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Original article

Synthesis and biological evaluation of analogs of the marine alkaloids granulatimide and isogranulatimide

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ABSTRACT

A series of pyrrolic analogs and two series of regioisomeric pyrazolic analogs of the marine alkaloids granulatimide and isogranulatimide were prepared. The synthesis of the two first ones was based on the condensation reaction of diversely 5-substituted 3-bromoindoles with pyrrole or pyrazole followed by addition of the intermediates on maleimide or dibromomaleimide, respectively, the so-obtained acyclic adducts being finally photocyclized to the desired analogs. Compounds of the last series were obtained by reacting different 5-substituted-indole-3-glyoxylates with N-Boc-pyrazole-3-acetamide and subsequent photochemical cyclization of the adducts. All the compounds were evaluated for their *in vitro* growth inhibitory properties toward eight cancer cell lines. Several compounds were also assayed for their ability to abrogate the G2-cell cycle checkpoint or to inhibit a panel of Ser/Thr kinases. Lastly, computer-assisted phase-contrast microscopy (quantitative videomicroscopy) revealed that the three most potent compounds (**4a**, **9a**, **9e**), with IC_{50} growth inhibitory concentrations ranging between 10 and 20 μM, displayed cytostatic, not cytotoxic, anticancer effects.

1. Introduction

During the cell division cycle, G1 and G2 checkpoints are activated in response to DNA damage and consequently block the cycle progression to allow time for DNA repair. Interestingly, in more than 50% of cancer cells, the G1 checkpoint is impaired as a consequence of p53 tumor suppressor mutation. Therefore, it has been proposed that a combination of DNA-damaging agent with a G2/M checkpoint inhibitor should force selectively cancer cells into a premature and lethal mitosis, such a combination being currently envisioned as an original and particularly promising chemotherapeutic approach [1–3].

In this biological context, granulatimide **1** and isogranulatimide **2**, natural alkaloids isolated from the ascidian *Didemnum granulatum*, have raised considerable interest as cell cycle G2/M checkpoint inhibitors (Fig. 1) [4–6]. From a mechanistic point of view, they have been identified as selective inhibitors of the Chk1 kinase [7], a key enzyme involved in the G2/M checkpoint [8]. Since this pioneering work, intensive structure–activity relationship studies have been carried out to investigate the structural parameters required for the biological activity [9,10]. In this way, the importance of the maleimide moiety, which establishes the two fundamental hydrogen bonds with Glu^{85} and Cys^{87} in the ATP binding pocket of the enzyme, has been pointed out.

In continuation of previous studies in which the imidazole ring of the natural compounds has been replaced by different aromatic rings, we report here, on the synthesis, *in vitro* antitumor properties, kinase inhibitory properties and G2/M checkpoint abrogation

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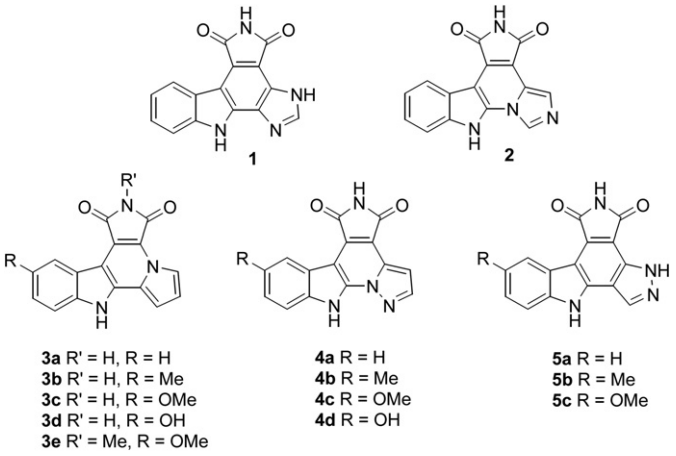


Fig. 1. Structures of the granulatimide and isogranulatimide natural alkaloids and analogues.

evaluation, of three families of granulatimide or isogranulatimide analogs. The first one **3a**–**e** consists in a completion of a pyrolic series previously reported by Hugon and co-workers [11] whereas in the two last ones, **4a**–**d** and **5a**–**c**, the imidazole ring of the natural compounds has been replaced by a pyrazole ring.

2. Chemistry

The synthesis of the already described compound **3a** was realized on the basis of the synthetic scheme previously proposed, except for the last step for which the same reagents, Pd black and nitrobenzene were involved, but with microwave irradiation instead of heating (Scheme 1). Under these conditions, the yield was improved from 16 to 44% [11].

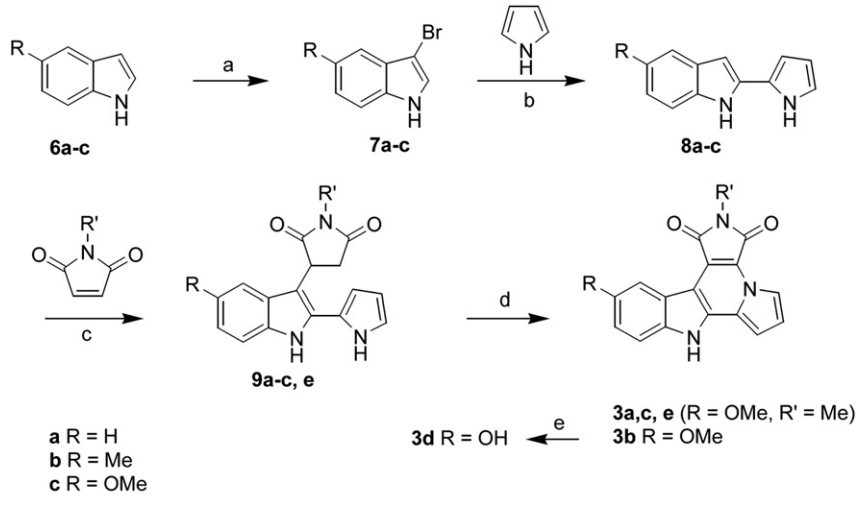
The 5-methyl or 5-methoxy-indole were coupled with pyrrole after prior transformation into 3-bromo derivatives **7b** and **7c** [12] to give the indolopyrrolic compounds **8b** and **8c**, in respectively 39 and 58% overall yield for the two steps. Condensation of these two compounds with maleimide, and in addition with methylmaleimide for compound **8c**, by using catalytic amounts of $SnCl_2$ in toluene, led to the Michael adducts **9b**, **9c** and **9e** (28, 59 and 61% yield, respectively). These compounds were cyclized under our conditions involving microwaves into the pyrrolic analogs **3b**, **3c** and **3e** in moderate yields, 49, 60 and 59%, respectively. Demethylation of compound **3c** by boron tribromide in dichloromethane furnished quantitatively the hydroxy-derivative **3d** previously prepared by Hugon and co-workers from its corresponding benzylic derivative (R = OBn) [13].

The synthetic route used to obtain the pyrrolic analogs was adapted to prepare the first series of pyrazolic analogs (Scheme 2).

Bromo derivatives **7a**–**c** were reacted with pyrazole, in the same conditions as before, the only change being the time of reaction which was longer (4h instead of 2h). N-indolylpyrazoles **10a**–**c** were obtained in modest to correct yields (61, 30 and 55%, respectively) through the formation of a C–N bond and not a C–C bond as previously observed with pyrrole and indole [11,13]. The conjugated base of these compounds generated *in situ* by action of LiHMDS was added on N-*t*-butyldimethylsilyl (TBDMS)-dibromomaleimide [14] to give the acyclic intermediates **11a**–**c** in low yields (24, 20 and 27%, respectively) resulting of the concomitant deprotection of the TBDMS protective group. Photocyclization of these last derivatives furnished the desired pyrazolic analogs **4a**–**c** in 58, 63 and 78% yields. Ultimately, the hydroxy-analog **4d** was obtained in good yield by demethylation of the methoxy derivative **4c** with BBr_3.

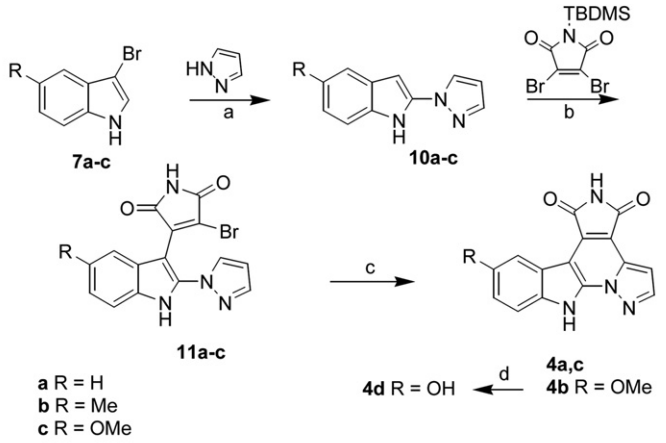
The synthetic route to the second series of regioisomeric pyrazolic analogs **5a**–**c** was based on the methodology reported by Faul *et al.* to prepare bisindolylmaleimides [15] and later on used by Piers *et al.* to publish an improved synthesis of isogranulatimide (Scheme 3) [16].

N-Boc-pyrazole-3-acetamide **14** was obtained by Boc-protection of pyrazole-3-acetamide, itself prepared from R.G. Jones' eight-step procedure [17]. The ethyl-5-methoxy (or 5-methyl)-3-glyoxylates, **12b** and **12c**, were readily obtained in 97 and 93% yield, respectively, from the corresponding indoles by using the two-step reaction scheme previously reported to prepare **12a** [18]. Following N-BOC-protection, the so-obtained compounds **13a**–**c** were reacted with compound **14** in the presence of tBuOK. The final photochemical cyclization of the indolylpyrazolylmaleimides **15a**–**c** under the same conditions as for the first series **3a**–**c** gave the N-pyrazolic-BOC protected pentacyclic derivatives **16a**–**c** in about 3% yield for the two steps. In a first approach, no attempt was done to



a- Br_2, DMF, rt, 2 h. b- TFA, CH_2Cl_2, rt, 4 h (61, 39, 58 % respectively for the two steps). c- $SnCl_2$, toluene, reflux, 24 h (35, 28, 59 and 61 % respectively). d- Pd Black, nitrobenzene, Micro wave 300 W, 190 °C, 15 min (44, 49, 59 and 60 % respectively). e- BBr_3, CH_2Cl_2, rt, 2 h (84 %).

