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Synthesis and anticancer activity of novel bisindolylhydroxymaleimide derivatives with potent GSK-3 kinase inhibition

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ABSTRACT

Synthesis and biological evaluation of a series of novel indole derivatives as anticancer agents is described. A bisindolylmaleimide template has been derived as a versatile pharmacophore with which to pursue chemical diversification. Starting from maleimide, the introduction of an oxygen to the headgroup (hydroxymaleimide) was initially investigated and the bioactivity assessed by screening of kinase inhibitory activity, identifying substituent derived selectivity. Extension of the hydroxymaleimide template to incorporate substitution of the indole nitrogens was next completed and assessed again by kinase inhibition identifying unique selectivity patterns with respect to GSK-3 and CDK kinases. Subsequently, the anticancer activity of bisindolylmaleimides were assessed using the NCI-60 cell screen, disclosing the discovery of growth inhibitory profiles towards a number of cell lines, such as SNB-75 CNS cancer, A498 and UO-31 renal, MDA MB435 melanoma and a panel of leukemia cell lines. The potential for selective kinase inhibition by modulation of this template is evident and will inform future selective clinical candidates.

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Maleimide substitution
Drug discovery
NCI anticancer screen

1. Introduction

Cancer is one of the leading causes of morbidity and mortality worldwide, causing about 13% of all human deaths and at least one fifth of all deaths in Europe and North America.[1,2] Classical cancer chemotherapy is associated with significant adverse effects due to the nature of the treatment: all rapidly dividing cells are treated the same leading to the well-known effects of myelosuppression, alopecia and many others.

More recently, targeted therapies have emerged using drugs that interfere with specific molecular targets involved in cancer progression. One such target is a series of enzymes responsible for signal transduction called protein kinases which modify other proteins by transferring phosphate groups from a high-energy donor molecule, such as adenosine triphosphate (ATP), to a specific substrate amino acid on the target protein. This phosphorylation results in a conformational change in the protein, affecting its function in areas such as enzyme activity, association with other proteins or cellular location.[3] In this way kinases regulate other proteins and the activities of cells, playing an important role in many intracellular signalling pathways including those that control cell growth and cell division. This role in cell signalling, makes them an object of study for drug design.[4,5]

Deregulated kinase activity has been implicated in a variety of human health conditions including disorders of the immune system, neurodegenerative disorders and diabetes, as well as cancer. Since the discovery that staurosporine **1** (Fig. 1) was a nanomolar inhibitor of PKC in 1986,[6] great interest has been generated into obtaining highly active and selective protein kinase inhibitors either through chemical synthesis or screening of new natural products.

The first kinase inhibitor to be approved by the FDA was imatinib **2** in 2001 (Fig. 1), as a treatment for chronic myeloid leukemia. By April 2015, a total of 28 small-molecule kinase inhibitors have been approved for clinical use by the FDA, half of which were approved in the past 3 years.[7] These include erlotinib **3**, which is marketed as a treatment for non-small cell lung cancer, and sunitinib **4**, which is marketed as a treatment for renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumor.[8,9] A large number are also currently undergoing human clinical trials for cancer and other conditions.

Many biologically relevant kinase inhibitors, including staurosporine **1**, have been shown to work by competing with ATP.[10–12] The interaction of staurosporine with the ATP-binding site has been proven through the resolution of crystal structures of staurosporine bound to CDK2 and PKA. These crystal structures show the indolocarbazole derivative being located in the ATP binding site, in the cleft between the two lobes of the protein kinase. The lactam/maleimide group mimics the hydrogen bonding pattern of the adenine base of ATP by forming two hydrogen bonds to the backbone of the hinge between the *N*-terminal and *C*-terminal domains of the kinase. The sugar moiety then forms hydrogen bonds within the ribose binding site. Due to the similarity between ATP binding sites in different kinases, staurosporine is a highly potent but unspecific kinase inhibitor. However, by structural modification it has been possible to obtain some selectivity by exploiting differences in the mode of ligand interaction in the ATP-binding pocket.[13]

Removal of the central ring leads to a group of compounds called the bisindolylmaleimides. Due to their increased conformational flexibility, the binding mode of bisindolylmaleimides is more complex than that of staurosporine.[14] Initially, GF 109203 X **5** and Ro 318220 **6** (Fig. 2) were reported to be potent and selective inhibitors of protein kinase C (IC_{50} = 10 nM and 20 nM, respectively).[15,16] However, they were later found to also potently inhibit several other kinases, such as MAPKAP kinase-1β (IC_{50} = 50 nM and 3 nM, respectively) and GSK-3β (IC_{50} = 360 nM and 6.8 nM, respectively).[17,18]

Another analogue, ruboxistaurin **7** (Fig. 2), has been reported to be a specific inhibitor of the PKC isoforms, PKCβ1 (IC_{50} = 4.7 nM) and PKCβ2 (IC_{50} = 5.9 nM).[19] It displays selectivity over other protein kinases and PKC isoforms such as PKCα (IC_{50} = 360 nM), PKCγ (IC_{50} = 300 nM), PKCε (IC_{50} = 600 nM) and PDK1 (IC_{50} = 750 nM) but has since been identified with PIM kinase inhibition (IC_{50} = 200 nM).[20] Ruboxistaurin entered clinical trials for the treatment of diabetic peripheral retinopathy. Enzastaurin **8** (Fig. 2) is a highly potent inhibitor of PKCβ (IC_{50} = 6 nM) and the PI3K/Akt pathway.[21] It shows moderate selectivity over other PKC isoforms including PKCα (IC_{50} = 39 nM), PKCγ (IC_{50} = 83 nM) and PKCε (IC_{50} = 110 nM). Enzastaurin also underwent clinical trials for cancers such as glioblastoma, non-small cell

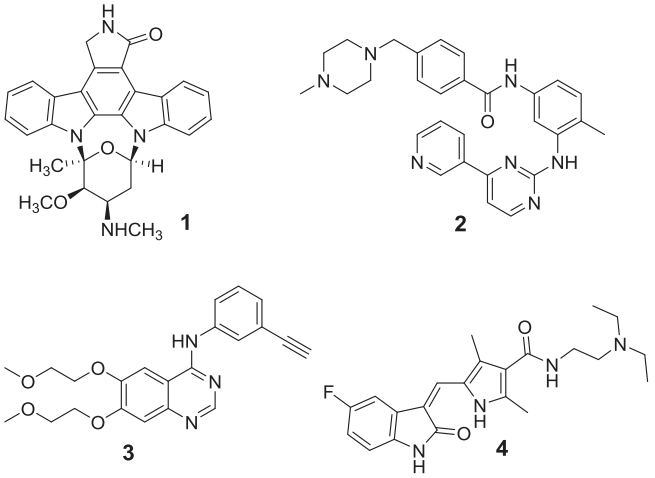


Fig. 1. Structures of common kinase inhibitors **1–4**.

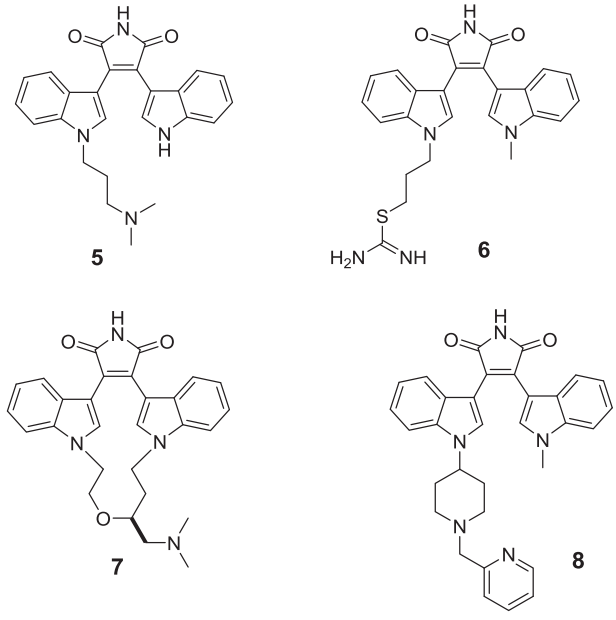


Fig. 2. The bisindolylmaleimide class of kinase inhibitors **5–8**.

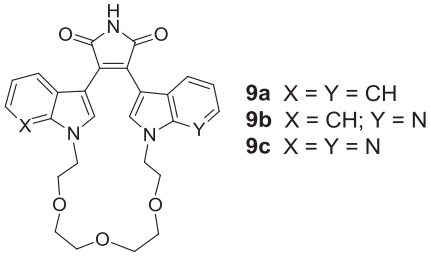


Fig. 3. Azaindole/indole bisindolylmaleimides with selectivity of kinase inhibition.

lung cancer and colorectal cancer.

Kuo et al. also investigated the synthesis of a panel of novel indole substituted bisindolylmaleimide derivatives which were found to be potent inhibitors of GSK-3β (Fig. 3).[22]

Although the bisindolyl analogue **9a** was highly active against GSK-3β (IC_{50} = 22 nM), it was not specific and was also active against several other kinases such as CDK1 and CDK2, VEGF-R and several PKC isoforms. Replacing one of the indole rings with 7-azaindole (compound **9b**) had little effect on the potency or selectivity at GSK-3β (IC_{50} = 17 nM) but the replacement of both indoles with 7-azaindole (compound **9c**) significantly increased the selectivity. The bis-7-azaindolylmaleimide **9c** showed limited activity against a panel of 50 other kinases indicating a specific inhibitor of GSK-3β (IC_{50} = 34 nM). Subsequent extension of this replacing one of the azaindoles with other aryl substituents yielded good potency but were compromised by CDK2/5 activity in a full kinase screen.[23,24]

It is therefore evident that indole substitution and azaindole incorporation can affect potent kinase inhibition of this pharmacophore. However, one key unexplored area in the SAR is the headgroup maleimide. We hereby describe the discreet exploration of this space via simple conversion to hydroxymaleimide coupled with novel indole substituents to probe the relationship of steric bulk on kinase binding and selectivity using a scope of kinase inhibition and cellular growth. A panel of 10 serine/threonine kinases was selected for initial assay to complement screening via the NCI 60 cell line screen for cancer cell growth.

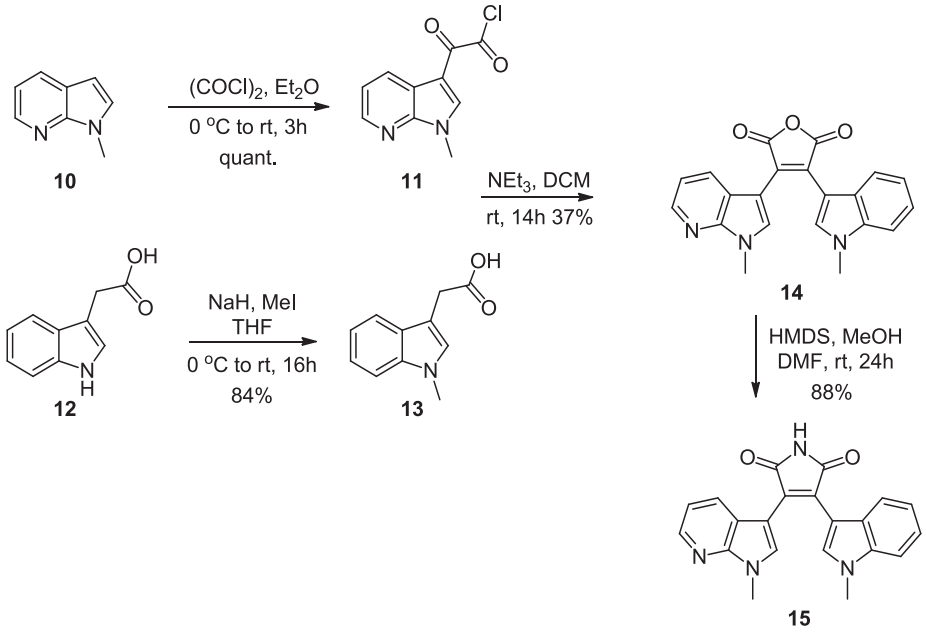
2. Results and discussion

2.1. Synthesis of azaindole indole maleimide and hydroxymaleimide

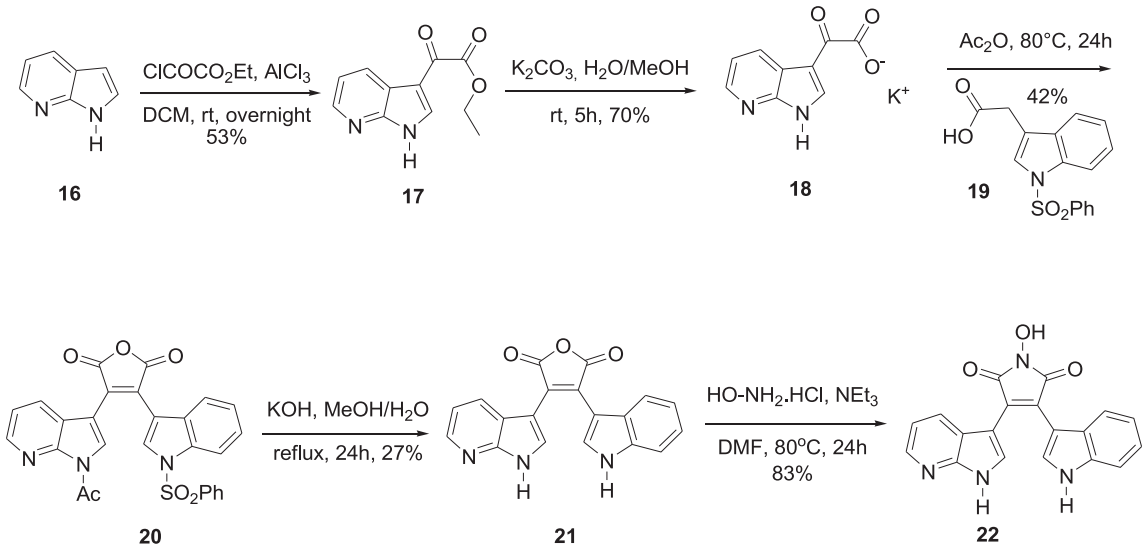
Our initial approach envisaged a bisindolylmaleimide and corresponding bisindolylhydroxymaleimide in order to probe whether atomic incorporation would differentiate activity and our starting point incorporated an indole, 7-azaindole and maleimide components. On initial scope of the literature of bisindolylmaleimides, it is surprising that the hydroxymaleimide model has very little precedent. To date there exist only three reported compounds with this substructure and none when a 7-azaindole is incorporated (although it is more common for indolocarbazoles).[25] Hence this is a field ripe for discovery especially since the reported compounds possess bioactivity (micromolar PKC and PKA inhibition).

The application of Perkin-type condensation methodology towards the synthesis of novel maleic anhydride **14** proved to be relatively straightforward (Scheme 1). Initially, *N*-methylindole-3-acetic acid **13** was formed by alkylation of indole-3-acetic acid **12**, with acid **13** formed in 84% yield.[26] Separately, a stirred solution of *N*-methyl-7-azaindole **10** was treated with oxalyl chloride and corresponding glyoxyl chloride **11** was isolated as an off-white solid in quantitative yield. Glyoxyl chloride **11** was then added to a stirred solution containing *N*-methylindole-3-acetic acid **13** and triethylamine in DCM, with isolation of maleic anhydride **14** as a bright red solid in 37% yield achieved by flash column chromatography (Scheme 1). Conversion of maleic anhydride **14** to maleimide **15** occurred in excellent yield of 88% via treatment with hexamethyldisilazane. Hence, compound **15** was formulated as the reference compound with some known bioactivity.[27]

Synthesis of a related hydroxymaleimide was undertaken in order to probe the effect of oxygen insertion into the headgroup and the effect of indole substitution (Scheme 2). 7-Azaindole **16** was converted to the ethyl glyoxylate **17** followed by hydrolysis to the glyoxylate salt **18**. Perkin condensation of this with benzenesulfonyl protected indole-3-acetic acid **19** yielded the protected maleic anhydride **20**.[26] This was subsequently deprotected and converted to the hydroxymaleimide **22** via treatment with hydroxylamine.



