Synthesis and biological evaluation of new dipyrrolo[3,4-a:3,4-c]carbazole-1,3,4,6-tetraones, substituted with various saturated and unsaturated side chains via palladium catalyzed cross-coupling reactions

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Received 21 October 2005; revised 10 January 2006; accepted 17 January 2006
Available online 3 February 2006

Abstract—The syntheses of a series of dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraones, substituted in 10-position with saturated and unsaturated side chains, via palladium catalyzed cross-coupling reactions, are described. These compounds can be considered as granulatimide bis-imide analogues. Their inhibitory activity toward Chk1 kinase and their antiproliferative activities in vitro in four tumor cell lines are reported.

1. Introduction

Granulatimide and isogranulatimide, natural compounds isolated from the ascidian *Didemnum granulatum*, are known to inhibit the G2 cell cycle checkpoint.[1–3] The G2 checkpoint is a particularly relevant target for anticancer drugs. Its role consists in blocking the cells in the G2 phase, in response to DNA damage, to allow time for DNA repair. In more than 50% of human tumors, the G1 checkpoint is lacking. Therefore, combination of DNA damaging agents with a G2 checkpoint inhibitor should drive selectively cancer cells to a lethal mitosis due to an accumulation of DNA lesions.

Several kinases are involved in the G2 checkpoint, among them, ATM, ATR, Chk1, and Chk2 play major roles.[4,5] Based on structural analogy with the indolocarbazole kinase inhibitors staurosporine and UCN-01,[6,7] granulatimide and isogranulatimide could be ATP competitive kinase inhibitors (Fig. 1). It has been reported that granulatimide and isogranulatimide are Chk1 inhibitors with IC_{50} values of 2 and 3 μM, respectively.[8] In the course of structure–activity relationship studies on granulatimide analogues, we have previously synthesized several families of compounds in which the imidazole heterocycle was replaced either by a pyrrole or by a maleimide ring, and in which the indole moiety has been replaced by a 7-azaindole.[9–12] Several substituents were introduced in different positions on the indole moiety.Other families of compounds have also been synthesized in which a sugar moiety is attached to the indole or to the azaindole.[11,13,14]

Dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraones can be considered as bis-imide granulatimide analogues. Indeed, we found that compound **A** (Fig. 1) was a potent Chk1 inhibitor (IC_{50} value of 15 nM toward Chk1, Table 1), more potent than granulatimide. In the course of structure–activity relationship studies on dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraones substituted in the 10-position, compounds **B** and **C** bearing a benzyloxy or a bromo substituent were previously synthesized.[10,11] Compared with compound **A**, the inhibitory potency of compound **B** toward Chk1 was strongly decreased, whereas that of compound **C** was in the same range (Table 1). Therefore,

Keywords: Isogranulatimide; Antitumor agents; Dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraone; Chk1 inhibitors.
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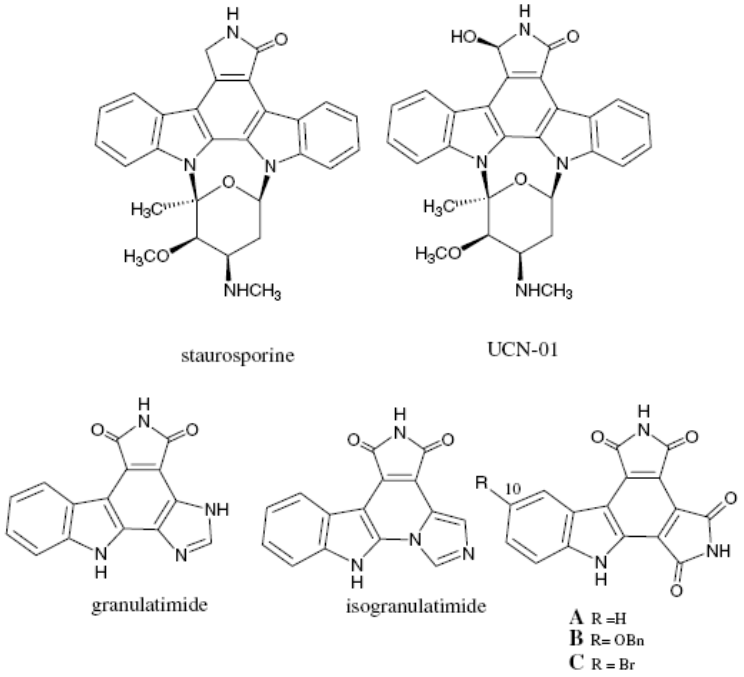


Figure 1.

Table 1. Percentages of Chk1 inhibition at a drug concentration of 10^{-5} M

Compound	% of Chk1 inhibition at 10^{-5} M	Chk1 inhibition (IC_{50}, μM)	L1210	DU145	A549	HCT116	HT29
Granulatimide	85.6	>2.54	2.8	2.8	11.4	6.1	5.7
Isogranulatimide	89.7	0.438	10	13.1	18.1	13	13.7
A	94.4	0.020	32.7	53.6	65.7	nd	9.7
B	53.9	>5.0	3.37	3.7	nd	nd	3.7
C	90.2	0.033	3.61	10.1	nd	nd	20
4	75.8	5.0	5.9	nd	nd	6.6	16.4
21	77.2	1.45	16.2	nd	nd	16.4	40.5
22	38.3	nd	2.3	nd	nd	1.7	4.7
24	88.8	0.144	17.8	nd	nd	31.2	38.1
28	91.1	0.008	1.8	nd	nd	2.2	3.29

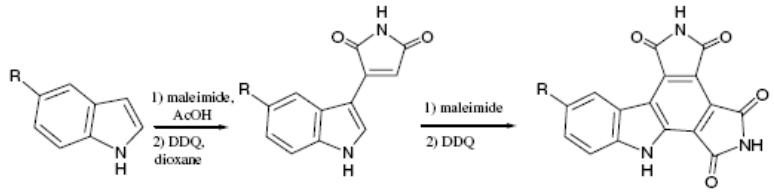
Chk1 inhibitory properties (IC_{50} in μM) and in vitro antiproliferative activities of granulatimide, isogranulatimide, and compounds **A**–**C**, **4**, **21**, **22**, **24**, and **28** toward four tumor cell lines: murine leukemia L1210, and human DU145 prostate carcinoma, A549 non-small cell lung carcinoma and HCT116 and HT29 colon carcinoma (IC_{50} μM). Due to its insolubility, the biological activities of compound **23** could not be evaluated.
nd, not determined.

the nature of the substituent in the 10-position has a major effect on the Chk1 inhibitory potency. Accordingly, to get an insight into the influence of the length, the functionality, and the orientation of side chains in the 10-position, we synthesized new dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraones bearing saturated and unsaturated side chains in the 10-position via palladium catalyzed cross-coupling reactions. The Chk1 inhibitory activities and the in vitro antiproliferative activities of these new compounds have been evaluated in the tumor cell lines, murine leukemia L1210, human DU145 prostate carcinoma, A549 non-small cell lung carcinoma, and HCT116 and HT29 colon carcinoma, and compared to those of granulatimide, isogranulatimide, and compounds **A**–**C**.

2. Chemistry

The general synthetic scheme in four steps of dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraones previously used for the synthesis of compounds **A**–**C** is outlined in Scheme 1. Indoles were coupled to maleimide in the presence of acetic acid. The Michaël adduct was oxidized. The third step was a Diels–Alder cycloaddition with a second molecule of maleimide. Finally, oxidation of the Diels–Alder adduct led to the required dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraone.