Scheme 1.



a- TFA, CH_2Cl_2, rt, 4 h (61, 30 and 55 % respectively for the two steps). b- LiHMDS, THF, - 15 °C then rt, 12 h (24, 20 and 27 % respectively). c- hν, CH_3CN, rt, 5 h (58, 63 and 78 % respectively). d- BBr_3, CH_2Cl_2, rt, 2 h (84 %).

Scheme 2.

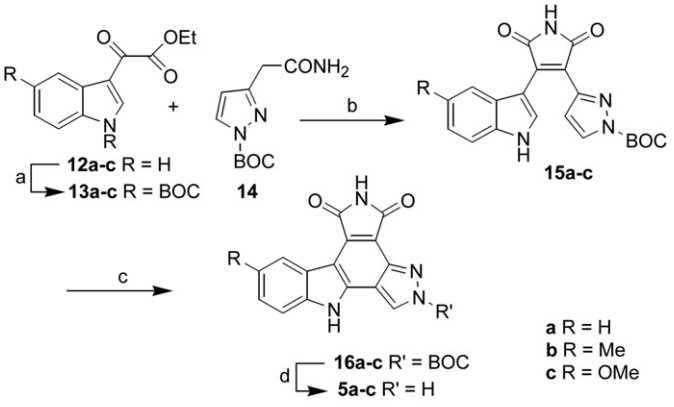
improve this low yield. The BOC group was finally cleaved to yield quantitatively compounds **5a**–**c**.

3. Pharmacology

3.1. In vitro growth inhibition in cancer cell lines

Growth inhibitory activity was determined by means of the MTT colorimetric assay in a panel of eight cancer cell lines including cell lines that display actual sensitivity to pro-apoptotic stimuli (MCF-7 [19], PC-3 [19], Hs683 [20,21], B16F10 [22]) versus cell lines that display various levels of resistance to pro-apoptotic stimuli (A549 [23], U373 [20,21], OE21 [24]). In addition, the p53 status (reported as either mutated or non-mutated) is indicated for most cell lines in Table 1.

The data from Table 1 reveal that the *in vitro* growth inhibitory activity associated with each compound under study is similar in (i) p53 wild-type (non-mutated) or p53 mutated cancer cells and (ii) in cancer cells displaying sensitivity versus a certain degree of resistance to pro-apoptotic stimuli. In contrast, heterogeneous patterns in terms of *in vitro* growth inhibitory activity have been observed with respect to the 23 compounds under study. Among the compounds of the three final series namely pyrrolic **3a**–**e**, pyrazolic **4a**–**d** and pyrazolic **5a**–**c**, the second group (including mainly **4a** and **4d**) exhibited the highest growth inhibitory activity with mean IC_{50} growth inhibitory concentrations of 11 and 16 μM respectively, e.g. similar to the activity displayed by granulatimide (Table 1). In contrast, isogranulatimide displayed weak in vitro growth inhibitory activity only (Table 1). The open structures **9a**–**c**, **e** and **11a**–**c**, precursors of pyrrolic **3** and pyrazolic **4** analogs, displayed *in vitro* antitumor activities of around 20 μM with the most active compound of both series being the methoxy derivative **9c** (mean IC_{50} concentration = 13 μM; Table 1). It is also interesting to mention that excepted for **11a**/**4a**, in main cases, the closure of the open structure into the corresponding final product has resulted in a lowering of the *in vitro* growth inhibition activity (Table 1).



a- BOC_2O, CH_2Cl_2, DMAP, rt, 4 h 92, 100 and 93 % respectively). b- 1) tBuOK, THF, rt, 12 h, 2) HCl. c- hν, CH_3CN, rt, 8 h.d- TFA, CH_2Cl_2, rt, 2 h.

Scheme 3.

3.2. The most active compounds are cytostatic, not cytotoxic

Of the most active compounds under study and for which we had sufficient amounts for further testing are included **4a**, **9a** and **9e**, which were assayed by means of quantitative videomicroscopy. The analyses were carried out with the A549 NSCLC and the U373 GBM cell lines, which both display various levels of resistance to pro-apoptotic stimuli as mentioned above. While p53 is mutated in U373 Glioblastoma cells, it is not in A549 NSCLC cells (Table 1). Fig. 2A shows that **9e** induced cytostatic effects on A549 NSCLC cells, as did **4a** and **9a** (data not shown). The same features were observed with **4a** (data not shown), **9a** (Fig. 2B) and **9e** (data not shown).

The morphological illustrations reported at high magnification in Fig. 2 further reveal that **9a** and **9e** induce cytostatic effects, i.e. a decrease in cell proliferation (while no cytotoxicity is observed) through marked enlargements of cancer cell sizes, a feature that could reflect modifications in the organization of the actin cytoskeleton according to data we obtained previously with other compound types of natural origin [26,27].

3.3. G2/M checkpoint abrogation

We examined the ability of the synthesized compounds to abrogate an activated G2/M checkpoint in HCT116 $p53^{-/-}$ cells. This checkpoint was activated following etoposide (VP-16) treatment and illegitimate entry into mitosis was monitored using an adaptation of the previously described mitotic trap assay [5,28]. Briefly, cells were treated with etoposide for 1 h then released in drug free media with or without inhibitor for 23 h. Nocodazole was added to the media, thus trapping the cells that could overcome or exit the G2/M checkpoint in a mitosis-like state. The percentage of mitotic cells, reflecting the ability of the assayed compound to act as a checkpoint abrogator, was evaluated by histone H3 phosphorylation labeling and DNA content was monitored using flow cytometry.

As presented in Table 2 following etoposide treatment, cells arrested their cell cycle at G2/M. In contrast, 75% of cells treated with etoposide and the potent Chk1 inhibitor AZD7762 (300 nM) did not arrest at G2/M, proceeded to mitosis, and were trapped in mitosis by nocodazole. When isogranulatimide and granulatimide were used in agreement with published results [5], moderate checkpoint abrogating effect was observed with about 20% of cells escaping the checkpoint and subsequently trapped in mitosis. Compound **4a** displayed a similar activity, while in contrast all other derivatives had a weaker activity in this biological assay. This result could not explain the cytotoxicity observed by **4a** in the sense that theoretically, G2 checkpoint inhibitors are not expected to be cytotoxic by themselves but in the presence of a DNA-damaging agent. It has to be pointed out that compound **3a** reported by Prudhomme and co-workers to be a potent Chk1 inhibitor (IC_{50} of 24 nM) did not display checkpoint abrogating effect in our

Table 1
In vitro growth inhibitory concentrations (IC_{50} indices).

Compounds	A549	U373	LoVo	MCF-7	HS683	PC-3	OE21	B16F10	Mean ± SEM
TP53 status[a]	Wt	Mut	Wt	Wt	Wt	Mut	nd	nd	
Granulatimide	18	6	3	46	23	11	–	5	14 ± 5
Isogranulatimide	79	>100	62	99	>100	91	–	35	>82
3a	18	33	11	49	52	50	75	19	38 ± 8
3b	14	22	52	42	45	75	>100	15	>46
3c	26	26	39	35	58	66	37	22	39 ± 6
3d	>100	>100	99	>100	>100	>100	>100	93	>99
3e	36	28	40	36	72	64	>100	27	>50
4a	8	0.2	0.4	25	3	22	2	25	11 ± 4
4b	8	57	4	9	64	75	5	37	32 ± 10
4c	8	>100	34	–	22	>100	35	–	>50
4d	15	8	7	–	23	36	–	7	16 ± 5
5a	>100	>100	44	–	93	>100	–	75	>85
5b	>100	>100	5	–	>100	>100	–	2	>68
5c	>100	51	42	–	45	>100	–	37	>63
8c	55	43	26	84	50	26	33	61	47 ± 7
9a	8	4	8	35	25	9	67	2	20 ± 8
9b	20	5	4	29	8	11	>100	0.5	>22
9c	4	15	4	19	6	6	53	0.6	13 ± 6
9e	7	10	25	7	28	37	42	6	20 ± 5
10a	>100	>100	86	>100	>100	>100	>100	>100	>98
10b	>100	>100	87	>100	>100	>100	>100	>100	>98
10c	>100	>100	70	>100	69	>100	>100	>100	>92
11a	25	27	24	27	29	27	27	13	25 ± 2
11b	29	27	27	15	29	26	28	8	24 ± 3
11c	29	27	26	11	30	26	30	8	23 ± 3

The IC_{50} indices (μM) represent the in vitro growth inhibitory concentrations at 50% obtained after having cultured the cells for 3 days in presence of the drug of interest.
[a] Whether each cancer cell line under study displays wild-type or mutated forms of TP53 is described in reference [25] (nd and – mean not determined).

experiment. Such a result was already observed by Golsteyn's group when testing different Chk1 inhibitors with IC_{50} ranging from 2.8 to 400 nM, only one indolocarbazole compound (S27888) scored positive in their checkpoint bypass assay [29].

3.4. Inhibition of kinases

In order to get an insight into the kinase selectivity, the final analogs **3**–**5** were tested on a selection of purified serine/threonine kinases, namely CDK5/p25, CK1, CLK1, DYRK1A, GSK-3, as described in Section 4. Dose–response curves allowed the determination of IC_{50} values relating to kinase activity inhibition, which are reported in Table 3. Results show weak to actual inhibitory activities, with poor selectivity in this small kinase panel. No clear-cut correlation with the anti-proliferative effects shown in Table 1 appeared except as in the case of compound **4a** and **4d**, for which their capacity to inhibit other kinases could be responsible for the cytotoxicity observed.

