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Synthesis and anti-leukaemic activity of pyrrolo[3,2,1-hi]indole-1,2- diones, pyrrolo[3,2,1-ij]quinoline-1,2-diones and other polycyclic isatin derivatives

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Abstract

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Keywords

pyrrolo, derivatives, isatin, polycyclic, other, quinoline, ij, diones, 3, indole, 2, hi, 1, synthesis, anti, leukaemic, activity, CMMB

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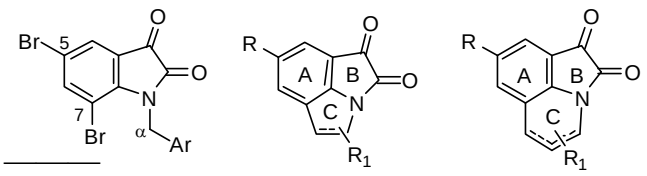
ABSTRACT

To further expand the structure-cytotoxic activity relationships of isatin derivatives and to reduce flexibility in substituent groups at nitrogen, 20 analogues incorporating a ring system between the N1 and C7 atoms of isatin were prepared using a variety of synthetic strategies. This yielded pyrroloindole-, pyrroloquinoline-, pyrroloacridine-, pyrrolophenanthridine- and benzopyrrolophenanthridine-based systems with embedded isatin moieties, the latter possessing a novel carbon skeleton. These compounds were subsequently assessed for their *in vitro* cytotoxicity against human U937 lymphoma cells, with the brominated pyrroloacridine dione **27** showing the most promising activity (IC_{50} 3.01 μM) after 24 h.

1. Introduction

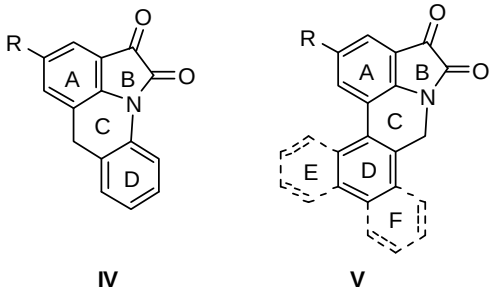
Isatin has been derivatised at the N1, C2, C3, C5, C6 and C7 positions in an attempt to increase its biological activity, in particular its cytotoxicity.[1] *N*-Substituted arylalkyl isatin derivatives of type **I** (Fig. 1) showed excellent potency but it was thought that restraining conformational mobility in the substituent would further improve activities in these isatins, based on improvements in the activity of other anti-cancer agents through conformational restrictions.[2] In order to achieve this, we initially considered target systems with a 5- or 6-membered ring present involving N1 and C7 in the isatin (systems of type **II** or **III** respectively; Fig. 1). In these systems the bromo substituent would be replaced by a similarly hydrophobic methylene or methine group and rotation about the *N*-αC bond would be restricted. Systems of type **II** and **III** correspond to pyrrolo[3,2,1-*hi*]indole-1,2-dione (6,5,5-tricyclic) and pyrrolo[3,2,1-*ij*]quinoline-1,2-dione (6,5,6-tricyclic) derivatives respectively. To facilitate comparison between previously reported *N*-alkylisatins[1c] and the new tricyclic isatins, a bromo substituent (R) was to be incorporated to improve cytotoxicity,[1b, 3] and R_1 was to be H, alkyl, or aryl. Later target systems envisaged incorporating elements of the aryl groups in **I** in further fused rings to afford targets **IV**(pyrrolo[3,2,1-*de*]acridine-1,2-diones) and **V** (pyrrolo[3,2,1-*de*]phenanthridine- and benzo[*j*]or[*l*]pyrrolo[3,2,1-*de*]phenanthridine-4,5-diones) (Fig. 2).



Ar = Ph; substituted Ph; naphthyl

R = H, Br
R_1 = H, alkyl, aryl

Fig. 1. *N*-Substituted isatins and initial ring fused target systems.



IV **V**

Fig. 2. Further ring fused target systems with an embedded isatin unit.

While the pyrrolo[3,2,1-*hi*]indole scaffold is well known in the scientific literature, there are only three currently

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known examples of pyrrolo[3,2,1-*hi*]indole-1,2-diones.[4] This may be attributed to the fact that 6,5,5-fused indole ring systems are highly strained.[5] Similarly, the pyrrolo[3,2,1-*ij*]quinoline nucleus is established[6] and many compounds containing this scaffold have also been reported to exhibit a broad spectrum of biological activities including analgesic, anti-pyretic, anti-inflammatory,[6a] anti-hyperlipidemic, anti-hypertensive,[7] anti-epileptic, anti-obesity[8] and anti-cancer[9] activities. Approximately 50 of the analogous isatin-based pyrrolo[3,2,1-*ij*]quinoline-1,2-diones have been reported in the literature to date. Structures of type **IV**[10] and the ABCDE ring system in type **V**[11] are rare in the literature, while the ABCDF ring system in type **V** represents a new heterocyclic scaffold. The ABCD ring system in type **V** is well known, however, and is present in many natural products including the *Amaryllidaceae* alkaloids.[6a, 12]

Two general approaches were considered for accessing the target systems. The first of these (route (i)) was based on constructing the 5-membered dione ring B from an amine precursor while the second (route (ii)), involved C7-N annulation from an isatin precursor (Fig. 3). The outcomes from these two approaches, along with the cytotoxic evaluation of the products, are now reported in this paper.

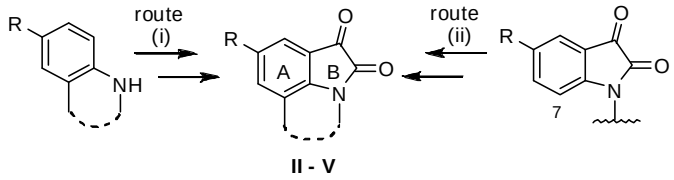


Fig. 3. General synthetic strategies to the target systems **II-V**.

2. Results and discussion

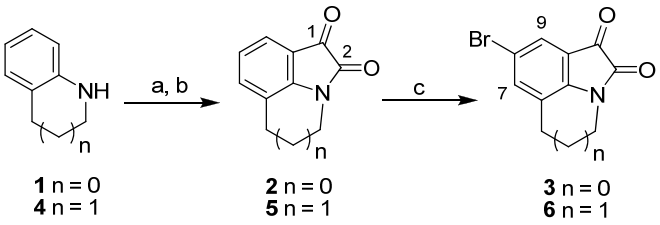
*2.1. Pyrroloindole-dione derivatives **II***

The Stolle isatin synthesis (route (i), Fig. 3), which involves a crucial intramolecular Friedel-Crafts acylation in the penultimate ring forming step, was attempted to access these derivatives with a 5-membered ring fusion. This synthetic approach has been employed to prepare a variety of isatins[13] including *N*-aryl and polycyclic isatins.[14] In 1979, Welstead Jr. *et al*. reported the first synthesis of the tricyclic isatin **2** from indoline (**1**) in 8% overall yield using the Stolle procedure.[4a] The yield was reportedly increased to 22% in 1996 by Norman *et al*.[15] In the current work, our attempts to synthesise pyrrolo[3,2,1-*hi*]indole-1,2-diones using free radical cyclisations and assorted heteroannulations were unsuccessful, however, the synthetic method based on the work by Norman *et al*. provided the desired tricyclic isatin **2**, albeit in low yield (Scheme 1). Subsequent bromination of **2** proceeded smoothly to afford **3** in moderate yield. Because of difficulties encountered in the cyclisation step, the synthesis of ring C-substituted pyrrolo[3,2,1-*hi*]indole-1,2-diones was not pursued. Semi-empirical level computational studies (AM1; Materials Studio 4.4) on **2** and its analogue with a C4-C5 double bond confirmed the expected significant strain in these compounds with positive heats of formation values (ΔH_f 10.9 and 43.2 kcal/mol respectively).

*2.2. Pyrroloquinoline-dione derivatives **III***

2.2.1 Via route(i)

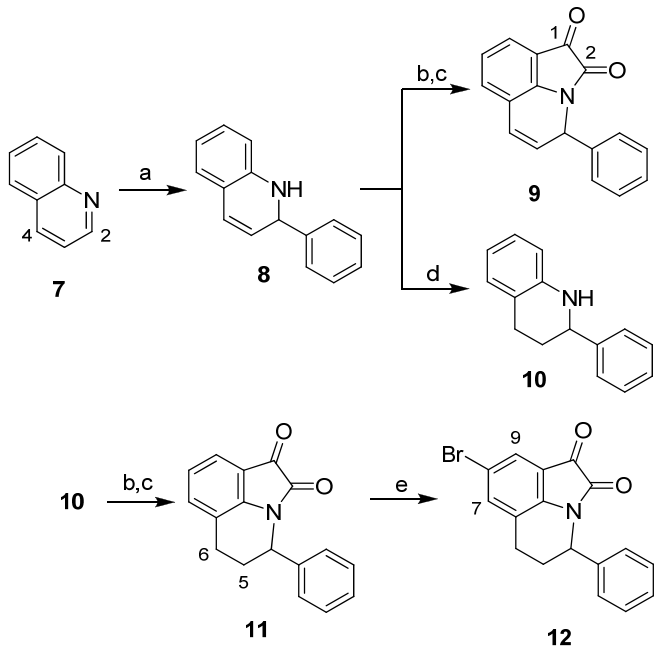
Pyrrolo[3,2,1-*ij*]quinoline-1,2-dione (**5**) was prepared by cyclising 1,2,3,4-tetrahydroquinoline (**4**) following a similar method described for the 5-membered analogue **2**. The compound was subsequently brominated to afford **6** in high yield (Scheme 1).



Scheme 1. (a) n = 0: $(COCl)_2$, DCM, RT, 3 h; n = 1: $(COCl)_2$, THF, reflux, 3 h; (b) n = 0: $AlCl_3$, 110 ºC, 20 min, 15% (over 2 steps); n = 1: $AlCl_3$, DCM, reflux, 3 h, 31% (over 2 steps); (c) Br_2, 95% EtOH, 70-75 ºC, **3** = 33%; **6** = 78%.

