3,4-*c*:3,4-*c*′]biscarbazole-5,7,8,9,-11,17(6*H*,10*H*,16*H*,18*H*)-hexaone (11A) and Benzo[1,2-*a*:4,5-*a*′]-dipyrrolo[3,4-*c*:3,4-*c*′]biscarbazole-1,3,9,10,12,18(2*H*,8*H*,11*H*,-17*H*)-hexaone (11B). Benzoquinone (149 mg, 1.38 mmol) was added to a suspension of 3-(indol-3-yl)maleimide **I** (50 mg, 0.23 mmol) in toluene (7 mL). The mixture was stirred at 140 °C in a sealed tube for 5 days. After cooling, the mixture was filtered and the residue was successively washed with water and EtOAc to give **11A** and **11B** as a mixture of isomers (43 mg, 0.08 mmol, 73% yield) and as a red solid. The isomeric ratio determined from the ^1H NMR spectrum on the doublets at 7.96 and 8.04 ppm was 1.4: 1: IR (KBr) $\upsilon_{C=O}$ 1722, 1770 cm^{-1}, υ_{NH} 3300, 3366 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) 7.45 (2H, pt, $J = 7.5$ Hz), 7.69 (2H, pt, $J = 7.5$ Hz), 7.96 (1H, d, $J = 8.0$ Hz), 8.04 (1H, d, $J = 8.0$ Hz), 9.00 (1H, d, $J = 8.0$ Hz), 9.08 (1H, d, $J = 8.0$ Hz), 11.65 (2H, s, NH), 12.49 (1H, s, NH), 12.82 (1H, s, NH); due to the insolubility of the compound, the ^{13}C NMR spectrum could not be recorded.

11-Methylbenzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3,4,7(2*H*,8*H*)-tetraone (12). Benzoquinone (1.3 g, 12.39 mmol) was added to a mixture of 3-(5-methyl-indol-3-yl)maleimide **II**[28] (70 mg, 0.31 mmol) in toluene (11 mL). The mixture was stirred at 140 °C in a sealed tube for 12 h. After cooling, the mixture was filtered. The solid was washed with water and EtOAc to give **12** (84 mg, 0.25 mmol, 82% yield) as a brown powder: mp >300 °C; IR (KBr) $\upsilon_{C=O}$ 1716, 1768 cm^{-1}, υ_{NH} 3293 cm^{-1}; HRMS (ESI+) $[M + H]^+$ calcd for $C_{19}H_{11}N_2O_4$ 331.0719, found 331.0710; ^1H NMR (400 MHz, DMSO-d_6) 2.57 (3H, s), 7.13 (1H, d, $J = 10.0$ Hz), 7.17 (1H, d, $J = 10.0$ Hz), 7.51 (1H, d, $J = 8.0$ Hz), 7.82 (1H, d, $J = 8.0$ Hz), 8.84 (1H, s), 11.56 (1H, s, NH), 12.57 (1H, s, NH); due to the insolubility of the compound, the ^{13}C NMR spectrum could not be recorded; HPLC purity, system I, 95.5%, detection wavelength 210 nm, $t_R = 10.7$ min; system II, >99%, detection wavelength 309 nm, $t_R = 22.8$ min.

11-Hydroxybenzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3,4,7(2*H*,8*H*)-tetraone (13). Benzoquinone (1.3 g, 12,39 mmol) was added to a mixture of 3-(5-hydroxy-indol-3-yl)maleimide **III**[26] (70 mg, 0.31 mmol) in toluene (10 mL). The mixture was stirred at 140 °C in a sealed tube for 12 h. After cooling, the mixture was filtered. The solid was washed with water and EtOAc to give **13** (71 mg, 0.21 mmol, 71% yield) as a brown powder: mp >300 °C; IR (KBr) $\upsilon_{C=O}$ 1717, 1765 cm^{-1}, $\upsilon_{NH,OH}$ 3255, 3419 cm^{-1}; HRMS (ESI+) $[M + H]^+$ calcd 333.0511, found 333.0527; ^1H NMR (400 MHz, DMSO-d_6) 7.11 (1H, d, $J = 10.0$ Hz), 7.15 (1H, d, $J = 10.0$ Hz), 7.18 (1H, dd, $J_1 = 9.0$ Hz, $J_2 = 2.0$ Hz), 7.76 (1H, d, $J = 9.0$ Hz), 8.48 (1H, d, $J = 2.0$ Hz), 9.52 (1H, s, OH), 11.53 (1H, s, NH), 12.46 (1H, s, NH); due to the insolubility of the compound, the ^{13}C NMR spectrum could not be recorded; HPLC purity, system I, >99%, detection wavelength 312 nm, $t_R = 10.2$ min; system II, >99%, detection wavelength 329 nm, $t_R = 14.9$ min.