For the synthesis of compound **4** bearing an ethyl group at the 10 position, a Sonogashira reaction between 5-iodoindole and trimethylsilylacetylene in the presence



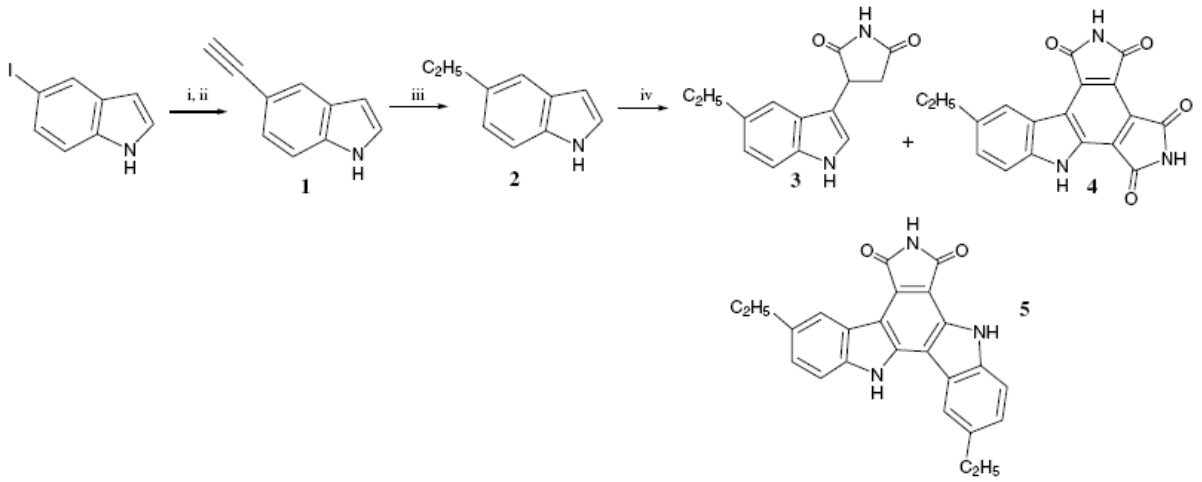
Scheme 1.

of $Pd(PPh_3)_4$, CuI, and triethylamine afforded the coupling product, which was further deprotected in NaOH/MeOH to give compound **1** in 87% yield. Catalytic hydrogenation of **1** led to 5-ethylindole **2** in 82% yield. The synthesis of compound **2** has already been reported in the literature.[15–21] However, to our knowledge, this approach has never been described (Scheme 2).

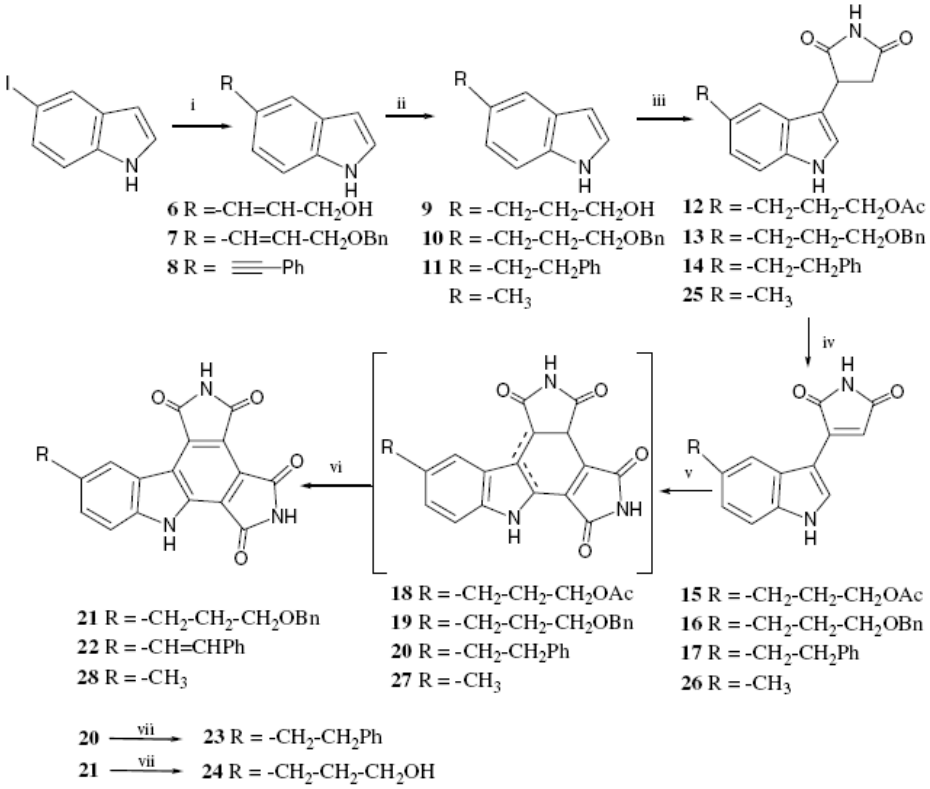
A Michaël addition was then carried out with maleimide in acetic acid leading to a mixture of the Michaël adduct **3** and to small amounts of compounds **4** and **5**. Compounds **4** and **5** were formed in situ, after oxidation of the Michaël adduct, via a Diels–Alder cycloaddition with maleimide and 5-ethylindole, respectively, followed by oxidation of the Diels–Alder adduct.

The synthesis of compounds **21**–**24** (Scheme 3) was carried out from 5-substituted indoles **6**–**8**, obtained from 5-iodoindole via a Heck or a Sonogashira cross-coupling reaction. The *E* configuration of the side-chain double bond of compounds **6** and **7** was determined from the ^{1}H NMR coupling constant ($J = 16$ Hz) between the two ethylenic protons at 6.27 and 6.63 ppm for compound **6**, and 6.31 and 6.73 ppm for compound **7**. Catalytic hydrogenation of the unsaturated side chains of **6**–**8** led to compounds **9**–**11**. To retain the benzyl group of compound **7**, the hydrogenation was performed in the presence of pyridine.[22,23] Michaël addition with maleimide in acetic acid yielded compounds **12**–**14**. The hydroxy group of the side chain of compound **9** was acetylated during the Michaël addition carried out in acetic acid. Oxidation to maleimides was achieved with DDQ in dioxane leading to compounds **15**–**17**. Diels–Alder reactions between compounds **15**–**17** and maleimide yielded the intermediates **18**–**20** which could possess an indoline or an indole structure. Indeed, in previous works, the isomerization of the Diels–Alder cycloadduct from indoline to indole was observed.[10,24] Compounds **18**–**20** were further oxidized. Compound **19** was easily oxidized with DDQ in dioxane to give compound **21**. The phenylethyl side chain of compound **20** was oxidized to a styryl chain in the presence of DDQ leading to compound **22**. The *E* configuration of the side-chain double bond of compound **22** was determined from the ^{1}H NMR coupling constant ($J = 16.5$ Hz) between the two ethylenic protons at 7.29 and 7.50 ppm. When the oxidation was performed by air oxidation in refluxing TFA, only oxidation of the cyclic moiety was observed leading to compound **23**. Oxidation of intermediate **18** using DDQ at reflux or at room temperature yielded degradation products. Removal of the benzyl group of compound **21** using refluxing TFA led to compound **24**.

To get an insight into the influence of the alkyl substituent at the 10 position on the biological activity, compound **28** bearing a methyl group was prepared according to the conventional method described on Scheme 3 from commercially available 5-methylindole. The last oxidation was carried out by air oxidation in TFA/dioxane.



Scheme 2. Reagents and conditions: (i) TMS-C≡CH, CuI, NEt_3, $Pd(PPh_3)_4$, CH_3CN, reflux, 4 h; (ii) 0.2 M NaOH, MeOH, rt, 2 h (87% for the two steps); (iii) 10% Pd/C, H_2, 1 bar, rt, 2 h (82%); (iv) maleimide, AcOH, reflux, 25 h (**3**, 30%; **4**, 6%; **5**, 2%).