To further investigate the cytotoxic SAR of the 6,5,6-fused tricyclic isatins, structures incorporating *N*-arylalkyl substituents were desired. The selective addition of an aryl substituent to the cyclised isatin **5** was expected to be difficult, hence, the required *N*-arylalkyl moiety would need to be incorporated into the structure prior to cyclisation. Reacting quinoline (**7**), with phenylmagnesium bromide (PhMgBr) formed 2-phenyl-1,2-dihydroquinoline (**8**) (Scheme 2). Due to the air-sensitivity and instability of 1,2-dihydroquinolines,[16] **8** was used in further reactions immediately after its preparation and purification was not attempted. Upon treatment with oxalyl chloride and $AlCl_3$, **8** was cyclised to form the novel tricyclic isatin **9** in trace amounts, which may be explained in part by the use of crude 2-phenyl-1,2-dihydroquinoline (**8**). The structure of **9** was confirmed through the ^{13}C NMR spectrum, which displayed two diagnostic carbonyl signals at 157.4 and 182.4 ppm, indicative of C2 and C1 respectively. Due to the low yield of **9** and the likelihood of bromine preferentially attacking the alkene rather than the aromatic ring, synthesis of the 8-bromo analogue was not attempted.

To prepare the saturated analogue of tricyclic isatin **9**, the dihydroquinoline **8** was reduced to 2-phenyl-1,2,3,4-tetrahydroquinoline (**10**), followed by treatment with oxalyl chloride and $AlCl_3$ to afford the tricyclic isatin **11** (Scheme 2), which was also indicated by the appearance of two carbonyl signals at 156.8 and 183.6 ppm in the ^{13}C NMR spectrum. The multiplicities and chemical shifts ascribed to the aromatic protons in the ^{1}H NMR spectrum also pointed to cyclisation having occurred to the activated C8 position in the tetrahydroquinoline nucleus rather than to the *ortho*-position in the pendant phenyl group. The tricyclic isatin **11** was then exposed to Br_2 to yield the racemic brominated tricyclic isatin **12** (Scheme 2).

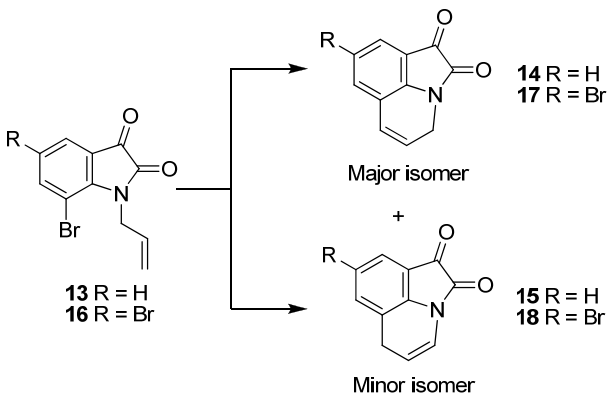


Scheme 2. (a) 1 M PhMgBr, THF, reflux, 18 h, 90% (crude); (b) $(COCl)_2$, THF, reflux, 3.5 h; (c) $AlCl_3$, $CHCl_3$, reflux, 18 h, **9** = 1% (over 2 steps); **10** = 5% (over 2 steps); (d) Na, EtOH, reflux, 2 h, 70%; (e) Br_2, 95% EtOH, 70-75 ºC, 50%.

2.2.2 Via route (ii)

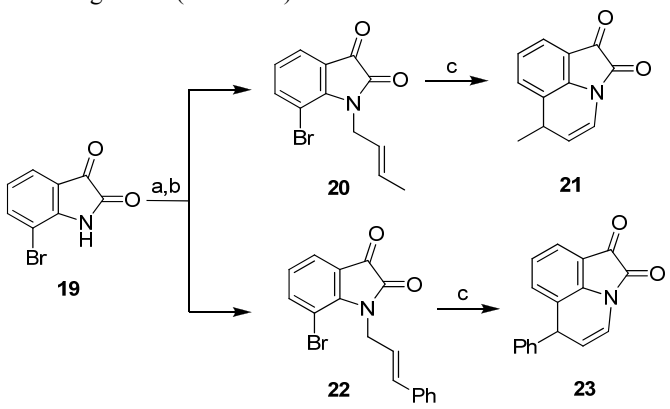
Many intramolecular Heck reactions have previously been applied to indoles to form tricyclic indoles.[5, 17] The formation of a 1,7-annulated isatin with an eight-membered ring through an intramolecular Heck reaction has also been reported,[18] however, the reaction has not been used to form 1,7-annulated isatins containing smaller ring sizes. Subjection of *N*-allyl-7-bromoisatin (**13**) to Heck conditions similar to those reported by Black *et al.*[5] provided the isomeric 6-*endo-trig* products **14** and **15** in a 3:1 ratio (Scheme 3). These products could be isolated by preparative TLC.

To obtain the 8-bromo analogues of **14** and **15** for cytotoxicity testing, *N*-allyl-5,7-dibromoisatin (**16**) was synthesised using the same conditions as for **13**. Subsequent exposure of **16** to intramolecular Heck conditions gave the brominated tricyclic isatins **17** and **18** in moderate yield (Scheme 3). Analysis by ^{1}H NMR spectroscopy revealed the 4*H* and 6*H* isomers (**17** and **18**) had formed in a 3:2 ratio, with the 4*H* isomer **17** again being the predominant.



Scheme 3. TBACl, K_2CO_3, $Pd(OAc)_2$, DMF, 85 ºC, 2 h, **14** = 39%; **15** = 13%; **17** = 29%; **18** = 20%.

The Heck reaction was also applied to other *N*-allylated isatins to further explore the synthesis and cytotoxicity of 6,5,6-tricyclics with phenyl or methyl substituents in ring C (Scheme 4). Thus, 7-bromoisatin (**19**) was reacted with crotyl bromide to yield **20**, followed by an intramolecular Heck reaction to yield the tricyclic isatin **21**, which was formed exclusively as determined by 1D and 2D NMR spectroscopy. Due to the low yield of **21**, synthesis of the analogous 8-bromo analogue was not attempted. 7-Bromoisatin (**19**) was also reacted with cinnamyl bromide to provide **22** and a subsequent intramolecular Heck reaction gave **23** (Scheme 4).

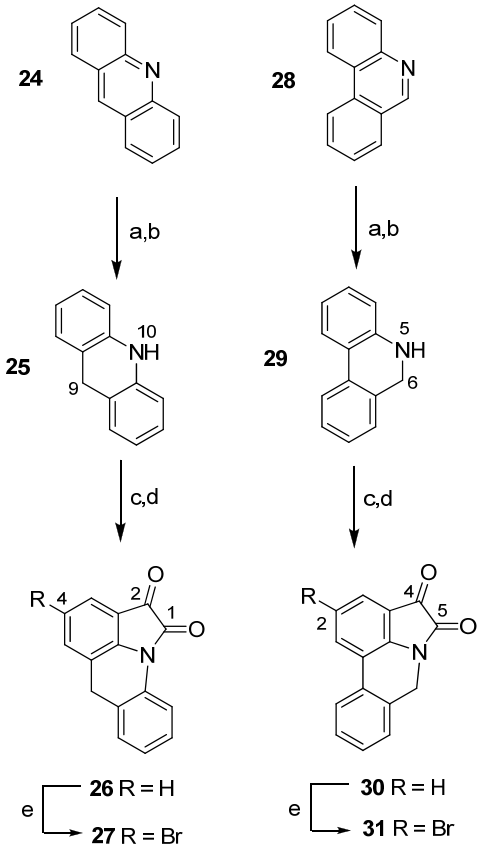


Scheme 4. (a) NaH, DMF, RT, 20 min; (b) crotyl or cinnamyl bromide, KI, 60 ºC, 18 h, **20** = 59% (over 2 steps); **22** = 97% (over 2 steps); (c) TBACl, K_2CO_3, $Pd(OAc)_2$, DMF, 85 ºC, 2 h, **21** = 8%; **23** = 17%.

*2.3. Pyrroloacridine-, pyrrolophenanthridine- and benzopyrrolophenanthridine-dione derivatives **IV-V***

In order to further explore the utility of the isatin scaffold for the development of novel cytotoxins and to incorporate the *N*-benzylic substituent moiety into a fused aromatic ring, it was decided to extend the quinoline nucleus to systems containing benzo or naphtho rings fused onto the heterocyclic ring of quinoline (Fig. 2). Specific targets included pyrroloacridine-diones **26** and **27**, which incorporated the cytotoxic acridine[19] and isatin moieties, as well as the pyrrolophenanthridine-diones **30** and **31** together with the benzopyrrolophenanthridine-dione derivative **36**, and the isomeric systems in **40** and **41**.

One compact route to the acridine- and phenanthridine-based isatins applies the Stolle isatin method (route (i), Fig. 3) to dihydroacridine **25** and dihydrophenanthridine **29**, which can be readily accessed in turn from acridine **24** or phenanthridrine **28** respectively, by reduction[20] with $NaCNBH_3$ (Scheme 5). It has been shown that the addition of a base and 4-(dimethylamino)pyridine (DMAP) to **29** was necessary to achieve good yields of amide derivatives.[21] In this case, the addition of *N*,*N*-diisopropylethylamine (DIPEA) and a catalytic amount of DMAP was employed to facilitate the formation of the oxamide intermediate, before being cyclised directly with $AlCl_3$ to yield **30**. The acridine-based isatin **26** was able to be prepared without the addition of DIPEA and DMAP in the intermediate amide formation step. Bromination of **26** and **30** proceeded smoothly in glacial AcOH to yield **27** and **31** (Scheme 5). The ^{1}H NMR spectrum revealed the absence of the H4 and H2 protons, respectively, in these bromo derivatives.