4,7-Dihydroxybenzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (14). A solution of $Na_2S_2O_4$ (89 mg, 0.51 mmol) in water (600 μL) was added to a mixture of compound **11** (55 mg, 0.17 mmol) in THF (2.8 mL). The mixture was stirred at room temperature for 2 h. After evaporation, the residue was purified by flash chromatography (eluent, cyclohexane/EtOAc 7:3) to give **14** (43 mg, 0.14 mmol, 80% yield) as a violet solid: mp >300 °C; IR (KBr) $\upsilon_{C=O}$ 1672,1738 cm^{-1}, $\upsilon_{NH,OH}$ 3053, 3211, 3412 cm^{-1}; HRMS (ESI+) $[M + H]^+$ calcd for $C_8H_{11}N_2O_4$ 319.0719, found 319.0720; ^1H NMR (400 MHz, DMSO-d_6) 7.03 (1H, d, $J = 8.5$ Hz), 7.20 (1H, d, $J = 8.5$ Hz), 7.40 (1H, dt, $J_1 = 7.0$ Hz, $J_2 = 1.0$ Hz), 7.56 (1H, dt, $J_1 = 7.0$ Hz, $J_2 = 1.0$ Hz), 8.01 (1H, d, $J = 8.0$ Hz), 9.00 (1H, d, $J = 8.0$ Hz), 10.84 (1H, br s), 11.78 (2H, s), 12.27 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) 111.4, 114.1, 117.8, 117.9, 120.0, 128.6, 139.7, 140.8, 146.2, 146.8 (C quat arom), 112.9, 113.1, 113.8, 120.8, 123.6, 126.1 (C tert arom), 170.2, 175.9 (C=O); HPLC purity, system I, 95.7%, detection wavelength 312 nm, $t_R = 11.0$ min; system II, 97.2%, detection wavelength 329 nm, $t_R = 16.0$ min.

4,7-Dihydroxy-11-methylbenzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3-(2*H*,8*H*)-dione (15). A solution of $Na_2S_2O_4$ (47 mg, 0.27 mmol)

in water (300 μL) was added to a mixture of compound **12** (30 mg, 0.09 mmol) in THF (1.5 mL). The mixture was stirred at room temperature for 3 h. After evaporation, the residue was purified by flash chromatography (eluent, cyclohexane/EtOAc 7:3) to give **15** (30 mg, 0.09 mmol, 100% yield) as a violet solid: mp >300 °C; IR (KBr) $\upsilon_{C=C}$ 1620 cm^{-1}, $\upsilon_{C=O}$ 1678,1740 cm^{-1}, $\upsilon_{NH,OH}$ 3300−3600 cm^{-1}; HRMS (ESI+) $[M + H]^+$ calcd for $C_{19}H_{13}N_2O_4$ 333.0875, found 333.0862; ^{1}H NMR (400 MHz, DMSO-d_6) 2.57 (3H, s), 7.01 (1H, d, J = 8.5 Hz), 7.18 (1H, d, J = 8.0 Hz), 7.38 (1H, d, J = 8.5 Hz), 7.88 (1H, d, J = 8.0 Hz), 8.79 (1H, s), 10.75 (1H, s), 11.78 (2H, s), 12.18 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) 21.5 (CH_3), 111.2, 114.1, 117.5, 117.7, 120.2, 128.7, 129.5, 138.0, 140.9, 146.2, 146.8 (C quat), 112.6, 113.0, 113.7, 123.3, 127.6 (C tert), 170.2, 175.9 (C═O); HPLC purity, system I, 95.5%, detection wavelength 210 nm, t_R = 11.5 min; system II, 98.7%, detection wavelength 329 nm, t_R = 16.6 min.