Scheme 3. Reagents and conditions: (i) allyl derivative, DMF, PPh_3, AgOAc, $Pd(OAc)_2$, 70 °C (for compounds **6** and **7**) or phenylacetylene, CuI, $Pd(PPh_3)_4$, NEt_3, CH_3CN, reflux (for compound **8**); (ii) 10% Pd/C, H_2 (1 bar), MeOH, pyridine; (iii) maleimide, AcOH, reflux; (iv) DDQ, dioxane, rt; (v) maleimide, xylene, reflux; (vi) TFA, dioxane, 80 °C (for compound **21** and **27**) or DDQ, dioxane, reflux (for compound **22**); (vii) TFA, reflux, 48 h.

3. Chk1 inhibition

The percentage of inhibition of Chk1 with the drugs at a concentration of 10^{-5}M was first evaluated, and, for the most active compounds, the IC_{50} values were determined (Table 1). Compared with **A**, compound **28**, bearing a methyl group, is a stronger Chk1 inhibitor, whereas compound **4**, bearing an ethyl group, is considerably less efficient. At first sight, it seems that a large hydrophobic substituent in the 10-position is not favorable to Chk1 inhibition. Compounds **21** and **24**, bearing a propyl side chain bearing a benzyloxy or a hydroxy function, exhibit higher inhibitory activities toward Chk1 than compound **4** bearing an ethyl group. Compared to the hydroxypropyl substituent, the benzyloxypropyl group induces a strong decrease of activity. The terminal hydroxy function of compound **24** may establish a hydrogen bond with the target enzyme that could be responsible for its better Chk1 inhibitory activity. Compound **22** bearing a styryl side chain is a weak Chk1 inhibitor. Unfortunately, due to its insolubility, the activity of compound **23** could not be evaluated, and the comparison with compound **22** was not possible.

4. In vitro cytotoxicities

The in vitro cytotoxicities of the newly synthesized compounds as well as those of granulatimide, isogranulatimide, and compounds **A**, **B**, and **C** were evaluated in four tumor cell lines: murine leukemia L1210, and four human tumor cell lines DU145 prostate carcinoma, A549 non-small cell lung carcinoma, and HCT116 and HT29 colon carcinoma. The results are reported in Table 1 as the concentrations required to reduce cell growth by 50% (IC_{50}). Isogranulatimide and the reference compound **A** do not exhibit strong cytotoxicities toward the cell lines tested. Compared with compound **A**, all the bis-imide analogues reported in this work are more cytotoxic against L1210 cells. Methyl and styryl substituents at the 10 position (compounds **22** and **28**) induced the strongest cytotoxicity toward all the cell lines tested. Compounds **21** and **24** possessing a flexible side chain are less cytotoxic than compound **22** bearing a more rigid side chain. Compounds **4** and **28** bearing, respectively, an ethyl and methyl substituent exhibit stronger cytotoxicities than compounds **21** and **24** in which the side chain is longer.

5. Conclusion

In summary, the synthesis of new bis-imide granulatimide analogues bearing various substituents at the 10 position has been performed. The substituents were introduced via pallado-catalyzed coupling allowing the attachment of saturated or unsaturated side chains with and without functionalities. The Chk1 inhibitory activi-

ties and the cytotoxicities of these new compounds toward various tumor cell lines were evaluated.

Concerning Chk1 inhibitory properties, the following sequence of efficiency is observed: **28** > **A** > **C** > **24** > **21** > **B** and **4** > **22**, whereas the sequence of cytoxicities toward L1210 cells is: **28**, **22**, **C** > **4** > **21**, **24** > **A**. It can be noticed that there is no parallelism between Chk1 inhibitory properties and cytotoxicities. Indeed, the role of a Chk1 inhibitor is to prevent the cell cycle arrest, essentially at the G2 checkpoint normally activated in response to DNA damage. Therefore, the absence of correlation between Chk1 inhibition and the cytotoxicity is not surprising.

Several parameters may account for the differences in the antiproliferative activities including the ability of the compound to enter cells and the stability of the imide heterocycles which are sensitive to nucleophiles. The effects of these parameters are now under investigation.

6. Experimental

6.1. Chemistry

IR spectra were recorded on a Perkin-Elmer 881 or Perkin-Elmer Paragon 500 spectrometers (ν in cm^{-1}). NMR spectra were performed on a Bruker AVANCE 400 (^1H: 400 MHz, ^{13}C: 100 MHz) and AVANCE 500 (^1H: 500 MHz, ^{13}C: 125 MHz) (chemical shifts δ in ppm, the following abbreviations are used: singlet (s), doublet (d), doubled doublet (dd), triplet (t), doubled triplet (dt), quartet (q), multiplet (m), br s (broad signal), tertiary carbons (C tert), and quaternary carbons (C quat). Low resolution mass spectra (ESI+ and APCI+) 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 or Kieselgel 60 (Merck) 0.063–0.200 mm column chromatography.

6.1.1. 5-Ethynyl-1*H*-indole (1). A mixture of 5-iodoindole (200 mg, 0.82 mmol), trimethylsilylacetylene (182 μL, 1.28 mmol), $Pd(PPh_3)_4$ (28 mg, 0.024 mmol), CuI (8 mg, 0.042 mmol), and triethylamine (230 μL) in acetonitrile (880 μL) was refluxed for 4 h. After cooling, water was added. After extraction with EtOAc, the organic phase was washed with brine and then was dried over $MgSO_4$. After filtration, the solvent was removed. 0.2 M NaOH (6.15 mL) and methanol (6 mL) were added. The mixture was stirred for 2 h at room temperature before filtration over Celite. The filtrate was evaporated and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 8:2) to give **1** (101 mg, 0.72 mmol, 87% yield) as a brown solid. Mp 64–66 °C. IR (KBr) $\nu_{C\equiv C}$ 2098 cm^{-1}, $\nu_{\equiv C-H}$ 3268 cm^{-1}, ν_{NH} 3427 cm^{-1}. Mass (CI+) 142 $[M+H]^+$.

^1H NMR (400 MHz, DMSO-d_6): 3.93 (1H, s, ≡CH), 6.47–6.50 (1H, m), 7.21 (1H, dd, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 7.41–7.46 (2H, m), 7.75 (1H, s), 11.34 (1H, br s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 77.5 (≡CH), 85.5 (C_{alkyne}), 101.3, 111.7, 124.1, 124.4, 126.6 (C tert arom), 111.8, 127.4, 135.7 (C quat arom).

6.1.2. 5-Ethyl-1*H*-indole (2). A mixture of **1** (93 mg, 0.66 mmol), methanol (10 mL), and 10% Pd/C (28 mg) was hydrogenated (1 bar) for 2 h. The mixture was filtrated over Celite and the filtrate was evaporated. The residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 8:2) to give **2** (79 mg, 0.54 mmol, 82% yield) as an oil. IR (NaCl) ν_{NH} 3250–3500 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): 1.25 (3H, t, J = 7.5 Hz), 2.69 (2H, q, J = 7.5 Hz), 6.37 (1H, s), 6.97 (1H, d,J = 8.5 Hz), 7.29–7.35 (2H, m), 7.37 (1H, s), 10.96 (1H, br s, NH).

^{13}C NMR (100 MHz, $CDCl_3$): 16.6 (CH_3), 29.1 (CH_2), 102.3, 110.8, 119.2, 122.7, 124.3 (C tert arom), 128.1, 134.3, 135.8 (C quat arom).