Scheme 1. Synthesis of maleimide **15** via Perkin-type condensation.



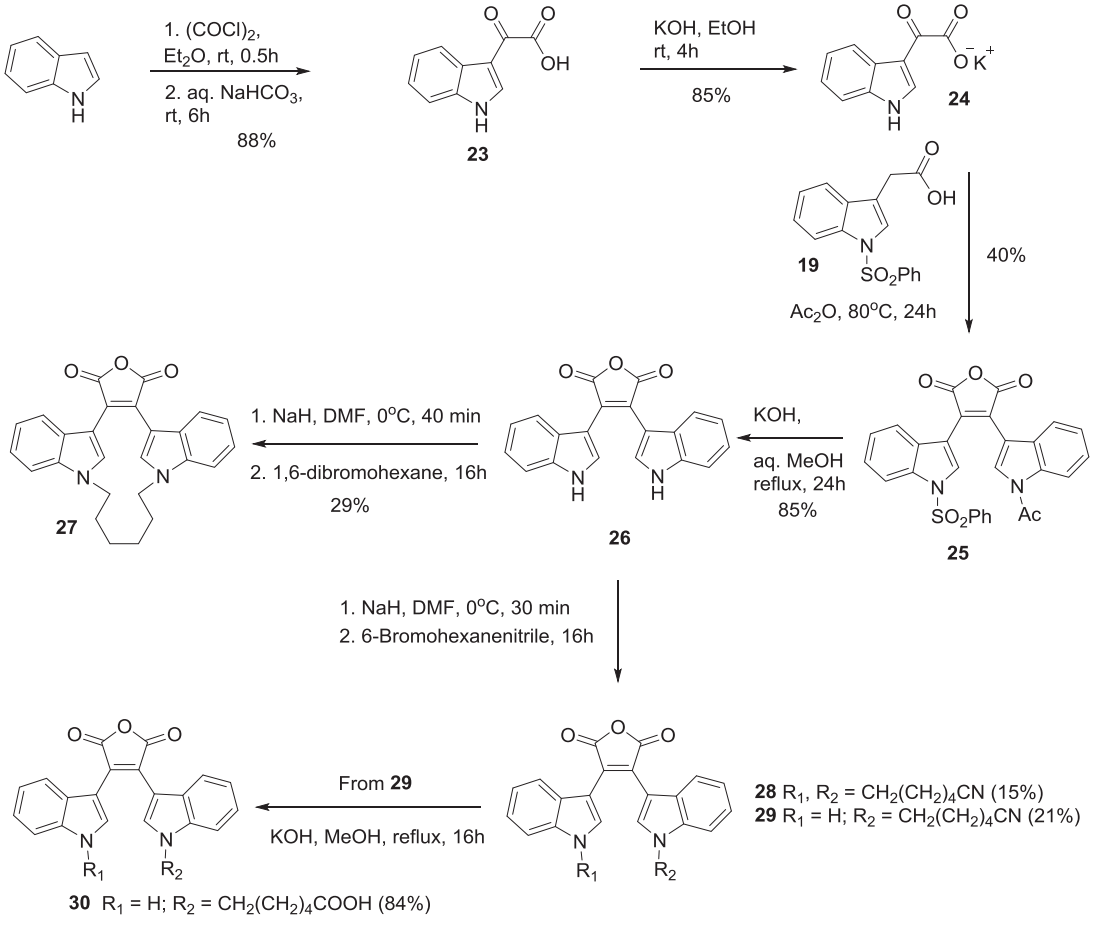
Scheme 2. Synthesis of maleimide **22** via Perkin-type condensation.

2.2. Evaluation of the kinase inhibitory activity of lead compounds 15 and 22

Due to the common reports of bisindolylmaleimides as kinase inhibitors and the reported polypharmacology of the class, as discussed in the introduction, the standard maleimide **15** and novel hydroxymaleimide **22** were evaluated by a preliminary kinase screen.[28]

GSK-3β has been shown to reduce apoptosis signals it may be useful for the treatment of Alzheimer's disease and protection against cell death.[29,30] In addition to the data above, there are reports of bisindolylmaleimide involvement with GSK-3β in the regulation of murine embryonic stem cell self-renewal so it is an obvious starting point.[27] Interestingly, inhibitors of GSK-3 have also been shown to enhance apoptotic signal transduction induced by TRAIL (TNF-related apoptosis inducing ligand TRAIL).[31]

The evaluation of derivatives for their inhibition of cancer-related protein kinases is also of relevance: *Hs*Haspin, *Hs*Aurora kinase B,



Scheme 3. Synthesis of key maleic anhydrides **27**–**30**.

*Hs*RIPK3, *Hs*CDK2, *Hs*CDK5, *Hs*CDK9, *Rn*DYRK1A, *Hs*PIM1 and *Ssc*CK1δ/ε. Haspin is expressed in a variety of tissues (e.g., testis, bone narrow, thymus, and spleen) and in proliferating cells, including in a number of neoplasms.[32–34] Aurora-B is a key component of the spindle assembly checkpoint and also functions in cytokinesis.[35] The receptor-interacting protein kinase-3 (RIPK3 or RIP3) is a critical regulator of necroptosis.[36–38] A recent study showed the important role of RIPK3 in maintaining *in vivo* tumor growth with RIPK3 knockout breast tumor cell growing at a significantly slower rate than vector control cells.[39] The Cyclin-Dependant Kinases (CDKs) play a direct role in the regulation of the cell cycle controlling cell proliferation. CDK2 has been shown to have a direct role in the cell cycle progression, while CDK subtypes 7–9 have been described to control RNA polymerase II mediated transcription and CDK5 is involved in neuronal function.[40] Dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A)[41] is involved in many major diseases, including cancer and neurodegenerative disorders.[41–43] PIM1 is a constitutively active enzyme and has been shown to have diverse biological roles in cell survival, proliferation, differentiation and apoptosis.[44] Overexpression of PIM-1 has been found in various hematopoietic malignancies as well as in solid tumors including colon, prostate and pancreas.[45] CK1 (casein kinase 1), when deregulated, is responsible for non-regulation of growth, proliferation, and apoptosis which may result in pathological conditions, such as tumorigenesis or neurological diseases.[46]

Results from one dose assay at 10 μM concentration confirmed that the maleimide was significantly more active than the hydroxymaleimide (see Table 1, Supporting Information). Significant kinase inhibition was seen for the maleimide **15** in all the CDKs (CDK2, CDK5 and CDK9) in addition to GSK-3 and PIM1 kinases. By contrast, the introduction of an oxygen into the headgroup and removal of the indole methyl substituents reduced the CDK activity completely with significant kinase inhibition only evident in PIM1 kinase and to a lesser extent in CK1 and GSK-3. This intriguing disparity and potential selectivity led to an exploration of the molecular space occupied by the headgroup and the indole substituents.

2.3. Synthesis of novel bisindolylmaleic anhydrides with symmetrical N-substituents

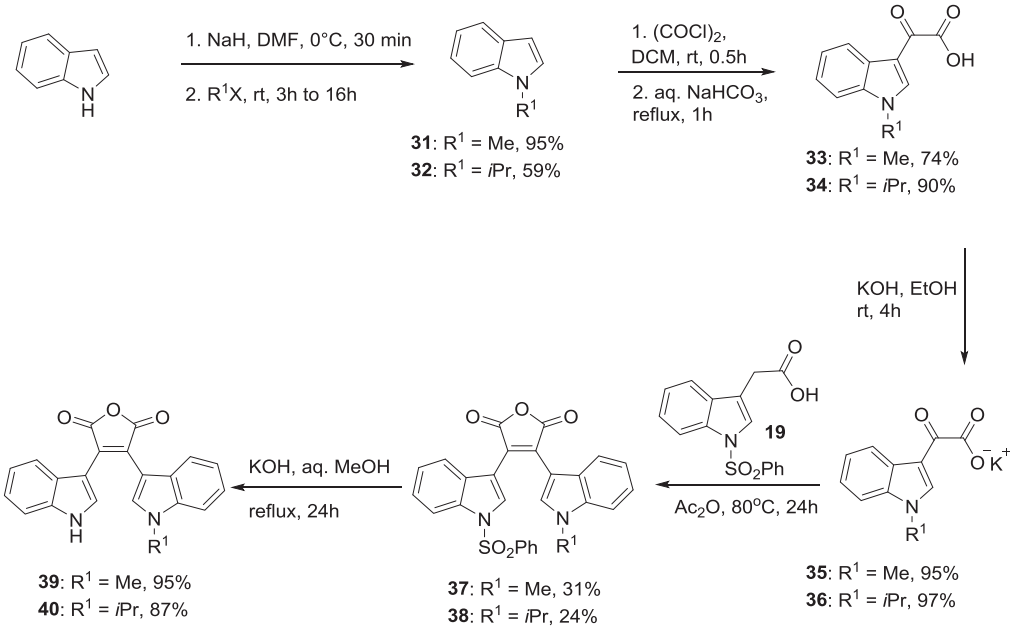
At the outset we decided to base our study on the novel bisindolylhydroxymaleimide series as this would probe the indole substituent effects without any competing/conflicting effects from the nitrogen of 7-azaindole. Initial synthesis begins from indole which is converted to the potassium salt of indole-3-glyoxylic acid **23** in excellent yield (Scheme 3).[47] After a deprotonation step, subsequent Perkin condensation in the presence of acetic anhydride with benzenesulfonyl protected indole-3-acetic acid **19** renders the fully protected maleic anhydride **25** in moderate yield. Complete hydrolysis can be achieved with aqueous base allowing for derivatisation of both indole nitrogens simultaneously.[48] To this end, the novel hexane bisindolyl maleic anhydride **27** was generated as a test macrocycle. Reaction of the same unsubstituted **26** with 6-bromohexanenitrile yielded both the mono- and bis-substituted hexanenitrile anhydrides **28** and **29**. Hydrolysis of **29** yields the monohexanoic acid derivative.

2.4. Synthesis of novel bisindolyl maleic anhydrides with unsymmetrical N-substituents

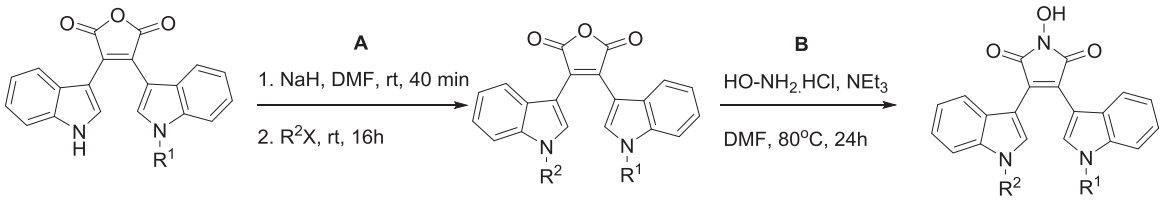
In order to probe the influence of indole nitrogen substitution on the anticancer and kinase activity of these compounds a synthetic route was devised to incorporate individual functionality on the indoles (Scheme 4). To achieve selective substitution of either nitrogen the strategy was adapted to incorporate an alkylation of the indole at an earlier stage and this was brought through a similar sequence. 1-Methyl (in order to correspond to **15**) and 1-isopropyl (to probe steric bulk) indole **31**–**32**, were generated from the starting indole and the corresponding potassium glyoxylate salts **35**–**36** were generated as above. These undergo Perkin condensation in the presence of acetic anhydride and indole acetic acid **19**, and subsequent base mediated deprotection yielding the mono-alkylated bisindolyl maleic anhydrides **39**–**40**.[49] These are then available for further alkylation to form fully functionalised maleic anhydrides (Scheme 5 or in the case of **44** base hydrolysis) to form anhydrides **41**–**46**. From observation of the ^{1}H NMR data, there is an interesting correlation between the substitution on the indole nitrogen and the chemical shift values of the representative maleic anhydrides (see Supplementary Information).

2.5. Synthesis of novel bisindolylhydroxymaleimides

Formation of the ultimate hydroxymaleimides **47**–**58** was achieved



Scheme 4. Synthesis of maleic anhydrides **39**–**40**.



Scheme 5. Synthesis of hydroxymaleimides **47–58** via novel maleic anhydrides **27**, **28**, **30**, **39–46**.

by condensation with hydroxylamine hydrochloride salt in the presence of trimethylamine and yields were generally excellent (Scheme 5 and Table 1).

2.6. Kinase screen

The full library of novel substituted bisindolylmaleimides was initially probed for kinase inhibitory activity against 10 kinase enzymes at a concentration of 10 μM (see Table 1, Supporting Information). It is very evident that small changes in the substitution pattern affect the ability to modulate phosphorylation in a discreet fashion. As mentioned above, there is a striking difference in the activity of related bisindolylmaleimides **15** and **22** and again it is evident that extension of the pharmacophore at the indole nitrogens influences inhibition. From the heat map it can be seen that CDK9, GSK-3 and, to a lesser extent, PIM1 kinase are all substantially affected by the panel, aside from **55** which is interesting given that it is the macrocyclic bisindolylmaleimide. This suggests that restricting the conformation in this way reduces the kinase activity (in contrast to the known kinase inhibitor ruboxistaurin **7**, see Fig. 2). It is also seen that **51** and **53** have a similar profile to **15**.

2.7. Kinase assay

Given the excellent selectivity and potency evident in the kinase screen, the full library of novel substituted bisindolylmaleimides progressed to kinase inhibitory assay (Table 2).[50] The most potent compound of the panel was the lead maleimide **15** with activity against GSK-3 30 nM, CDK2/5/9 80–800 nM and PIM1 2 μM.

On evaluating GSK-3 inhibition, the hydroxymaleimides are active irrespective of indole substituent (save for the inactive macrocyclic **55**) which suggests a mode of binding for this template whereby incorporation of anchoring subunits on the indole nitrogens (as in **5**, **6** and **8**, Fig. 2) may be fruitful.

The removal of indole substitution and incorporation of the hydroxymaleimide headgroup rendered GSK-3 and PIM1 selective inhibition albeit at 4–4.5 μM (**15** *vs* **22**). Comparing **47** and **48** which differ in only by the presence of a methyl substituent, the influence of the indole N—H gives rise to more potency against GSK-3 (200 nM). However this effect is not a constant as on extension of the alkyl substituent further potency is seen for **49**, **50**, **53** and **54** showing the positive influence of at least one bulky substituent. Interestingly **51** and **54** have submicromolar inhibition of CDK-9 kinase which could be exploited as a cancer target.[51]

In comparing the methyl against the propyl subsets (**47–49** vs **52–54**) it is evident that the increased steric bulk of the propyl substituent is tolerated by GSK-3. Extending the alkyl chain further to hexanenitrile **50**, maintains potency against GSK-3 and kinase selectivity over CDKs. Conversion of **50** to carboxylic acid **51** improves the potency to CDK-9 rather than GSK-3 with the introduction of DYRK inhibition while removal of a methyl from **51** to yield **57** reverses this effect. Interestingly, the bishexanenitrile substitution pattern of **56** imbues selectivity of action over CDKs and with inhibition of GSK kinase at 750 nM and is a lead for future studies.

Finally, in order to confirm the selectivity imposed by the N-OH group extension of the maleimide, a direct comparison with the corresponding maleimide **58** returns the potency with consequent polykinase inhibition (GSK, DYRK, CDK-2/9). It is clearly evident that the incorporation of the oxygen limits the CDK and DYRK inhibition seen with maleimides.

2.8. Full anticancer evaluation

Selected compounds were identified for further studies into their effect on cancer cells through the NCI anticancer screening programme. Compound **15**, **47–48**, **50–53**, and **55–58** were chosen in order to scope the breadth of structural diversity and kinase inhibition.[52] The compounds were initially screened (again at 10 μM) in a cellular based assay against the NCI 60-cell line panel with some interesting results compared with other headgroups (Table 3).[53,54] The majority gave high mean growth across the 60 cell lines at 10 μM (70–100%) but intriguingly the restriction of cellular growth appears related to kinase inhibition given the potencies seen in Table 3. Specific growth percentages for two renal, three melanoma and one leukaemia cell lines are given and it is evident that the indole substitution affects activity.Table 4.