4,7,11-Trihydroxybenzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (16). To a suspension of compound **13** (90 mg, 0.27 mmol) in THF (4 mL) was added a solution of $Na_2S_2O_4$ (142 mg, 0.81 mmol) in water (4 mL). The mixture was stirred at room temperature for 3 h. After evaporation, the residue was purified by flash chromatography (eluent, EtOAc/cyclohexane 8:2) to give **16** (40 mg, 0.12 mmol, 44% yield) as a violet solid: mp >280 °C; IR (KBr) $\upsilon_{C=O}$ 1661,1743 cm^{-1}, $\upsilon_{NH,OH}$ 3286, 3415 cm^{-1}; HRMS (ESI+) $[M + H]^+$ calcd for $C_{18}H_{11}N_2O_5$ 335.0668, found 335.0668; ^{1}H NMR (400 MHz, DMSO-d_6) 6.97 (1H, d, J = 8.5 Hz), 7.02 (1H, dd, J_1 = 8.5 Hz, J_2 = 2.0 Hz), 7.13 (1H, d, J = 8.5 Hz), 7.77 (1H, d, J = 8.5 Hz), 8.38 (1H, d, J = 2.0 Hz), 9.28 (1H, s), 10.71 (1H, s), 11.75 (1H, s), 11.78 (1H, s), 12.06 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) 108.1, 112.9, 113.4, 113.6, 116.1 (C tert arom), 111.3, 114.1, 116.8, 117.7, 120.8, 129.0, 133.8, 141.1, 146.2, 146.8, 152.1 (C quat arom), 170.3, 175.9 (C═O); HPLC purity, system I, 97.6%, detection wavelength 254 nm, t_R = 9.3 min; system II, 99.0%, detection wavelength 327 nm, t_R = 14.2 min.

1*H*-Benzo[*a*]furo[3,4-*c*]carbazole-1,3,4,7(8*H*)-tetraone (17). To a suspension of 3-(indol-3-yl)furane-2,5-dione (30 mg, 0.14 mmol) in toluene (7 mL) was added benzoquinone (611 mg, 5.66 mmol). The mixture was stirred at 140 °C in a sealed tube for 48 h. After cooling and filtration, the solid was washed with water and EtOAc to give **17** (10 mg, 0.031 mmol, 23% yield) as a brown solid: mp >300 °C; IR (KBr) $\upsilon_{C=O}$ 1715, 1771, 1820, 1846 cm^{-1}, υ_{NH} 3398 cm^{-1}; HRMS (ESI+) $[M + Na]^+$ calcd for $C_{18}H_7NO_5Na$ 340.0222, found 340.0237; ^{1}H NMR (400 MHz, DMSO-d_6) 7.22 (2H, s), 7.50 (1H, t, J = 7.0 Hz), 7.75 (1H, t, J = 8.0 Hz), 7.99 (1H, d, J = 8.0 Hz), 8.89 (1H, d, J = 8.0 Hz), 12.93 (1H, s, NH); due to the insolubility of the compound, the ^{13}C NMR spectrum could not be recorded.

7-*tert*-Butylcarbonyloxy-4-hydroxybenzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (18). To a solution of diphenol **14** (50 mg, 0.16 mmol) in CH_3CN (1.5 mL) was added K_2CO_3 (66 mg, 1.48 mmol). The mixture was stirred at room temperature for 20 min and then pivaloyl chloride (120 μL, 0.96 mmol) was added dropwise. The mixture was stirred for 8 h at 33 °C. Water was added and the orange precipitate was filtered and washed with water to give **18** (60 mg, 0.15 mmol, 94% yield) as a red-orange solid: mp >280 °C; IR (KBr) $\upsilon_{C=O}$ 1636, 1684, 1759 cm^{-1}, $\upsilon_{NH,OH}$ 3000−3700 cm^{-1}; HRMS (ESI+) $[M + H]^+$ calcd for $C_{23}H_{19}N_2O_5$ 403.1294, found 403.1283; ^{1}H NMR (400 MHz, $CDCl_3$): 1.58 (9H, s), 7.10 (1H, d, J = 8.5 Hz), 7.20 (1H, d, J = 8.5 Hz), 7.34−7.39 (2H, m), 7.50 (1H, t, J = 7.0 Hz), 7.68 (1H, br s, NH), 8.85 (1H, d, J = 7.5 Hz), 9.76 (1H, br s), 11.98 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) 26.7 (CH_3), 112.6, 113.2, 121.5, 123.2, 124.1, 127.0 (C tert), 113.2, 117.6, 117.8, 119.4, 120.2, 128.3, 138.5, 138.9, 139.8, 151.9 (C quat), 169.7, 175.7, 177.2 (C═O); HPLC purity, system I, >99%, detection wavelength 230 nm, t_R = 14.2 min; system II, >99%, detection wavelength 309 nm, t_R = 17.8 min.