6.1.3. 3-(2,5-Dioxopyrrolidin-3-yl)-5-ethyl-1*H*-indole (3), 10-ethyl-1,3,4,6-tetrahydro-2*H*, 5*H*,7*H*-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraone (4), and 2,10-diethyl-6,8-dihydro-5*H*,7*H*,13*H*-indolo[3,2-*a*]pyrrolo[3,4-*c*]carbazole-6,8-dione (5). A mixture of compound **2** (79 mg, 0.54 mmol), maleimide (58 mg, 0.60 mmol), and acetic acid (1 mL) was refluxed for 15 h. Maleimide (58 mg, 0.60 mmol) was added and the mixture was refluxed for 10 h. After evaporation, EtOAc was added to the residue, the mixture was filtered off, and the solid residue was washed with EtOAc before purification by flash chromatography (eluent: cyclohexane/THF 7:3) to give **4** (11.8 mg, 0.035 mmol, 6% yield) and **5** (3.8 mg, 0.010 mmol, 2% yield) as orange solids. The filtrate was evaporated and purified by flash chromatography (eluent: cyclohexane/EtOAc from 7:3 to 5:5) to give **3** (39.6 mg, 0.163 mmol, 30% yield) as a brown solid.

6.1.3.1. Compound 3. Mp 220–222 °C. IR (KBr) $\nu_{C=O}$ 1685, 1775 cm^{-1}, ν_{NH} 3150–3450 cm^{-1}. Mass (CI+) 243 $[M+H]^+$.

^1H NMR (400 MHz, DMSO-d_6): 1.24 (3H, t, J = 7.5 Hz), 2.69 (2H, q, J = 7.5 Hz), 2.79 (1H, dd, J_1 = 18.0 Hz, J_2 = 5.5 Hz), 3.21 (1H, dd, J_1 = 18.0 Hz, J_2 = 9.5 Hz), 4.34 (1H, dd, J_1 = 9.5 Hz, J_2 = 5.5 Hz), 7.00 (1H, dd, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 7.25 (1H, s), 7.29–7.34 (2H, m), 10.93 (1H, br s, NH), 11.32 (1H, br s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 16.6 (CH_3), 28.5, 37.4 (CH_2), 39.0 (CH), 111.5, 116.7, 121.8, 123.3 (C tert arom), 110.6, 126.1, 134.0, 135.0 (C quat arom), 178.1, 180.0 (C=O).

6.1.3.2. Compound 4. Mp >300 °C. IR (KBr) $\nu_{C=O}$ 1720, 1761 cm^{-1}, ν_{NH} 3100–3600 cm^{-1}. Mass (ESI+) 356 $[M+Na]^+$.

^1H NMR (400 MHz, DMSO-d_6): 1.33 (3H, t,J = 7.5 Hz), 2.85 (2H, q, J = 7.5 Hz), 7.56 (1H, dd, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 7.69 (1H, d, J = 8.5 Hz), 8.83

(1H, s), 11.53 (1H, s, NH), 11.56 (1H, s, NH), 12.63 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 16.2 (CH_3), 28.4 (CH_2), 112.6, 123.9, 130.4 (C tert arom), 117.8, 119.3, 119.5, 124.2, 125.5, 131.4, 136.9, 137.1, 142.5 (C quat arom), 166.4 (2C), 168.7, 169.3 (C=O).

6.1.3.3. Compound 5. Mp > 290 °C. IR (KBr) $\nu_{C=O}$ 1700, 1735 cm^{-1}, ν_{NH} 3150–3380 cm^{-1}. Mass (CI+) 382 $[M+H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): 1.36 (3H, t, J = 7.5 Hz), 1.42 (3H, t, J = 7.5 Hz), 2.87 (2H, q, J = 7.5 Hz), 2.92 (2H, q, J = 7.5 Hz), 7.41–7.45 (2H, m), 7.68 (1H, d, J = 8.0 Hz), 7.69 (1H, d, J = 8.5 Hz), 8.62 (1H, s), 8.82 (1H, s), 11.02 (1H, s, NH), 12.04 (1H, s, NH), 12.17 (1H, s, NH).

6.1.4. 5-(3-Hydroxyprop-1-enyl)-1*H*-indole (6). To a mixture of 5-iodoindole (200 mg, 0.82 mmol) and allyl alcohol (168 µL, 2.47 mmol) in DMF (1 mL) were added PPh_3 (18.6 mg, 0.071 mmol), $Pd(OAc)_2$ (14 mg, 0.062 mmol), and AgOAc (137 mg, 0.82 mmol). The mixture was stirred at 70 °C for 6 h. After cooling and filtration over Celite, the solid residue was washed successively with MeOH and THF, and the filtrate was evaporated. Water was added to the residue. After extraction with EtOAc, the organic phase was dried over $MgSO_4$. After filtration, the solvent was removed and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 7:3) to give **6** (49 mg, 0.283 mmol, 34% yield) as an orange oil. IR (NaCl) $\nu_{C=C}$ 1620, 1650 cm^{-1}, $\nu_{NH,\ OH}$ 3090–3650 cm^{-1}. Mass (CI+) 174 $[M+H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): 4.15 (2H, dt, J_1 = 5.5 Hz, J_2 = 1.5 Hz), 4.80 (1H, t, J = 5.5 Hz, OH), 6.27 (1H, dt, J_1 = 16.0 Hz, J_2 = 5.5 Hz), 6.42–6.44 (1H, m), 6.63 (1H, d, J = 16.0 Hz), 7.27 (1H, dd, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 7.34 (1H, t, J = 2.5 Hz), 7.37 (1H, d, J = 8.5 Hz), 7.56 (1H, s), 11.11 (1H, br s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 61.9 (CH_2), 101.3, 111.5, 118.4, 119.3, 125.7, 126.9, 130.4 (CH), 127.8, 128.0, 135.5 (C quat arom).

6.1.5. 5-(3-Benzyloxyprop-1-enyl)-1*H*-indole (7). To a mixture of 5-iodoindole (1.00 g, 4.11 mmol) and allylbenzylether (1.90 mL, 12.3 mmol) in DMF (5 mL) were added PPh_3 (93 mg, 0.355 mmol), $Pd(OAc)_2$ (46 mg, 0.205 mmol), and AgOAc (686 mg, 4.11 mmol). The mixture was stirred at 70 °C for 20 h. After cooling and filtration over Celite, the solid residue was washed successively with MeOH and THF, and the filtrate was evaporated. Brine was added to the residue. After extraction with EtOAc, the organic phase was dried over $MgSO_4$. After filtration, the solvent was removed and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 9:1) to give **7** (476 mg, 1.81 mmol, 44% yield) as a pale yellow oil. IR (NaCl) $\nu_{C=C}$ 1618, 1652 cm^{-1}, ν_{NH} 3120–3520 cm^{-1}. Mass (CI+) 264 $[M+H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): 4.20 (2H, dd, J_1 = 6.0 Hz, J_2 = 1.0 Hz), 4.56 (2H, s), 6.31 (1H, dt, J_1 = 16.0 Hz, J_2 = 6.0 Hz), 6.44–6.47 (1H, m), 6.73 (1H, d, J = 16.0 Hz), 7.30–7.43 (8H, m), 7.62 (1H, s), 11.16 (1H, br s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 70.6, 71.1 (CH_2), 101.4, 111.6, 118.8, 119.4, 122.5, 125.8, 127.3, 127.5 (2C), 128.2 (2C), 133.6 (C tert arom), 127.5, 127.8, 135.7, 138.6 (C quat arom).

6.1.6. 5-(2-Phenylethynyl)-1*H*-indole (8). A mixture of 5-iodoindole (100 mg, 0.41 mmol), phenylacetylene (71 µL, 0.65 mmol), CuI (4 mg, 0.021 mmol), $Pd(PPh_3)_4$ (14 mg, 0.014 mmol), and triethylamine (117 µL) in acetonitrile (440 µL) was refluxed for 23 h. After evaporation, the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 9:1) to give **8** (79 mg, 0.36 mmol, 88% yield) as a light brown solid. Mp 135 °C. IR (KBr) $\nu_{C\equiv C}$ 2204 cm^{-1}, $\nu_{C=C}$ 1592 cm^{-1}, ν_{NH} 3409 cm^{-1}. Mass (CI+) 218 $[M+H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): 6.51 (1H, s), 7.29 (1H, dd, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 7.40–7.49 (5H, m), 7.55–7.60 (2H, m), 7.82 (1H, s), 11.38 (1H, br s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 86.6, 91.6, 112.3, 123.2, 127.6, 135.7 (C_{alkyne} and C quat arom), 101.4, 111.9, 123.8, 124.3, 126.7, 128.1, 128.7, 131.1 (C tert arom).