Evaluation of compound **47** with one methyl *N*-substituent and one unsubstituted indole identified an average mean growth of 78.0% across all 60 cell lines. Inhibition was observed for leukaemia cell lines (growth reduced to 31% in SR) and also for melanoma cell line MDA-MB-435 (17%), renal cancer cell line UO-31 (41%) and breast cancer cell line MCF7 (31%). Addition of a second methyl substituent significantly reduced activity with compound **48** being almost completely inactive; although some growth inhibition was observed for leukaemia cell line SR (75%), melanoma cell line LOX IMVI (73%) and renal cancer cell line UO-31 (74%). Compounds with an isopropyl substituent (**52** and **53**) were mostly inactive apart from a similar selectivity towards LOX IMVI (59% and 62%, respectively) and UO-31 (68% and 56%, respectively) and for most leukaemia cell lines. Compounds with one extended alkyl chain with a nitrile or carboxylic acid functional group displayed little activity with nitrile **50** being slightly more active than acids **51** and **57**. These compounds again displayed moderate

Table 1
Structure and synthetic yields of **41–58** via alkylation (**A**) and hydroxymaleimide formation (**B**).

Compound	Precursor maleic anhydride	R^1	R^2	Yield (A)	Yield (B)
47	**39**	Me	H	–	83%
48	**39**	Me	Me	80% (**41**)	84%
49	**39**	Me	Et	72% (**42**)	69%
50	**39**	Me	$(CH_2)_5CN$	40% (**43**)	82%
51	**43**	Me	$(CH_2)_5COOH$	76% (**44**)*	79%
52	**40**	*i*Pr	H	–	77%
53	**40**	*i*Pr	Me	46% (**45**)	93%
54	**40**	*i*Pr	Et	51% (**46**)	80%
55	**27**	$–CH_2(CH_2)_4CH_2–$		–	82%
56	**28**	$(CH_2)_5CN$	$(CH_2)_5CN$	–	87%
57	**30**	$(CH_2)_5COOH$	H	–	86%
58	**28**	$(CH_2)_5CN$	$(CH_2)_5CN$	–	90%**

* Using the method as per the formation of **30** in Scheme 3.

** Using HMDS rather than hydroxylamine form the corresponding maleimide for reference purposes.

Table 2
IC_{50} values from the kinase inhibition assay (values quoted in μM).

Compound	*Hs* HASPIN	*Hs* AURKB	*Hs* RIPK3	*Hs* CDK2/CyclinA	*Hs* CDK5/p25	*Hs* CDK9/CyclinT	*Hs* DYRK1A	*Ssc* GSK-3α/β	*Hs* PIM1	*Ssc* CK1δ/ε
15	>10	>10	>10	**0.10**	**0.80**	**0.08**	>10	**0.03**	**2.0**	>10
22	>10	>10	>10	>10	>10	>10	>10	**4.0**	**4.5**	>10
47	>10	>10	>10	>10	>10	**2.0**	>10	**0.20**	**5.0**	>10
48	>10	>10	>10	>10	>10	**9.0**	>10	**3.0**	>10	>10
49	>10	>10	>10	>10	>10	**1.5**	>10	**0.30**	>10	>10
50	>10	>10	>10	>10	>10	**2.10**	**5.00**	**0.27**	>10	>10
51	>10	>10	>10	**4.0**	>10	**0.70**	**1.00**	**3.20**	>10	>10
52	>10	>10	>10	>10	>10	>10	>10	**1.5**	>10	>10
53	>10	>10	>10	**7.0**	>10	**1.5**	>10	**0.20**	>10	>10
54	>10	>10	>10	>10	>10	**0.70**	>10	**0.40**	>10	>10
55	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
56	>10	>10	>10	>10	>10	>10	>10	**0.75**	>10	>10
57	>10	>10	>10	>10	>10	**2.00**	**2.00**	**0.75**	>10	>10
58	>10	>10	>10	**2.50**	>10	**0.42**	**0.62**	**0.12**	>10	>10

Values in Bold font represent activity < 10 μM; values which are shaded in pink are < 1 μM.

Table 3
Summary of activity of selected bisindolylmaleimides in the NCI 60 cell line screen.

Comp No. (NSC)	Mean Growth at 10 μM (%)	Growth of selected cell lines (%)					
		A498	SK-MEL-2	MDA-MB-435	LOX IMVI	UO-31	SR
15* (762129)	**29**	−20	−13	13	41	23	12
47 (775309)	**78**	79	76	17	51	41	31
48 (774887)	**98**	86	102	95	73	74	75
50 (776694)	**91**	77	94	68	60	51	67
51 (776691)	**96**	92	–	102	80	63	90
52 (775308)	**94**	94	87	102	59	68	86
53 (775307)	**91**	99	87	104	62	56	–
55 (781334)	**103**	88	120	111	79	80	92
56* (776693)	**68**	69	68	16	30	44	18
57 (776692)	**98**	89	99	102	80	61	100
58 (781333)	**87**	54	96	105	70	73	–

* Compounds taken forward to five dose assay by NCI.

Table 4
Selected GI_{50}, TGI and LC_{50} data for **15** and **56**.

Cancer subtype	Cell line	GI_{50} (μM)		TGI (μM)		LC_{50} (μM)	
		15	56	15	56	15	56
Leukaemia	HL-60(TB)	4.05	2.54	17.9	6.44	> 100	> 100
	SR	1.04	3.55	18.4	9.48	> 100	> 100
Melanoma	MDA-MB-435	0.648	2.74	19.3	8.31	> 100	> 100
	SK-MEL-5	2.15	2.92	5.80	10.6	21.8	42.8
	UACC-62	4.22	3.23	22.0	16.0	96.7	84.4
CNS	SNB-75	0.291	2.84	7.97	20.0	94.6	> 100
Breast	HS-578T	0.847	3.97	4.06	> 100	> 100	> 100
Renal	A498	0.320	2.36	2.34	86.0	> 100	> 100

inhibition of LOX IMVI and UO-31.

Interestingly, addition of a second nitrile chain significantly increased activity with compound **56** displaying a mean growth of 68% and being selected to progress to five dose assay. This derivative exhibited considerable activity towards a number of individual cell lines, including leukaemia cell lines HL-60(TB) (15%) and SR (18%), melanoma cell lines LOX IMVI (30%) and MDA-MB-435 (16%), renal cancer cell line UO-31 (44%) and breast cancer cell lines MCF7 (40%) and MDA-MB-231/ATCC (40%). Replacement of the hydroxymaleimide moiety with an unsubstituted maleimide surprisingly reduced activity, with compound **58** showing a mean growth of 87%.

The derivative with both indole nitrogens linked by a 6-carbon

chain **55** was found to be almost completely inactive with a mean growth of 103% and little deviation from the mean growth across the panel. This was a rather disappointing result as maleimide analogue has been reported to inhibit of a number of kinases including PKCα (*K*i = 0.3 μM), p70 S6K (*K*i = 0.9 μM) and GSK-3 β (*K*i = 0.7 μM), and is analogous to ruboxistaurin.[19,55]

However, the most active anticancer agent in the screen was compound **15** with a mean growth response of just 29%. This compound was taken forward to five dose assays (along with **56**).

A summary of the effects of compound **15** on the NCI 60 cell line panel identifies that the mean GI_{50} of the compound is 5 μM and that the anticancer effects are not particularly specific for any cancer sub-type or cell phenotype (see Supplementary Information). It is clearly evident that there is a cytostatic effect at concentrations around 10 μM for all cell lines but this does not translate into a cytotoxic effect at higher concentrations in the majority of cases. Specific cell lines with sub-micromolar growth inhibition values include: SNB-75 (GI_{50} = 291 nM); MDA-MB-435 (GI_{50} = 648 nM); A498 (GI_{50} = 320 nM); HS 578 T (GI_{50} = 847 nM). From this it can be seen that the spread of activity over CNS, Melanoma, Renal and Breast cancer cell lines and the activity against SNB-75 and A498 is of particular interest for future studies.

Bishexanenitrile derivative **56** had a relatively high mean growth on one dose screening but low micromolar GI_{50} values were observed for a number of cell lines, especially in leukaemia and melanoma cancer types. It is of interest to note that a number of values are lower for **56** over **15** on transfer to cellular assay. Most cell lines required a concentration of greater than 100 μM for the death of 50% of cell population (LC50), with only 2 cell lines, SK-MEL-5 and UACC-62, showing any appreciable cytotoxicity.

3. Conclusions

In summary, we have explored the molecular space around the bisindolylmaleimide pharmacophore, generating 28 novel compounds and the development of novel anticancer leads. Our initial test compounds identified a dichotomy of activity with the maleimide headgroup **15** offering potent kinase inhibitory activity against all CDKs and GSK-3 while the hydroxymaleimide and removal of the indole methyl groups **22** offers some selectivity of action albeit at lower potency.

In order to assess the effect of substitution on potency and selectivity, a series of novel bisindolylhydroxymaleimides functionalized at the indole nitrogens were consequently synthesised and assessed for kinase inhibitory and anticancer activity. The move from azaindole to indole does not significantly affect potency or the GSK-3 kinase activity but does remove all CDK2 and CDK5 inhibition (but not CDK9). Converging towards a ruboxistaurin macrocyclic system is disappointingly associated with a complete loss of kinase activity and indeed anticancer activity as seen in the NCI screen but the majority of other substitutions are well tolerated. In addition to the significant potency of **15**, bishexanitrile substituted **56** is a key lead compound for future development with evident GSK-3 inhibition in the absence of other kinase activity in the screen. The anticancer activity of **15** in the NCI 60 cell line screen has uncovered sub-micromolar growth inhibition of cancer cells across the full panel and significant inhibition of SNB-75 CNS cancer, A498 and UO-31 renal and MDA-MB-435 melanoma cell lines. The fact that **56** progressed to five dose assay suggests that GSK-3 kinase has a likely role in its effect on cancer cell growth.

Overall, our findings confirm that the insertion of an oxygen into the bisindolylmaleimide pharmacophore at the key headgroup is well tolerated and capable of low nanomolar kinase inhibition in particular with GSK-3 kinase. It is evident that this new binding platform is capable of imbuing discrete inhibitory activity on related target kinases and will be the focus of our future research.

4. Experimental section

4.1. General procedures

Solvents were distilled prior to use as follows: dichloromethane was distilled from phosphorous pentoxide; ethyl acetate was distilled from potassium carbonate; ethanol and methanol were distilled from magnesium in the presence of iodine; toluene was distilled from sodium and benzophenone; hexane was distilled prior to use; tetrahydrofuran was freshly distilled from sodium and benzophenone. Diethyl ether was obtained pure from Riedel-de Haën. Organic phases were dried using anhydrous magnesium sulfate. All commercial reagents were used without further purification unless otherwise stated. Infrared spectra were recorded as a thin film on sodium chloride plates for liquids or potassium bromide (KBr) disc for solids on a Perkin Elmer Spectrum 100 FT-IR spectrometer. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer. ^{1}H (400 MHz) NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra were recorded on a Bruker Avance III 600 MHz NMR spectrometer equipped with a dual CH cryoprobe. All spectra were recorded at room temperature (~20 °C) in deuterated chloroform ($CDCl_3$) with tetramethylsilane (TMS) as an internal standard, or deuterated dimethylsulfoxide (DMSO-d_6). ^{1}H NMR spectra recorded in deuterated dimethylsulfoxide (DMSO-d_6) were assigned using the DMSO-d_6 peak as the reference peak. Chemical shifts (δ_H and δ_C) are expressed in parts per million (ppm) relative to the reference peak. Coupling constants (*J*) are expressed in Hertz (Hz). Splitting patterns in ^{1}H NMR spectra are designated as s (singlet), br s (broad singlet), d (doublet), br d (broad doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), ddd (doublet of doublet of doublets), ddt (doublet of doublet of triplets) and m (multiplet). Low resolution mass spectra were recorded on a Waters Quattro Micro triple quadrupole spectrometer (QAA1202) in electrospray ionisation (ESI) mode using 50% acetonitrile–water containing 0.1% formic acid as eluent. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier Time of Flight spectrometer (KD160) in electrospray ionisation (ESI) mode using 50% acetonitrile–water containing 0.1% formic acid as eluent. All synthetic compounds were confirmed to be > 95% pure by LCMS analysis using a 14 min gradient method (90:10 to 10:90 water:acetonitrile with 0.1% formic acid as additive) on a Waters Alliance 2695 HPLC and a Waters Xterra Phenyl 3.5 μm (2.1 × 100 mm) HPLC column. Melting points were measured in a uni-melt Thomas Hoover capillary melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was carried out on precoated silica gel plates (Merck 60 PF254), and visualisation was achieved by UV light detection (254 nm).

4.2. Kinase inhibition assays

Kinase activities were assayed in appropriate kinase buffer, with either protein or peptide as substrate in the presence of 15 μM [γ-^{33}P] ATP (3000 Ci/mmol; 10 mCi/ml) in a final volume of 30 μL following the assay described.[28,50] Controls were performed with appropriate dilutions of dimethylsulfoxide. Full-length kinases are used unless specified. Peptide substrates were obtained from ProteoGenix (Schiltigheim, France).

The buffers used for kinase assays are the following: (A) 10 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, 50 μg/mL heparin; (B) 60 mM β-glycerophosphate, 30 mM p-nitrophenyl-phosphate, 25 mM MOPS (pH 7), 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 0.1 mM sodium orthovanadate; (D) 25 mM MOPS, pH7.2, 12.5 mM β-glycerolphosphate, 25 mM $MgCl_2$, 5 mM EGTA, 2 mM EDTA, 0.25 mM DTT; (H) MOPS 25 mM pH 7.5, 10 mM $MgCl_2$; (R) 1.67 mM MOPS pH 7.2, 0.83 mM β-glycerophosphate, 1.33 mM $MgCl_2$, 0.83 mM $MnCl_2$, 0.33 mM EGTA, 0.13 mM EDTA, 16.67 μg/ml BSA, 0.017 mM DTT.

A panel of ten native or recombinant protein kinases was used

during this study: (i) *Hs*RIPK3 (human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed in buffer R with 0.1 μg/μl of MBP as substrate; (ii) *Hs*PIM1 (human proto-oncogene, recombinant, expressed in bacteria) was assayed in buffer B with 0.8 μg/μl of histone H1 (Sigma #H5505) as substrate; (iii) *Hs*Haspin-k_d (human, kinase domain, amino acids 470 to 798, recombinant, expressed in bacteria) was assayed in buffer H with 0.007 μg/μl of Histone H3 (1–21) peptide (ARTKQTARKSTGGKAPRKQLA) as substrate; (iv) *Hs*CDK2/CyclinA (human, cyclin-dependent kinase-2, kindly provided by Dr. A. Echalier-Glazer, Leicester, UK) was assayed in buffer A (supplemented with 0.15 mg/ml BSA and 0.23 mg/ml DTT) with 0.8 μg/μl of histone H1 as substrate; (v) *Hs*CDK9/CyclinT (human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed in buffer A (supplemented with 0.15 mg/ml BSA and 0.23 mg/ml DTT) with 0.27 μg/μl of the following peptide: YSPTSPSYSPTSPSYSPTSPSKKKK, as substrate; (vi) *Hs*CDK5/p25 (human, recombinant, expressed in bacteria) was assayed in buffer B, with 0.8 μg/μl of histone H1 as substrate; (vii) *Hs*AuroraB (human, recombinant, expressed by baculovirus in Sf9 insect cells, SignalChem, product #A31-10G) was assayed in buffer D with 0.2 μg/μl of MBP as substrate; (viii) *Ssc*GSK-3α/ β (*Sus scrofa domesticus*, glycogen synthase kinase-3, affinity purified from porcine brain) was assayed in buffer A (supplemented with 0.15 mg/ml BSA and 0.23 mg/ml DTT), with 0.010 μg/μl of GS-1 peptide, a GSK-3-selective substrate (YRRAAVPPSPSLSRHSSPHQSpEDEEE, "Sp" stands for phosphorylated serine); (ix) *Ssc*CK1δ/ε (*Sus scrofa domesticus*, casein kinase 1δ/ε, affinity purified from porcine brain) was assayed in buffer B, with 0.022 μg/μl of the following peptide: RRKHAAIGSpAYSITA as CK1-specific substrate; (x) *Rn*DYRK1A-k_d (*Rattus norvegicus,* amino acids 1 to 499 including the kinase domain, recombinant, expressed in bacteria, DNA vector kindly provided by Dr. W. Becker, Aachen, Germany) was assayed in buffer A (supplemented with 0.5 mg/mL BSA and 0.23 mg/ml DTT) with 0.033 μg/μl of the following peptide: KKISGRLSPIMTEQ as substrate.