4. Conclusion

In conclusion, the current study reports about the *in vitro* growth inhibitory activity of pyrazolic analogs of the marine granulatimide alkaloid in a panel of eight cancer cell lines and a pyrolic series was also completed. The data show that the *in vitro* growth inhibitory activity of the most potent compounds was ~10 μM in the above-mentioned panel of cancer cell lines. The most active compounds displayed similar growth inhibitory activity whether the cancer cells (i) were p53 wild-type (non-mutated) or p53 mutated cancer cells and (ii) displayed sensitivity versus a certain degree of resistance to pro-apoptotic stimuli. In term of cancer cell growth inhibition these compounds were cytostatic, not cytotoxic, and one compound revealed a G2 checkpoint abrogating effect similar to that observed with the natural alkaloids. The most active compounds from the present study therefore deserve further chemical derivatization and pharmacological characterization to bring them at a drugable level.

5. Experimental section

5.1. Chemistry

^{1}H and ^{13}C NMR spectra were performed on Bruker Avance 300 or Avance 500 (cryoprobe TCI $^{1}H\{^{13}C, ^{31}P\}$) spectrometers with the chemical shifts of the remaining protons of the deuterated solvents serving as internal standards. IR spectra were obtained on a Perkin-Elmer 883 spectrophotometer. Mass spectra were recorded on a GCT premier waters mass spectrometer for CI and high-resolution mass spectra (HRMS) were recorded on a UPLC Xevo G2 Q TOF Waters spectrometer for ESI. Reagents were purchased from commercial sources and used as received. Flash-chromatography was performed on silica gel (15–40 μM) by means of the solvent systems indicated below. The purity of tested compounds was established to be >95% by HPLC analysis using a Waters acquity liquid chromatograph and a BECH 18 column (2.1 mm × 50 mm × 1.7 μM). Detection wavelength was 318 nm. Solvents were (A) water, 0.1% TFA; (B) acetonitile, 0.1% TFA. The method was A/B 80:20 for 0.2 min, then a gradient mode up to A/B 0:100 for 4 min, then A/B 0:100 for 1 min then a gradient up to A/B 80:20 for 0.2 min.

5.1.1. 3-Bromo-5-methyl-1H-indole (**7b**)

A solution of 5-methylindole (200 mg, 1.52 mmol) in anhydrous DMF anhydre (7.5 mL) was degased and added dropwise at room temperature to a solution of bromine (78 μL, 1.52 mmol) in anhydrous and degassed DMF (7.5 mL). After 2 h stirring in the dark, the reaction was poured into ice-cold water (80 mL), aqueous ammonia (1.4 mL) and Na_2SO_3 (1 g). After extraction with CH_2Cl_2 (3 × 60 mL), the organic layers were dried over $MgSO_4$. After removal of the solvent, the crude product was used without further purification in the next step. ^{1}H NMR (acetone-d_6) 2.87 (s, 3H); 6.54 (dd, 1H, J = 1.5 Hz and 8.3 Hz); 6.73 (d, 1H, J = 0.6 Hz); 6.85 (d, 1H, J = 8.4 Hz); 7.01 (d, 1H, J = 2.4 Hz); 10.87 (br. s, 1H). ^{13}C NMR (acetone-d_6) δ 22.5, 91.0, 113.5, 119.6, 126.0, 126.2, 128.8, 131.0, 136.1

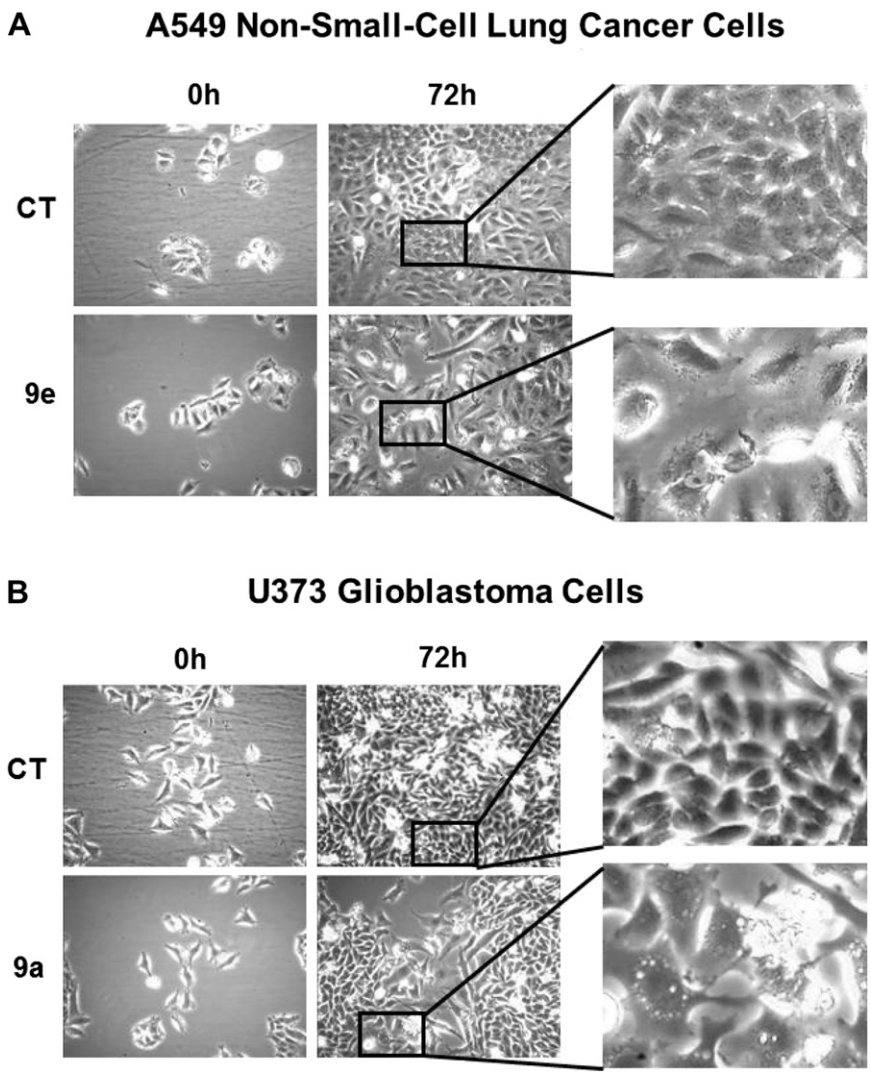


Fig. 2. Quantitative videomicroscopy on A549 NSCLC and U373 GBM cell lines.

*5.1.2. 5-Methyl-2-(1H-pyrrol-2-yl)-1H-indole (**8b**)*

To a solution of compound **7b** (1.52 mmol) in distilled CH_2Cl_2 (15 mL) was added pyrrole (211 μL, 3 mmol) and TFA (40 μL). The mixture was stirred at room temperature for 30 min. After addition of aqueous ammonia up to alkaline pH, the reaction media was concentrated and the crude product was purified by flash-chromatography (petroleum ether/AcOEt 85:15) to give the expected compound as a light-sensitive white solid (152 mg, 39% yield), mp 168 °C. HRMS (CI+) $[M+H]^+$ calcd 197.1079, found 197.1060. ^{1}H NMR (acetone-d_6) 2.36 (s, 3H); 6.17 (td, 1H, $J = 2.6$ Hz and 3.4 Hz); 6.54 (m, 1H); 6.86 (ddd, 1H, $J = 1.3$ Hz, $J = 2.7$ Hz and 5.2 Hz); 7.21 (d, 1H, $J = 8.2$ Hz); 7.26 (d, 1H, $J = 0.7$ Hz); 10.23 (br. s, 1H); 10.50 (br. s, 1H). ^{13}C NMR (acetone-d_6) 22.5, 97.0, 107.2, 110.7, 112.1, 120.7, 121.1, 124.2, 127.4, 129.9, 131.6, 134.5, 136.9. IR (KBr) 3359, 2924, 1775, 1707, 1353, 1184.799 cm^{-1}.

*5.1.3. 5-Methoxy-2-(1H-pyrrol-2-yl)-1H-indole (**8c**)*

Same procedure as for compound **8b** involving **7c** [12] (770 mg, 3.4 mmol), distilled CH_2Cl_2 (35 mL), pyrrole (475 μL, 6.8 mmol) and TFA (100 μL). The crude product was purified by flash-

Table 2
Evaluation of the G2/M checkpoint abrogation.

Compounds	Mitotic index
3a	9.6
4a	23.9
4b	13.9
4d	3.9
5b	0
8b	5.0
8c	8.3
11b	5.5
Isogranulatimide	17.2
Granulatimide	19.4
AZD	75.0
Control	0

Table 3
Inhibition of kinases.

Compounds	CDK5	CK1	CLK1	DYRK1A rat	GSK-3
3a	2.1	10	0.52	1.3	0.57
3b	7.5	>10	1.5	8.3	1.7
3c	>10	>10	10	>10	1.7
3d	2.6	1.1	0.26	0.2	0.42
4a	4.0	3.2	0.3	2.1	0.49
4b	>10	7.1	3.5	>10	0.045
4c	>10	>10	>10	>10	0.7
4d	0.51	0.16	0.09	0.18	0.041
5a	0.91	2.6	0.42	1.7	0.9
5b	0.31	0.23	0.13	1.4	0.17

IC_{50} values were determined from dose–response curves on purified protein kinases assayed in the presence of various compounds.

chromatography (petroleum ether/AcOEt 85:15) to give the expected compound as a light-sensitive white solid (417 mg, 58% yield), mp 147 °C. HRMS (ESI$^+$) $[M+H]^+$ calcd 213.1028, found 213.1019. ^{1}H NMR (acetone-d_6) 3.78 (s, 3H); 6.19 (td, 1H, $J=2.7$ Hz and 3.3 Hz); 6.58 (m, 2H); 6.71 (dd, 1H, $J=2.4$ Hz and 8.7 Hz); 6.87 (td, 1H, $J=1.5$ Hz and 2.7 Hz); 7.01 (d, 1H, $J=2.4$ Hz); 7.23 (d, 1H, $J=8.7$ Hz); 10.23 (br. s, 1H); 10.48 (br. s, 1H). ^{13}C NMR (acetone-d_6) 56.73, 97.41, 103.34, 107.17, 110.80, 112.62, 112.96, 120.68, 127.35, 131.73, 133.57, 135.02, 156.09. IR (KBr) 3417, 3386, 1608, 1489, 1450, 1241, 1226 cm^{-1}.