6.1.7. 5-(3-Hydroxypropyl)-1*H*-indole (9). A mixture of compound **6** (225 mg, 1.30 mmol) and 10% Pd/C (23 mg) in methanol (20 mL) was hydrogenated (1 bar) for 3 h. After filtration over Celite, the filtrate was evaporated and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 7:3) to give **9** (134 mg, 0.765 mmol, 59% yield) as a yellow-orange oil. IR (NaCl) $\nu_{NH,\ OH}$ 3100–3650 cm^{-1}. Mass (CI+) 176 $[M+H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): 1.73–1.82 (2H, m), 2.69 (2H, t, J = 7.5 Hz), 3.46 (2H, dt, J_1 = 6.5 Hz, J_2 = 5.0 Hz), 4.46 (1H, t,J = 5.0 Hz, OH), 6.35–6.37 (1H, m), 6.95 (1H, dd, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 7.29–7.33 (2H, m), 7.34–7.36 (1H, m), 10.95 (1H, br s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 31.9, 35.2, 60.3 (CH_2), 100.6, 111.1, 119.0, 122.0, 125.2 (C tert arom), 127.8, 132.3, 134.4 (C quat arom).

6.1.8. 5-(3-Benzyloxypropyl)-1*H*-indole (10). A mixture of compound **7** (388 mg, 1.47 mmol), 10% Pd/C (39 mg), and pyridine (145 µL, 1.79 mmol) in methanol (22 mL) was hydrogenated (1 bar) for 4 h. After filtration over Celite, the filtrate was evaporated and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 9:1) to give **10** (363 mg, 1.37 mmol, 93% yield) as a brown oil. IR (NaCl) ν_{NH} 3180–3500 cm^{-1}. Mass (CI+) 266 $[M+H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): 1.86–1.95 (2H, m), 2.73 (2H, t, J = 7.5 Hz), 3.47 (2H, t, J = 6.5 Hz), 4.49

(2H, s), 6.35–6.38 (1H, m), 6.95 (1H, d, $J = 8.5$ Hz), 7.29–7.42 (8H, m), 10.98 (1H, br s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 31.8, 31.9, 69.0, 71.8 (CH_2), 100.6, 111.1, 119.1, 121.9, 125.2, 127.3, 127.5 (2C), 128.2 (2C) (C tert arom), 127.8, 131.7, 134.4, 138.7 (C quat arom).

6.1.9. 5-(2-Phenylethyl)-1*H*-indole (11). A mixture of **8** (366 mg, 1.68 mmol) and 10% Pd/C (134 mg) in methanol (25 mL) was hydrogenated (1 bar) for 20 h. After filtration over Celite, the filtrate was evaporated and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 9:1) to give **11** (236 mg, 1.07 mmol, 63% yield) as a white solid. Mp 75 °C. IR (KBr) $\nu_{C=C}$ 1580, 1600 cm^{-1}, ν_{NH} 3410 cm^{-1}. Mass (ESI+) 244 $[M+Na]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): 2.90–3.00 (4H, m), 6.35–6.38 (1H, m), 7.01 (1H, dd, $J_1 = 8.5$ Hz, $J_2 = 1.5$ Hz), 7.18–7.23 (1H, m), 7.25–7.34 (6H, m), 7.40 (1H, s), 10.99 (1H, br s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 37.5, 38.1 (CH_2), 100.6, 111.0, 119.1, 121.9, 125.2, 125.7, 128.2 (2C), 128.4 (2C) (C tert arom), 127.7, 131.7, 134.5, 141.9 (C quat arom).

6.1.10. 5-(3-Acetoxypropyl)-3-(2,5-dioxopyrrolidin-3-yl)-1*H*-indole (12). A mixture of **9** (134 mg, 0.765 mmol), maleimide (80 mg, 0.82 mmol), and acetic acid (685 μL) was refluxed for 20 h. After evaporation, the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc from 7:3 to 4:6) to give **12** (102 mg, 0.324 mmol, 42% yield) as a yellow oil. IR (film): $\nu_{C=C}$ 1712, 1772 cm^{-1}; ν_{NH} 3090–3690 cm^{-1}. Mass (CI+) 315 $[M+H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): 1.88–1.97 (2H, m), 2.01 (3H, s), 2.72 (2H, t, $J = 7.5$ Hz), 2.80 (1H, dd, $J_1 = 18.0$ Hz, $J_2 = 5.5$ Hz), 3.21 (1H, dd, $J_1 = 18.0$ Hz, $J_2 = 9.5$ Hz), 4.04 (2H, t, $J = 6.5$ Hz), 4.35 (1H, dd, $J_1 = 9.5$ Hz, $J_2 = 5.5$ Hz), 7.00 (1H, dd, $J_1 = 8.5$ Hz, $J_2 = 1.5$ Hz), 7.26 (1H, s), 7.30–7.35 (2H, m), 10.97 (1H, s, NH), 11.33 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 20.7 (CH_3), 30.5, 31.7, 37.4, 63.3 (CH_2), 39.0 (CH), 111.6, 117.5, 122.2, 123.4 (C tert arom), 110.6, 126.2, 131.2, 135.1 (C quat arom), 170.4, 178.1, 179.9 (C=O).

6.1.11. 5-(3-Benzyloxypropyl)-3-(2,5-dioxopyrrolidin-3-yl)-1*H*-indole (13). A mixture of compound **10** (352 mg, 1.33 mmol), maleimide (135 mg, 1.39 mmol), and acetic acid (1.1 mL) was refluxed for 20 h. After evaporation, the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc from 7:3 to 4:6) to give **13** (237 mg, 0.654 mmol, 49% yield) as a dark brown solid. Mp 110 °C. IR (KBr) $\nu_{C=O}$ 1685, 1780 cm^{-1}, ν_{NH} 3100–3500 cm^{-1}. Mass (ESI+) 385 $[M+Na]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): 1.84–1.93 (2H, m), 2.73 (2H, t, $J = 7.5$ Hz), 2.79 (1H, dd, $J_1 = 18.0$ Hz, $J_2 = 5.5$ Hz), 3.21 (1H, dd, $J_1 = 18.0$ Hz, $J_2 = 9.5$ Hz), 3.48 (2H, t, $J = 6.5$ Hz), 4.34 (1H, dd, $J_1 = 9.5$ Hz, $J_2 = 5.5$ Hz), 4.50 (2H, s), 6.99 (1H, dd, $J_1 = 8.5$ Hz, $J_2 = 1.5$ Hz), 7.26 (1H, s), 7.29–7.42 (7H, m), 10.95 (1H, d, $J = 1.5$ Hz, NH), 11.34 (1H, br s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 31.9, 32.0, 37.4, 69.0, 71.9 (CH_2), 39.0 (CH), 111.5, 117.4, 122.3, 123.4, 127.3, 127.5 (2C), 128.2 (2C) (C tert arom), 110.6, 126.2, 131.8, 135.1, 138.7 (C quat arom), 178.1, 180.0 (C=O).