4.3. NCI-60 anticancer screening

The experimental [56]methodology involves initial growth of the tumor cell lines in RPMI 1640 medium containing 5% foetal bovine serum and 2 mM l-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 μL of medium at plating densities ranging from 5000 to 40,000 cells/well, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of candidate compounds.

After 24 h, two plates of each cell line are fixed *in situ* with trichloroacetic acid (TCA), to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Candidate compounds are dissolved in DMSO at 400-fold the desired final maximum test concentration and stored frozen prior to use. The single dose screen is carried out at a concentration of 10 μM.

Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 μL of cold 50% (w/v) TCA and incubated for 60 min at 4 °C. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. Absorbance is read on an automated plate reader at a wavelength of 515 nm, and using the absorbance measurements of time zero (Tz), control growth (C), and test growth in the presence of the drug at a concentration of 10 μM, the percentage growth is calculated.[52]

4.4. Chemical data

For the synthesis of compounds (**13**), (**15–19**), (**23–24**), (**26–27**), (**31–35**), (**37**), (**39**) and (**41**) see the Supplementary Information.

4.4.1. 3-(1-Methyl-1H-indol-3-yl)-4-(1-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl)furan-2,5-dione (14)

A solution of 1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine **10** (1.808 g, 13.7 mmol) in diethyl ether (80 mL) was cooled to 0 °C in an ice bath. Oxalyl chloride (1.39 mL, 16.4 mmol) was then added in a dropwise manner over a period of 20 min.[57] The resulting mixture was stirred for 3 h, while being allowed to slowly return to room temperature in that time. The solvent and excess oxalyl chloride was removed under reduced pressure and the solid residue was dissolved in DCM (60 mL). This was then added to a stirred solution containing 2-(1-methyl-1*H*-indol-3-yl)acetic acid **13** (2.592 g, 13.7 mmol) and triethylamine (3.82 mL, 27.4 mmol) in DCM (30 mL). The reaction mixture was stirred at room temperature for 14 h, after which time the solvent was removed under reduced pressure. The dark red residue was subjected to flash column chromatography (hexane/ethyl acetate, 70:30), yielding maleic anhydride **14** as a red solid (1.796 g, 37%): m.p. 222–224 °C; ν_{max}/cm^{-1} (KBr) 2919, 1819, 1747, 1523, 1371, 1254; δ_H (300 MHz, $CDCl_3$) 3.91 [3H, s, NCH_3], 3.94 [3H, s, NCH_3], 6.75 [1H, q, *J* 8.0, 4.7, C-$H_{5'}$], 6.77–6.79 [2H, m, C-$H_{5,6}$], 7.13–7.19 [1H, m, C-H_7], 7.30 [1H, dd, *J* 8.0, 1.5, C-$H_{4'}$], 7.34 [1H, d, *J* 8.3, C-H_4], 7.85 [1H, s, C-H_2], 7.86 [1H, s, C-$H_{2'}$], 8.25 [1H, dd, *J* 4.7, 1.5, C-$H_{6'}$]; δ_C (75 MHz, $CDCl_3$) 31.9 (CH_3, NCH_3), 33.6 (CH_3, NCH_3), 103.6 (C, aromatic C), 104.8 (C, aromatic C), 109.9 (CH, aromatic CH), 116.5 (CH, aromatic CH), 118.6 (C, aromatic C), 120.9 (CH, aromatic CH), 122.3 (CH, aromatic CH), 123.0 (CH, aromatic CH), 125.5 (C, aromatic C), 126.4 (C, aromatic C), 128.1 (C, aromatic C), 130.5 (CH, aromatic CH), 133.6 (CH, aromatic CH), 134.0 (CH, aromatic CH), 137.0 (C, aromatic C), 144.0 (CH, aromatic CH), 147.8 (C, aromatic C), 166.6 (C, C=O), 166.8 (C, C=O); *m/z* (ES+) 358.1 $[M+H]^+$ (100%); HRMS (ES+): Exact mass calculated for $(C_{21}H_{16}N_3O_3)^+$ 358.1192. Found 358.1185.

4.4.2. 3-(1-Acetyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-4-(1-(phenylsulfonyl)-1H-indol-3-yl)furan-2,5-dione 20

A suspension of potassium 2-oxo-2-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl) acetate **18** (4.05 g, 17.7 mmol) and 2-(1-(phenylsulfonyl)-1*H*-indol-3-yl)acetic acid **19** (5.56 g, 17.63 mmol) were stirred in acetic anhydride (40 mL) at 80 °C for 24 h. Following this, the mixture was vacuum filtered. The collected solid was stirred in boiling ethyl acetate (100 mL) for 20 min before being filtered hot to remove insoluble impurities and the protected maleic anhydride recrystallized from ethyl acetate/hexane as a bright yellow solid (3.292 g). Further work-up of the mother liquor yielded a yellow/brown solid which was purified by flash column chromatography (80:20, hexane/ethyl acetate) to yield a further batch of the protected maleic anhydride (0.532 g, combined yield 3.824 g, 42%): m.p. 123–125 °C; ν_{max}/cm^{-1} (KBr) 3432, 3139, 2929, 1831, 1764, 1724, 1645, 1547, 1375; δ_H (300 MHz, DMSO-d_6) 3.01 (s, 3H, OAc), 6.50 (q, 1H, *J* = 8.0, 4.7 Hz, C-H_5), 6.89 (dd, 1H, *J* = 8.0, 1.7 Hz, C-$H_{6'}$), 6.99 (m, 2H, C-$H_{3''}$, $H_{5''}$), 7.28 (ddd, 1H, *J* = 8.5, 1.8 Hz, C-$H_{7'}$), 7.67 (t, 2H, *J* = 8.0 Hz, C-$H_{4'}$, $H_{5'}$), 7.80 (d, 1H, *J* = 7.5 Hz, C-$H_{4''}$), 7.96 (d, 1H, *J* = 8.5 Hz, C-H_4), 8.06 (m, 2H, C-$H_{2''}$, $H_{6''}$), 8.23 (s, 1H, C-$H_{2'}$), 8.25 (dd, 1H, *J* = 4.7, 1.5 Hz, C-H_6), 8.47 (s, 1H, C-H_2); *m/z* (ES-) 468.2 ($M-AcO^-$) 90%; HRMS (ES+): Exact mass calculated for $(C_{25}H_{15}N_3O_5S)^+$ 468.0654. Found 468.0661.

4.4.3. 3-(1H-Indol-3-yl)-4-(1H-pyrrolo[2,3-b]pyridin-3-yl)furan-2,5-dione 21

A suspension of 3-(1-acetyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-4-(1-(phenylsulfonyl)-1*H*-indol-3-yl)furan-2,5-dione **20** (1.64 g, 3.21 mmol) and potassium hydroxide (1.07 g, 19.2 mmol) in aqueous methanol (4:1 MeOH:Water, 50 mL) was heated to reflux for 24 h. Following this, the mixture was concentrated under reduced pressure, water (20 mL) was added to the residue, the pH was adjusted to 5 using 2 M HCl and the mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (1 × 15 mL), water (2 × 20 mL), brine (1 × 30 mL), dried and concentrated under reduced pressure to yield a glassy red solid.

Purification by flash column chromatography (70:30–60:40, hexane/ethyl acetate gradient elution) to yield the deprotected maleic anhydride as an orange solid (0.283 g, 27%): m.p. 299–301 °C; ν_{max}/cm^{-1} (KBr) 3435, 3020, 2882, 1839, 1820, 1751, 1631, 1532; δ_H (400 MHz, DMSO-d_6) 6.71–6.75 (m, 2H, C-$H_{5'}$, C-H_4), 6.79 (q, 1H, J = 8.0, 4.7 Hz, C-H_5), 7.05 (ddd, 1H, J = 8.2, 1.3 Hz, C-$H_{6'}$), 7.24 (dd, 1H, J = 8.1, 1.7 Hz, C-$H_{7'}$), 7.45 (d, 1H, J = 8.0 Hz, C-$H_{4'}$), 7.91 (s, 1H, C-$H_{2'}$), 7.95 (s, 1H, C-H_2), 8.17 (dd, 1H, J = 4.6, 1.5 Hz, C-H_6), 12.02 (brs, 1H, indole N—H), 12.39 (brs, 1H, 7-azaindole N—H); δ_C (100 MHz, DMSO-d_6) 103.7 (C, aromatic C), 104.6 (C, aromatic C), 112.2 (CH, aromatic CH), 116.1 (CH, aromatic CH), 117.5 (C, aromatic C), 120.0 (CH, aromatic CH), 121.0 (CH, aromatic CH), 122.2 (CH, aromatic CH), 124.6 (C, aromatic C), 127.1 (C, aromatic C), 129.1 (C, aromatic C), 129. 3 (CH, aromatic CH), 130.5 (CH, aromatic CH), 130.8 (CH, aromatic CH), 136.2 (C, aromatic C), 143.6 (CH, aromatic CH), 148.4 (C, aromatic C), 166.2 (C, C = O), 166.4 (C, C = O); m/z (ES+) 330.2 ($M+H^+$) 100%; HRMS (ES+): Exact mass calculated for $(C_{19}H_{12}N_3O_3)^+$ 330.0879. Found 378.0871.

4.4.4. 1-Hydroxy-3-(1H-indol-3-yl)-4-(1H-pyrrolo[2,3-b]pyridin-3-yl)-1H-pyrrole-2,5-dione **22**

To a mixture of 3-(1*H*-indol-3-yl)-4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl) furan-2,5-dione **21** (0.132 g, 0.4 mmol) and hydroxylammonium hydrochloride (0.109 g, 1.57 mmol) in DMF (1 mL) was added triethylamine (0.21 mL, 1.51 mmol). The mixture was allowed to stir for 24 h at 80 °C. Following this, 1 M HCl (10 mL) was added. The mixture was extracted with ethyl acetate (3 × 5 mL). The combined organic extracts were washed with water (2 × 10 mL), brine (1 × 10 mL), dried and concentrated under reduced pressure to yield the hydroxymaleimide as a deep red solid (0.112 g, 83%): m.p. > 300 °C; ν_{max}/cm^{-1} (KBr) 3340, 1777, 1721, 1703, 1645, 1539, 1419, 1133, 1100, 1026, 726; δ_H (400 MHz, DMSO-d_6) 6.65 (t, 1H, J = 7 Hz, C-$H_{5'}$), 6.70 (s, 1H, C-$H_{7'}$), 6.72 (q, 1H, J = 8.1, 4.6 Hz, C-H_5), 7.00 (t, 1H, J = 7.6 Hz, C-$H_{6'}$), 7.15 (dd, 1H, J = 8.0, 1.2 Hz, C-H_4), 7.41 (d, 1H, J = 8.0 Hz, C-$H_{4'}$), 7.86 (s, 1H, C-$H_{2'}$), 7.87 (s, 1H, C-H_2), 8.11 (dd, 1H, J = 4.5, 1.2 Hz, C-H_6), 10.48 (brs, 1H, indole N—H), 11.81 (brs, 1H, 7-azaindole N—H), 12.24 (brs, 1H, *N*-OH); δ_C (100 MHz, DMSO-d_6) 104.3 (C, aromatic C), 105.1 (C, aromatic C), 112.0 (CH, aromatic CH), 115.1 (CH, aromatic CH), 117.7 (C, aromatic C), 119.6 (CH, aromatic CH), 120.7 (CH, aromatic CH), 121.9 (CH, aromatic CH), 123.4 (C, aromatic C), 125.0 (C, aromatic C), 125.2 (C, aromatic C), 129.0 (CH, aromatic CH), 129.5 (CH, aromatic CH), 129.7 (CH, aromatic CH), 136.0 (C, aromatic C), 143.2 (CH, aromatic CH), 148.3 (C, aromatic C), 168.2 (C, C = O), 168.3 (C, C=O); m/z (ES-) 343.3 ($M-H^-$) 100%; HRMS (ES+): Exact mass calculated for $(C_{19}H_{13}N_4O_3)^+$ 345.0988. Found 345.0981.

4.4.5. 3-(1-Acetyl-1H-indol-3-yl)-4-[1-(phenylsulfonyl)-1H-indol-3-yl] furan-2,5-dione **25**

A mixture of potassium 2-(1*H*-indol-3-yl)-2-oxoacetate **24** (3.677 g, 15.8 mmol), 2-[1-(phenylsulfonyl)-1*H*-indol-3-yl]acetic acid **19** (5.020 g, 15.8 mmol) and acetic anhydride (30 mL) was stirred at 80 °C for 24 h. The yellow suspension was then cooled to room temperature and filtered. The solid collected was stirred in boiling ethyl acetate for 30 min before hot filtration removed a white side product. The yellow filtrate was concentrated to dryness giving a yellow crystalline solid (2.426 g). The acetic anhydride solution from the initial filtration was concentrated under reduced pressure. The residue was then washed with saturated sodium bicarbonate solution (50 mL) and extracted into ethyl acetate (3 × 30 mL). The organic layer was filtered before being washed with saturated sodium bicarbonate solution (30 mL), followed by water (30 mL) and brine (30 mL) and then dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude residue was recrystallised from ethyl acetate to give additional product (0.544 g, combined yield 2.970 g, 40%): m.p. 223–225 °C; ν_{max}/cm^{-1} (KBr) 3139, 1830, 1762, 1718, 1373, 1176; δ_H (300 MHz, DMSO-d_6) 2.69 (3H, s, CH_3), 6.44 [1H, t, J 7.4, C(6′)-H], 6.59 [1H, d, J 7.8, C(7′)-H], 6.95 [1H, t, J 7.3, C(6)-H], 7.03 [1H, d, J 7.6, C(7)-H], 7.16 [1H, t, J 7.8, C(5′)-H], 7.27 [1H, t, J 7.7, C(5)-H], 7.61–7.71 [2H, m, C(3″, 5″)-H], 7.75–7.85 [1H, t, J 7.4, C(4″)-H], 7.93 [1H, d, J 8.6, C(4)-H], 7.98–8.06 [2H, m, C(2″, 6″)-H], 8.19 [1H, s, C(2′)-H], 8.26 [1H, d, J 8.3, C(4′)-H], 8.30 [1H, s, C(2)-H]; δ_C (75 MHz, DMSO-d_6) 23.9 (CH_3), 109.3 (C, aromatic C), 111.2 (C, aromatic C), 113.1 (CH, aromatic CH), 115.9 (CH, aromatic CH), 120.5 (CH, aromatic CH), 121.6 (CH, aromatic CH), 123.1 (CH, aromatic CH), 123.7 (CH, aromatic CH), 125.4 (CH, aromatic CH), 125.7 (CH, aromatic CH), 126.6 (C, aromatic C), 126.9 (2CH, 2 × aromatic CH), 127.6 (C, aromatic C), 129.6 (CH, aromatic CH), 130.1 (2CH, 2 × aromatic CH, C, aromatic C), 130.8 (CH, aromatic CH), 131.4 (C, aromatic C), 133.6 (C, aromatic C), 134.7 (C, aromatic C), 135.2 (CH, aromatic CH), 136.4 (C, aromatic C), 164.9 (C=O), 165.1 (C=O), 169.7 (C=O); m/z (ESI^+) 511.1 [$(M+H)^+$ 10%]; HRMS (ESI^+): Exact mass calculated for $(C_{28}H_{19}N_2O_6S)^+$ 511.0964. Found 511.0965.