4-Hydroxy-7-hydroxysulfonyloxybenzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (19). To a solution of diphenol **14** (50 mg, 0.16 mmol) in pyridine (1.5 mL) was added sulfur trioxide/pyridine complex (205 mg, 1.29 mmol). The mixture was stirred for 24 h at 33 °C. Water was added. After removal of the solvents, the residue was purified by flash chromatography (eluent, MeOH/EtOAc 2:8) to give **19** (63 mg, 0.16 mmol, 37% yield) as a red solid; 50 mg of diphenol **14** could be recovered. Compound **19**: mp >280 °C; IR (KBr) $\upsilon_{C=C}$ 1619, 1635 cm^{-1}, $\upsilon_{C=O}$ 1681, 1752 cm^{-1}, $\upsilon_{NH,OH}$ 3300−3600 cm^{-1}; HRMS (ESI−) $[M - H]^-$ calcd for $C_{18}H_9N_2O_7$ 397.0130, found 397.0148; ^{1}H NMR (400 MHz, DMSO-d_6) 7.13 (1H, d, J = 8.5 Hz), 7.45 (1H, dt, J_1 = 7.0 Hz, J_2 = 1.0 Hz), 7.62 (1H, dt, J_1 = 8.0 Hz, J_2 = 1.0 Hz), 7.64 (1H, d, J = 8.5 Hz), 7.81 (1H, d, J = 8.0 Hz), 9.03 (1H, d, J = 8.0 Hz), 11.63 (1H, s), 11.95 (1H, br s), 12.26 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) 112.0, 113.2, 121.1, 121.8, 124.0, 126.9 (C tert), 112.3, 117.7, 118.2, 118.9, 120.0, 128.3, 139.1, 139.4, 141.7, 150.4 (C quat), 170.1, 175.9 (C═O); HPLC purity, system I, >99%, detection wavelength 230 nm, t_R = 10.1 min; system II, 97.2%, detection wavelength 329 nm, t_R = 15.3 min.

4-Hydroxy-11-methyl-7-hydroxysulfonyloxybenzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (20). To a solution of **15** (100 mg, 0.30 mmol) in pyridine (1.5 mL) was added SO_3/pyridine complex (305 mg, 2.61 mmol). The mixture was stirred for 24 h at 33 °C. Water was added, and the solvents were removed. The residue was purified by flash chromatography (eluent, MeOH/EtOAc 20:80) to give **20** (60 mg, 0.146 mmol, 49% yield) as a red solid: mp >280 °C; IR (KBr) $\upsilon_{C=C,C=O}$ 1619, 1638, 1680, 1749 cm^{-1}, $\upsilon_{NH,OH}$ 3300−3600 cm^{-1}; HRMS (ESI−) $[M - H]^-$ calcd for $C_{19}H_{11}N_2O_7S_1$ 411.0287, found 411.0282; ^{1}H NMR (400 MHz, DMSO-d_6) 2.59 (3H, s), 7.11 (1H, d, J = 8.5 Hz), 7.45 (1H, d, J = 8.0 Hz), 7.62 (1H, d, J = 8.5 Hz), 7.69 (1H, d, J = 8.5 Hz), 8.83 (1H, s), 11.53 (1H, s), 11.93 (1H, s), 12.26 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) 21.4 (CH_3), 111.7, 113.1, 121.7, 123.6, 128.4 (C tert), 112.1, 117.6, 118.2, 118.5, 120.2, 128.3, 129.9, 137.4, 139.6, 141.6, 150.4 (C quat), 170.1, 175.9 (C═O).