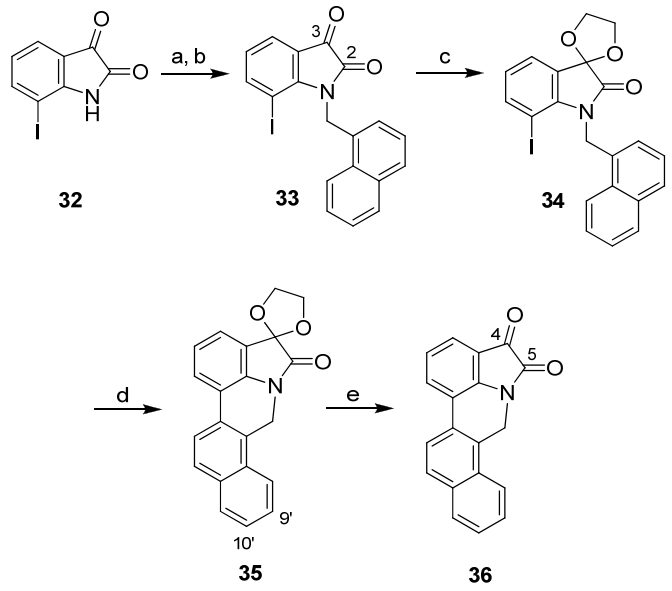


Scheme 5. (a) AcOH, EtOH, reflux, 30 min; (b) $NaCNBH_3$, reflux, 1.5 h, **25** = 47% (over 2 steps); **29** = 77% (over 2 steps); (c) **26** = $(COCl)_2$, THF, reflux, 3.5 h; **30** = $(COCl)_2$, DMAP, DIPEA, THF, reflux, 3.5 h; (d) $AlCl_3$, $CHCl_3$, reflux, 1.5 h, **26** = **30** = 15% (over 2 steps); (e) Br_2, AcOH, 70-75 ºC, **27** = 41%, **31** = 69%.

An alternative approach (route (ii), Fig. 3) which incorporated Pd-mediated intramolecular cyclisation[6a] was used to prepare the other benzo-fused pyrrolophenanthridine targets. These phenanthridine-embedded isatins could be prepared through the Pd-catalysed biaryl intramolecular coupling[22] of *N*-arylalkylisatins such as 7-iodo-*N*-(1-naphthylmethyl)isatin (**33**) and 7-iodo-*N*-(2-naphthylmethyl)isatin (**37**). The precursor **33** was readily accessed by *N*-alkylation of **32**, and the reactive C3 carbonyl[12, 22] was then protected as a cyclic ketal to afford **34** (Scheme 6).

Reaction of the isatin **34** with $Pd(OAc)_2$ enabled an intramolecular biaryl coupling to take place with the possibility of two regioisomers being formed. The ^{1}H NMR spectral data indicated the isomer produced in high yield was the benzopyrrolophenanthridine **35**. The aromatic proton signals for H9' (7.56 ppm) and H10' (7.50 ppm) in **35** were observed as triplets which were coupled to one another.

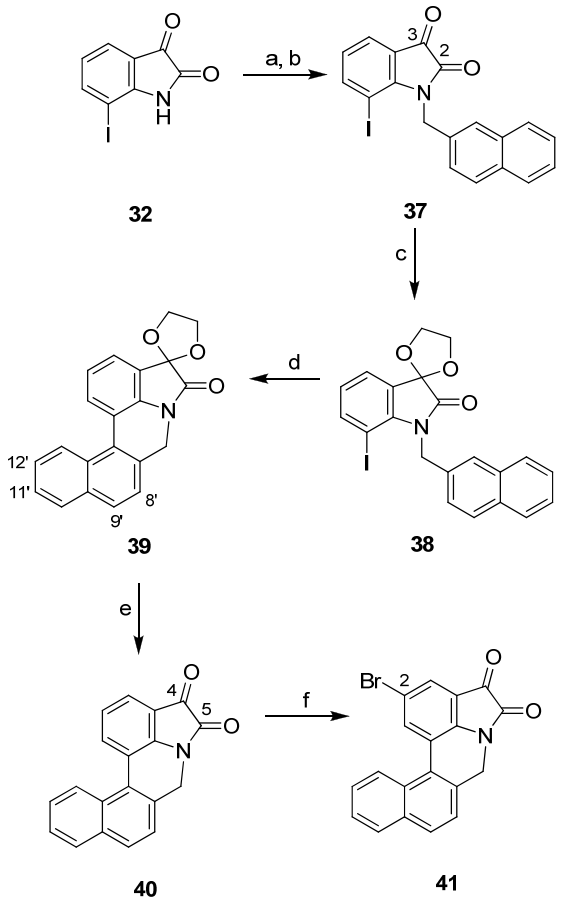
Under acidic conditions the ketal moiety in **35** was hydrolysed to yield the target **36**, a new heterocyclic ring system, as a dark red powder (Scheme 6). The red colour of the compound is strong evidence that the ketal had been destroyed and that the C4 carbonyl, an important element in the chromophore of the isatin core, had been restored. Bromination of **36** was unsuccessful due to the poor solubility of the substrate in organic solvents.



Scheme 6. (a) NaH, DMF, RT, 20 min; (b) 1-(chloromethyl)naphthalene, KI, 60 ºC, 18 h, 58% (over 2 steps); (c) $(CH_2OH)_2$, PTSA, PhMe, reflux, 48 h, 75%; (d) $Pd(OAc)_2$, TBAB, NaOAc, DMF, 90 ºC, 5 h, 86%; (e) 6 M HCl/THF (1:1), reflux, 5 h, 67%.

The isomeric benzopyrrolophenanthridine dione **40** could also be accessed by biaryl coupling using 7-iodo-*N*-(2-naphthylmethyl)isatin (**37**) (Scheme 7). Again, the *N*-naphthylmethylisatin **37** was subjected to Dean-Stark conditions to protect the ketone as a cyclic ketal prior to biaryl coupling. As with the related compound **36**, the intramolecular biaryl coupling of **38** could lead to two regioisomers. A pair of coupled doublets (H8' and H9' at 7.21 and 7.74 ppm respectively) and two triplet signals coupled to each other (H11' and H12' at 7.50 and 7.56 ppm respectively) were observed in the ^{1}H NMR spectrum, which is consistent with the exclusive formation of isomer **39** (Scheme 7). The regioselectivity of this reaction is in accordance with results for similar pyrrolophenanthridine reactions reported by Torres *et al*.[12]

Acid-promoted cleavage of the ketal functionality in **39** afforded, in high yield, the benzopyrrolophenanthridine **40** (Scheme 7). The benzopyrrolophenanthridine **40** had higher solubility in organic solvents relative to the isomer **36** and consequently was readily brominated, to produce the 2-bromo derivative **41** (Scheme 7); no evidence for bromination of the naphthalene moiety was observed.



Scheme 7. (a) NaH, DMF, RT, 20 min; (b) 2-(bromomethyl)naphthalene, KI, 60 ºC, 18 h, 36% (over 2 steps); (c) $(CH_2OH)_2$, PTSA, PhMe, reflux, 64 h, 92%; (d) $Pd(OAc)_2$, TBAB, NaOAc, DMF, 90 ºC, 5 h, 67%; (e) 6 M HCl/THF (1:1), reflux, 5 h, 90%; (f) NBS, CH_3CN, RT, 18 h, 39%.

2.4. Biological results

Preliminary cytotoxicity testing of the 20 tricyclic/polycyclic isatins was carried out on the U937 human histiocytic lymphoma cell line using the CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (MTS assay) (Table 1). This cell line has been utilised previously within our research group[1b-d] to screen the *in vitro* cytotoxicity of isatin derivatives.

Table 1

Cytotoxicity of the tricyclic/polycyclic isatin derivatives against U937 cells after 24 h exposure.

Compound	IC_{50} (μM)[a]	Compound	IC_{50} (μM)
Isatin	565[1b]	**18**	28.6 (± 2.9)
5-Bromoisatin	64.5[1b]	**21**	33.2 (± 9.5)
2	> 577	**23**	77.8 (± 6.2)
3	111 (± 53)	**26**	7.21 (± 0.74)
5	278 (± 23)	**27**	3.01 (± 1.1)
6	8.36 (± 3.5)	**30**	46.4 (± 12)
9	106 (± 30)	**31**	19.7 (± 2.6)
11	99.3 (± 3.9)	**36**	> 114
12	15.3 (± 11)	**40**	23.4 (± 9.8)
14	57.1 (± 14)	**41**	18.2 (± 4.7)
15	59.3 (± 8.6)	**Vinblastine**	6.88[1c]
17	19.3 (± 0.70)		

[a]IC_{50} values were calculated from sigmoidal dose–response curves (variable slope) generated using GraphPad Prism 5.00 (GraphPad Software Inc., San Diego, CA, USA). Values are the mean of at least two independent experiments performed in triplicate ± SEM.

The 6,5,5-fused tricyclic isatin, pyrrolo[3,2,1-*hi*]indole-1,2-dione (**2**) was inactive against U937 cells with an IC_{50} value of > 577 μM (beyond testing limits), while the brominated analogue **3** displayed an IC_{50} value of 111 μM, a 5-fold increase in cytotoxicity. However, isatin and 5-bromoisatin have IC_{50} values of 565 μM and 64.5 μM respectively, against the same cell line,[1b] establishing that the tricyclic analogues **2** and **3** are less active than the parent isatin molecules. Conversely, the 6,5,6-fused tricyclic isatin, pyrrolo[3,2,1-*ij*]quinoline-1,2-dione (**5**), displayed at least a 2-fold increase in cytotoxicity compared to **2** and is more active than the parent molecule, isatin. The brominated derivative **6** exhibited a 33-fold increase in activity when compared to **5**. A similar pattern was observed for polycyclic compounds where the brominated derivatives displayed greater cytotoxicity (up to 2.4 times) than their parent molecules (i.e. **27** vs. **26**; **31** vs. **30**; **41** vs. **40**).

The introduction of a double bond into compounds **14** and **15** led to an approximate 6-fold increase in cytotoxicity when compared to the parent molecule **5**. The inclusion of a phenyl substituent at the C6 position in **23** resulted in decreased cytotoxicity compared to the parent compound **15**, while a methyl substituent at C6 in **21** increased activity compared to compound **15**.

Although more than one structural change was made in compound **12 (**IC_{50} 15.3 μM**)**, it is apparent that restricting rotation in the *N*-benzyl group through ring formation in this way had a detrimental effect on cytotoxic potency in comparison to *N*-benzyl-5,7-dibromoisatin (IC_{50} 1.14 μM; U937).[1c] Similarly, incorporation of a methylene group and part of the naphthyl ring in the ring fusion as in compound **41** (IC_{50} 18.2 μM) resulted in a deterioration of activity compared to 5,7-dibromo-*N*-(2-naphthylmethyl)isatin (IC_{50} 0.74 μM; U937).[1d]

The addition of the extra ring system in **40** increased the cytotoxicity 2-fold compared to **30**, while the introduction of an additional ring in derivative **36** decreased cytotoxic activity at least 2.5-fold compared to **30**. The pyrroloacridine **26** was greater than 6-fold more cytotoxic than the pyrrolophenanthridine **30** and both acridines (**26** and **27**) are highly cytotoxic (IC_{50} < 10 μM). These two compounds were significantly more cytotoxic than any of the tricyclic derivatives shown in Table 1 with the most active compound (**27**) having an IC_{50} value of 3.01 μM against U937 cells after 24 h, which is more potent than the anti-cancer drug vinblastine against the same cell line (Table 1).