4.4.6. 6,6′-(3,3′-(2,5-Dioxo-2,5-dihydrofuran-3,4-diyl)bis(1H-indole-3,1-diyl))dihexanenitrile **28**

To a solution of 3,4-di(1*H*-indol-3-yl)furan-2,5-dione **26** (5.004 g, 15.2 mmol) in anhydrous DMF (80 mL) at 0 °C under a nitrogen atmosphere was added sodium hydride (60 wt% oil dispersion, 0.640 g, 16.0 mmol). The dark purple solution was stirred for 30 min while warming to room temperature before 6-bromohexanenitrile (2.62 mL, 3.47 g, 19.7 mmol) was added. The solution was stirred for 16 h before being poured into water (200 mL) and extracted with ethyl acetate (3 × 60 mL). The combined organic layers were washed with 1 M aqueous HCl (2 × 60 mL) followed by water (60 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude material was purified using column chromatography on silica gel with 20:80–30:70 ethyl acetate/hexane to give 2 fractions found to be the mono alkylated product **29** and also dialkylated 6,6′-[3,3′-(2,5-dioxo-2,5-dihydrofuran-3,4-diyl)bis(1*H*-indole-3,1-diyl)]dihexanenitrile **28**.

6,6′-(3,3′-(2,5-Dioxo-2,5-dihydrofuran-3,4-diyl)bis(1*H*-indole-3,1-diyl))dihexanenitrile **28** was isolated as a red solid (1.658 g, 21%): m.p. 120–122 °C; ν_{max}/cm^{-1} (KBr) 3435, 2939, 2243, 1815, 1749, 1525, 1257, 747; δ_H (300 MHz, DMSO-d_6) 1.27–1.40 [4H, m, 2 × C(4″)-H_2], 1.57 [4H, quin, J 7.4, 2 × C(3″)-H_2], 1.76 [4H, quin, J 7.3, 2 × C(5″)-H_2], 2.45 [4H, t, J 7.0, 2 × C(2″)-H_2], 4.27 [4H, t, 2 × C(6″)-H_2], 6.75 [2H, t, J 7.4, C(6, 6′)-H], 6.87 [2H, d, J 7.9, C(7, 7′)-H], 7.10 [2H, t, J 7.7, C(5, 5′)-H], 7.55 [2H, d, J 8.3, C(4, 4′)-H], 7.90 [2H, s, C(2, 2′)-H]; δ_c (75 MHz, DMSO-d_6) 16.0 (2 × CH_2), 24.3 (2 × CH_2), 25.1 (2 × CH_2), 28.7 (2 × CH_2), 45.6 (2 × CH_2), 104.2 (2C, 2 × aromatic C), 110.6 (2CH, 2 × aromatic CH), 120.0 (2CH, 2 × aromatic CH), 120.5 (2 × CN), 121.5 (2CH, 2 × aromatic CH), 122.2 (2CH, 2 × aromatic CH), 125.3 (2C, 2 × aromatic C), 127.6 (2C, 2 × aromatic C), 133.1 (2CH, 2 × aromatic CH), 136.0 (2C, 2 × aromatic C), 166.3 (2 × C=O); m/z (ESI^+) 519.2 [$(M+H)^+$ 10%]; HRMS (ESI^+): Exact mass calculated for $(C_{32}H_{31}N_4O_3)^+$ 519.2396. Found 519.2401.

6-{3-[4-(1*H*-Indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H*-indol-1-yl}hexanenitrile **29** was isolated as a red solid (0.953 g, 15%): m.p. 102–104 °C; ν_{max}/cm^{-1} (KBr) 3391, 2935, 2246, 1819, 1748, 1529, 1251, 743; δ_H (300 MHz, DMSO-d_6) 1.32 [2H, m, C(4″)-H_2], 1.57 [2H, quin, J 7.3, C(3″)-H_2], 1.74 [2H, quin, J 7.3, C(5″)-H_2], 2.45 [2H, t, J 7.1, C(2″)-H_2], 4.26 [2H, t, J 7.0, C(6″)-H_2], 6.69 [1H, ddd, J 7.9, 6.9, 0.9, C(6)-H], 6.74–6.82 [2H, m, C(6′, 7)-H], 6.95 [1H, d, J 7.8, C(7′)-H], 7.04 [1H, ddd, J 8.2, 7.1, 1.2, C(5)-H], 7.10 [1H, ddd, J 8.2, 7.2, 1.1, C(5′)-H], 7.43 [1H, d, J 8.2, C(4)-H], 7.55 [1H, d, J 8.2, C(4′)-H], 7.86 [1H, s, C(2′)-H], 7.89 [1H, s, C(2)-H], 11.92 (1H, bs, NH); δc (75 MHz, DMSO-d_6) 16.0 (CH_2), 24.3 (CH_2), 25.2 (CH_2), 28.7 (CH_2), 45.6 (CH_2), 104.2 (C, aromatic C), 104.9 (C, aromatic C), 110.5 (CH, aromatic CH), 112.1 (CH, aromatic CH), 119.8 (CH, aromatic CH), 120.0 (CH, aromatic CH), 120.5 (CN), 121.2 (CH, aromatic CH), 121.4 (CH, aromatic CH), 122.1 (CH, aromatic CH), 122.2 (CH, aromatic CH), 124.7 (C, aromatic C), 125.5 (C, aromatic C), 127.4 (C, aromatic C),

128.2 (C, aromatic C), 130.6 (CH, aromatic CH), 133.0 (CH, aromatic CH), 135.9 (C, aromatic C), 136.2 (C, aromatic C), 166.36 (C=O), 166.43 (C=O); *m/z* (ESI$^+$) 424.2 [(M + H)$^+$ 100%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{26}H_{22}N_3O_3)^+$ 424.1661. Found 424.1659.

4.4.7. 6-{3-[4-(1H-Indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1H-indol-1-yl}hexanoic acid **30**

6-{3-[4-(1*H*-Indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1-indol-1-yl}hexanenitrile **29** (0.402 g, 0.950 mmol) was dissolved in a mixture of methanol (20 mL) and 10% aqueous potassium hydroxide solution (20 mL). The solution was heated to reflux for 16 h before being allowed to cool and the solvent evaporated under reduced pressure. The residue was dissolved in water (20 mL) and acidified to pH 2 using 20% aqueous HCl, and then extracted with ethyl acetate (20 mL × 2). The organic layer was washed with water (20 mL × 2) followed by brine (20 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give desired compound as a red solid (0.335 g, 84%): 198–199 °C; ν_{max}/cm^{-1} (KBr) 3386, 2924, 1816, 1740, 1699, 1531, 1261; δH (300 MHz, DMSO-d_6) 1.23 [2H, quin, *J* 7.5, C(4″)-H2], 1.51 [2H, quin, *J* 7.5, C(3″)-H2], 1.72 [2H, quin, *J* 7.2, C(5″)-H2], 2.17 [2H, t, *J* 7.3, C(2″)-H2], 4.24 [2H, t, *J* 7.0, C(6″)-H2], 6.67 [1H, t, *J* 7.5, C(6)-H], 6.76 [1H, t, *J* 7.8, C(6′)-H], 6.77 [1H, d, *J* 7.5, C(7)-H], 6.93 [1H, d, *J* 8.0, C(7′)-H], 7.04 [1H, t, *J* 8.0, C(5)-H], 7.09 [1H, t, *J* 7.8, C(5′)-H], 7.43 [1H, d, *J* 8.1, C(4)-H], 7.53 [1H, d, *J* 8.3, C(4′)-H], 7.84 [1H, s, C(2′)-H], 7.89 [1H, s, C(2)-H], 11.92 (1H, bs, NH), 11.99 (1H, bs, COOH); δc (75 MHz, DMSO-d_6) 24.0 (CH2), 25.6 (CH2), 29.3 (CH2), 33.5 (CH2), 45.7 (CH2), 104.2 (C, aromatic C), 104.9 (C, aromatic C), 110.5 (CH, aromatic CH), 112.1 (CH, aromatic CH), 119.8 (CH, aromatic CH), 120.0 (CH, aromatic CH), 121.2 (CH, aromatic CH), 121.4 (CH, aromatic CH), 122.07 (CH, aromatic CH), 122.12 (CH, aromatic CH), 124.7 (C, aromatic C), 125.5 (C, aromatic C), 127.4 (C, aromatic C), 128.2 (C, aromatic C), 130.6 (CH, aromatic CH), 133.0 (CH, aromatic CH), 135.9 (C, aromatic C), 136.1 (C, aromatic C), 166.4 (C=O), 166.5 (C=O), 174.3 (COOH); *m/z* (ESI-) 441.2 [(M − H)$^-$ 100%]; HRMS (ESI+): Exact mass calculated for $(C_{26}H_{23}N_2O_5)^+$ 443.1607. Found 443.1611.

4.4.8. Synthesis of N-isopropyl maleic anhydride intermediate

4.4.8.1. Potassium 2-(1-isopropyl-1H-indol-3-yl)-2-oxoacetate ***36***. A solution of 2-(1-isopropyl-1*H*-indol-3-yl)-2-oxoacetic acid **34** (3.499 g, 15.1 mmol) in ethanol (50 mL) was treated with potassium hydroxide (85 wt%, 0.993 g, 15.1 mmol) and the mixture was stirred at room temperature for 4 h. The solvent was removed under reduced pressure and the residue dried overnight at 40 °C to give the desired product as a pale pink solid (3.961 g, 97%): m.p. 80–84 °C; ν_{max}/cm^{-1} (KBr) 3456, 2981, 1649, 1607, 1301, 1203, 738; δ_H (300 MHz, DMSO-d_6) 1.48 [d, 6H, *J* 6.6, CH(C<u>H</u>$_3$)$_2$], 4.80 [1H, sept, *J* 6.7, C<u>H</u>(CH$_3$)$_2$], 7.17 [1H, ddd, *J* 8.2, 7.2, 1.1, C(5)-H], 7.22 [1H, ddd, *J* 8.5, 7.1, 1.4, C(6)-H], 7.58 [1H, d, *J* 7.6, C(7)-H], 8.14 [1H, s, C(2)-H], 8.19 [1H, d, *J* 7.3, C(4)-H]; δ_c (75 MHz, DMSO-d_6) 22.2 (2 × CH$_3$), 47.2 [<u>C</u>H(CH$_3$)$_2$], 110.5 (CH, aromatic CH), 113.4 (C, aromatic C), 121.4 (CH, aromatic CH), 121.5 (CH, aromatic CH), 122.2 (CH, aromatic CH), 126.5 (C, aromatic C), 134.0 (CH, aromatic CH), 135.8 (C, aromatic C), 169.8 (C=O), 193.1 (C=O); *m/z* (ESI$^+$) 232.3 [(M + 2H)$^+$, 100%]; HRMS (ESI+): Exact mass calculated for $(C_{13}H_{17}N_2O_3)^+$ 249.1239 [(M + NH$_4$ + H)$^+$]. Found 249.1239.

4.4.8.2. 3-(1-Isopropyl-1H-indol-3-yl)-4-[1-(phenylsulfonyl)-1H-indol-3-yl]furan-2,5-dione ***38***. A mixture of potassium 2-(1-isopropyl-1*H*-indol-3-yl)-2-oxoacetate **36** (3.502 g, 13.0 mmol), 2-[1-(phenylsulfonyl)-1*H*-indol-3-yl]acetic acid **19** (4.096 g, 13.0 mmol) and acetic anhydride (30 mL) was stirred at 80 °C for 24 h. The yellow suspension was then allowed to cool to room temperature and the solvent was evaporated under reduced pressure. The residue was washed with saturated aqueous sodium bicarbonate (50 mL) and extracted into ethyl acetate (2 × 30 mL). The organic layer was washed with saturated aqueous sodium bicarbonate (30 mL), followed by water (30 mL) and brine (30 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was then purified using column chromatography on silica gel with 10% ethyl acetate/hexane to give a yellow foamy residue which was dissolved in chloroform (15 mL) and diethyl ether was added until a yellow solid precipitated which was collected by vacuum filtration (1.582 g, 24%): m.p. 102–105 °C; ν_{max}/cm^{-1} (KBr) 3561, 3135, 2977, 1821, 1755, 1631, 1536, 1372, 1185; δ_H (300 MHz, DMSO-d_6) 1.35 [6H, d, *J* 6.6, CH(C<u>H</u>$_3$)$_2$], 4.80 [1H, sept, *J* 6.6, C<u>H</u>(CH$_3$)$_2$], 6.39 [1H, t, *J* 7.6, C(6′)-H], 6.70 [1H, d, *J* 8.0, C(7′)-H], 6.83–6.93 [2H, m, C(6, 7)-H], 7.05 [1H, t, *J* 7.7, C(5′)-H], 7.26 [1H, ddd, *J* 8.3, 6.9, 1.5, C(5)-H], 7.54 [1H, d, *J* 8.4, C(4′)-H], 7.63–7.69 [2H, m, C(3″, 5″)-H], 7.80 [1H, m, C(4″)-H], 7.91 [1H, s, C(2′)-H], 7.95 [1H, d, *J* 8.3, C(4)-H], 8.04–8.07 [2H, m, C(6″, 2″)-H], 8.15 [1H, s, C(2)-H]; δ_c (75 MHz, DMSO-d_6) 22.0 (2 × CH$_3$), 47.5 [C<u>H</u>(CH$_3$)$_2$], 104.1 (C, aromatic C), 110.9 (CH, aromatic CH), 112.0 (C, aromatic C), 113.2 (CH, aromatic CH), 120.7 (CH, aromatic CH), 121.0 (CH, aromatic CH), 121.7 (CH, aromatic CH), 122.5 (CH, aromatic CH), 122.8 (C, aromatic C), 123.4 (CH, aromatic CH), 125.0 (C, aromatic C), 125.4 (CH, aromatic CH), 126.9 (2CH, 2 × aromatic CH), 127.6 (C, aromatic C), 128.6 (CH, aromatic CH), 130.0 (2CH, 2 × aromatic CH), 130.9 (CH, aromatic CH), 133.7 (C, aromatic C), 134.2 (C, aromatic C), 135.0 (CH, aromatic CH), 135.7 (C, aromatic C), 136.6 (C, aromatic C), 165.5 (C=O), 165.8 (C=O); *m/z* (ESI+) 511.1 [(M + H)$^+$ 50%]; HRMS (ESI+): Exact mass calculated for $(C_{29}H_{23}N_2O_5S)^+$ 511.1328. Found 511.1310.