5.1.4. 3-(5-Methyl-2-(1H-pyrrol-2-yl)-1H-indol-3-yl)pyrrolidine-2,5-dione (**9b**)

A mixture of compound **8b** (60 mg, 0.31 mmol), maleimide (59 mg, 0.62 mmol), a catalytic amount of $SnCl_2$ in toluene (17 mL) was refluxed for 24 h. After removal of the solvent, the residue was purified by flash-chromatography (petroleum ether/AcOEt 90:10 then 70:30) to give the expected compound as a off-white solid (24.4 mg, 28% yield), mp 168 °C. HRMS (ESI$^+$) $[M+H]^+$ calcd 294.1243, found 294.1267. ^{1}H NMR (acetone-d_6) 2.36 (s, 3H); 3.04 (dd, 1H, $J=5.1$ Hz and 18.3 Hz); 3.28 (dd, 1H, $J=9.9$ Hz and 18.3 Hz); 4.59 (dd, 1H, $J=5.1$ Hz and 9.9 Hz); 6.25 (td, 1H, $J=2.4$ Hz and 3.6 Hz); 6.49 (ddd, 1H, $J=1.5$ Hz, 2.7 Hz and 3.0 Hz); 6.95 (dd, 1H, $J=1.5$ Hz and 8.1 Hz); 6.98 (td, 1H, $J=1.5$ Hz and 2.7 Hz); 7.15 (br. s, 1H); 7.29 (d, 1H, $J=8.1$ Hz); 10.19 (br. s, 1H); 10.33 (br. s, 1H); 10.41 (br. s, 1H). ^{13}C NMR (acetone-d_6) 22.73, 38.46, 41.43, 107.90, 110.39, 111.00, 113.05, 119.58, 121.4, 125.02, 125.35, 128.64, 130.20, 133.02, 136.69, 179.05, 182.01. IR (KBr) 3359, 2924, 1775, 1707, 1353, 1184, 799, 731 cm^{-1}.

5.1.5. 3-[5-Methoxy-2-(1H-pyrrol-2-yl)-1H-indol-3-yl]-pyrrolidine-2,5-dione (**9c**)

Same procedure as for compound **9b** involving **8c** (320 mg, 1.50 mmol), maleimide (290 mg, 3.00 mmol), a catalytic amount of $SnCl_2$ and toluene (90 mL) Purification of the crude product by flash-chromatography (CH_2Cl_2/MeOH 98:2) gave the expected compound as a pale green solid (276 mg, 59% yield), mp 142 °C. HRMS (ESI$^+$) $[M+H]^+$ calcd 310.1192, found 310.1167. ^{1}H NMR (acetone-d_6) 3.09 (dd, 1H, $J=18.0$ Hz and 5.1 Hz); 3.31 (dd, 1H, $J=18.0$ Hz and 9.9 Hz); 3.76 (s, 3H); 4.63 (dd, 1H, $J=4.8$ Hz and 9.9 Hz); 6.26 (td, 1H, $J=2.4$ Hz and 3.6 Hz); 6.50 (m, 1H); 6.79 (dd, 1H, $J=2.4$ Hz and 8.7 Hz); 6.85 (d, 1H, $J=2.4$ Hz); 6.98 (td, 1H, $J=1.5$ Hz and 2.7 Hz); 7.30 (d, 1H, $J=8.7$ Hz); 10.15 (br. s, 1H); 10.39 (br. s, 2H). ^{13}C NMR (acetone-d_6) 91.41, 113.75, 120.04, 121.83, 124.25, 126.20, 128.51, 137.7. IR (KBr) 3353, 2940, 2833, 1775, 1708, 1488, 1218, 1182 cm^{-1}

5.1.6. 3-(5-Methoxy-2-(1H-pyrrol-2-yl)-1H-indol-3-yl)-1-methylpyrrolidine-2,5-dione (**9e**)

Same procedure as for compound **9b** involving compound **2b** (120 mg, 0.56 mmol), N-methylmaleimide (124 mg, 1.12 mmol), a catalytic amount of $SnCl_2$ and toluene (36 mL). Purification by flash-chromatography (petroleum ether/AcOEt 80:20) gave the expected compound as a pale green solid (110 mg, 61% yield), mp 132 °C. HRMS (ESI$^+$) $[M+H]^+$ calcd 324.1348, found 324.1351. ^{1}H NMR (acetone-d_6) 3.01 (dd, 1H, $J=18.0$ Hz and 4.8 Hz); 3.04 (s, 3H); 3.27 (dd, 1H, $J=9.6$ Hz and 18.0 Hz); 3.74 (s, 3H); 4.55 (dd, 1H, $J=4.8$ Hz and 9.6 Hz); 6.24 (td, 1H, $J=2.7$ Hz and 3.3 Hz,); 6.46 (m, 1H,); 6.65 (d, 1H, $J=2.4$ Hz); 6.77 (dd, 1H, $J=8.7$ Hz and 2.4 Hz); 6.98 (dt, 1H, $J=2.7$ Hz and 1.5 Hz); 7.30 (d, 1H, $J=8.7$ Hz); 10.18 (br. s, 1H); 10.42 (br.s, 1H). ^{13}C NMR (acetone-d_6) 24.19, 35.32, 38.12, 54.92, 100.23, 106.52, 108.63, 109.16, 111.23, 112.11, 119.64, 123.40, 126.87, 131.48, 131.69, 154.19, 176.6, 179.3. IR (KBr) 3337, 1692, 1438, 1282, 1218, 1119, 1030 cm^{-1}.

5.1.7. 8-Methyl-11H-1,5,11,11b-tetraaza-benzo[a]trindene-4,6-dione (**3b**)

A suspension of **9b** (50 mg, 0.17 mmol) and Pd black (18.1 mg, 0.171 mmol) in nitrobenzene (2.5 mL) was irradiated at 300 W for 30 min. After cooling, petroleum ether (5 mL) was added and the mixture was filtered on silica gel to remove nitrobenzene (petroleum ether, then petroleum ether/AcOEt 90:10 and petroleum ether/AcOEt 1/1) to give the expected compound as a dark red solid (24 mg, 49% yield), mp >260 °C. HRMS (ESI$^+$) $[M+H]^+$ calcd 290.0930, found 290.0922. ^{1}H NMR (acetone-d_6) 2.52 (s, 3H); 7.05 (d, 2H, $J=2.1$ Hz); 7.25 (td, 1H, $J=6.6$ Hz and 1.2 Hz); 7.48 (d, 1H, $J=8.4$ Hz); 8.30 (t, 1H, $J=2.1$ Hz); 8.48 (dd, 1H, $J=2.4$ Hz and 0.9 Hz). ^{13}C NMR (DMSO-d_6) 22.7, 101.2, 106.2, 113.1, 116.2, 117.8, 120.3, 124.6, 126.8, 130.5, 132.2 (2C), 136.0, 139.3, 167.8, 170.5. IR (KBr) 3443–3224, 1710, 1490, 1216, 1084, 1216 cm^{-1}. HPLC: t_R 2.28 min.

5.1.8. 8-Methoxy-11H-3a,5,11-triaza-benzo-[a]-trindene-4,6-dione (**3c**)

Same procedure as for compound **3b** involving **9c** (140 mg, 0.45 mmol) and Pd black (48 mg, 0.45 mmol) in nitrobenzene (7 mL). The filtration on silica gel (petroleum ether/AcOEt 1:0 up to 0:1) gave the expected product as a dark red solid (82 mg, 60% yield), mp >260 °C. HRMS (ESI$^+$) $[M+H]^+$ calcd 306.0879, found 306.0884. ^{1}H NMR (acetone-d_6) 3.92 (s, 3H); 7.03 (dd, 1H, $J=8.7$ Hz and 2.7 Hz); 7.05 (d, 1H, $J=1.8$ Hz); 7.05 (d, 1H, $J=2.4$ Hz); 7.50 (dd, 1H, $J=8.7$ Hz and 0.6 Hz); 8.25 (d, 1H, $J=2.7$ Hz); 8.30 (dd, 1H, $J=2.4$ Hz and 1.8 Hz); 9.84 (br. s, 1H); 11.52 (br. s, 1H). ^{13}C NMR (DMSO-d_6) 55.96, 100.23, 104.26, 105.37, 112.92, 114.46 (2C), 116.40, 117.67, 121.31, 122.74, 125.02, 134.13, 134.64, 154.99, 166.91, 169.84. IR (KBr) 3316, 2923, 2852, 1747, 1707, 1486, 1459, 1208, 1025 cm^{-1}. HPLC: t_R 2.05 min.