3. Conclusions

The successful synthesis of two pyrrolo[3,2,1-*hi*]indole-1,2-diones, together with eleven pyrrolo[3,2,1-*ij*]quinoline-1,2-diones, two pyrrolo[3,2,1-*de*]acridine-1,2-diones and two pyrrolo[3,2,1-*de*]phenanthridine-4,5-diones via the Stolle isatin synthesis or intramolecular Heck reactions is described. Three other benzo[*j*]or[*l*]pyrrolo[3,2,1-*de*]phenanthridine-4,5-diones, the last system being novel, were also prepared utilizing Pd-mediated intramolecular biaryl coupling methodology from a pre-formed substituted isatin.

In vitro toxicity of the synthsised compounds against U937 cells was assessed and generally, the order of cytotoxicity for the compounds described was pyrroloacridines > (benzo)pyrrolophenanthridines > pyrrolo[3,2,1-*ij*]quinoline-1,2-diones > pyrrolo[3,2,1-*hi*]indole-1,2-diones. Bromination at the C5 position of the isatin nucleus increased cytotoxicity relative to the non-brominated analogues in all cases by 2 to 33-fold, similar to previously reported literature.[1a, b] The fused acridine-isatin derivative **27** was identified as a new cytotoxic structural lead for potential future development.

4. Experimental section

4.1. General

Isatin, 1,2,3,4-tetrahydroquinoline, phenanthridine and acridine were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). DCM refers to dichloromethane; PS refers to petroleum spirit (b.p. 40-60 °C). Celite (Celite 545, particle size 0.02-0.1 mm, Merck) was used to remove metal catalysts from reaction mixtures. Flash column chromatography was performed on silica gel 60 (230-400 mesh). Melting points were obtained using a Reichert melting point apparatus and are uncorrected. Geometry optimisation (ΔH_f) of tricyclic isatins was performed using VAMP at semi-empirical level (AM1) on Materials Studio 4.4 (Accelrys Inc., San Diego, CA, USA). Nuclear magnetic resonance (NMR) spectra were acquired on a Varian Unity 300 MHz spectrometer or Varian Inova 500 spectrometer. The symbol † denotes coincident peaks. Low resolution electron ionisation mass spectra (LREI-MS) were obtained using a Shimadzu QP5050 spectrometer; high resolution electron ionisation mass spectra (HREI-MS) were obtained using a Fisons/VG Autospec spectrometer; high resolution electrospray ionisation spectra (HRESI-MS) were obtained using a Waters Q-TOF Ultima spectrometer.

4.2 Synthesis

*4.2.1 4,5-Dihydropyrrolo[3,2,1-hi]indole-1,2-dione (**2**)*

The compound was prepared according to the method of Norman *et al.*[15] to yield **2** as a red powder (845 mg, 15%), m.p. 195-197 °C (lit.[15] 203-207 ºC). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.38 (t, *J* = 7.5 Hz, 2H), 4.09 (t, *J* = 7.5 Hz, 2H), 6.98 (t, *J* = 7.5 Hz, 1H), 7.26 (d, *J* = 7.0 Hz, 1H), 7.48 (d, *J* = 7.0 Hz, 1H). The ^{1}H NMR spectral data coincided with those reported in the literature.[15] LREI-MS: *m/z* (%): 173 (22) [M^+], 145 (100) [M-CO], 117 (63).

*4.2.2 7-Bromo-4,5-dihydropyrrolo[3,2,1-hi]indole-1,2-dione (**3**)*

According to the method of Vine *et al.*,[1b] the cyclised isatin **2** (100 mg, 577 μmol) was dissolved in 95% EtOH (4 mL) and heated to 75 ºC. Br_2 (276 mg, 88.7 μL, 1.73 mmol) was added drop wise while the solution was still hot. The reaction mixture was then cooled on ice, filtered and concentrated. The resulting solid was purified by flash chromatography (100% DCM) to yield **3** as dark red crystals (47.8 mg, 33%), m.p. 162-164 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.38 (t, *J* = 7.5 Hz, 2H), 4.07 (t, *J* = 7.5 Hz, 2H), 7.49 (s, 1H), 7.66 (s, 1H). ^{13}C NMR (DMSO-d_6, 126 MHz): δ 30.7, 46.7, 113.7, 115.3, 124.7, 128.3, 136.1, 155.9, 159.3, 183.1. LREI-MS: *m/z* (%): 251/253 (13) [M^+]^{79}Br/^{81}Br, 223/225 (55) [M-CO], 195/197 (32) [M-CO], 168 (61), 116 (66), 100 (100). HRESI-MS: *m/z* calcd for $C_{10}H_7{}^{79}BrNO_2$ $[M+H]^+$: 251.9660; found 251.9655.

*4.2.3 5,6-Dihydro-4H-pyrrolo[3,2,1-ij]quinoline-1,2-dione (**5**)*

The compound was prepared according to the method of Kojima *et al.*[23] using DCM instead of CS_2 as the solvent, to yield **5** as red crystals (437 mg, 31%), m.p. 195-197 °C (lit.[23] 198-200 °C). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.02-2.06 (m, 2H), 2.77 (t, *J* = 6.0 Hz, 2H), 3.75 (t, *J* = 6.0 Hz, 2H), 6.99 (t, *J* = 7.5 Hz, 1H), 7.32 (d, *J* = 7.5 Hz, 1H), 7.37 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 20.2, 23.9, 38.4, 115.7, 121.9, 123.0, 123.2, 137.1, 147.6, 156.9, 183.9. LREI-MS: *m/z* (%): 187 (70) [M^+], 159 (60) [M-CO], 130 (100). HREI-MS: *m/z* calcd for $C_{11}H_9NO_2$ [M^+]: 187.0633; found 187.0638.

*4.2.4 8-Bromo-5,6-dihydro-4H-pyrrolo[3,2,1-ij]quinoline-1,2-dione (**6**)*

The compound was prepared according to the method for **3** using the isatin **5** (150 mg, 801 μmol) and Br_2 (384 mg, 123 μL, 2.40 mmol) as starting materials. The resulting solid was purified by flash chromatography (100% DCM) to yield **6** as dark red crystals (166 mg, 78%), m.p. 201-203 °C (lit.[24] 185-187 °C). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.02-2.07 (m, 2H), 2.77 (t, *J* = 6.0 Hz, 2H), 3.75 (t, *J* = 5.5 Hz, 2H), 7.47 (s, 1H), 7.49 (s, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 20.3, 23.9, 38.7, 116.3, 117.1, 124.3, 125.9, 139.5, 146.7, 156.4, 182.8. LREI-MS: *m/z* (%): 265/267 (60) [M^+]^{79}Br/^{81}Br, 237/239 (100) [M-CO], 209 (45), 130 (90). HREI-MS: *m/z* calcd for $C_{11}H_8{}^{79}BrNO_2$ [M^+]: 264.9738; found 264.9738.

*4.2.5 2-Phenyl-1,2-dihydroquinoline (**8**)*

The compound was prepared by reduction of **7** according to the method of Goldstein and Dambek[25] to yield **8** as an orange/brown oil (8.67 g, 90%), b.p. *ca* 760 mm 155 °C.

*4.2.6 4-Phenyl-4H-pyrrolo[3,2,1-ij]quinoline-1,2-dione (**9**)*

Dihydroquinoline **8** (1.04 g, 5.02 mmol) in anhydrous THF (6 mL) was added portion wise over 30 min to a refluxing solution of oxalyl chloride (1.27 g, 873 μL, 10.0 mmol) in anhydrous THF (4 mL). The reaction mixture was heated at reflux for 3.5 h before the solvent was removed by rotary evaporation. The dark residue was dissolved in anhydrous $CHCl_3$ (30 mL) and heated at reflux. $AlCl_3$ (2.01 g, 15.1 mmol) was added portion wise over 5 h and the solution was heated at reflux for a further 18 h. The solvent was once again removed by rotary evaporation, the residue was cooled on ice, concentrated HCl (10 mL) was added, followed by H_2O (10 mL) and $CHCl_3$ (50 mL). The phases were separated and the organic layer was washed with H_2O (2 × 50 mL), dried over $MgSO_4$, filtered and the solvent removed by rotary evaporation. The resulting solid was purified by flash chromatography (100% DCM) to yield **9** as dark red crystals (15.7 mg, 1%), m.p. 74-76 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.83 (dd, *J* = 3.2 Hz, 10.0 Hz, 1H), 5.88 (d, *J* = 3.2 Hz, 1H), 6.52 (d, *J* = 10.0 Hz, 1H), 6.99 (t, *J* = 8.0 Hz, 1H), 7.29 (d, *J* = 7.5 Hz, 1H), 7.33 (d, *J* = 6.5 Hz, 1H), 7.35-7.38 (m, 3H), 7.44 (d, *J* = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 56.0, 114.8, 118.0, 120.1, 123.6, 124.1, 127.3,† 127.4, 128.7, 129.0,† 133.7, 139.2, 147.3, 157.4, 182.4. LREI-MS: *m/z* (%): 261(32) [M^+], 232 (43), 204 (100), 156 (59). HRESI-MS: *m/z* calcd for $C_{17}H_{12}NO_2$ $[M+H]^+$: 262.0868; found 262.0858.

*4.2.7 2-Phenyl-1,2,3,4-tetrahydroquinoline (**10**)*

The compound was prepared by reduction of **8** according to the method of Goldstein and Dambek[25] to yield **10** as a yellow/orange oil (3.18 g, 70%), b.p. *ca* 760 mm 192 °C (lit.[25] b.p. *2* mm 176 °C). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.93-2.02 (m, 2H), 2.73-2.78 (m, 2H), 3.97 (bs, 1H), 4.44 (dd, *J* = 2.5 Hz, 9.0 Hz, 1H), 6.46-6.67 (m, 2H), 6.94-7.03 (m, 2H), 7.22-7.41 (m, 5H). The ^{1}H NMR spectral data coincided with those reported in the literature.[16b] LREI-MS: *m/z* (%): 209 (96) [M^+], 132 (100) [M-Ph].