Molecular Modeling. For docking experiments, all molecular mechanics calculations were performed by the Macromodel[43] molecular modeling software. We used as model the complex structure of Chk1/staurosporine[18] downloaded from the Protein Data Bank (PDB ID: 1NVR).

Energy minimization was done with the AMBER force field[44,45] using the truncated Newton conjugate gradient method (TNCG). For the superimpositions, all the Cartesian positions of the atoms of the minimized structures were shown on the same view.

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.[46] Inhibitors were diluted with a Tecan Evo150 robot. Protein kinase activity in the presence or absence of compounds was measured in a buffer containing 10 mM HEPES (pH 7.4), 12.5 mM β-glycerophosphate, 2.5 mM EGTA, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, and 66.7 ng/μL of a peptide substrate. The cosubstrate (ATP with 0.2 μCi P^{33}-γ-ATP) to a final concentration of 15 μM was added with a Precision 2000 (Biotek Robotic). The reaction mixture was incubated for 20 min at 30 °C and then stopped by adding 1.0% orthophosphoric acid and transferred to a phosphocellulose filter microplate (Whatman P81). The plate was washed three times with 1.0% orthophosphoric acid and one time with acetone and then dried. The remaining activity was measured on a Topcount with 25 μL of scintillation solution (Packard UltimaGold). IC_{50} values are determined as part of a standardized high-throughput screen for protein kinase inhibitors. Curves used to calculate an IC_{50} are derived from measures of inhibition taken at 12 different concentrations spanning 6 logs of compound dilution. Data points are measured in duplicate and the average of the two points are used. In all tests, a reference compound is used to determine interexperimental variability. If the values of the reference compound are outside a predetermined limit, then the experiment is not validated.

Growth Inhibition Assays. Tumor cells were provided by American Type Culture Collection (Frederik, MD). They were cultivated in RPMI 1640 medium (Life Science technologies, Cergy-Pontoise, France) supplemented with 10% fetal calf serum,

2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH 7.4). Compounds were dissolved in DMSO (10^{-2} M), and then the solutions were diluted in RPMI medium (1/100 v/v, 10^{-4} M). Cytotoxicity was measured by the microculture tetrazolium assay as described.[47] For the cellular tests, compounds were dissolved in DMSO (10^{-2} M), and then the solutions were diluted in RPMI medium (1/100 v/v) to give 10^{-4} M solutions. All the compounds for which the cytotoxicities could be determined are soluble at 10^{-4} M in the DMSO/RPMI (1/100 v/v) mixture. Except compound **2**, for which the biological tests could not be done, due to its insolubility, all the other compounds were soluble at 1 μM in DMSO/water 0.1/99.9 v/v. Cells were continuously exposed to graded concentrations of the compounds for four doubling times, and then 15 μL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was 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 that reduced by 50% the optical density of treated cells with respect to untreated controls.

Src and Other Kinases Inhibition Assays. Src Inhibition Assays. Inhibitors were diluted with a Tecan Evo150 robot. The kinase assay was performed with 4 μL of inhibitor (10% DMSO), 10 μL of kinase assay buffer 4× concentrated (80 mM $MgCl_2$, 200 mM HEPES, 0.4 mM EDTA, 2 mM DTT), 10 μL substrate peptide (KVEKIGEGYYGVVYK, 370 nM), and 6 μL of Src kinase (stock GTP purified diluted with 1× kinase assay buffer to 200 nM). Cosubstrate (10 μL, 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, stopped by adding 200 μL of 0.85% orthophosphoric acid, and then transferred to a phosphocellulose filter microplate (Whatman P81). The plate was washed three times with 200 μL of 0.85% orthophosphoric acid dried with 200 μL of acetone. The remaining activity was measured on a Topcount with 25 μL of scintillation solution (Packard UltimaGold).

Other Kinases Inhibition Assays. Inhibition assays toward the 19 other kinases were performed by Upstate's kinase profiler screening service (Dundee, Scotland).

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