6.1.12. 3-(2,5-Dioxopyrrolidin-3-yl)-5-(2-phenylethyl)-1*H*-indole (14). A mixture of compound **11** (213 mg, 0.96 mmol), maleimide (102 mg, 1.05 mmol), and acetic acid (0.8 mL) was refluxed for 21 h. After evaporation, the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc from 7:3 to 4:6) to give **14** (125 mg, 0.39 mmol, 41% yield) as an off-white solid. Mp 208–218 °C. IR (KBr) $\nu_{C=O}$ 1690, 1775 cm^{-1}, ν_{NH} 3100–3260, 3260–3500 cm^{-1}. Mass (ESI+) 341 $[M+Na]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): 2.75 (1H, dd, $J_1 = 18.0$ Hz, $J_2 = 5.5$ Hz), 2.88–3.00 (4H, m), 3.19 (1H, dd, $J_1 = 18.0$ Hz, $J_2 = 9.5$ Hz), 4.33 (1H, dd, $J_1 = 9.5$ Hz, $J_2 = 5.5$ Hz), 7.04 (1H, dd, $J_1 = 8.5$ Hz, $J_2 = 1.5$ Hz), 7.18–7.23 (1H, m), 7.25–7.33 (7H, m), 10.95 (1H, br s, NH), 11.32 (1H, br s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 37.4, 37.5, 38.0 (CH_2), 39.0 (CH), 111.4, 117.5, 122.4, 123.4, 125.7, 128.2 (2C), 128.4 (2C) (C tert arom), 110.6, 126.1, 131.6, 135.1, 141.8 (C quat arom), 178.0, 179.9 (C=O).

6.1.13. 5-(3-Acetoxypropyl)-3-(2,5-dihydro-2,5-dioxopyrrol-3-yl)-1*H*-indole (15). A solution of DDQ (82 mg, 0.361 mmol) in dioxane (2.7 mL) was slowly added to solution of compound **12** (108 mg, 0.344 mmol) in dioxane (2.7 mL). The mixture was stirred at room temperature for 3 h. After filtration, the filtrate was evaporated and the residue was purified by flash chromatography (eluent cyclohexane/EtOAc 7:3) to give compound **15** (75 mg, 0.240 mmol, 70% yield) as a yellow solid. Mp = 165 °C. IR (KBr) $\nu_{C=C}$ 1610 cm^{-1}, $\nu_{C=O}$ 1690, 1740, 1760 cm^{-1}, ν_{NH} = 3090–3490 cm^{-1}. Mass (ESI+) 335 $[M+Na]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): 1.94–2.03 (2H, m), 2.05 (3H, s), 2.81 (2H, t, $J = 7.5$ Hz), 4.05 (2H, t, $J = 6.5$ Hz), 6.86 (1H, d, $J = 1.0$ Hz), 7.14 (1H, dd, $J_1 = 8.5$ Hz, $J_2 = 1.5$ Hz), 7.46 (1H, d, $J = 8.5$ Hz), 7.83 (1H, s), 8.36 (1H, d, $J = 3.0$ Hz), 10.77 (1H, s, NH), 11.96 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 20.7 (CH_3), 30.5, 31.6, 63.4 (CH_2), 112.3, 115.0, 119.7, 123.8, 130.9 (C tert arom), 105.1, 125.8, 134.5, 135.2, 139.5 (C quat), 170.5, 173.2, 173.5 (C=O).

6.1.14. 5-(3-Benzyloxypropyl)-3-(2,5-dihydro-2,5-dioxopyrrol-3- yl)-1*H*-indole (16). A solution of DDQ (152 mg, 0.67 mmol) in dioxane (5 mL) was slowly added to a solution of compound **13** (231 mg, 0.64 mmol) in

dioxane (5 mL). The mixture was stirred at room temperature for 3 h. After filtration, the filtrate was evaporated and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 7:3) to give compound **16** (197 mg, 0.55 mmol, 86% yield) as a yellow solid. Mp 160 °C. IR (KBr) $\nu_{C=C}$ = 1610 cm^{-1}, $\nu_{C=O}$ 1705, 1760 cm^{-1}, ν_{NH} 3150–3400 cm^{-1}. Mass (ESI+) 383 $[M+Na]^+$.

^1H NMR (400 MHz, DMSO-d_6): 1.91–2.00 (2H, m), 2.82 (2H, t, J = 7.5 Hz), 3.49 (2H, t, J = 6.5 Hz), 4.50 (2H, s), 6.84 (1H, d, J = 1.0 Hz), 7.13 (1H, dd, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 7.28–7.41 (5H, m), 7.45 (1H, d, J = 8.5 Hz), 7.81 (1H, s), 8.35 (1H, d, J = 3.0 Hz), 10.77 (1H, s, NH), 11.96 (1H, d, J = 2.5 Hz, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 31.8, 32.0, 69.1, 71.9 (CH_2), 112.2, 114.9, 119.7, 123.8, 127.3, 127.5 (2C), 128.2 (2C), 130.9 (C tert arom), 105.1, 125.8, 135.1 (2C), 138.7, 139.5 (C quat), 173.2, 173.5 (C=O).

6.1.15. 3-(2,5-Dihydro-2,5-dioxo-pyrrol-3-yl)-5-(2-phenylethyl)-1*H*-indole (17). A solution of DDQ (87 mg, 0.383 mmol) in dioxane (2.9 mL) was slowly added to a solution of compound **14** (117 mg, 0.367 mmol) in dioxane (2.9 mL). The mixture was stirred at room temperature for 3 h. After filtration, the filtrate was evaporated and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 7:3) to give **17** (102 mg, 0.322 mmol, 88% yield) as an orange solid. Mp 212 °C. IR (KBr) $\nu_{C=C}$ 1605 cm^{-1}, $\nu_{C=O}$ 1690, 1760 cm^{-1}, ν_{NH} 3120–3460 cm^{-1}. Mass (ESI+) 339 $[M+Na]^+$.

^1H NMR (400 MHz, DMSO-d_6): 2.94–3.01 (2H, m), 3.02–3.08 (2H, m), 6.84 (1H, s), 7.17 (1H, d, J = 8.5 Hz), 7.19–7.25 (1H, m), 7.29–7.34 (4H, m), 7.45 (1H, d, J = 8.5 Hz), 7.86 (1H, s), 8.35 (1H, d, J = 2.0 Hz), 10.77 (1H, s, NH), 11.96 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 37.4, 38.1 (CH_2), 112.2, 114.9, 119.8, 123.9, 125.7, 128.2 (2C), 128.4 (2C), 130.9 (CH), 105.1, 125.8, 134.9, 135.2, 139.5, 141.9 (C quat), 173.2, 173.5 (C=O).

6.1.16. 10-(3-Benzyloxypropyl)-1,3,4,6-tetrahydro-2*H*,5*H*,7*H*-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraone (21). A mixture of compound **16** (191 mg, 0.53 mmol), maleimide (56.7 mg, 0.58 mmol) in *p*-xylene (9 mL) was refluxed for 24 h. After cooling, the mixture was filtered off, the solid residue was washed with *p*-xylene and then was dried to give 223 mg of intermediate **19**. Compound **19** (50 mg) in dioxane (3.6 mL) was stirred at 80 °C in the presence of TFA (265 µL, 3.44 mmol) for 3 days. After evaporation, water was added to the residue and the mixture was filtered off. The solid residue was washed with water and then was dried to give **21** (41.5 mg, 0.092 mmol, 77% yield) as an orange solid. Mp > 290 °C. IR (KBr) $\nu_{C=O}$ 1721, 1759, 1774 cm^{-1}, ν_{NH} 3080–3590 cm^{-1}. HRMS (ESI+) $[M+Na]^+$ calcd for $C_{26}H_{19}N_3NaO_5$ 476.1222, found 476.1202.

^1H NMR (400 MHz, DMSO-d_6): 1.94–2.03 (2H, m), 2.90 (2H, t, J = 7.5 Hz), 3.53 (2H, t, J = 6.5 Hz), 4.53 (2H, s), 7.27–7.42 (5H, m), 7.56 (1H, dd, J_1 = 8.5 Hz, J_2 = 1.0 Hz), 7.70 (1H, d, J = 8.5 Hz), 8.86 (1H, s), 11.56 (1H, s, NH), 11.59 (1H, s, NH), 12.68 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 31.5, 31.9, 68.8, 71.9 (CH_2), 112.7, 124.6, 127.3, 127.5 (2C), 128.2 (2C), 130.9 (C tert arom), 117.8, 119.3, 119.5, 124.2, 125.5, 131.4, 135.0, 137.0, 138.6, 142.6 (C quat arom), 166.4, 166.5, 168.7, 169.3 (C=O).