*4.2.8 5,6-Dihydro-4-phenyl-4H-pyrrolo[3,2,1-ij]quinoline-1,2-dione (**11**)*

The compound was prepared according to the method for **9** using **10** (2.00 g, 9.56 mmol) and oxalyl chloride (2.42 g,

1.66 mL, 19.1 mmol) as starting materials. The resulting solid was purified by flash chromatography (100% DCM) to yield **11** as dark red crystals (120 mg, 5%), m.p. 139-140 °C (lit.[23] 129-130 °C). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.16-2.22 (m, 1H), 2.26-2.29 (m, 1H), 2.47-2.53 (m, 1H), 2.63-2.66 (m, 1H), 5.47 (app. bs, 1H), 7.05 (t, *J* = 7.5 Hz, 1H), 7.16 (d, *J* = 7.5 Hz, 2H), 7.27 (t, *J* = 7.0 Hz, 1H), 7.32 (t, *J* = 7.0 Hz, 2H), 7.35 (d, *J* = 7.5 Hz, 1H), 7.47 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 19.8, 27.7, 51.6, 115.6, 122.0, 123.2, 123.2, 125.4,[†] 127.8, 128.9,[†] 137.2, 139.1, 147.8, 156.8, 183.6. LREI-MS: *m/z* (%): 263 (79) [M^+], 206 (100). HREI-MS: *m/z* calcd for $C_{17}H_{13}NO_2$ [M^+]: 263.0946; found 263.0949.

*4.2.9 8-Bromo-5,6-dihydro-4-phenyl-4H-pyrrolo[3,2,1-ij]quinoline-1,2-dione (**12**)*

The compound was prepared according to the method for **6** using the isatin **11** (65.2 mg, 248 μmol) and Br_2 (119 mg, 38.3 μL, 7.44 μmol) as starting materials. The resulting solid was purified by flash chromatography (100% DCM) to yield **12** as red crystals (42.3 mg, 50%), m.p. 60-62 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.15-2.19 (m, 1H), 2.26-2.29 (m, 1H), 2.50-2.53 (m, 1H), 2.62-2.66 (m, 1H), 5.46 (s, 1H), 7.14 (d, *J* = 7.5 Hz, 2H), 7.27-7.29 (m, 1H), 7.32 (d, *J* = 7.5 Hz, 2H), 7.50 (s, 1H), 7.59 (s, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 19.7, 27.6, 38.7, 116.2, 116.7, 124.2, 125.3,[†] 125.9, 128.0, 129.0,[†] 132.3, 139.4, 146.7, 156.0, 182.3. LREI-MS: *m/z* (%): 341/343 (42) [M^+]^{79}Br/^{81}Br, 313/315 (30) [M-CO], 284/286 (72), 204 (43), 102 (100). HRESI-MS: *m/z* calcd for $C_{17}H_{13}{}^{79}BrNO_2$ [M+H]$^+$: 342.0130; found 342.0106.

*4.2.10 1-Allyl-7-bromo-1H-indole-2,3-dione (**13**)*

A mixture of 7-bromoisatin (**19**) (250 mg, 1.11 mmol) and NaH (62.3 mg, 1.55 mmol) was dissolved in anhydrous DMF (6 mL) and stirred at RT for 20 min before the addition of KI (37.2 mg, 222 μmol) and allyl bromide (295 mg, 211 μL, 2.44 mmol). The reaction mixture was heated at 60 °C and stirred at this temperature for 18 h. After cooling, EtOAc (25 mL) was added and the resulting solution was extracted with 0.5 M HCl (25 mL), followed by brine (25 mL). The orange organic layer was dried over $MgSO_4$ and the solvent was removed by rotary evaporation to yield a sticky red residue. The resulting solid was purified by flash chromatography (100% DCM) to yield **13** as orange crystals (236 mg, 81%), m.p. 112-114 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.80-4.81 (m, 2H), 5.245 (d, *J* = 11.5 Hz, 1H), 5.246 (d, *J* = 16.0 Hz, 1H), 5.95-6.02 (m, 1H), 7.00 (t, *J* = 7.5 Hz, 1H), 7.59 (d, *J* = 7.5 Hz, 1H), 7.69 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 43.4, 104.5, 117.7, 120.9, 124.9, 125.3, 132.0, 144.2, 148.0, 158.7, 182.6. LREI-MS: *m/z* (%): 265/267 (66) [M^+]^{79}Br/^{81}Br, 224/226 (100) [M-CH_2CHCH_2], 168/170 (50) (M-CH_2CHCH_2-$(CO)_2$). HREI-MS: *m/z* calcd for $C_{11}H_8{}^{79}BrNO_2$ [M^+]: 264.9738; found 264.9736.

*4.2.11 4H-Pyrrolo[3,2,1-ij]quinoline-1,2-dione (**14**) and 6H-pyrrolo[3,2,1-ij]quinoline-1,2-dione (**15**)*

The isatin **13** (150 mg, 564 μmol), TBACl (157 mg, 564 μmol), K_2CO_3 (234 mg, 1.69 mmol) and $Pd(OAc)_2$ (12.7 mg, 56.4 μmol) were dissolved in anhydrous DMF (7 mL) and heated at 85 °C for 2 h. Upon cooling, DCM (50 mL) was added and the solution was extracted with H_2O (3 × 15 mL) and brine (1 × 15 mL). The organic layer was dried over $MgSO_4$, the suspension filtered and the solvent removed by rotary evaporation. The resulting solid was purified by flash chromatography and preparative TLC (100% DCM) to yield **14** (41.1 mg, 39%) and **15** (13.6 mg, 13%) as dark red crystals, m.p. 194-196 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): **14**: δ 4.62 (t, *J* = 3.0 Hz, 2H), 5.84 (dt, *J* = 10.5 Hz, 3.5 Hz, 1H), 6.40 (dt, *J* = 10.5 Hz, 2.5 Hz, 1H), 6.93 (t, *J* = 7.5 Hz, 1H), 7.16 (d, *J* = 8.0 Hz, 1H), 7.30 (d, *J* = 7.5 Hz, 1H); **15**: δ 3.60 (d, *J* = 2.5 Hz, 2H), 5.29-5.32 (m, 1H), 6.94-6.95 (m, 1H), 7.19 (t, *J* = 7.5 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.47 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): **14**: δ 41.2, 114.7, 118.3, 121.7, 123.2, 123.8, 124.1, 133.7, 148.5, 157.6, 182.6. **15**: δ 24.8, 108.5, 116.2, 118.0, 119.1, 123.6, 125.9, 136.7, 144.6, 153.2, 182.8. LREI-MS: *m/z* (%): **14**: 185 (20) [M^+], 156 (48), 85 (36), 71 (36), 71 (64), 57 (100), 43 (80). **15**: 185 (31) [M^+], 156 (100), 128 (38). HREI-MS: *m/z* calcd for $C_{11}H_7NO_2$ [M^+]: 185.0477; found 185.0471 (4*H* isomer, **14**), 185.0474 (*6H* isomer, **15**).

*4.2.12 1-Allyl-5,7-dibromo-1H-indole-2,3-dione (**16**)*

The compound was prepared according to the method for **13** using 5,7-dibromoisatin (1.50 g, 4.92 mmol), NaH (165 mg, 6.89 mmol), KI (163 mg, 984 μmol) and allyl bromide (1.31 g, 937 μL, 10.8 mmol) as starting materials. The resulting solid was purified by flash chromatography (100% DCM) to yield **16** as red crystals (1.48 g, 87%), m.p. 98-100 °C, (lit.[1c] 103-105 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.78 (d, *J* = 4.8 Hz, 2H), 5.23 (d, *J* = 17.1 Hz, 1H), 5.25 (d, *J* = 9.9 Hz, 1H), 5.90-6.03 (m, 1H), 7.69 (br s, 1H), 7.85 (br s, 1H). The ^{1}H NMR spectral data were close to those reported in the literature.[1c] LREI-MS: *m/z* 343/345/347 [M^+]^{79}Br^{79}Br/^{79}Br ^{81}Br;/^{81}Br ^{81}Br.

*4.2.13 8-Bromo-4H-pyrrolo[3,2,1-ij]quinoline-1,2-dione (**17**) and 8-bromo-6H-pyrrolo[3,2,1-ij]quinoline-1,2-dione (**18**)*

These compounds were prepared according to the method for **14** and **15** using the isatin **16** (150 mg, 435 μmol), TBACl (121 mg, 435 μmol), K_2CO_3 (181 mg, 1.31 mmol) and $Pd(OAc)_2$ (9.80 mg, 43.5 μmol) as starting materials. The resulting solid was purified by flash chromatography ($CHCl_3$/PS, 2:1) and preparative TLC (100% DCM) to yield **17** (33.4 mg, 29%) and **18** (22.4 mg, 20%) as dark red crystals, m.p. 215-217 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): **17**: δ 4.63 (brd, *J* = 2.5 Hz, 2H), 5.89 (dt, *J* = 11 Hz, 2.5 Hz, 1H), 6.35 (brd, *J* = 11 Hz, 1H), 7.29 (s, 1H), 7.42 (s, 1H); **18**: δ 3.59 (app. bs, 2H), 5.29-5.32 (m, 1H), 6.93 (d, *J* = 8.5 Hz, 1H), 7.50 (s, 1H), 7.59 (s, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): **17**: δ 41.2, 115.8, 116.6, 120.2, 120.8, 124.7, 126.5, 135.8, 146.4, 156.8, 181.3. **18**: δ 24.8, 108.5, 118.2, 119.2, 121.4, 122.8, 126.6, 139.3, 148.5, 158.4, 182.0. LREI-MS: *m/z* (%): **17**: 263/265 (38) [M^+] ^{79}Br/^{81}Br, 234/236 (100). **18**: 263/265 (27) [M^+] ^{79}Br/^{81}Br, 234/236 (100), 180 (91). HRESI-MS: *m/z* calcd for $C_{11}H_7{}^{79}BrNO_2$ [M+H]$^+$: 263.9660; found 263.9659 (4*H* isomer, **17**), *m/z* calcd for $C_{11}H_7{}^{81}BrNO_2$ [M+H]$^+$: 265.9640; found 265.9804 (*6H* isomer, **18**).