5.1.9. 8-Hydroxy-11H-1,5,11,11b-tetraaza-benzo[a]trindene-4,6-dione (**3d**)

To a solution of **3c** (15 mg, 0.05 mmol) in CH_2Cl_2 (6 mL) was added BBr_3 (1 M in CH_2Cl_2, 2 mL, 2 mmol). After 4 h stirring at room temperature, the mixture was poured into a saturated solution of $NaHCO_3$ (20 mL). Filtration of the mixture gave quantitatively the expected compound as a dark red solid (12.3 mg, 84% yield), mp >260 °C. SM (ESI$^+$) $[M+H]^+$ calcd 292.0722, found 292.0706. ^{1}H NMR (DMSO-d_6) 6.89 (dd, 1H, $J=2.5$ Hz and 8.6 Hz); 7.04 (s, 1H); 7.42 (d, 1H, $J=8.7$ Hz); 7.99 (d, 1H, $J=2.4$ Hz); 8.22 (dd, 1H, $J=1.4$ Hz and 2.7 Hz). ^{13}C NMR (DMSO-d_6) 99.6, 103.5, 107.0, 112.1, 113.8, 114.5, 115.8, 117.4, 120.4, 122.5, 124.5, 132.8, 134.1, 152.2, 166.4, 169.4. IR (KBr) 1740, 1710 cm^{-1}. HPLC: t_R 1.99 min.

5.1.10. 8-Methoxy-5-methyl-11H-3a,5,11-triaza-benzo[a]trindene-4,6-dione (**3e**)

Same procedure as for compound **3b** involving **9e** (30 mg, 0.094 mmol), Pd black (10 mg, 0.094 mmol) and nitrobenzene (2 mL), the mixture was irradiated at 300 W for 1.5 h. Purification by filtration on silica gel gave the expected compound as a dark red solid (17.7 mg, 59% yield), mp >260 °C. MS (ESI$^+$) $[M+H]^+$ calcd 320.1035, found 320.1040. ^{1}H NMR (acetone-d_6) 3.18 (s, 3H); 3.93 (s, 3H); 7.04 (m, 3H); 7.50 (d, 1H, $J=8.8$ Hz); 8.26 (d, 1H, $J=2.5$ Hz), 8.29 (m, 1H). ^{13}C NMR (acetone-d_6) 23.95, 55.82, 100.22, 104.24, 104.88, 112.97, 114.37, 114.67, 116.50, 116.89, 120.79, 122.56, 124.89, 134.08, 134.46, 154.97, 165.67, 168.68. IR (KBr) 3297, 1682, 1436, 1377, 1222, 1043 cm^{-1}. HPLC: t_R 2.65 min.

5.1.11. 2-Pyrazol-1-yl-1H-indole (**10a**)

Same procedure as for compound **8b** involving bromoindole (838.5 mg, 4.3 mmol), distilled CH_2Cl_2 (35 mL), pyrazole (576 mg, 8.6 mmol) and TFA (120 μL). The mixture was stirred at room

temperature for 24 h. Purification by flash-chromatography (petroleum ether/AcOEt 85:15) gave the expected compound as a white solid (480 mg, 61% yield), mp 147 °C. HRMS (ESI^+) $[M+H]^+$ calcd 184.0875, found 184.0882. ^1H NMR (acetone-d_6) 6.53 (dd, 1H, $J = 1.8$ Hz and 2.4 Hz); 6.59 (br. s, 1H); 7.05 (td, 1H, $J = 1.5$ Hz and 7.6 Hz); 7.13 (td, 1H, $J = 1.5$ Hz and 7.6 Hz); 7.48 (dd, 1H, $J = 0.8$ Hz and 7.6 Hz); 7.55 (dd, 1H, $J = 0.6$ Hz and 7.6 Hz); 7.71 (d, 1H, $J = 1.8$ Hz); 8.31 (d, 1H, $J = 2.4$ Hz); 10.85 (br. s, 1H). ^{13}C NMR (acetone-d_6) 88.8, 109.5, 113.2, 121.8, 122.0, 123.3, 129.7, 129.9, 135.9, 138.0, 142.5. IR 3122, 1694, 1621, 1586, 1566, 1470, 1393, 1233, 1046, 919, 749 cm^{-1}.

5.1.12. 5-Methyl-2-pyrazol-1-yl-1H-indole (**10b**)

Same procedure as for compound **8b** involving **7b** (320.0 mg, 1.52 mmol), distilled CH_2Cl_2 (15 mL), pyrazole (211 μL, 3 mmol) and TFA (40 μL). Purification by flash-chromatography (petroleum ether/AcOEt 85:15) gave the expected compound as a white light-sensitive solid (90 mg, 30% yield), mp 153 °C. HRMS (ESI^+) $[M+H]^+$ calcd 198.1031, found 198.1026. ^1H NMR (acetone-d_6) 2.39 (s, 3H); 6.50 (dd, 1H, $J = 0.8$ Hz and 2.2 Hz); 6.52 (dd, 1H, $J = 1.8$ Hz and 2.5 Hz); 6.95 (dd, 1H, $J = 1.2$ Hz and 8.2 Hz); 7.32 (dd, 1H, $J = 0.7$ Hz and 1.5 Hz); 7.35 (m, 1H); 7.69 (d, 1H, $J = 1.5$ Hz); 8.29 (dd, 1H, $J = 0.5$ Hz and 2.5 Hz); 10.72 (s, 1H). ^{13}C NMR (acetone-d_6) 22.6, 88.6, 109.4, 112.9, 121.6, 124.8, 129.6, 130.2, 130.8, 134.2, 138.0, 142.4. IR (KBr) 3382, 1597, 1471, 1390, 1048, 798, 748 cm^{-1}.

5.1.13. 5-Methoxy-2-pyrazol-1-yl-1H-indole (**10c**)

Same procedure as for compound **8b** involving **7c** (770.0 mg, 3.4 mmol), distilled CH_2Cl_2 (35 mL), pyrazole (460 mg, 6.8 mmol) and TFA (100 μL). The mixture was stirred in the dark for 12 h. Purification by flash-chromatography (petroleum ether/AcOEt 8:2) gave the expected product as a white solid (396 mg, 55% yield), mp 117 °C. HRMS (ESI^+) $[M+H]^+$ calcd 214.0980, found 214.0979. ^1H NMR (acetone-d_6) 3.80 (s, 3H, OMe); 6.51 (m, 2H); 6.78 (dd, 1H, $J = 2.4$ Hz and 8.7 Hz); 7.06 (d, 1H, $J = 2.4$ Hz); 7.37 (d, 1H, $J = 8.7$ Hz); 7.70 (d, 1H, $J = 1.8$ Hz); 8.26 (d, 1H, $J = 2.4$ Hz); 10.75 (br. s, 1H). ^{13}C NMR (acetone-d_6) 56.78, 89.02, 103.89, 109.42, 113.42, 113.88, 129.60, 130.48, 130.85, 138.38, 142.41, 156.62. IR (KBr) 3192, 1592, 1571, 1499, 1452, 1397, 1236, 1226 cm^{-1}.

5.1.14. 3-(2-(1H-Pyrazol-1-yl)-1H-indol-3-yl)-4-bromo-1H-pyrrole-2,5-dione (**11a**)

To a solution of **10a** (65 mg, 0.36 mmol) in THF (1 mL) was added dropwise at −15 °C, LiHMDS (1 M solution in THF, 1.1 mL, 1.08 mmol). After 45 min stirring, a solution of compound 3,4-dibromo-1-(*tert*-butyldimethylsilyl)-1H-pyrrole-2,5-dione [13] (140 mg, 0.38 mmol) in THF (1 mL) was added and the mixture was let to come back up to rt for 12 h. The reaction media was then, poured into ice-cold 10% HCl and it was extracted with AcOEt (3 × 20 mL). The organic layers were washed first, with a saturated solution of $NaHCO_3$ and then with brine. After drying over $MgSO_4$ and removal of the solvent, the crude product was purified by flash-chromatography (petroleum ether/AcOEt 80:20 up to 50:50) to give the expected compound as a red solid (30 mg, 24% yield), mp 114 °C. HRMS (ESI^+) $[M+H]^+$ calcd 356.9987, found 356.9998. ^1H NMR (acetone-d_6) 6.51 (dd, 1H, $J = 1.8$ Hz and 2.5 Hz); 7.18 (m, 1H); 7.26 (m, 1H); 7.57 (t, 2H, $J = 9.0$ Hz), 7.73 (d, 1H, $J = 1.8$ Hz); 8.24 (d, 1H, $J = 2.5$ Hz); 10.15 (br. s, 1H); 11.57 (br. s, 1H). ^{13}C NMR (DMSO-d_6) 91.2, 108.1, 111.9, 120.4, 122.5, 123.0, 125.2, 130.6, 133.2, 134.9, 138.5, 141.8, 167.0, 169.1. IR (KBr) 3304, 1723, 1628, 1564, 1460, 1331, 1022 cm^{-1}.

5.1.15. 3-Bromo-4-(5-methyl-2-(1H-pyrazol-1-yl)-1H-indol-3-yl)-1H-pyrrole-2,5-dione (**11b**)

A solution of compound **10b** (120 mg, 0.61 mmol) in THF (8 mL) was added dropwise at room temperature, under nitrogen atmosphere, to a solution of EtMgBr (1 M in THF, 2.4 mL, 2.4 mmol) and the mixture was stirred for 30 min. A solution of 3,4-dibromo-1-(*tert*-butyldimethylsilyl)-1H-pyrrole-2,5-dione (220 mg, 0.61 mmol) in THF (2 mL) was then added and stirring was continued for 12 h. The reaction media was poured into NH_4Cl (10% aqueous solution, 10 mL) and was extracted with AcOEt (2 × 15 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed. The crude product was purified by chromatography on alumina (petroleum ether/CH_2Cl_2/MeOH 8:2:0 up to 0:95:5) to give the expected compound as a red solid (40 mg, 20% yield), mp 148 °C. HRMS (ESI^+) $[M+H]^+$ calcd 371.0144, found 371.0151. ^1H NMR (acetone-d_6) 2.42 (s, 3H); 6.50 (dd, 1H, $J = 4.5$ Hz and 1.8 Hz); 7.10 (dd, 1H, $J = 8.04$ Hz and 1.2 Hz); 7.38 (s, 1H); 7.43 (d, 1H, $J = 8.4$ Hz); 7.72 (d, 1H, $J = 1.2$ Hz); 8.20 (d, 1H, $J = 2.7$ Hz). ^{13}C NMR (acetone-d_6) 20.93, 91.29, 108.02, 111.61, 120.47, 123.76, 124.43, 126.19, 130.15, 130.23, 131.99, 135.40, 141.66, 166.19, 168.48. IR (KBr) 3322-2927, 1718, 1619, 1496, 1396, 1336, 1031, 651 cm^{-1}.