6.1.17. (*E*)-10-Styryl-1,3,4,6-tetrahydro-2*H*,5*H*,7*H*-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraone (22). A mixture of **17** (107 mg, 0.338 mmol) and maleimide (36 mg, 0.371 mmol) in *p*-xylene (5.8 mL) was refluxed for 24 h. After cooling, the mixture was filtered off, and the solid residue was washed with *p*-xylene and then was dried to give 124.6 mg of intermediate **20**. Compound **20** (123 mg) in dioxane (4 mL) was refluxed for 3 days in the presence of DDQ (138 mg, 0.61 mmol). After evaporation, water was added to the residue and the mixture was filtered off. The solid residue was successively washed with water and EtOAc, and then was dried to give **22** (37 mg, 0.091 mol, 27% yield) as a red solid. Mp > 290 °C. IR (KBr) $\nu_{C=C}$ 1600 cm^{-1}, $\nu_{C=O}$ 1720, 1775 cm^{-1}, ν_{NH} 3150–3400 cm^{-1}. HRMS (ESI+) $[M+Na]^+$ calcd for $C_{24}H_{13}N_3NaO_4$ 430.0804, found 430.0803.

^1H NMR (400 MHz, DMSO-d_6): 7.29 (1H, d, J = 16.5 Hz), 7.32 (1H, t, J = 7.5 Hz), 7.44 (2H, t, J = 7.5 Hz), 7.50 (1H, d, J = 16.5 Hz), 7.72 (2H, d, J = 7.5 Hz), 7.79 (1H, d, J = 8.5 Hz), 8.04 (1H, d, J = 8.5 Hz), 9.14 (1H, s), 11.59 (1H, s, NH), 11.65 (1H, s, NH), 12.84 (1H, s, NH).

Due to its insolubility, the ^{13}C NMR spectrum could not be recorded.

6.1.18. 10-(2-Phenylethyl)-1,3,4,6-tetrahydro-2*H*,5*H*,7*H*-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraone (23). A mixture of **17** (79 mg, 0.234 mmol) and maleimide (26.7 mg, 0.275 mmol) in *p*-xylene (4.3 mL) was refluxed for 24 h. After cooling, the mixture was filtered off, and the solid residue was washed with *p*-xylene and then was dried to give 81 mg of intermediate **20**. Compound **20** (30 mg) in TFA (56 µL, 0.73 mmol) was refluxed for 48 h. After evaporation, water was added to the residue. The mixture was filtered off and the solid residue was washed with water and then was dried to give **23** (24.0 mg, 0.059 mmol, 68% yield) as an orange solid. Mp > 290 °C. IR (KBr) $\nu_{C=C}$ 1607 cm^{-1}, $\nu_{C=O}$ 1721, 1773 cm^{-1}, ν_{NH} 3140–3460 cm^{-1}. HRMS (ESI+) $[M+Na]^+$ calcd for $C_{24}H_{15}N_3NaO_4$ 432.0960, found 432.0956.

^1H NMR (400 MHz, DMSO-d_6): 3.00–3.07 (2H, m), 3.09–3.17 (2H, m), 7.19–7.25 (1H, m), 7.29–7.35 (4H, m), 7.58 (1H, dd, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 7.69 (1H, d, J = 8.5 Hz), 8.88 (1H, s), 11.55 (1H, s, NH), 11.58 (1H, s, NH), 12.68 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 37.2, 37.5 (CH_2), 112.5, 124.5, 125.8, 128.2 (2C), 128.4 (2C), 130.9 (C tert

arom), 117.8, 119.3, 119.4, 124.2, 125.5, 131.4, 134.7, 136.9, 141.4, 142.6 (C quat arom), 166.4 (2C), 168.6, 169.2 (C=O).

6.1.19. 10-(3-Hydroxypropyl)-1,3,4,6-tetrahydro-2*H*,5*H*,7*H*-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraone (24). A solution of compound **22** (30.0 mg, 0.066 mmol) in TFA (4 mL) was refluxed for 48 h. After cooling, the mixture was co-evaporated with toluene. EtOAc was added to the residue and the mixture was filtered off to give **24** (19.1 mg, 0.053 mmol, 80% yield) as an orange solid. Mp > 290 °C. IR (KBr) $\nu_{C=O}$ 1721, 1756, 1773 cm^{-1}, ν_{NH} 3100–3640 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{19}H_{14}N_3O_5$ 364.0933, found 364.0935.

^1H NMR (400 MHz, DMSO-d_6): 2.11–2.20 (2H, m), 2.92 (2H, t, J = 7.5 Hz), 4.48 (2H, t, J = 6.5 Hz), 7.58 (1H, dd, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 7.71 (1H, d, J = 8.5 Hz), 8.84 (1H, s), 11.55 (1H, br s, NH), 11.58 (1H, br s, NH), 12.68 (1H, br s, NH).

Due to its insolubility, the ^{13}C NMR spectrum could not be recorded.

6.1.20. 3-(2,5-Dioxopyrrolidin-3-yl)-5-methyl-1*H*-indole (25). A mixture of 5-methylindole (1.00 g, 7.62 mmol), maleimide (740 mg, 7.62 mmol), and acetic acid (7.7 mL) was refluxed for 24 h. After evaporation, the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 6:4) to give compound **25** (586 mg, 2.57 mmol, 34% yield) as a yellow solid. Mp 220–225 °C. IR (KBr) $\nu_{C=O}$ 1685, 1775 cm^{-1}, ν_{NH} 3100–3500 cm^{-1}. Mass (ESI+) 251 $[M+Na]^+$.

^1H NMR (400 MHz, DMSO-d_6): 2.40 (3H, s, CH_3), 2.78 (1H, dd, J_1 = 18.0 Hz, J_2 = 5.5 Hz), 3.20 (1H, dd, J_1 = 18.0 Hz, J_2 = 9.5 Hz), 4.33 (1H, dd, J_1 = 9.5 Hz, J_2 = 5.5 Hz), 6.96 (1H, dd, J_1 = 8.0 Hz, J_2 = 1.5 Hz), 7.22 (1H, s), 7.29 (1H, d, J = 8.0 Hz), 7.30 (1H, d, J = 2.5 Hz), 10.93 (1H, s, NH), 11.33 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 21.3 (CH_3), 37.4 (CH_2), 39.0 (CH), 111.4, 117.9, 122.9, 123.4 (C tert arom), 110.3, 126.2, 127.1, 134.8 (C quat arom), 178.1, 179.9 (C=O).

6.1.21. 3-(2,5-Dihydro-2,5-dioxo-pyrrol-3-yl)-5-methyl-1*H*-indole (26). A solution of DDQ (602 mg, 2.65 mmol) in dioxane (24 mL) was slowly added to a solution of compound **25** (550 mg, 2.41 mmol) in dioxane (24 mL). The mixture was stirred at room temperature overnight. After filtration, the filtrate was evaporated and the residue was purified by flash chromatography (cyclohexane/EtOAc 7:3) to give compound **26** (434 mg, 1.92 mmol, 80% yield) as an orange solid. Mp 210–230 °C. IR (KBr) $\nu_{C=C}$ 1610 cm^{-1}, $\nu_{C=O}$ 1700, 1750 cm^{-1}, ν_{NH} 3080–3450 cm^{-1}. Mass (CI+) 227 $[M+H]^+$.