*4.2.14 7-Bromo-1H-indole-2,3-dione (**19**)*

Chloral hydrate (5.01 g, 30.2 mmol) and Na_2SO_4 (35.0 g, 246 mmol) were dissolved in H_2O (70 mL) in a 300 mL beaker and heated to 35 °C. 2-Bromoaniline (4.75 g, 3.12 mL, 27.6 mmol) in H_2O (20 mL) and concentrated HCl (3 mL) were warmed and added to the reaction mixture. Hydroxylamine HCl (6.10 g, 87.8 mmol) in H_2O (27.5 mL) was warmed and added before the mixture was heated at 85 °C for 3 h. The mixture was cooled, filtered and washed with H_2O (100 mL) to yield the intermediate (5.75 g, 86%) as a fluffy light brown powder. Concentrated H_2SO_4 (100 mL) was heated in a 1 L beaker to 60 °C. The intermediate was added in portions over 15 min so the temperature did not exceed 65 °C. The reaction mixture was then heated at 80 °C for

15 min before being poured into ice (500 mL). The precipitate was washed with H_2O (100 mL) to yield **19** as a fine dark red powder (2.94 g, 47%), m.p. 192-194 °C (lit.[26] 195-200 °C). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.02 (t, *J* = 7.5 Hz, 1H), 7.51 (d, *J* = 7.5 Hz, 1H), 7.78 (d, *J* = 7.5 Hz, 1H), 11.31 (bs, 1H). The ^{1}H NMR spectral data coincided with those reported in the literature.[27] LREI-MS: *m/z* 225/227 $[M^+]^{79}Br/^{81}Br$.

*4.2.15 (E)-7-Bromo-1-(but-2-enyl)-1H-indole-2,3-dione (**20**)*

The compound was prepared according to the method for **13** using 7-bromoisatin (**19**) (500 mg, 2.21 mmol), NaH (74.2 mg, 3.09 mmol), KI (73.4 mg, 442 μmol) and crotyl bromide (656 mg, 500 μL, 4.86 mmol) as starting materials. The resulting solid was purified by flash chromatography (100% DCM) to yield **20** as orange crystals (363 mg, 59%), m.p. 71-73 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.69 (d, *J* = 6.0 Hz, 3H), 4.73 (d, *J* = 4.5 Hz, 2H), 5.61-5.64 (m, 1H), 5.74-5.80 (m, 1H), 6.99 (t, *J* = 7.5 Hz, 1H), 7.58 (d, *J* = 7.5 Hz, 1H), 7.69 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 17.8, 42.6, 104.2, 120.7, 124.6, 124.7, 124.9, 129.9, 143.9, 147.9, 158.5, 182.7. LREI-MS: *m/z* (%): 279/281 (42) $[M^+]^{79}Br/^{81}Br$, 224/226 (100), 168/170 (24), 75 (39). HRESI-MS: *m/z* calcd for $C_{12}H_{11}{}^{79}BrNO_2$ $[M+H]^+$: 279.9973; found 279.9969.

*4.2.16 6-Methyl-6H-pyrrolo[3,2,1-ij]quinoline-1,2-dione (**21**)*

The compound was prepared according to the method for **14** and **15** using the isatin **20** (161 mg, 575 μmol), K_2CO_3 (239 mg, 1.73 mmol), TBACl (160 mg, 575 μmol) and $Pd(OAc)_2$ (12.9 mg, 57.5 μmol) as starting materials. The resulting solid was purified by flash chromatography and preparative TLC (100% DCM) to yield **21** as a dark red powder (9.30 mg, 8%), m.p. 96-97 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.43 (d, *J* = 7.5 Hz, 3H), 3.73-3.75 (m, 1H), 5.21 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H), 6.90 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H), 7.19 (t, *J* = 7.5 Hz, 1H), 7.43 (d, *J* = 7.5 Hz, 1H), 7.45 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 23.6, 29.8, 114.7, 116.0, 116.3, 123.4, 124.6, 126.0, 136.1, 143.8, 153.2, 182.8. LREI-MS: *m/z* (%): 199 (16) $[M^+]$, 184 (16) $[M\text{-}CH_3]$, 156 (60) $[M\text{-}CH_3\text{-}CO]$, 85 (60), 71 (100), 69 (86). HRESI-MS: *m/z* calcd for $C_{12}H_{10}NO_2$ $[M+H]^+$: 200.0712; found 200.0659.

*4.2.17 (E)-7-Bromo-1-(3-phenyl-allyl)-1H-indole-2,3-dione (**22**)*

The compound was prepared according to the method for **13** using 7-bromoisatin (**19**) (751 mg, 3.33 mmol), NaH (186 mg, 4.66 mmol), KI (111 mg, 670 μmol) and cinnamyl bromide (1.45 g, 1.09 mL, 7.33 mmol) as starting materials. The resulting solid was purified by flash chromatography (100% DCM) to yield **22** as an orange powder (551 mg, 48%), m.p. 149-151 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.96 (d, *J* = 6.0 Hz, 2H), 6.30-6.36 (m, 1H), 6.66 (d, *J* = 16 Hz, 1H), 7.01 (t, *J* = 7.0 Hz, 1H), 7.24 (t, *J* = 7.5 Hz, 1H) 7.30 (t, *J* = 7.5 Hz, 2H), 7.35 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.0 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 47.8, 104.2, 120.8, 123.0, 124.7, 125.1, 126.5,† 128.0, 128.6,† 133.5, 136.2, 144.0, 147.7, 158.6, 182.5. LREI-MS: *m/z* (%): 341/343 (16) $[M^+]^{79}Br/^{81}Br$, 224/226 (56) $[M\text{-}CH_2CHCHC_6H_5]$, 117 (100). HREI-MS: *m/z* calcd for $C_{17}H_{12}{}^{79}BrNO_2$ $[M^+]$: 341.0051; found 341.0055.

*4.2.18 6-Phenyl-6H-pyrrolo[3,2,1-ij]quinoline-1,2-dione (**23**)*

The compound was prepared according to the method for **14** and **15** using the isatin **22** (130 mg, 380 μmol), K_2CO_3 (158 mg, 1.14 mmol), TBACl (106 mg, 380 μmol) and $Pd(OAc)_2$ (8.50 mg, 38.0 μmol) as starting materials. The resulting solid was purified by flash chromatography ($CHCl_3$/PS, 3:2) and preparative TLC (100% DCM) to yield **23** as orange/red crystals (17.2 mg, 17%), m.p. 130-132 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.88 (bs, 1H), 5.33 (dd, *J* = 4.0 Hz, 8.0 Hz, 1H), 7.07 (d, *J* = 8.0 Hz, 1H), 7.12 (t, *J* = 7.5 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 1H), 7.25 (d, *J* = 7.5 Hz, 2 H), 7.29 (d, *J* = 7.0 Hz, 1H), 7.36 (t, *J* = 7.5 Hz, 2H), 7.48 (d, *J* = 7.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 41.7, 112.8, 116.2, 116.5, 123.7, 126.1, 127.5, 128.3,† 128.9, 129.0,† 137.5, 143.4, 147.6, 153.4, 182.5. LREI-MS: *m/z* (%): 261 (16) $[M^+]$, 233 (33) [M-CO], 168 (50), 156 (50), 100 (100). HRESI-MS: *m/z* calcd for $C_{17}H_{12}NO_2$ $[M+H]^+$: 262.0868; found 262.0855.

*4.2.19 9,10-Dihydroacridine (**25**)*

A mixture of acridine (**24**) (10.0 g, 55.8 mmol) and AcOH (6.01 g, 5.73 mL, 100 mmol) were added to absolute EtOH (100 mL) and heated at reflux for 30 min before the addition of $NaCNBH_3$ (6.66 g, 106 mmol). The solution was heated at reflux for a further 1.5 h, cooled and the solvent removed by rotary evaporation. The white residue was basified with aqueous NH_3 (200 mL) and extracted with Et_2O (3 × 100 mL). The organic extract was evaporated and the white precipitate was recrystallised from 95% EtOH to yield **25** as cream coloured needles (4.76 g, 47%), m.p. 171-172 °C (lit.[28] 166-169 °C). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.06 (s, 2H), 5.95 (bs, 1H), 6.67 (d, *J* = 8.0 Hz, 2H), 6.86 (t, *J* = 7.5 Hz, 2H), 7.06-7.11 (m, 4H). The ^{1}H NMR spectral data coincided with those reported in the literature.[29] LREI-MS: *m/z* (%): 180 (100) $[M\text{-}H]^+$.

*4.2.20 6H-Pyrrolo[3,2,1-de]acridine-1,2-dione (**26**)*

The compound was prepared according to the method for **9** using 9,10-dihydroacridine (**25**) (4.50 g, 24.8 mmol) and oxalyl chloride (6.30 g, 4.33 mL, 49.6 mmol) as starting materials. The resulting solid was purified by flash chromatography (100% $CHCl_3$) to yield **26** as dark red crystals (857 mg, 15%), m.p. 204-206 °C, (lit.[10b] 208-210 °C). The ^{1}H NMR spectral data coincided with those reported in the literature.[10b] ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.21 (s, 2H), 7.14-7.33 (m, 4H), 7.45 (d, *J* = 7.5 Hz, 1H), 7.52 (d, *J* = 7.5 Hz, 1H), 8.68 (d, *J* = 8.0 Hz, 1H). LREI-MS: *m/z* (%): 235 (29) $[M^+]$, 207 (100) [M-CO].

*4.2.21 4-Bromo-6H-pyrrolo[3,2,1-de]acridine-1,2-dione (**27**)*

The compound was prepared according to the method for **6** using **26** (100 mg, 425 μmol), glacial AcOH (5 mL) and Br_2 (205 mg, 65.9 μL, 1.28 mmol) as starting materials. The resulting solid was purified by flash chromatography (100% $CHCl_3$) to yield **27** as a dark red powder (54.6 mg, 41%), m.p. 190-192 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.20 (s, 2H), 7.22 (t, *J* = 7.5 Hz, 1H), 7.41-7.47 (m, 3H), 7.55 (t, *J* = 7.0 Hz, 1H), 8.57 (d, *J* = 8.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 28.1, 116.1, 118.9,† 119.1, 123.4, 123.9, 125.7, 131.1, 131.3, 132.5, 136.8, 145.1, 155.4, 181.8. LREI-MS: *m/z* (%): 313/315 (25) $[M^+]^{79}Br/^{81}Br$, 285/287 (100) [M-CO], 206 (55) [M-CO-Br], 177 (55), 151 (43), 103 (48), 89 (85), 75 (80). HRESI-MS: *m/z* calcd for $C_{15}H_9{}^{79}BrNO_2$ $[M+H]^+$: 313.9817; found 313.9807.