5.1.16. 3-Bromo-4-(5-methoxy-2-(1H-pyrazol-1-yl)-1H-indol-3-yl)-1H-pyrrole-2,5-dione (**11c**)

Same procedure as for compound **11a** involving **10c** (410 mg, 1.92 mmol), THF (7 mL), LiHMDS (1 M in THF, 5.76 mmol, 5.76 mL), 3,4-dibromo-1-(*tert*-butyldimethylsilyl)-1H-pyrrole-2,5-dione (740 mg, 2.0 mmol), THF (10 mL), 10% HCl (35 mL). After work-up, the crude product was purified by flash-chromatography (petroleum ether/AcOEt 80/20 up to 50/50) to give the expected compound as a red solid (200 mg, 27% yield), mp 105 °C. HRMS (ESI^+) $[M+H]^+$ calcd 387.0093, found 387.0087. ^1H NMR (acetone-d_6) 3.83 (s, 3H); 6.51 (dd, 1H, $J = 1.8$ Hz and 2.6 Hz); 6.91 (dd, 1H, $J = 2.5$ Hz and 8.8 Hz); 7.12 (d, 1H, $J = 2.5$ Hz); 7.46 (dd, 1H, $J = 0.6$ Hz and 8.8 Hz); 7.73 (dd, 1H, $J = 0.5$ Hz and 1.8 Hz); 8.20 (dd, 1H, $J = 0.6$ Hz and 2.6 Hz); 10.11 (br. s, 1H); 11.36 (br. s, 1H). ^{13}C NMR (acetone-d_6) 56.8, 93.7, 104.8, 109.8, 114.5, 114.7, 125.2, 128.3, 130.3, 132.0, 137.3, 141.1, 143.5, 157.0, 167.9, 170.2. IR (KBr), 3435–3213, 1717, 1617, 1491, 1329, 1211, 654 cm^{-1}.

5.1.17. 11H-1,5,11,11b-tetraaza-benzo[a]trindene-4,6-dione (**4a**)

A solution of **11a** (18 mg, 0.05 mmol) in CH_3CN (11 mL) was irradiated at 350 nm for 5 h. Filtration of the mixture gave the expected compound as an orange solid (8.1 mg, 58% yield), mp >260 °C. HRMS (ESI^+) $[M+H]^+$ calcd 277.0726, found 277.0714. ^1H NMR (DMSO-d_6) 7.11 (d, 1H, $J = 2.2$ Hz); 7.40 (t, 1H, $J = 7.5$ Hz); 7.49 (t, 1H, $J = 7.6$ Hz); 7.67 (d, 1H, $J = 8.2$ Hz); 8.45 (d, 1H, $J = 2.2$ Hz); 8.60 (d, 1H, $J = 7.3$ Hz); 11.26 (br. s, 1H); 13.66 (br. s, 1H). ^{13}C NMR (DMSO-d_6) 98.54, 100.56, 112.31, 113.22, 121.26, 122.76, 123.70, 126.52, 130.64, 132.82, 137.59, 139.59, 146.30, 168.02, 169.65. IR (KBr) 3452–3148, 1755, 1707, 1635, 1588, 1467, 1351, 750 cm^{-1}. HPLC: t_R 1.69 min.

5.1.18. 8-Methyl-11H-1,5,11,11b-tetraaza-benzo[a]trindene-4,6-dione (**4b**)

A solution of **11b** (20 mg, 0.054 mmol) in toluene (11 mL) was irradiated at 350 nm for 8 h. After removal of the solvent, the crude product was purified by flash-chromatography (CH_2Cl_2/MeOH 95:5 then MeOH/AcOH 99:1) to give the expected compound as an orange solid (10 mg, 63% yield), mp >260 °C. HRMS (ESI^+) $[M+H]^+$ calcd 291.0882, found 291.0886. ^1H NMR (DMSO-d_6) 7.09 (d, 1H, $J = 2.0$ Hz); 7.31 (ddd, 1H, $J = 8.3$ Hz and 2.1 Hz); 7.55 (d, 1H, $J = 8.3$ Hz); 8.40 (dd, 1H, $J = 2.1$ Hz); 8.43 (d, 1H, $J = 2.0$ Hz); 11.24 (br. s, 1H); 13.54 (br. s, 1H). ^{13}C NMR (DMSO-d_6) 21.84, 98.43, 99.91, 112.55, 112.80, 121.59, 123.10, 127.45, 128.90, 131.47, 133.77, 135.58, 139.41, 145.10, 169.45, 170.11. IR (KBr) 3446, 3250, 1751, 1701, 1629, 1590, 1488 cm^{-1}. HPLC: t_R 1.90 min.

*5.1.19. 8-Methoxy-11H-1,5,11,11b-tetraaza-benzo[a]trindene-4,6-dione (**4c**)*

Same procedure as for compound **4b** involving **11c** (20 mg, 0.052 mmol) in toluene (11 mL) and irradiation for 5 h. Filtration of the mixture gave the expected compound as an orange solid (12.4 mg, 78% yield), mp >260 °C. HRMS (ESI^+) $[M+H]^+$ calcd 307.0831, found 307.0819. ^1H NMR (DMSO-d_6) 3.38 (s, 3H); 7.09 (d, 1H, $J=2.5$ Hz); 7.12 (dd, 1H, $J=8.5$ Hz and 2.5 Hz); 7.57 (d, 1H, $J=8.5$ Hz); 8.15 (d, 1H, $J=2.5$ Hz); 8.42 (d, 1H, $J=2.5$ Hz); 11.25 (s, 1H); 13.51 (s, 1H). ^{13}C NMR (DMSO-d_6) 56.00, 98.52, 100.20, 106.22, 112.36, 113.89, 114.80, 122.09, 128.98, 131.92, 133.76, 139.52, 145.06, , 155.65, 169.47, 170.17. IR (KBr) 3446, 3250, 1751, 1701, 1629, 1590, 1488 cm^{-1}. HPLC: t_R 1.72 min.

*5.1.20. 8-Hydroxy-11H-1,5,11,11b-tetraaza-benzo[a]trindene-4,6-dione (**4d**)*

Same procedure as for compound **3d** involving **4c** (50 mg, 0.16 mmol), CH_2Cl_2 (15 mL) BBr_3 (1 M) in CH_2Cl_2 (6 mL, 6 mmol), stirring for 4 h and $NaHCO_3$ (20 mL). Filtration gave the expected compound as a red solid (39 mg, 84% yield), mp >260 °C. HRMS (ESI^+) $[M+H]^+$ calcd 293.0675, found 293.0677. ^1H NMR (DMSO-d_6) 6.88 (dd, 1H, $J=2.4$ Hz and 8.4 Hz); 6.97 (d, 1H, $J=2.4$ Hz); 7.42 (d, 1H, $J=8.4$ Hz); 8.00 (d, 1H, $J=2.4$ Hz); 8.29 (d, 1H, $J=2.4$ Hz). ^{13}C NMR (DMSO-d_6) 94.73, 98.14, 108.29 (2C), 113.93, 115.26, 119.99, 122.51, 129.06, 130.76, 133.72, 144.60, 153.29, 169.51, 170.52. IR (KBr) 3500-2976, 1701, 1636, 1585, 1483, 1193, 771 cm^{-1}. HPLC: t_R 1.15 min.

*5.1.21. Ethyl 2-(5-methyl-1H-indol-3-yl)-2-oxoacetate (**12b**)*

To a solution of 5-methylindole (200 mg, 1.52 mmol) in Et_2O (7 mL) was added dropwise at room temperature under nitrogen atmosphere, oxalyl chloride (0.17 mL, 1.97 mmol) and the mixture was stirred for 4 h. After removal of the solvent, EtOH (3.5 mL) and Et_3N (2.4 mL, 1.82 mmol) were added to the residue. The mixture was refluxed for 5 h and then stirred at room temperature for 12 h. Filtration gave the expected compound as a white solid (340 mg, 97% yield), mp 202 °C. HRMS (ESI^+) $[M+H]^+$ calcd 232.0974, found 232.0977. ^1H NMR (acetone-d_6) 1.37 (t, 3H, $J=7.1$ Hz); 2.46 (s, 1H); 4.38 (q, 2H, $J=7.2$ Hz); 7.14 (dd, 1H, $J=1.5$ Hz and 8.3 Hz); 7.45 (d, 1H, $J=8.3$ Hz); 8.12 (m, 1H); 8.38 (s, 1H); 11.24 (br. s, 1H). ^{13}C NMR (DMSO-d_6) 13.9, 21.2, 61.5, 111.9, 112.3, 120.8, 125.2, 125.7, 131.8, 134.9, 138.0, 163.6, 178.9. IR (KBr) 3236, 1723, 1626, 1610, 1503, 1429, 1266, 1135 cm^{-1}.