^1H NMR (400 MHz, DMSO-d_6): 2.48 (3H, s, CH_3), 6.82 (1H, s), 7.11 (1H, dd, J_1 = 8.5 Hz, J_2 = 1.0 Hz), 7.43 (1H, d, J = 8.5 Hz), 7.81 (1H, s), 8.34 (1H, d, J = 3.0 Hz), 10.76 (1H, br s, NH), 11.94 (1H, br s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 21.2 (CH_3), 112.2, 114.8, 120.1, 124.4, 130.8 (CH), 104.9, 125.8, 130.4, 134.9, 139.5 (C quat), 173.2, 173.4 (C=O).

6.1.22. 10-Methyl-1,3,4,6-tetrahydro-1,3,4,6-tetraoxo-2*H*,5*H*,7*H*-dipyrrolo[3,4-*a*:3,4-*c*]carbazole (28). A mixture of compound **26** (361 mg, 1.60 mmol) and maleimide (163 mg, 1.68 mmol) in *p*-xylene (27.5 mL) was refluxed for 36 h. Maleimide was added (155 mg) and the mixture was refluxed for 48 h. After cooling, the mixture was filtered off, and the solid residue was washed with *p*-xylene and then was dried to give 473 mg of intermediate **27**. Compound **27** (100 mg) in dioxane (19 mL) was refluxed for 18h in the presence of TFA (309 µL). After cooling, the mixture was filtered off. The orange solid (53 mg) was refluxed for 48 h in dioxane (9.5 mL) in the presence of TFA (154 µL). After cooling, the mixture was evaporated, water was added to the residue, and the mixture was filtered off. The solid residue was successively washed with water and EtOAc, and then was dried to give **28** (22.9 mg, 0.072 mmol, 21% yield). Mp > 300 °C. IR (KBr) $\nu_{C=O}$ 1710, 1720, 1750, 1770 cm^{-1}, ν_{NH} 3100–3350 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{17}H_{10}N_3O_4$ 320.0671, found 320.0665.

^1H NMR (400 MHz, DMSO-d_6): 2.52 (3H, s), 7.45 (1H, d, J = 8.0 Hz), 7.57 (1H, d, J = 8.0 Hz), 8.66 (1H, s), 11.48 (1H, s, NH), 11.50 (1H, s, NH), 12.49 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 21.2 (CH_3), 112.4, 125.0, 131.3 (C tert arom), 117.6, 119.2, 119.4, 123.9, 125.4, 130.4, 131.2, 136.7, 142.2 (C quat arom), 166.3, 166.4, 168.6, 169.1 (C=O).

6.2. Chk1 inhibition

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.[25]

6.3. 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.[26] 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 was DMSO. Results are expressed as IC_{50}, concentration which reduced by 50% the optical density of treated cells with respect to untreated controls.

Acknowledgments

The authors are grateful to Bertrand Légeret, University Blaise Pascal, Clermont-Ferrand, for recording the mass spectra, Jean Boutin and Gilles Ferry (Molecular and Cellular Pharmacology, IdRS) for purification of Chk1, and Séverine Ugnon-Café and Céline Bossard for kinase assays.

References and notes

1. Berlinck, R. G. S.; Britton, R.; Piers, E.; Lim, L.; Roberge, M.; Moreira da Rocha, R.; Andersen, R. J. *J. Org. Chem.* **1998**, *63*, 9850–9856.
2. Roberge, M.; Berlinck, R. G. S.; Xu, L.; Anderson, H. J.; Lim, L. Y.; Curman, D.; Stringer, C. M.; Friend, S. H.; Davies, P.; Vincent, I.; Haggarty, S. J.; Kelly, M. T.; Britton, R.; Piers, E.; Andersen, R. J. *Cancer Res.* **1998**, *58*, 5701–5706.
3. Andersen, R. J.; Roberge, M.; Sanghera, J.; Leung, D.; International Patent, 1999, WO99/47522, CA 131: 243451.
4. Nyberg, K. A.; Michelson, R. J.; Putnam, C. W.; Weinert, T. A. *Annu. Rev. Genet.* **2002**, *36*, 617–656.
5. Anderson, H. J.; Andersen, R. J.; Roberge, M. In Meijer, L., Jézéquel, A., Roberge, M., Eds. *Prog. Cell Cycle Res.* **2003**, *5*, 423–430.
6. Zhao, B.; Bower, M. J.; McDevitt, P. J.; Zhao, H.; Davis, S. T.; Johanson, K. O.; Green, S. M.; Concha, N. O.; Zhou, B. B. S. *J. Biol. Chem.* **2002**, *277*, 46609–46615.
7. Fischer, P. M.; Endicott, J.; Meijer, L.. In Meijer, L., Jézéquel, A., Roberge, M., Eds. *Prog. Cell Cycle Res.* **2003**, *5*, 235–248.
8. Jiang, X.; Zhao, B.; Britton, R.; Lim, L. Y.; Leong, D.; Sanghera, J. S.; Zhou, B. B. S.; Piers, E.; Andersen, R. J.; Roberge, M. *Mol. Cancer Ther.* **2004**, *3*, 1221–1227.
9. Hugon, B.; Pfeiffer, B.; Renard, P.; Prudhomme, M. *Tetrahedron Lett.* **2003**, *44*, 3927–3930.
10. Hugon, B.; Pfeiffer, B.; Renard, P.; Prudhomme, M. *Tetrahedron Lett.* **2003**, *44*, 3935–3937.
11. Hénon, H.; Messaoudi, S.; Hugon, B.; Anizon, F.; Pfeiffer, B.; Prudhomme, M. *Tetrahedron* **2005**, *61*, 5599–5614.
12. Hénon, H.; Anizon, F.; Pfeiffer, B.; Prudhomme, M. *Synthesis*, in press.
13. Hugon, B.; Pfeiffer, B.; Renard, P.; Prudhomme, M. *Tetrahedron Lett.* **2003**, *44*, 4607–4611.
14. Hénon, H.; Anizon, F.; Pfeiffer, B.; Prudhomme, M. *Tetrahedron* **2006**, *62*, 1116–1123.
15. Prelog, V.; Vejdelek, Z. *Helv. Chim. Acta* **1948**, *31*, 1178–1182.
16. Muratake, H.; Natsume, M. *Heterocycles* **1989**, *29*, 771–782.
17. Glennon, R. A.; Hong, S.-S.; Bondarev, M.; Law, H.; Dukat, M.; Rakhit, S.; Power, P.; Fan, E.; Kinneau, D.; Kamboj, R.; Teitler, M.; Herrick-Davis, K.; Smith, C. *J. Med. Chem.* **1996**, *39*, 314–322.
18. Starostenko, N. E.; Dat, F. T.; Serova, I. A.; Kamenetskii, A. V.; Suvorov, N. N. *Chem. Heterocycl. Compd. (Engl. Transl.)* **1991**, *27*, 508–511.
19. Suvorov, N. N.; Starostenko, N. E.; Zeiberlikh, F. N. *J. Org. Chem. USSR (Engl. Transl.)* **1980**, *16*, 2236–2241.
20. Campanati, M.; Franceschini, S.; Piccolo, O.; Vaccari, A. *J. Catal.* **2005**, *232*, 1–9.
21. Campanati, M.; Vaccari, A.; Piccolo, O. *Catal. Today* **2000**, *60*, 289–295.
22. Sajiki, H.; Kuno, H.; Hirota, K. *Tetrahedron Lett.* **1998**, *39*, 7127–7130.
23. Sajiki, H.; Hirota, K. *Tetrahedron* **1998**, *54*, 13981–13996.
24. Bergman, J.; Janosik, T.; Koch, E.; Pelcman, B. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2615–2621.
25. Davies, S. P.; Reddy, H.; Caivano, M.; Cohen, P. *Biochem. J.* **2000**, *351*, 95–105.
26. Léonce, S.; Pérez, V.; Casabianca-Pignède, M. R.; Anstett, M.; Bisagni, E.; Atassi, G. *Invest. New Drugs* **1996**, *14*, 169–180.