*4.2.22 5,6-Dihydrophenanthridine (**29**)*

The compound was prepared according to the method for **25** using phenanthridine (**28**) (5.00 g, 27.9 mmol), AcOH (1.68 g, 1.60 mL, 27.9 mmol) and $NaCNBH_3$ (3.33 g, 53.1 mmol) as the starting materials. The crude white precipitate was recrystallised

from 95% EtOH to yield **29** as yellow crystals (3.89 g, 77%), m.p. 64-66 °C (lit.[30] 89-90 °C). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.72 (s, 1H), 4.39 (s, 2H), 6.66-6.68 (m, 1H), 6.82-6.86 (m, 1H), 7.09-7.12 (m, 2H), 7.20-7.23 (m, 1H), 7.30-7.33 (m, 1H), 7.68-7.70 (m, 2H). The ^{1}H NMR spectral data coincided with those reported in the literature.[31] LREI-MS: *m/z* (%): 181 (65) [M^+], 180 (100) [M-H], 152 (33) [M-H-CO].

*4.2.23 7H-Pyrrolo[3,2,1-de]phenanthridine-4,5-dione (**30**)*

A mixture of 5,6-dihydrophenanthridine (**29**) (500 mg, 2.76 mmol), oxalyl chloride (701 mg, 482 µL, 5.52 mmol), DMAP (33.7 mg, 276 µmol) and DIPEA (357 mg, 480 µL, 2.76 mmol) was dissolved in anhydrous THF (10 mL) and heated at reflux for 3.5 h. The solution was evaporated and the residue was dissolved in anhydrous $CHCl_3$ (40 mL) and heated at reflux. $AlCl_3$ (1.10 g, 8.28 mmol) was added portion wise over 30 min and the solution was heated at reflux for a further 1.5 h. Upon cooling, the solution was extracted with H_2O (2 × 25 mL) and brine (1 × 25 mL). The organic extract was dried over $MgSO_4$, filtered and evaporated. The resulting solid was purified using flash chromatography (100% DCM) to yield **30** as dark red crystals (100 mg, 15%), m.p. 212-214 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.06 (s, 2H), 7.09 (t, *J* = 7.5 Hz, 1H), 7.19 (d, *J* = 7.5 Hz, 1H), 7.33 (t, *J* = 7.0 Hz, 1H), 7.37 (t, *J* = 7.5 Hz, 1H), 7.42 (d, *J* = 7.5 Hz, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 42.6, 116.5, 118.9, 122.8, 124.3, 124.7, 126.9, 128.1, 128.4, 128.7, 129.6, 130.7, 146.6, 157.9, 183.2. LREI-MS: *m/z* (%): 235 (50) [M^+], 206 (100), 179 (33). HREI-MS: *m/z* calcd for $C_{15}H_9NO_2$ [M^+]: 235.0633; found 235.0625.

*4.2.24 2-Bromo-7H-pyrrolo[3,2,1-de]phenanthridine-4,5-dione (**31**)*

The compound was prepared according to the method for **6** using **30** (84.0 mg, 357 µmol), glacial AcOH (5 mL) and Br_2 (171 mg, 55.0 µL, 1.07 mmol) as starting materials. The resulting solid was purified by flash chromatography (100% $CHCl_3$) to yield **31** as dark red powder (77.1 mg, 69%), m.p. 239-241 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.08 (s, 2H), 7.19-7.22 (m, 1H), 7.37-7.39 (m, 2H), 7.53 (s, 1H), 7.70-7.73 (m, 1H), 7.98 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 42.4, 117.1, 117.3, 120.7, 122.8, 125.6, 126.9, 128.0, 128.5, 128.7, 130.1, 132.8, 145.2, 156.8, 181.7. LREI-MS: *m/z* (%): 313/315 (52) [M^+]^{79}Br/^{81}Br, 284/286 (100), 257 (37), 177 (48), 150 (44). HREI-MS: *m/z* calcd for $C_{15}H_8{}^{81}BrNO_2$ [M^+]: 314.9718; found 314.9731.

*4.2.25 7-Iodo-1H-indole-2,3-dione (**32**)*

The compound was prepared according to the method for **19** using chloral hydrate (6.67 g, 40.3 mmol), Na_2SO_4 (46.2 g, 325 mmol), 2-iodoaniline (8.00 g, 36.5 mmol) and hydroxylamine HCl (8.13 g, 117 mmol) as starting materials, to yield **32** as a dark red/brown powder (5.77 g, 58%), m.p. 205-206 °C (lit.[32] 208 °C). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 6.89 (t, *J* = 7.5 Hz, 1H), 7.49 (d, *J* = 7.5 Hz, 1H), 7.94 (d, *J* = 8.0 Hz, 1H), 11.00 (bs, 1H). The ^{1}H NMR spectral data coincided with those reported in the literature.[33] LREI-MS: *m/z* 273 [M^+].

*4.2.26 7-Iodo-1-(naphthalen-1-ylmethyl)-1H-indole-2,3-dione (**33**)*

This compound was prepared according to the method for **13** using **32** (6.00 g, 22.0 mmol), NaH (739 mg, 30.8 mmol), KI (730 mg, 4.40 mmol) and 1-(chloromethyl)naphthalene (8.11 g, 6.87 mL, 48.4 mmol) as starting materials. The resulting solid was purified by flash chromatography (100% $CHCl_3$) to yield **33** as a red powder (5.29 g, 58%), m.p. 177-178 °C, R_f 0.21. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.89 (s, 2H), 6.90 (t, *J* = 8.0 Hz, 1H), 7.10 (d, *J* = 7.0 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 1H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.62 (t, *J* = 7.5 Hz, 1H), 7.72 (d, *J* = 7.0 Hz, 1H), 7.81 (d, *J* = 8.5 Hz, 1H), 7.92 (dd, *J* = 3.5 Hz, 8.5 Hz, 2H), 7.99 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 42.1, 74.1, 120.6, 121.9, 122.3, 125.4, 125.5, 125.6, 126.1, 126.6, 128.1, 129.0, 130.0, 131.0, 133.9, 151.0, 151.1, 158.9, 182.4. LREI-MS: *m/z* (%): 413 (58) [M^+], 272 (87) [M-$CH_2C_{10}H_7$], 141 (100). HRESI-MS: *m/z* calcd for $C_{19}H_{12}INO_2$ [$M]^+$: 412.9913; found 412.9915.

*4.2.27 7'-Iodo-1'-(naphthalen-1-ylmethyl)spiro[[1,3]-dioxolane-2,3'-indolin]-2'-one (**34**)*

According to the method of Ribeiro *et al.*,[34] the isatin **33** (4.96 g, 12.0 mmol), ethylene glycol (14.9 g, 13.4 mL, 240 mmol) and PTSA (496 mg, 10% of starting material weight) were dissolved in PhMe (40 mL) and heated at reflux under Dean-Stark conditions for 24 h. No evidence of product had formed so more ethylene glycol was added (7.45 g, 6.70 mL, 120 mmol) and the solution was heated at reflux for a further 24 h before the solvent was removed by rotary evaporation. The resulting solid was purified by flash chromatography (100% DCM) to yield **34** as a beige powder (4.14 g, 75%), m.p. 236-238 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.38 (t, *J* = 7.0 Hz, 2H), 4.63 (t, *J* = 6.5 Hz, 2H), 5.77 (s, 2H), 6.83 (d, *J* = 7.5 Hz, 1H), 7.05 (d, *J* = 7.5 Hz, 1H), 7.37 (t, *J* = 7.5 Hz, 1H), 7.44 (d, *J* = 7.5 Hz, 1H), 7.53 (t, *J* = 7.0 Hz, 1H), 7.60 (t, *J* = 8.0 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.90 (d, *J* = 7.5 Hz, 1H), 8.00 (d, *J* = 8.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 41.9, 66.3,† 72.8, 101.2, 122.3, 122.7, 125.2, 125.4, 125.9, 126.0, 126.5, 127.5, 127.8, 129.1, 130.4, 131.9, 134.0, 144.7,† 174.5. LREI-MS: *m/z* (%): 457 (21) [M^+], 316 (100) [M-$CH_2C_{10}H_7$], 272 (37) [M-$CH_2C_{10}H_7$-$(CH_2)_2O$], 141(34). HRESI-MS: *m/z* calcd for $C_{21}H_{17}INO_3$ [M+H]$^+$: 458.0253; found 458.0233.

*4.2.28 Spiro[benzo[j]pyrrolo[3,2,1-de]phenanthridine-4,2'-[1,3]dioxolan]-5(7H)-one (**35**)*

The ketal **34** (2.00 g, 4.37 mmol), $Pd(OAc)_2$ (98.0 mg, 437 µmol), TBAB (1.55 g, 4.81 mmol) and NaOAc (1.79 g, 21.9 mmol) were dissolved in anhydrous DMF (44 mL) and heated at 90 ºC for 5 h. Upon cooling, H_2O (200 mL) was added and the solution was extracted with EtOAc (3 × 100 mL). The organic extract was dried over $MgSO_4$, filtered and evaporated. The resulting solid was purified using flash chromatography (100% $CHCl_3$) to yield **35** as a light brown powder (1.24 g, 86%), m.p. 222-224 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.36 (t, *J* = 6.5 Hz, 2H), 4.63 (t, *J* = 6.5 Hz, 2H), 5.27 (s, 2H), 7.08 (t, *J* = 7.0 Hz, 1H), 7.25 (d, *J* = 7.5 H, 1H), 7.50 (t, *J* = 8.0 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.79-7.84 (m, 3H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 40.7, 65.9,† 103.2, 117.4, 120.0, 122.3, 122.4, 123.4, 123.5, 124.3, 124.5, 125.2, 126.4, 127.3, 128.7, 128.8, 130.3, 133.0, 139.3, 183.2. LREI-MS: *m/z* (%): 329 (33) [M^+], 300 (100), 256 (33), 229 (30), 201 (57), 128 (30), 114 (33). HRESI-MS: *m/z* calcd for $C_{21}H_{16}NO_3$ [M^+]: 330.1130; found 330.1117.