*5.1.22. Ethyl 2-(5-methoxy-1H-indol-3-yl)-2-oxoacetate (**12c**)*

Same procedure as for **12b** involving 5-methoxy-indole (150 mg, 1.02 mmol), Et_2O (2.5 mL), oxalyl chloride (0.12 mL, 1.32 mmol), EtOH (2.5 mL) and Et_3N (0.17 mL, 1.22 mmol) Filtration gave the expected compound as a pale pink solid (235 mg, 93% yield), mp 218 °C. HRMS (ESI^+) $[M+H]^+$ calcd 248.0923, found 248.0923. ^1H NMR (acetone-d_6) 1.38 (t, 3H, $J=7.1$ Hz); 3.86 (s, 1H); 4.38 (q, 2H, $J=7.1$ Hz); 6.93 (dd, 1H, $J=2.5$ Hz and 8.8 Hz); 7.47 (d, 1H, $J=8.9$ Hz); 7.83 (d, 1H, $J=2.5$ Hz); 8.37 (t, 1H, $J=1.6$ Hz); 11.23 (br. s, 1H). ^{13}C NMR (acetone-d_6) 13.9, 55.2, 61.5, 103.0, 112.2, 113.3, 1134, 126.3, 131.3, 138.0, 156.0, 163.3, 178.9. IR (KBr) 3159, 1721, 1621, 1475, 1208, 1129, 1025 cm^{-1}.

*5.1.23. tert-Butyl-3-(2-ethoxy-2-oxoacetyl)-1H-indole-1-carboxylate (**13a**)*

To a solution of ethyl 2-(1H-indol-3-yl)-2-oxoacetate [17] (500 mg, 2.30 mmol) anhydrous CH_2Cl_2 (2 mL) were added di-*tert*-butyl-di-carbonate (990 mg, 4.60 mmol) and DMAP (5.6 mg, 0.46 mmol). After 30 min stirring, 1 N HCl (2 mL) was added. The organic layer was separated, dried over $MgSO_4$ and concentrated to give the expected compound as a pale pink solid (670 mg, 92% yield), mp 130 °C. HRMS (ESI^+) $[M+H]^+$ calcd 317.1263, found 317.1265. ^1H NMR ($CDCl_3$) 1.45 (t, 3H, $J=7.1$ Hz); 1.71 (s, 9H); 4.44 (q, 2H, $J=7.1$ Hz); 7.40 (m, 1H); 8.17 (m, 1H); 8.39 (m, 1H); 8.79 (s, 1H). ^{13}C NMR ($CDCl_3$) 13.6, 27.4 (3C), 62.1, 86.0, 114.97, 115.00, 121.5, 124.6, 126.0, 126.7, 134.6, 137.1, 148.0, 161.5, 179.2. IR (KBr) 1747, 1723, 1659, 1543, 1481, 1365, 1258, 1141 cm^{-1}.

*5.1.24. tert-Butyl-3-(2-ethoxy-2-oxoacetyl)-5-methyl-1H-indole-1-carboxylate (**13b**)*

Same procedure as for compound **13a** involving **12c** (530 mg, 2.3 mmol), anhydrous CH_2Cl_2 (3 mL), di-*tert*-butyl-di-carbonate (1.01 g, 4.6 mmol), DMAP (5.6 mg, 0.046 mmol), 1 N HCl (3 mL). The expected compound was obtained quantitatively as a white solid (760 mg), mp 186 °C. HRMS (ESI^+) $[M+H]^+$ calcd 332.1498, found 332.1485. ^1H NMR (acetone-d_6) 1.41 (t, 1H, $J=7.7$ Hz); 1.73 (s, 9H); 2.47 (s, 3H); 4.43 (q, 2H, $J=7.1$ Hz); 7.29 (dd, 1H, $J=1.7$ Hz and 8.6 Hz); 8.07 (d, 1H, $J=8.6$ Hz); 8.12 (m, 1H); 8.69 (s, 1H). ^{13}C NMR ($CDCl_3$) 15.3, 28.4 (3C tBu), 57.0, 63.8, 87.6, 106.0, 116.6, 117.9, 130.2, 131.7, 139.0, 148.6, 150.1, 159.5, 164.1, 181.6. IR (KBr) 1745, 1725, 1657, 1479, 1457, 1365, 1264, 1150 cm^{-1}.

*5.1.25. tert-Butyl-3-(2-ethoxy-2-oxoacetyl)-5-methoxy-1H-indole-1-carboxylate (**13c**)*

Same procedure as for compound **13a** involving **12b** (190 mg, 0.84 mmol), anhydrous CH_2Cl_2 (2 mL), di-*tert*-butyl-di-carbonate (370 mg, 1.68 mmol), DMAP (2 mg, 0.017 mmol), 1 N HCl (2 mL). The expected compound was obtained as a yellow solid (270 mg, 93% yield), mp 164 °C. HRMS (ESI^+) $[M+H]^+$ calcd 347.1369, found 347.1374. ^1H NMR (acetone-d_6) 1.41 (t, 3H, $J=7.1$ Hz); 1.72 (s, 9H); 3.88 (s, 3H); 4.43 (q, 2H, $J=7.1$ Hz); 7.06 (dd, 1H, $J=2.6$ Hz and 9.1 Hz); 7.82 (d, 1H, $J=2.6$ Hz); 8.08 (d, 1H, $J=9.1$ Hz); 8.70 (s, 1H). ^{13}C NMR ($CDCl_3$) 15.3, 28.4 (3C), 57.0, 63.8, 87.6, 106.0, 116.6, 117.9, 130.2, 131.7, 139.0, 148.6, 150.1, 159.5, 164.1, 181.6. IR (KBr) 1742, 1662, 1611, 1587, 1481, 1454, 1368, 1269, 1147, 1101 cm^{-1}.

*5.1.26. tert-Butyl 3-(2-amino-2-oxoethyl)-1H-pyrazole-1-carboxylate (**14**)*

Same procedure as for compound **13a** involving 2-(1H-Pyrazol-3-yl)-acetamide [17] (750 mg, 6 mmol), anhydrous CH_2Cl_2 (20 mL), di-*tert*-butyl-di-carbonate (2.8 g, 13 mmol), DMAP (15 mg, 0.12 mmol). After 4 h stirring, the solvent was removed to give the expected compound (1.5 g), which was used in the next step without further purification. ^1H NMR (DMSO-d_6) 1.57 (s, 9H); 3.44 (s, 2H); 6.42 (d, 1H, $J=2.8$ Hz); 8.15 (d, 1H, $J=2.8$ Hz). ^{13}C NMR (DMSO-d_6) 27.4, 35.2, 84.5, 109.4, 131.6, 147.1, 152.1, 170.4.

*5.1.27. tert-Butyl-3-(4-(1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1H-pyrazole-1-carboxylate (**15a**) and tert-Butyl-4,6-dioxo-4,5,6,11-tetrahydro-2H-pyrazolo[4,3-a]pyrrolo[3,4-c] carbazole-2-carboxylate (**16a**)*

To a solution of **14** (225 mg, 1.0 mmol) and **13a** (350 mg, 1.1 mmol) in THF (5 mL) was added at 0 °C, tBuOK (336 mg, 3.0 mmol). After 12 h stirring, 37% HCl (1.2 mL) and AcOEt (10 mL) were added. The organic layer was separated, washed with water and dried over $MgSO_4$. Purification of the crude product by flash-chromatography (CH_2Cl_2/MeOH 95:5) gave the expected compound as a yellow solid (38 mg, 10% yield). MS (CI) $[M+H]^+$ 379.1 (100%) 279.1 (13.5%). ^1H NMR (acetone-d_6) 1.69 (s, 9H); 6.79 (m, 1H); 6.95 (d, 1H, $J=8.3$ Hz); 7.09 (t, 1H, $J=7.5$ Hz); 7.33 (t, 1H, $J=7.2$ Hz); 7.84 (s, 1H); 8.10 (d, 1H, $J=9.0$ Hz); 8.20 (s, 1H); 11.25 (br. s, 1H); 13.26 (br. s, 1H). IR (KBr) 3322, 1730, 1701, 1642, 1542, 1454, 1350, 1150, 1090 cm^{-1}. A solution of compound **15a** (35 mg, 0.092 mmol) in CH_3CN (20 mL) was irradiated at 350 nm for 8 h. After removal of the solvent, the residue was used without further

purification in the next step. HRMS (ESI^+) $[M+H]^+$ calcd 377.1250, found 377.1238.

*5.1.28. tert-Butyl-3-(4-(5-methyl-1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1H-pyrazole-1-carboxylate (**15b**) and tert-Butyl-8-methyl-4,6-dioxo-4,5,6,11-tetrahydro-2H-pyrazolo[4,3-a] pyrrolo[3,4-c]carbazole-2-carboxylate (**16b**)*

Same procedure as for compound **15a** involving **14** (171 mg, 0.76 mmol), **13b** (171 mg, 0.84 mmol), tBuOK (255 mg, 2.28 mmol), THF (5 mL), MS (DCI-NH_3) of **15b** 392.0 (45%), 359.0 (100%). A solution of the crude compound in CH_3CN (15 mL) was irradiated at 350 nm for 8 h. After removal of the solvent, the crude product was purified by flash-chromatography (CH_2Cl_2/MeOH 98:2) to give the expected compound as a yellow solid (8.0 mg, 3% yield from **15b**), mp >260 °C. MS (CI) $[M+H]^+$ 408.1 (22%), 391.1 (70%), 308.0 (100%), 291.1 (70%). ^1H NMR (DMSO-d_6) 1.79 (s, 9H); 7.40 (d, 1H, $J=9.0$ Hz); 8.03 (d, 1H, $J=8.1$ Hz); 8.79 (s, 1H); 8.91 (s, 1H); 11.37 (br. s, 1H); 14.20 (br. s, 1H). IR (KBr) 3348, 1757, 1740, 1708, 1636, 1599, 1492, 1309, 1223, 1079, 800, 760 cm^{-1}.

*5.1.29. tert-Butyl-3-(4-(5-methoxy-1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1H-pyrazole-1-carboxylate (**15c**) and tert-Butyl-8-methoxy-4,6-dioxo-4,5,6,11-tetrahydro-2H-pyrazolo[4,3-a] pyrrolo[3,4-c]carbazole-2-carboxylate (**16c**)*

Same procedure as for compound **15a** involving **14** (131 mg, 0.58 mmol), ^{13}C (222 mg, 0.64 mmol), tBuOK (195 mg, 1.74 mmol) and THF (2 mL). MS (CI) $[M+H]^+$ of **15c** 409.0 (100%). A solution of the crude product in CH_3CN (12 mL) was irradiated at 350 nm for 8 h. After removal of the solvent, this new crude product was used in the next step.MS (DCI-NH_3) of **16c** 407.1 (100%); 124.1 (23%).