4.4.8.3. 3-(1H-Indol-3-yl)-4-(1-isopropyl-1H-indol-3-yl)furan-2,5-dione ***40***. To a stirred suspension of 3-(1-isopropyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione **38** (1.353 g, 2.37 mmol) in a mixture of methanol (60 mL) and water (15 mL) was added potassium hydroxide (85 wt%, 1.053 g, 15.9 mmol). The mixture was then heated to reflux for 24 h before being allowed to cool and the solvent evaporated under reduced pressure. The residue was acidified to pH 2 using 10% aqueous HCl and extracted with ethyl acetate (3 × 25 mL). the combined organic layers were washed with water (3 × 30 mL) and brine (30 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give the desired product as red solid (0.764 g, 87%): m.p. 186–188 °C; ν_{max}/cm^{-1} (KBr) 3118, 2971, 1812, 1745, 1627, 1529, 1247; δ_H (300 MHz, DMSO-d_6) 1.38 [6H, d, *J* 6.7, CH(C<u>H</u>$_3$)$_2$], 4.81 [1H, sept, *J* 6.6, C<u>H</u>(CH$_3$)$_2$], 6.68–6.71 [2H, m, C(6, 7)-H], 6.85 [1H, ddd, *J* 8.1, 6.9, 0.7, C(6′)-H], 7.03–7.17 [3H, m, C(5, 5′, 7′)-H], 7.44 [1H, d, *J* 8.1, C(4)-H], 7.59 [1H, d, *J* 8.2, C(4′)-H], 7.75 [1H, s, C(2′)-H], 7.92 [1H, d, *J* 2.9, C(2)-H], 11.94 (1H, bs, N−H); δ_c (75 MHz, DMSO-d_6) 22.1 (2 × CH$_3$), 47.2 (CH), 104.4 (C, aromatic C), 104.8 (C, aromatic C), 110.6 (CH, aromatic CH), 112.2 (CH, aromatic CH), 119.9 (CH, aromatic CH), 120.2 (CH, aromatic CH), 121.4 (CH, aromatic CH), 121.6 (CH, aromatic CH), 122.1 (2CH, 2 × aromatic CH), 124.2 (C, aromatic C), 125.6 (C, aromatic C), 127.2 (C, aromatic C), 128.5 (C, aromatic C), 129.5 (CH, aromatic CH), 130.9 (CH, aromatic CH), 135.5 (C, aromatic C), 136.3 (C, aromatic C), 166.3 (C = O), 166.5 (C=O); *m/z* (ESI-) 369.3 [(M − H)$^-$ 100%]; HRMS (ESI+): Exact mass calculated for $(C_{23}H_{19}N_2O_3)^+$ 371.1396. Found 371.1395.

4.4.9. Substitutions on methyl intermediate

4.4.9.1. 3-(1-Ethyl-1H-indol-3-yl)-4-(1-methyl-1H-indol-3-yl)furan-2,5-dione ***42***. To a solution of 3-(1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl) furan-2,5-dione **39** (0.262 g, 0.76 mmol) in anhydrous DMF (15 mL) under nitrogen was added sodium hydride (60 wt% oil dispersion, 0.037 g, 1.1 mmol) and the mixture was allowed to stir for 40 min at room temperature. Iodoethane (0.09 mL, 0.17 g, 1.1 mmol) was then added and the reaction was stirred for a further 16 h. Water (30 mL) was then added and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were then washed with 1 M aqueous HCl (2 × 20 mL), followed by water (20 mL) and brine (20 mL)

before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude residue was purified using column chromatography on silica gel with 20% ethyl acetate/hexane to give desired product as a red solid (0.204 g, 72%): m.p. = 214–216 °C; ν_{max}/cm^{-1} (KBr) 3420, 3133, 1816, 1758, 1626, 1525, 1219, 737; δ_H (300 MHz, DMSO-d_6) 1.31 (3H, t, *J* 7.2, $CH_2C\underline{H}_3$), 3.89 (3H, s, CH_3), 4.26 (2H, q, *J* 7.2, $C\underline{H}_2CH_3$), 6.67–6.74 [2H, m, C(6′, 7′)-H], 6.81 [1H, t, *J* 7.9, C(6)-H], 7.04 [1H, d, *J* 8.0, C(7)-H], 7.08–7.16 [2H, m, (5, 5′)-H], 7.50 [1H, d, *J* 8.4, C(4′)-H], 7.54 [1H, d, *J* 8.4, C(4)-H], 7.81 [1H, s, C(2)-H], 7.97 [1H, s, C(2′)-H]; δ_c (75 MHz, DMSO-d_6) 15.1 ($CH_2\underline{C}H_3$), 33.0 (CH_3), 40.8 ($\underline{C}H_2CH_3$), 103.9 (C, aromatic C), 104.2 (C, aromatic C), 110.4 (CH, aromatic CH), 110.5 (CH, aromatic CH), 120.05 (CH, aromatic CH), 120.08 (CH, aromatic CH), 121.5 (CH, aromatic CH), 121.6 (CH, aromatic CH), 122.2 (2CH, 2 × aromatic CH), 124.9 (C, aromatic C), 125.7 (C, aromatic C), 127.1 (C, aromatic C), 127.7 (C, aromatic C), 132.5 (CH, aromatic CH), 134.4 (CH, aromatic CH), 135.6 (C, aromatic C), 136.8 (C, aromatic C), 166.3 (C═O), 166.5 (C═O); *m/z* (ESI^+) 371.1 [$(M+H)^+$ 100%]. HRMS (ESI^+): Exact mass calculated for $(C_{23}H_{19}N_2O_3)^+$ 371.1396. Found 371.1389.

*4.4.9.2. 6-{3-[4-(1-Methyl-1H-indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1H-indol-1-yl}hexanenitrile **43***. To a solution of 3-(1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **39** (0.505 g, 1.48 mmol) in anhydrous DMF (20 mL) under nitrogen was added sodium hydride (60 wt% oil dispersion, 0.070 g, 1.77 mmol) and the mixture was stirred for 40 min at room temperature. 6-Bromohexane nitrile (0.30 mL, 0.38 g, 2.2 mmol) was then added and the reaction was stirred for a further 16 h. Water (40 mL) was then added and the mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were then washed with 1 M aqueous HCl (2 × 30 mL), followed by water (30 mL) and brine (30 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude residue was purified using column chromatography on silica gel with 30% ethyl acetate/hexane to give desired product as a red solid (0.258 g, 40%): m.p. 78–79 °C; ν_{max}/cm^{-1} (KBr) 3411, 2936, 2244, 1816, 1750, 1529, 1254, 1100, 741; δH (400 MHz, DMSO-d_6) 1.27–1.35 [2H, m, C(4″)-H2], 1.56 [2H, quin, *J* 7.4, C(3″)-H2], 1.73 [2H, quin, *J* 7.4, C(5″)-H2], 2.44 [2H, t, *J* 6.9, C(2″)-H2], 3.89 (3H, s, CH3), 4.24 [2H, t, *J* 6.9, C(6″)-H2], 6.69–6.72 [2H, m, C(6′, 7′)-H], 6.79 [1H, t, *J* 7.6, C(6)-H], 7.01 [1H, d, *J* 8.15, C(7)-H], 7.07–7.13 [2H, m, (5, 5′)-H], 7.49 [1H, d, *J* 8.15, C(4′)-H], 7.54 [1H, d, 8.49, C(4)-H], 7.82 [1H, s, C(2)-H], 7.97 [1H, s, C(2′)-H]; δc (75 MHz, DMSO-d_6) 16.0 (CH2), 24.3 (CH2), 25.1 (CH2), 28.7 (CH2), 33.0 (CH3), 45.6 (CH2), 103.9 (C, aromatic C), 104.3 (C, aromatic C), 110.50 (CH, aromatic CH), 110.54 (CH, aromatic CH), 120.0 (CH, aromatic CH), 120.1 (CH, aromatic CH), 120.5 (CN), 121.4 (CH, aromatic CH), 121.5 (CH, aromatic CH), 122.1 (CH, aromatic CH), 122.2 (CH, aromatic CH), 125.0 (C, aromatic C), 125.7 (C, aromatic C), 127.0 (C, aromatic C), 127.9 (C, aromatic C), 132.9 (CH, aromatic CH), 134.3 (CH, aromatic CH), 135.9 (C, aromatic C), 136.7 (C, aromatic C), 166.3 (C═O), 166.5 (C═O); *m/z* (ESI+) 438.2 [$(M+H)^+$ 12%]; HRMS (ESI+): Exact mass calculated for $(C_{27}H_{24}N_3O_3)^+$ 438.1818. Found 438.1802.

*4.4.9.3. 6-{3-[4-(1-Methyl-1H-indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1H-indol-1-yl}hexanoic acid **44***. 6-{3-[4-(1-Methyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H*-indol-1-yl}hexanenitrile **43** (0.103 g, 0.235 mmol) was dissolved in a mixture of methanol (8 mL) and 10% aqueous potassium hydroxide solution (8 mL). The solution was heated to reflux for 16 h before being allowed to cool and the solvent evaporated. The residue was dissolved in water (10 mL) and acidified to pH 2 using 20% aqueous HCl, and was then extracted with ethyl acetate (10 mL × 2). The organic layer was washed with water (10 mL × 2) followed by brine (10 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give desired compound as a red solid (0.082 g, 76%): m.p. 95–97 °C; ν_{max}/cm^{-1} (KBr) 3050, 2937, 1816, 1751, 1705, 1611, 1528, 1255, 740; δH (300 MHz, DMSO-d_6) 1.18–1.29 [2H, m, C(4″)-H2], 1.51 [2H, quin, *J* 7.4, C(3″)-H2], 1.71 [2H, quin, *J* 7.3, C(5″)-H2], 2.17 [2H, t, *J* 7.4, C(2″)-H2], 3.90 (3H, s, CH3), 4.23 [2H, t, *J* 6.7, C(6″)-H2], 6.68 [2H, m, C(6′, 7′)-H], 6.78 [1H, t, *J* 7.6, C(6)-H], 7.00 [1H, d, *J* 8.0, C(7)-H], 7.06–7.14 [2H, m, (5, 5′)-H], 7.48 [1H, d, *J* 8.3, C(4′)-H], 7.53 [1H, d, *J* 8.3, C(4)-H], 7.81 [1H, s, C(2)-H], 7.99 [1H, s, C(2′)-H], 11.99 (COOH); δc (75 MHz, DMSO-d_6) 24.1 (CH2), 25.9 (CH2), 29.3 (CH2), 33.0 (CH3), 33.8 (CH2), 45.7 (CH2), 103.9 (C, aromatic C), 104.2 (C, aromatic C), 110.48 (CH, aromatic CH), 110.52 (CH, aromatic CH), 120.0 (2CH, 2 × aromatic CH), 121.4 (2CH, 2 × aromatic CH), 122.1 (2CH, 2 × aromatic CH), 125.0 (C, aromatic C), 125.7 (C, aromatic C), 127.0 (C, aromatic C), 127.8 (C, aromatic C), 132.9 (CH, aromatic CH), 134.3 (CH, aromatic CH), 135.9 (C, aromatic C), 136.7 (C, aromatic C), 166.3 (C═O), 166.5 (C═O), 174.5 (COOH); *m/z* (ESI+) 457.3 [$(M+H)^+$ 100%]; HRMS (ESI+): Exact mass calculated for $(C_{27}H_{25}N_2O_5)^+$ 457.1763. Found 457.1755.

4.4.10. Substitutions on isopropyl intermediate

*4.4.10.1. 3-(1-Isopropyl-1H-indol-3-yl)-4-(1-methyl-1H-indol-3-yl)furan-2,5-dione **45***. To a solution of 3-(1*H*-indol-3-yl)-4-(1-isopropyl-1*H*-indol-3-yl)furan-2,5-dione **40** (0.302 g, 0.81 mmol) in anhydrous DMF (15 mL) under nitrogen was added sodium hydride (60 wt% oil dispersion, 0.037 g, 1.5 mmol) and the mixture was stirred for 40 min at room temperature. Iodomethane (0.06 mL, 0.14 g, 0.97 mmol) was then added and the reaction was allowed to stir for a further 16 h. Water (30 mL) was then added and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were then washed with 1 M aqueous HCl (2 × 20 mL), followed by water (20 mL) and brine (20 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude residue was purified using column chromatography on silica gel with 20% ethyl acetate/hexane to give desired product as a red solid (0.140 g, 46%): m.p. 164–167 °C; ν_{max}/cm^{-1} (KBr) 3118, 3048, 2970, 1812, 1745, 1627, 1529, 1247, 739; δ_H (300 MHz, DMSO-d_6) 1.37 [6H, d, *J* 6.7, 2 × CH_3], 3.90 (3H, s, CH_3), 4.81 [1H, sept, *J* 6.6, $C\underline{H}(CH_3)_2$], 6.60 [1H, d, *J* 8.0, C(7)-H], 6.72 [1H, t, *J* 8.0, C(6)-H], 6.87 [1H, t, *J* 7.8, C(6′)-H], 7.09–7.19 [3H, m, C(5, 5′, 7′)-H], 7.50 [1H, d, *J* 8.4, C(4)-H], 7.59 [1H, d, *J* 8.4, C(4′)-H], 7.73 [1H, s, C(2′)-H], 8.01 [1H, s, C(2)-H]; δ_c (75 MHz, DMSO-d_6) 22.0 (2 × CH_3), 33.0 [$\underline{C}H(CH_3)_2$], 47.2 (CH_3), 103. 9 (C, aromatic C), 104.5 (C, aromatic C), 110.5 (CH, aromatic CH), 110.6 (CH, aromatic CH), 120.1 (CH, aromatic CH), 120.2 (CH, aromatic CH), 121.5 (CH, aromatic CH), 121.6 (CH, aromatic CH), 122.15 (CH, aromatic CH), 122.18 (CH, aromatic CH), 124.5 (C, aromatic C), 125.7 (C, aromatic C), 126.9 (C, aromatic C), 128.1 (C, aromatic C), 129.1 (CH, aromatic CH), 134.65 (CH, aromatic CH), 134.69 (C, aromatic C), 136.9 (C, aromatic C), 166.2 (C═O), 166.5 (C═O); *m/z* (ESI^+) 385.3 [$(M+H)^+$, 100%]; HRMS (ESI+): Exact mass calculated for $(C_{24}H_{21}N_2O_3)^+$ 385.1552. Found 385.1540.

*4.4.10.2. 3-(1-Ethyl-1H-indol-3-yl)-4-(1-isopropyl-1H-indol-3-yl)furan-2,5-dione **46***. To a solution of 3-(1*H*-indol-3-yl)-4-(1-isopropyl-1*H*-indol-3-yl)furan-2,5-dione **40** (0.167 g, 0.45 mmol) in anhydrous DMF (10 mL) under nitrogen was added sodium hydride (60 wt% oil dispersion, 0.019 g, 0.83 mmol) and the mixture was stirred for 40 min at room temperature. Iodoethane (0.06 mL, 0.12 g, 0.69 mmol) was then added and the reaction was allowed to stir for a further 16 h. Water (20 mL) was then added and the mixture was extracted with ethyl acetate (3 × 15 mL). The combined organic layers were then washed with 1 M aqueous HCl (2 × 15 mL), followed by water (15 mL) and brine (15 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude residue was purified using column chromatography on silica gel with 20% ethyl acetate/hexane to give desired product as an orange solid (0.091 g, 51%): m.p. 165–167 °C; ν_{max}/cm^{-1} (KBr) 2978, 1817,

1746, 1524, 1211, 741; δ_H (400 MHz, DMSO-d_6) 1.34 (3H, t, *J* 7.1, $CH_2C\underline{H}_3$) 1.40 [6H, d, *J* 6.7, 2 × CH_3], 4.30 (2H, q, *J* 7.2, $C\underline{H}_2CH_3$), 4.82 [1H, sept, *J* 6.6 $C\underline{H}(CH_3)_2$], 6.75–6.86 [3H, m, C(6, 6′, 7)-H), 7.04 [1H, d, *J* 7.7, C(7′)-H] 7.10–7.16 [2H, m, C(5, 5′)-H), 7.56 [1H, d, *J* 8.1, C(4)-H], 7.60 [1H, d, *J* 8.3, C(4′)-H], 7.80 [1H, s, C(2′)-H], 7.93 [1H, s, C(2)-H]; δ_c (75 MHz, DMSO-d_6) 15.1 (CH_3), 22.1 (2 × CH_3), 40.9 (CH_2), 47.2 (CH), 104.1 (C, aromatic C), 104.4 (C, aromatic C), 110.5 (CH, aromatic CH), 110.7 (CH, aromatic CH), 120.1 (CH, aromatic CH), 120.2 (CH, aromatic CH), 121.65 (CH, aromatic CH), 121.73 (CH, aromatic CH), 122.1 (CH, aromatic CH), 122.2 (CH, aromatic CH), 125.0 (C, aromatic C), 125.3 (C, aromatic C), 127.4 (C, aromatic C), 127.9 (C, aromatic C), 129.3 (CH, aromatic CH), 132.9 (CH, aromatic CH), 135.5 (C, aromatic C), 135.8 (C, aromatic C), 166.3 (C═O), 166.4 (C═O); *m/z* (ESI^+) 399.3 [$(M+H)^+$, 100%]; HRMS (ESI+): Exact mass calculated for $(C_{25}H_{23}N_2O_3)^+$ 399.1709. Found 399.1706.