*5.1.30. 3H-Pyrazolo[4,3-a]pyrrolo[3,4-c]carbazole-4,6(5H,11H)-dione (**5a**)*

To a solution of crude **16a** (0.092 mmol) in CH_2Cl_2 (4 mL) was added TFA (4 mL, 51.6 mmol). After 4 h stirring, the solvent was removed and a saturated solution of $NaHCO_3$ (8 mL) was added to the residue. Filtration gave quantitatively the expected compound as a yellow solid (25.4 mg), mp >260 °C. HRMS ((ESI^+) $[M+H]^+$) calcd 276.2496, found 276.2492. ^1H NMR (acetone-d_6) 7.34 (t, 1H, $J=7.8$ Hz); 7.51 (d, 1H, $J=7.8$ Hz); 7.69 (d, 1H, 7.8 Hz); 8.55 (s, 1H); 8.90 (d, 1H, 7.8 Hz). ^{13}C NMR (DMSO-d_6) 108.20, 110.67, 112.12 (2C), 113.41, 120.85, 122.05, 124.05, 126.07, 132.32, 133.50, 137.81, 140.42, 167.12, 171.20. IR (KBr) 3437, 1687, 1642, 1438, 1208, 1139, 844, 725 cm^{-1}. HPLC: t_R 1.49 min.

*5.1.31. 8-Methyl-3H-pyrazolo[4,3-a]pyrrolo[3,4-c]carbazole-4,6(5H,11H)-dione (**5b**)*

Same procedure as for compound **5a** involving **16b** (5 mg, 0.013 mmol), CH_2Cl_2 (1 mL), TFA (1 mL, 12.9 mmol), $NaHCO_3$ (2 mL). Filtration gave quantitatively the expected compound as a yellow solid (3.8 mg), mp >260 °C. HRMS ((ESI^+) $[M+H]^+$) calcd 291.0882, found 291.0888. ^1H NMR (DMSO-d_6) 2.50 (s, 3H); 7.31 (d, 1H, $J=8.7$ Hz); 7.56 (d, 1H, 8.7 Hz); 8.53 (s, 1H); 8.67 (s, 1H); 11.06 (br. s, 1H); 12.76 (br. s, 1H); 14.07 (br. s, 1H). ^{13}C NMR (DMSO-d_6) 21.85, 107.46, 110.61, 111.79 (2C), 122.03 (2C), 123.80, 127.61, 129.79 (2C), 132.99, 137.91, 138.49, 169.96, 171.53. IR (KBr) 3429, 1739, 1699, 1638, 1478, 1370, 1207, 1141, 802 cm^{-1}. HPLC: t_R 1.69 min.

*5.1.32. 8-Methoxy-3H-pyrazolo[4,3-a]pyrrolo[3,4-c]carbazole-4,6(5H,11H)-dione (**5c**)*

Same procedure as for compound **5a** involving crude **16c** (0.58 mmol), CH_3CN (15 mL). Purification by flash-chromatography (CH_2Cl_2/MeOH 95:5) gave the expected product as a yellow solid (5.0 mg, 3% yield from ^{13}C), mp >260 °C. HRMS (ESI^+) $[M+H]^+$ calcd 307.0831, found 307.0846. ^1H NMR (DMSO-d_6) 3.82 (s, 3H); 6.25 (d, 1H, $J=2.4$ Hz); 6.91 (dd, 1H, $J=9$ Hz and 2.4 Hz); 7.96 (d, 1H, $J=9$ Hz); 8.13 (s, 1H); 11.24 (br. s, 1H); 13.27 (br. s, 2H). ^{13}C NMR (DMSO-d_6) 56.00, 106.94, 107.44, 110.87, 112.74 (2C), 113.34, 115.13, 122.47, 128.14, 133.11, 134.92, 138.16, 154.67, 169.96, 171.61. IR (KBr) 3436, 3307, 1742, 1694, 1646, 1600, 1483, 1340, 1209 cm^{-1}. HPLC: t_R 1.72 min.

6. Pharmacology

6.1. Determination of in vitro growth inhibition

The influence of each compound under study on the *in vitro* growth rates of various cancer cell lines was determined using the colorimetric MTT ((3-[4,5-dimethylthiazol-2yl])-diphenyl tetrazolium bromide, Sigma, Belgium) assay as detailed previously [19,21,22,24]. The use of the MTT colorimetric assay is based on the capability of living cells to reduce the yellow product MTT (3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to a blue product, formazan, by a reduction reaction occurring in the mitochondria. The number of living cells after 72 h of culture in the presence (or absence: control) of the various compounds is directly proportional to the intensity of the blue, which is quantitatively measured by spectrophotometry (Biorad Model 680XR; Biorad, Nazareth, Belgium) at a 570 nm wavelength (with a reference of 630 nm). Each experiment was carried out in six replicates.

The cell lines were incubated for 24 h in 96-microwell plates (at a concentration of 10,000 to 40,000 cells/mL culture medium depending on the cell type) to ensure adequate plating prior to cell growth determination. The cells were cultured in RPMI (Invitrogen, Merelbeke, Belgium) media supplemented with 10% heat inactivated fetal calf serum (Invitrogen). All culture media were supplemented with 4 mM glutamine, 100 μg/mL gentamicin, and penicillin–streptomycin (200 U/mL and 200 μg/mL; Invitrogen).

We have made use of seven human and one mouse cancer cell lines. The seven human cancer cell lines include the OE21 esophageal cancer (obtained from the European Collection of Cell Culture (ECACC); code 96062201), the A549 non-small-cell lung cancer (obtained from the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSMZ); code ACC107), the HS683 oligodendroglioma (obtained from the American Type Culture Collection (ATCC); code HTB-138), the U373 glioblastoma (ECACC; code 89081403), the PC-3 prostate cancer (DSMZ; code ACC465), the MCF-7 breast cancer (DSMZ; code ACC115) and the LoVo colon cancer (DSMZ; code ACC350) cell lines, while the mouse cancer cell line relates to the B16F10 melanoma (ATCC; code CRL-6475).

6.2. Computer-assisted phase-contrast microscopy (quantitative videomicroscopy)

Quantitative videomicroscopy was employed to determine as whether **4a**, **9a** and **9e** displayed cytotoxic or rather cytostatic effects in the human A549 NSCLC and the U373 GBM cell lines, as detailed elsewhere [30,31]. Cancer cells were monitored for 72 h in the absence (control) or the presence of each compound analyzed at its MTT test-related IC_{50} growth inhibitory concentrations. Movies were built on the obtained time-lapse image sequences and enabled a rapid screening for cell viability [30,31]. Experiments were conducted in triplicate.

6.3. Cell culture and checkpoint abrogation evaluation

HCT116 $p53^{-/-}$ cells (a generous gift of B. Vogelstein) were grown in DMEM (Invitrogen) supplemented with 10% FCS. Etoposide (Sigma) was applied to the cells for 1 h at a concentration of 40 μM, before being washed extensively. Checkpoint recovery

experiment was performed essentially as described previously [6]. The AZD7762 Chk1 inhibitor was generously provided by Dr Sonya Zabludoff (AstraZeneca). Cells were harvested and fixed with cold 70% ethanol. They were subsequently stained with either anti-phospho H3 Ser-10 antibodies or the monoclonal 3.12.I.22 [28] that were revealed using Alexa Fluor 488 conjugated antibodies. DNA was stained with propidium iodide (Sigma). DNA content and mitotic index were determined using an Accuri C6 flow cytometer.

6.4. Inhibition of kinases

Kinase activities were assayed in triplicate using Buffer D (10 mM $MgCl_2$, 1 mM EGTA (ethyleneglycoltetraacetic acid), 1 mM DTT (dithiothreitol), 25 mM Tris/HCl, 50 mg/ml Heparin) at 30 °C, at a final ATP concentration of 15 mM. Blank values were subtracted and activities expressed as % of the maximal activity in the absence of inhibitors. Controls were performed with appropriate dilutions of DMSO. IC_{50} values were calculated from dose–response curves. The kinase peptide substrates were obtained from Proteogenix (Oberhausbergen, France).

DYRK1A (rat, recombinant, expressed in *E. coli* as a GST fusion protein) was purified by affinity chromatography on glutathione-agarose and assayed using Woodtide (KKISGRLSPIMTEQ) (1.5 μg/assay) as a substrate, in the presence of 15 μM [γ-^{33}P] ATP (3000 Ci/mmol; 10 mCi/ml) in a final volume of 30 μl. After 30 min incubation at 30 °C, the reaction was stopped by harvesting onto P81 phosphocellulose papers (Whatman) using a FilterMate harvester (Packard) and were washed in 1% phosphoric acid. Scintillation fluid was added and the radioactivity measured in a Packard counter.

CLK1 (mouse, recombinant, expressed in *E. coli* as a GST fusion protein) was assayed with RS peptide (GRSRSRSRSRSR) (1 μg/assay).

CDK5/p25 (human, recombinant) was prepared as previously described [32]. Its kinase activity was assayed in buffer C, with 1 mg histone H1/ml.

GSK-3α/β (porcine brain, native) was assayed in Buffer A and using a GSK-3 specific substrate (GS-1: YRRAAVPPSPSLSRHSSPHQSpEDEEE) (pS stands for phosphorylated serine) [33].

CK1δ/ε (porcine brain, native) was assayed in three-fold diluted buffer C, using 25 μM CKS peptide (RRKHAAIGpSAYSITA), a CK1-specific substrate [34]

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