4.4.11. Synthesis of bisindolyl hydroxymaleimides

General procedure (i): To a solution of maleic anhydride (1 eq.) in anhydrous DMF (30 mL/mmol) was added hydroxylamine hydrochloride (5 eq.) followed by triethylamine (5 eq.). The mixture was then heated to 70 °C and stirred at this temperature for 24 h. After cooling, water (100 mL/mmol) was added and the mixture was extracted with ethyl acetate (3 × 40 mL/mmol). The organic layer was washed with 1 M aqueous HCl (2 × 50 mL/mmol), followed by water (50 mL/mmol) and brine (50 mL/mmol) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated to give a dark red solid.

4.4.11.1. 1-Hydroxy-3-(1H-indol-3-yl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione ***47***. Following general procedure (i) starting from 3-(1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **39** (0.104 g, 0.29 mmol) in anhydrous DMF (8 mL) with hydroxylamine hydrochloride (0.101 g, 1.5 mmol) and triethylamine (0.20 mL, 0.15 g, 1.5 mmol) gave desired product as a dark red solid (83%): m.p. 137–139 °C; ν_{max}/cm^{-1} (KBr) 3332, 2930, 1767, 1706, 1612, 1529, 1460, 1023; δ_H (300 MHz, DMSO-d_6) 3.87 (3H, s, CH_3), 6.61–6.74 [3H, m, C(6, 6′, 7′)-H], 6.83 [1H, d, *J* 8.0, C(7)-H], 6.99 [1H, t, *J* 7.5, C(5)-H], 7.04 [1H, t, *J* 7.5, C(5′)-H], 7.39 [1H, d, *J* 8.0, C(4)-H], 7.44 [1H, d, *J* 8.1, C(4′)-H], 7.77 [1H, d, *J* 7.8, C(2)-H], 7.87 [1H, s, C(2′)-H], 10.45 (1H, s, *N*-OH), 11.74 (1H, bs, NH); δ_c (75 MHz, DMSO-d_6) 32.9 (CH_3), 104.5 (C, aromatic C), 105.5 (C, aromatic C), 110.2 (CH, aromatic CH), 111.9 (CH, aromatic CH), 119.4 (CH, aromatic CH), 119.7 (CH, aromatic CH), 120.8 (CH, aromatic CH), 121.0 (CH, aromatic CH), 121.7 (CH, aromatic CH), 121.8 (CH, aromatic CH), 123.9 (C, aromatic C), 124.0 (C, aromatic C), 125.4 (C, aromatic C), 125.6 (C, aromatic C), 129.3 (CH, aromatic CH), 133.2 (CH, aromatic CH), 135.9 (C, aromatic C), 136.5 (C, aromatic C), 168.5 (2 × C═O); *m/z* (ESI^-) 356.2 [$(M-H)^-$ 50%]. HRMS (ESI^-): Exact mass calculated for $(C_{21}H_{14}N_3O_3)^-$ 356.1035. Found 356.1025.

4.4.11.2. 1-Hydroxy-3,4-bis(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione ***48***. Following general procedure (i) starting from 3,4-bis(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **41** (0.092 g, 0.26 mmol) in anhydrous DMF (10 mL) with hydroxylamine hydrochloride (0.090 g, 1.3 mmol) and triethylamine (0.18 mL, 0.13 g, 1.3 mmol) gave desired product as a dark red solid (0.081 g, 84%): m.p. > 300 °C; ν_{max}/cm^{-1} (KBr) 3224, 1764, 1694, 1610, 1519, 1367, 1097; δ_H (300 MHz, DMSO-d_6) 3.86 (6H, s, 2 × CH_3), 6.67 [2H, t, *J* 7.4, C(6, 6′)-H], 6.76 [2H, d, *J* 7.9, C(7, 7′)-H], 7.05 [2H, t, *J* 7.4, C(5, 5′)-H], 7.44 [2H, d, *J* 8.2, C(4, 4′)-H], 7.84 (2H, s, C(2, 2′)-H], 10.44 (1H, s, *N*-OH); δ_c (75 MHz, DMSO-d_6) 32.9 (2 × CH_3), 104.6 (2C, 2 × aromatic C), 110.2 (2CH, 2 × aromatic CH), 119.6 (2CH, 2 × aromatic CH), 121.1 (2CH, 2 × aromatic CH), 121.8 (2CH, 2 × aromatic CH), 123.7 (2C, 2 × aromatic C), 125.7 (2C, 2 × aromatic C), 133.2 (2CH, 2 × aromatic CH), 136.5 (2C, 2 × aromatic C), 168.4 (2 × C═O); *m/z* (ESI^+) 372.1 [$(M+H)^+$ 100%]. HRMS (ESI^+): Exact mass calculated for $(C_{22}H_{18}N_3O_3)^+$ 372.1348. Found 372.1348.

4.4.11.3. 3-(1-Ethyl-1H-indol-3-yl)-1-hydroxy-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione ***49***. Following general procedure (i) starting from 3-(1-ethyl-1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **42** (0.103 g, 0.27 mmol) in anhydrous DMF (10 mL) with hydroxylamine hydrochloride (0.094 g, 1.3 mmol) and triethylamine (0.2 mL, 0.14 g, 1.3 mmol) gave desired product as a dark red solid (0.072 g, 69%): m.p. = 236–238 °C; ν_{max}/cm^{-1} (KBr) 3239, 2932, 1763, 1699, 1523, 1219, 739; δ_H (300 MHz, DMSO-d_6) 1.30 (3H, t, *J* 7.6, $CH_2C\underline{H}_3$), 3.87 (3H, s, CH_3), 4.24 (2H, q, *J* 7.1 $C\underline{H}_2CH_3$), 6.62–6.69 [2H, m, C(6′, 7′)-H], 6.75 [1H, t, *J* 7.6, C(6)-H], 6.96 [1H, d, *J* 8.15, C(7)-H], 7.03–7.11 [2H, m, (5, 5′)-H], 7.45 [1H, d, *J* 8.15, C(4′)-H], 7.50 [1H, d, 8.49, C(4)-H], 7.74 [1H, s, C(2)-H], 7.90 [1H, s, C(2′)-H], 10.47 (*N*-OH); δ_c (75 MHz, DMSO-d_6) 15.2 ($CH_2C\underline{H}_3$), 32.9 (CH_3), 40.7 ($C\underline{H}_2CH_3$), 104.4 (C, aromatic C), 104.8 (C, aromatic C), 110.18 (CH, aromatic CH), 110.23 (CH, aromatic CH), 119.65 (CH, aromatic CH), 119.67 (CH, aromatic CH), 121.2 (CH, aromatic CH), 121.3 (CH, aromatic CH), 121.8 (2CH, 2 × aromatic CH), 123.4 (C, aromatic C), 124.1 (C, aromatic C), 125.2 (C, aromatic C), 126.0 (C, aromatic C), 131.6 (CH, aromatic CH), 133.4 (CH, aromatic CH), 135.4 (C, aromatic C), 136.6 (C, aromatic C), 168.37 (C═O), 168.41 (C═O); *m/z* (ESI^+) 386.2 [$(M+H)^+$ 100%]. HRMS (ESI^+): Exact mass calculated for $(C_{23}H_{20}N_3O_3)^+$ 386.1505. Found 386.1509.

4.4.11.4. 6-{3-[1-Hydroxy-4-(1-methyl-1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-1H-indol-1-yl}hexanenitrile ***50***. Following general procedure (i) starting from 6-(3-(4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl)-1*H*-indol-1-yl)hexanenitrile **43** (0.103 g, 0.235 mmol) in anhydrous DMF (8 mL) with hydroxylamine hydrochloride (0.079 g, 1.18 mmol) and triethylamine (0.16 mL, 0.12 g, 1.2 mmol) gave desired product as a dark red solid (0.087 g, 82%): m.p. 123–125 °C; ν_{max}/cm^{-1}(KBr) 3229, 2935, 2244, 1769, 1708, 1529, 1099, 742; δH (300 MHz, DMSO-d_6) 1.22–1.39 [2H, m, C(4″)-H2], 1.57 [2H, quin, *J* 7.27, C(3″)-H2], 1.73 [2H, quin, *J* 7.04, C(5″)-H2], 2.45 [2H, t, *J* 7.2, C(2″)-H2], 3.88 (3H, s, CH3), 4.23 [2H, t, *J* 6.9, C(6″)-H2], 6.62–6.68 [2H, m, C(6′, 7′)-H], 6.73 [1H, t, *J* 7.7, C(6)-H], 6.94 [1H, d, *J* 7.8, C(7)-H], 7.01–7.10 [2H, m, (5, 5′)-H], 7.45 [1H, d, *J* 8.0, C(4′)-H], 7.50 [1H, d, *J* 8.2, C(4)-H], 7.75 [1H, s, C(2)-H], 7.90 [1H, s, C(2′)-H], 10.43 (1H, s, NOH); δc (75 MHz, DMSO-d_6) 16.0 (CH2), 24.3 (CH2), 25.2 (CH2), 28.8 (CH2), 32.9 (CH3), 45.9 (CH2), 104.4 (C, aromatic C), 104.9 (C, aromatic C), 110.2 (CH, aromatic CH), 110.3 (CH, aromatic CH), 119.6 (2 × CH, 2 aromatic CH), 120.6 (CN), 121.1 (CH, aromatic CH), 121.2 (CH, aromatic CH), 121.77 (CH, aromatic CH), 121.81 (CH, aromatic CH), 123.5 (C, aromatic C), 124.2 (C, aromatic C), 125.3 (C, aromatic C), 126.0 (C, aromatic C), 132.0 (CH, aromatic CH), 133.3 (CH, aromatic CH), 135.7 (C, aromatic C), 136.6 (C, aromatic C), 168.3 (C═O), 168.4 (C═O); *m/z* (ESI-) 451.3 [$(M-H)^-$ 50%]; HRMS (ESI-): Exact mass calculated for $(C_{27}H_{23}N_4O_3)^-$ 451.1770. Found 451.1783.

4.4.11.5. 6-{3-[1-Hydroxy-4-(1-methyl-1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-1H-indol-1-yl}hexanoic acid ***51***. Following general procedure (i) starting from 6-{3-[4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H*-indol-1-yl}hexanoic acid **44** (0.101 g, 0.221 mmol) in anhydrous DMF (8 mL) with hydroxylamine hydrochloride (0.076 g, 1.11 mmol) and triethylamine (0.15 mL, 0.11 g, 1.1 mmol) gave desired compound as a dark red solid (0.085 g, 79%): m.p. 196–197 °C; ν_{max}/cm^{-1} (KBr) 3114, 2935, 1710, 1609, 1529, 1098, 741; δH (300 MHz, DMSO-d_6) 1.16–1.29 [2H, m, C(4″)-H2], 1.51 [2H, quin, *J* 7.4, C(3″)-H2], 1.70 [2H, quin, *J* 7.1, C(5″)-H2], 2.17 [2H, t, 7.3, C(2″)-H2], 3.87 (3H, s, CH3), 4.21 [2H, t, *J* 6.7, C(6″)-H2], 6.58–6.66 [2H, m, C(6′, 7′)-H], 6.72 [1H, t, *J* 7.4, C(6)-H], 6.93 [1H, d, *J* 8.0, C(7)-H], 7.00–7.09 [2H, m, (5, 5′)-H], 7.43 [1H, d, *J* 8.3, C(4′)-H], 7.48 [1H, d, *J* 8.3, C(4)-H], 7.73 [1H, s, C(2)-H], 7.90 [1H, s, C(2′)-

H], 10.43 (1H, bs, NH), 11.99 (1H, bs, COOH); δc (75 MHz, DMSO-d_6) 24.0 (CH_2), 25.6 (CH_2), 29.3 (CH_2), 32.9 (CH_3), 33.5 (CH_2), 45.6 (CH_2), 104.4 (C, aromatic C), 104.8 (C, aromatic C), 110.17 (CH, aromatic CH), 110.24 (CH, aromatic CH), 119.57 (CH, aromatic CH), 119.59 (CH, aromatic CH), 121.10 (CH, aromatic CH), 121.12 (CH, aromatic CH), 121.8 (2CH, 2 × aromatic CH), 123.5 (C, aromatic C), 124.3 (C, aromatic C), 125.3 (C, aromatic C), 126.0 (C, aromatic C), 132.0 (CH, aromatic CH), 133.3 (CH, aromatic CH), 135.7 (C, aromatic C), 136.6 (C, aromatic C), 168.4 (C═O), 168.4 (C═O), 174.3 (COOH); *m/z* (ESI +) 472.2 $[(M+H)^+$ 100%]; HRMS (ESI+): Exact mass calculated for $(C_{27}H_{26}N_3O_5)^+$ 472.1872. Found 472.1864.

4.4.11.6. 1-Hydroxy-3-(1H-indol-3-yl)-4-(1-isopropyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione ***52***. Following general procedure (i) starting from 3-(1*H*-indol-3-yl)-4-(1-isopropyl-1*H*-indol-3-yl)furan-2,5-dione **40** (0.120 g, 0.31 mmol) in anhydrous DMF (8 mL) with hydroxylamine hydrochloride (0.11 g, 1.6 mmol) and triethylamine (0.23 mL, 0.17 g, 1.6 mmol) gave desired product as a dark red solid (0.097 g, 77%): m.p. 106–109 °C; ν_{max}/cm^{-1} (KBr) 3118, 3135, 2970, 1812, 1745, 1627, 1529, 1247; δ_H (300 MHz, DMSO-d_6) 1.38 (6H, d, *J* 6.6, 2 × CH_3), 4.78 [1H, sept, *J* 6.7 C<u>H</u>$(CH_3)_2$], 6.63 (2H, m, C(6, 7)-H], 6.78 [1H, t, *J* 7.2, C(6′)-H], 6.96–7.10 [3H, m, C(5, 5′, 7′)-H], 7.39 [1H, d, *J* 8.1, C(4)-H], 7.53 [1H, d, *J* 8.3, C(4′)-H], 7.69 [1H, s, C(2′)-H], 7.83 [1H, d, *J* 2.8, C(2)-H], 10.42 (1H, s, *N*-OH), 11.74 (1H, s, N−H); δ_c (75 MHz, DMSO-d_6) 22.1 [CH(<u>C</u>$H_3)_2$], 47.0 [<u>C</u>H$(CH_3)_2$], 105.0 (C, aromatic C), 105.2 (C, aromatic C), 110.3 (CH, aromatic CH), 111.9 (CH, aromatic CH), 119.5 (CH, aromatic CH), 119.8 (CH, aromatic CH), 121.1 (CH, aromatic CH), 121.3 (CH, aromatic CH), 121.71 (CH, aromatic CH), 121.73 (CH, aromatic CH), 123.6 (C, aromatic C), 124.6 (C, aromatic C), 124.9 (C, aromatic C), 125.9 (C, aromatic C), 128.2 (CH, aromatic CH), 129.8 (CH, aromatic CH), 135.3 (C, aromatic C), 136.1 (C, aromatic C), 168.37 (C = O), 168.44 (C═O); *m/z* (ESI^+) 386.4 $[(M+H)^+$, 100%]; HRMS (ESI+): Exact mass calculated for $(C_{23}H_{20}N_3O_3)^+$ 386.1505. Found 386.1495.

4.4.11.7. 1-Hydroxy-3-(1-isopropyl-1H-indol-3-yl)-4-(1-methyl-1H-Indol-3-yl)-1H-pyrrole-2,5-dione ***53***. Following general procedure (i) starting from 3-(1-isopropyl-1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **45** (0.099 g, 0.26 mmol) in anhydrous DMF (8 mL) with hydroxylamine hydrochloride (0.089 g, 1.29 mmol) and triethylamine (0.18 mL, 0.13 g, 1.29 mmol) gave desired product as a dark red solid (0.096 g, 93%): m.p. 84–86 °C; ν_{max}/cm^{-1} (KBr) 3118, 2930, 1712, 1654; δ_H (300 MHz, DMSO-d_6) 1.37 (6H, d, *J* 6.6, 2 × CH_3), 4.78 [1H, sept, *J* 6.6, C<u>H</u>$(CH_3)_2$], 6.52 [1H, d, *J* 7.7, C(7)-H] 6.63 [1H, t, *J* 7.2, C(6)-H], 6.80 [1H, t, *J* 7.3, C(6′)-H], 7.03–7.11 [3H, m, C(5, 5′, 7)-H], 7.45 [1H, d, *J* 8.2, C(4)-H], 7.54 [1H, d, *J* 8.5, C(4′)-H], 7.67 [1H, s, C(2′)-H], 7.93 [1H, s, C(2)-H], 10.48 (1H, s, N-OH); δ_c (75 MHz, DMSO-d_6) 22.1 (2 × CH_3), 32.9 [<u>C</u>H$(CH_3)_2$], 47.0 (CH_3), 104.3 (C, aromatic C), 105.1 (C, aromatic C), 110.2 (CH, aromatic CH), 110.3 (CH, aromatic CH), 119.7 (CH, aromatic CH), 119.8 (CH, aromatic CH), 121.3 (2CH, 2 × aromatic CH), 121.75 (CH, aromatic CH), 121.77 (CH, aromatic CH), 123.4 (C, aromatic C), 124.6 (C, aromatic C), 124.9 (C, aromatic C), 126.0 (C, aromatic C), 128.2 (CH, aromatic CH), 133.6 (CH, aromatic CH), 135.3 (C, aromatic C), 136.7 (C, aromatic C), 168.3 (C═O), 168.4 (C═O); *m/z* (ESI^+) 400.2 $[(M+H)^+$, 100%]; HRMS (ESI +): Exact mass calculated for $(C_{24}H_{22}N_3O_3)^+$ 400.1661. Found 400.1651.

4.4.11.8. 3-(1-Ethyl-1H-indol-3-yl)-1-hydroxy-4-(1-isopropyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione ***54***. Following general procedure (i) starting from 3-(1-ethyl-1*H*-indol-3-yl)-4-(1-isopropyl-1*H*-indol-3-yl)furan-2,5-dione **46** (0.068 g, 0.17 mmol) in anhydrous DMF (8 mL) with hydroxylamine hydrochloride (0.059 g, 0.85 mmol) and triethylamine (0.12 mL, 0.87 g, 0.85 mmol) gave desired product as a dark red solid (0.056 g, 80%): m.p. 83–85 °C; ν_{max}/cm^{-1} (KBr) 3125, 2975, 1769, 1713, 1527, 1214, 741; δ_H (300 MHz, DMSO-d_6,) 1.34 (3H, t, *J* 7.2, CH_2C<u>H</u>$_3$) 1.39 (6H, d, *J* 6.6, 2 × CH_3), 4.27 (2H, q, *J* 7.2, C<u>H</u>$_2$$CH_3$), 4.79 [1H, sept, *J* 6.6 C<u>H</u>$(CH_3)_2$], 6.66–6.72 [2H, m, C(6, 7)-H], 6.76 [1H, t, *J* 7.6, C(6′)-H], 6.95 [1H, d, *J* 7.8, C(7′)-H], 7.04–7.11 [2H, m, C(5, 5′)-H], 7.50 [1H, d, *J* 8.3, C(4)-H], 7.55 [1H, d, *J* 8.4, C(4′)-H], 7.74 [1H, s, C(2′)-H], 7.85 [1H, s, C(2)-H], 10.47 (1H, s, *N*-OH), δ_c (75 MHz, DMSO-d_6) 15.2 (CH_3), 22.1 (2 × CH_3), 40.7 (CH_2), 47.0 (CH), 104.6 (C, aromatic C), 105.0 (C, aromatic C), 110.2 (CH, aromatic CH), 110.4 (CH, aromatic CH), 119.7 (CH, aromatic CH), 119.8 (CH, aromatic CH), 121.38 (CH, aromatic CH), 121.44 (CH, aromatic CH), 121.7 (CH, aromatic CH), 121.8 (CH, aromatic CH), 123.9 (C, aromatic C), 124.3 (C, aromatic C), 125.3 (C, aromatic C), 125.7 (C, aromatic C), 128.4 (CH, aromatic CH), 131.9 (CH, aromatic CH), 135.4 (C, aromatic C), 135.6 (C, aromatic C), 168.3 (C═O), 168.4 (C═O); *m/z* (ESI^+) 414.2 $[(M+H)^+$, 100%]; HRMS (ESI+): Exact mass calculated for $(C_{25}H_{24}N_3O_3)^+$ 414.1818. Found 414.1814.

4.4.11.9. 19-Hydroxy-6,7,8,9,10,11-hexahydro-5,21:12,17-dimetheno-18H-dibenzo[i,o]pyrrolo[3,4-l][1,8]diazacyclohexadecine-18,20(19H)-dione ***55***. Following general procedure (i) starting from 6,7,8,9,10,11-hexahydro-5,21:12,17-dimethenodibenzo[*i,o*]furo[3,4-*l*][1,8] diazacyclohexadecine-18,20-dione **27** (0.151 g, 0.37 mmol) in anhydrous DMF (10 mL) with hydroxylamine hydrochloride (0.127 g, 1.8 mmol) and triethylamine (0.26 mL, 0.19 g, 1.8 mmol) gave desired product as a purple solid (0.129 g, 82%): m.p. > 300 °C; ν_{max}/cm^{-1} (KBr) 3373, 2924, 1768, 1712, 1619, 1534, 1470, 1390, 735; δ_H (300 MHz, DMSO-d_6) 1.06 [4H, bs, C(8, 9)-H_2], 1.80 [4H, bs, C(7, 10)-H_2], 4.12–4.22 [4H, m, C(6, 11)-H_2], 7.14 [2H, ddd, *J* 7.9, 7.2, 1.0, C(2, 15)-H], 7.21 [2H, ddd, *J* 8.2, 7.1, 1.2, C(3, 14)-H], 7.39 [2H, s, C(22, 23)-H], 7.51 [2H, d, *J* 7.8, C(4, 13)-H], 7.81 [2H, dd, *J* 7.1, 0.9, C(1, 16)-H], 10.47 (*N*-O<u>H</u>); δ_c (75 MHz, DMSO-d_6) 22.7 (2 × CH_2), 27.3 (2 × CH_2), 44.2 (2 × CH_2), 102.5 (2C, 2 × aromatic C), 110.5 (2CH, 2 × aromatic CH), 120.2 (2CH, 2 × aromatic CH), 121.4 (2CH, 2 × aromatic CH), 121.7 (2CH, 2 × aromatic CH), 126.8 (2C, 2 × aromatic C), 128.6 (2C, 2 × aromatic C), 131.9 (2CH, 2 × aromatic CH), 135.2 (2C, 2 × aromatic C), 167.7 (2 × C═O); *m/z* (ESI+) 426.2 $[(M+H)^+$ 10%]; HRMS (ESI+): Exact mass calculated for $(C_{26}H_{24}N_3O_3)^+$ 426.1818. Found 426.1827.

4.4.11.10. 6,6′-[3,3′-(1-Hydroxy-2,5-dioxo-2,5-dihydro-1H-pyrrole-3,4-diyl)bis(1H-indole-3,1-diyl)]dihexanenitrile ***56***. Following general procedure (i) starting from 6,6′-[3,3′-(2,5-dioxo-2,5-dihydrofuran-3,4-diyl)bis(1*H*-indole-3,1-diyl)]dihexanenitrile **28** (0.084 g, 0.162 mmol) in anhydrous DMF (8 mL) with hydroxylamine hydrochloride (0.056 g, 0.809 mmol) and triethylamine (0.11 mL, 0.08 g, 0.81 mmol) gave desired product as a dark red solid (0.076 g, 87%): m.p. 83–84 °C; ν_{max}/cm^{-1} (KBr) 3257, 2936, 2244, 1771, 1713, 1530, 1392, 1159, 743; δH (300 MHz, DMSO-d_6) 1.25–1.38 [4H, m, 2 × C(4″)-H2], 1.57 [4H, quin, *J* 7.4, 2 × C(3″)-H2], 1.75 [4H, quin, *J* 7.2, 2 × C(5″)-H2], 2.45 [4H, t, *J* 7.1, 2 × C(2″)-H2], 4.25 [4H, t, *J* 6.9, × C(6″)-H2], 6.68 [2H, t, *J* 7.4, C(6, 6′)-H], 6.80 [2H, d, *J* 7.7, C(7, 7′)-H], 7.05 [2H, t, *J* 7.6, C(5, 5′)-H], 7.50 [2H, d, *J* 8.3, C(4, 4′)-H], 7.82 [2H, s, C(2, 2′)-H], 10.43 (*N*-OH); δc (75 MHz, DMSO-d_6) 16.0 (2 × CH_2), 24.3 (2 × CH_2), 25.2 (2 × CH_2), 28.7 (2 × CH_2), 45.4 (2 × CH_2), 104.7 (2C, 2 × aromatic C), 110.3 (2CH, 2 × aromatic CH), 119.6 (2CH, 2 × aromatic CH), 120.5 (2 × CN), 121.2 (2CH, 2 × aromatic CH), 121.8 (2CH, 2 × aromatic CH), 124.0 (2C, 2 × aromatic C), 125.6 (2C, 2 × aromatic C), 132.1 (2CH, 2 × aromatic CH), 135.8 (2C, 2 × aromatic C), 168.3 (2 × C═O); *m/z* (ESI+) 534.3 $[(M+H)^+$ 40%]; HRMS (ESI+): Exact mass calculated for $(C_{32}H_{32}N_5O_3)^+$ 534.2505. Found 534.2496.

4.4.11.11. 6-{3-[1-Hydroxy-4-(1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-1Hindol-1-yl}hexanoic acid ***57***. Following general procedure (i) starting from 6-{3-[4-(1*H*indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H*-indol-1-yl}hexanoic acid **30** (0.120 g,

0.271 mmol) in anhydrous DMF (10 mL) with hydroxylamine hydrochloride (0.094 g, 1.36 mmol) and triethylamine (0.19 mL, 0.14 g, 1.4 mmol) gave desired product as a dark red solid (0.107 g, 86%): m.p. 133–135 °C; ν_{max}/cm^{-1} (KBr) 3372, 2935, 1768, 1703, 1612, 1527, 1391, 1097, 735; δH (400 MHz, DMSO-d_6) 1.18–1.27 [2H, m, C(4″)-H2], 1.51 [2H, quin, *J* 7.5, C(3″)-H2], 1.71 [2H, quin, *J* 7.3, C(5″)-H2], 2.18 [2H, t, *J* 7.4, C(2″)-H], 4.22 [2H, t, *J* 6.9, C(6″)-H], 6.60 [1H, t, *J* 7.6, C(6)-H], 6.67–6.73 [2H, m, C(6′, 7)-H], 6.87 [1H, d, *J* 8.2, C(7′)-H], 6.98 [1H, t, *J* 7.6, C(5)-H], 7.04 [1H, t, *J* 7.7, C(5′)-H], 7.38 [1H, d, *J* 8.2, C(4)-H], 7.48 [1H, d, *J* 8.3, C(4′)-H], 7.77 [1H, s, C(2′)-H], 7.82 [1H, d, *J* 2.9, C(2)-H], 10.45 (1H, s, NOH), 11.75 (1H, bd, *J* 2.4, NH), 12.02 (1H, bs, COOH); δc (75 MHz, DMSO-d_6) 24.0 (CH_2), 25.6 (CH_2), 29.3 (CH_2), 33.5 (CH_2), 45.6 (CH_2), 104.7 (C, aromatic C), 105.4 (C, aromatic C), 110.2 (CH, aromatic CH), 111.8 (CH, aromatic CH), 119.4 (CH, aromatic CH), 119.6 (CH, aromatic CH), 120.9 (CH, aromatic CH), 121.1 (CH, aromatic CH), 121.7 (CH, aromatic CH), 121.8 (CH, aromatic CH), 123.8 (C, aromatic C), 124.6 (C, aromatic C), 125.0 (C, aromatic C), 125.9 (C, aromatic C), 129.5 (CH, aromatic CH), 132.0 (CH, aromatic CH), 135.8 (C, aromatic C), 136.0 (C, aromatic C), 168.39 (C═O), 168.44 (C═O), 174.3 (COOH); *m/z* (ESI+) 458.2 [$(M+H)^+$ 100%]; HRMS (ESI+): Exact mass calculated for $(C_{26}H_{24}N_3O_5)^+$ 458.1716. Found 458.1700.

4.4.11.12. 6,6′-[3,3′-(2,5-Dioxo-2,5-dihydro-1H-pyrrole-3,4-diyl)bis(1H-indole-3,1-diyl)]dihexanenitrile ***58***. To a solution of 6,6′-[3,3′-(2,5-dioxo-2,5-dihydrofuran-3,4-diyl)bis(1*H*-indole-3,1-diyl)]dihexanenitrile **28** (1.302 g, 2.51 mmol) in anhydrous DMF (10 mL) was added 1,1,1,3,3,3 hexamethyldisilazane (3.53 mL, 0.77 g, 28.4 mmol), followed by methanol (0.34 mL, 0.27 g, 8.4 mmol) and the reaction mixture was stirred at room temperature for 8 h. Water (40 mL) was then added and the product extracted into ethyl acetate (3 × 30 mL). The combined organic layers were washed with water (3 × 40 mL) followed by brine (40 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give a red solid (1.165 g, 90%): m.p. 82–84 °C; ν_{max}/cm^{-1} (KBr) 3274, 2936, 2244, 1704, 1533, 1334, 1160, 743; δH (300 MHz, $CDCl_3$) 1.39–1.51 [4H, m, 2 × C(4″)-H_2], 1.65 [4H, quin, *J* 7.3, 2 × C(3″)-H_2], 1.87 [4H, quin, *J* 7.3, 2 × C(5″)-H_2], 2.30 [4H, t, *J* 7.0, 2 × C(2″)-H_2], 4.15 [4H, t, *J* 7.0, 2 × C(6″)-H_2], 6.76 [2H, t, *J* 7.4, C(6, 6′)-H], 6.99 [2H, d, *J* 8.0, C(7, 7′)-H], 7.11 [2H, t, *J* 7.6, C(5, 5′)-H], 7.28 [2H, d, *J* 8.3, C(4, 4′)-H], 7.43 (1H, s, NH), 7.63 [1H, s, C(2, 2′)-H]; δc (75 MHz, $CDCl_3$) 17.0 (2 × CH_2), 25.0 (2 × CH_2), 25.9 (2 × CH_2), 29.2 (2 × CH_2), 46.3 (2 × CH_2), 105.9 (2C, 2 × aromatic C), 109.5 (2CH, 2 × aromatic CH), 119.4 (2 × CN), 120.1 (2CH, 2 × aromatic CH), 122.3 (2CH, 2 × aromatic CH), 122.4 (2CH, 2 × aromatic CH), 126.2 (2C, 2 × aromatic C), 127.8 (2C, 2 × aromatic C), 131.7 (2CH, 2 × aromatic CH), 136.2 (2C, 2 × aromatic C), 172.3 (2 × C═O); *m/z* (ESI+) 518.2 [$(M+H)^+$ 100%]; HRMS (ESI+): Exact mass calculated for $(C_{32}H_{32}N_5O_2)^+$ 518.2556. Found 518.2554.

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Author contributions

HJW/KOS/MMC/LTP performed the research in relation to the synthesis and characterization on novel compounds. TR/SB/SR designed and performed the kinase assays. PM reviewed the draft manuscript; FOM designed the research and drafted the manuscript.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmc.2018.07.012. These data include MOL files and InChiKeys of the most important compounds described in this article.

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