*4.2.29 7H-Benzo[j]pyrrolo[3,2,1-de]phenanthridine-4,5-dione (**36**)*

The cyclised ketal **35** (1.10 g, 3.34 mmol) was dissolved in 6 M HCl/THF (1:1, 35 mL) and heated at reflux for 5 h. Upon cooling, H_2O (200 mL) was added, the solution filtered and washed with more H_2O to yield **36** as a fine dark red powder

(641 mg, 67%), m.p. 251-253 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.44 (s, 2H), 7.14 (t, *J* = 7.5 Hz, 1H), 7.45 (d, *J* = 7.5 Hz, 1H), 7.62 (t, *J* = 7.0 Hz, 1H), 7.66 (t, *J* = 7.5 Hz, 1H), 7.95 (d, *J* = 8.5 Hz, 1H), 8.01 (t, *J* = 7.5 Hz, 2H), 8.16 (d, *J* = 9.0 Hz, 1H), 8.25 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 40.8, 115.7, 118.7, 119.6, 122.4, 123.4, 124.4, 124.7, 127.0, 127.8, 129.2, 129.9, 130.2, 130.8, 133.3, 134.2, 146.5, 157.8, 182.9. LREI-MS: *m/z* (%): 285 (55) [M^+], 256 (100), 229 (38), 201 (32), 128 (23), 114 (40), 100 (43). HRESI-MS: *m/z* calcd for $C_{19}H_{12}NO_2$ $[M+H]^+$: 286.0868; found 286.0829.

*4.2.30 7-Iodo-1-(naphthalen-2-ylmethyl)-1H-indole-2,3-dione (**37**)*

The compound was prepared according to the method for **13** using **32** (2.50 g, 9.16 mmol), NaH (307 mg, 12.8 mmol), KI (304 mg, 1.83 mmol) and 2-(bromomethyl)naphthalene (3.03 g, 13.7 mmol) as starting materials. The resulting solid was purified by flash chromatography (100% $CHCl_3$) to yield **37** as red/brown crystals (1.37 g, 36%), m.p. 154-155 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.64 (s, 2H), 6.88 (t, *J* = 7.5 Hz, 1H), 7.38 (d, *J* = 7.5 Hz, 1H), 7.46 (dd, *J* = 3.0 Hz, 6.0 Hz, 2H), 7.61 (s, 1H), 7.68 (d, *J* = 6.5 Hz, 1H), 7.75 (dd, *J* = 3.0 Hz, 5.5 Hz, 1H), 7.81-7.85 (m, 2H), 7.94 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 43.8, 73.7, 120.8, 124.4, 124.9, 125.5, 125.7, 126.0, 126.4, 127.7, 127.8, 128.7, 132.7, 133.3,† 150.9, 151.1, 159.2, 182.4. LREI-MS: *m/z* (%): 413 (56) [M^+], 272 (85) [M-$CH_2C_{10}H_7$], 141 (100), 115 (47). HRESI-MS: *m/z* calcd for $C_{19}H_{13}INO_2$ $[M+H]^+$: 413.9991; found 413.9950.

*4.2.31 7'-Iodo-1'-(naphthalen-2-ylmethyl)spiro[[1,3]-dioxolane-2,3'-indolin]-2'-one (**38**)*

The compound was prepared according to the method for **34** using the isatin **37** (1.10 g, 2.66 mmol), ethylene glycol (4.95 g, 4.45 mL, 79.8 mmol) and PTSA (110 mg, 10% of starting material weight) as starting materials. The resulting solid was purified by flash chromatography (100% DCM) to yield **38** as a beige powder (1.12 g, 92%), m.p. 156-158 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.36-4.39 (m, 2H), 4.62-4.64 (m, 2H), 5.51 (s, 2H), 6.82 (t, *J* = 8.0 Hz, 1H), 7.35 (dd, *J* = 1.5 Hz, 8.5 Hz, 1H), 7.41 (dd, *J* = 1.0 Hz, 7.0 Hz, 1H), 7.43-7.45 (m, 2H), 7.57 (s, 1H), 7.71 (dd, *J* = 1.0 Hz, 8.5 Hz, 1H), 7.75-7.77 (m, 1H), 7.80-7.82 (m, 2H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 43.7, 66.3,† 72.6, 101.1, 124.5, 124.7, 124.9, 125.2, 125.7, 126.1, 127.4, 127.6, 127.9, 128.5, 132.6, 133.4, 134.1, 144.4, 144.5, 174.5. LREI-MS: *m/z* (%): 457 (23) [M^+], 316 (100) [M-$CH_2C_{10}H_7$], 272 (45) [M-$CH_2C_{10}H_7$-$(CH_2)_2O$], 141(37), 115 (35). HRESI-MS: *m/z* calcd for $C_{21}H_{17}INO_3$ $[M+H]^+$: 458.0253; found 458.0215.

*4.2.32 Spiro[benzo[l]pyrrolo[3,2,1-de]phenanthridine-4,2'-[1,3]dioxolan]-5(7H)-one (**39**)*

The compound was prepared according to the method for **35** using the ketal **38** (1.00 g, 2.19 mmol), $Pd(OAc)_2$ (49.2 mg, 219 µmol), TBAB (777 mg, 2.41 mmol) and NaOAc (902 mg, 11.0 mmol) as starting materials. The resulting solid was purified using flash chromatography (100% $CHCl_3$) to yield **39** as a beige powder (485 mg, 67%), m.p. 161-163 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.36 (t, *J* = 6.5 Hz, 2H), 4.61 (t, *J* = 6.5 Hz, 2H), 5.05 (s, 2H), 7.14 (t, *J* = 8.0 Hz, 1H), 7.21 (d, *J* = 8.5 Hz, 1H), 7.30 (d, *J* = 7.5 Hz, 1H), 7.50 (t, *J* = 7.5 Hz, 1H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.74 (d, *J* = 8.0 Hz, 1H), 7.85 (d, *J* = 9.0 Hz, 1H), 8.17 (d, *J* = 8.5 Hz, 1H), 8.63 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 44.5, 66.1,† 119.1, 121.6, 123.3, 124.1, 125.1, 125.3, 125.5, 126.1, 127.3, 129.2, 129.3, 129.4, 129.8, 130.2, 134.4, 141.9,†174.3. LREI-MS: *m/z* (%): 329 (44) [M^+], 300 (100), 256 (31), 229 (28), 201 (18), 128 (33), 114 (32), 100 (28). HRESI-MS: *m/z* calcd for $C_{21}H_{16}NO_3$ $[M+H]^+$: 330.1130; found 330.1081.

*4.2.33 7H-Benzo[l]pyrrolo[3,2,1-de]phenanthridine-4,5-dione (**40**)*

The compound was prepared according to the method for **36** using the cyclised ketal **39** (420 mg, 1.28 mmol) in 6 M HCl/THF (1:1, 13.4 mL) as starting materials to yield **40** as a fine dark red powder (330 mg, 90%), m.p. 205-207 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.15 (s, 2H), 7.18 (t, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.48 (d, *J* = 7.5 Hz, 1H), 7.55 (t, *J* = 8.5 Hz, 1H), 7.60 (td, *J* = 1.0 Hz, 8.5 Hz, 1H), 7.80 (d, *J* = 8.5 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 8.37 (d, *J* = 8.0 Hz, 1H), 8.55 (d, *J* = 8.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 44.3, 117.2, 120.2, 123.6, 123.9, 124.2, 124.6, 124.7, 126.3, 127.6, 129.2, 129.8, 129.9, 130.8, 134.2, 135.6, 149.2, 158.8, 183.1. LREI-MS: *m/z* (%): 285 (48) [M^+], 256 (100), 228 (35), 201 (26), 128 (13), 114 (39), 100 (48). HRESI-MS: *m/z* calcd for $C_{19}H_{12}NO_2$ $[M+H]^+$: 286.0868; found 286.0860.

*4.2.34 2-Bromo-7H-benzo[l]pyrrolo[3,2,1-de]phenanthridine-4,5-dione (**41**)*

According to the bromination method of Zysman-Colman *et al.*,[35] the cyclised phenanthridine **40** (100 mg, 351 µmol) was dissolved in anhydrous CH_3CN (1 mL) at 0 °C. NBS (62.5 mg, 351 µmol) in anhydrous CH_3CN (1 mL) was added and the reaction mixture was warmed to RT and stirred for 18 h. DCM (30 mL) was added and the solution was extracted with H_2O (3 × 20 mL). The organic extract was dried over $MgSO_4$, filtered and evaporated. The resulting solid was purified using flash chromatography (100% $CHCl_3$) to yield **41** as a fine dark red powder (49.2 mg, 39%), m.p. 197-199 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.09 (s, 2H), 7.20 (d, *J* = 8.0 Hz, 1H), 7.47-7.57 (m, 2H), 7.63 (app. bs, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.87 (d, *J* = 7.0 Hz, 1H), 8.30-8.35 (m, 1H), 8.41 (bs, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 44.2, 116.6, 118.1, 122.0, 122.7, 124.0, 124.6, 126.1, 126.5, 128.1, 129.4, 129.6, 130.7, 134.1, 135.6, 137.5, 147.9, 157.9, 181.7. LREI-MS: *m/z* (%): 363/365 (24) [M^+]^{79}Br/^{81}Br, 334/336 (38), 256 (53), 227 (61), 200 (35), 100 (65). HRESI-MS: *m/z* calcd for $C_{19}H_{11}{}^{79}BrNO_2$ $[M+H]^+$: 363.9973; found 363.9991.

4.3 Cytotoxicity Testing

Human U937 histiocytic lymphoma cells were obtained from the American Type Culture Collection (ATCC, VA, USA). Cells were routinely maintained in RPMI-1640 medium, containing 2 mM L-glutamine, 5.6 % (2 g/L) sodium bicarbonate and 5 % fetal calf serum (at 37 °C, 95 % humidified atmosphere and 5 % CO_2). Cytotoxicity of the isatin derivatives was determined using the CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (MTS assay) (Promega Co., Madison, WI, USA) as described previously.[1c] The concentration required to inhibit 50 % of the metabolic activity of the cell population (IC_{50}) was calculated from sigmoidal dose-response curves using GraphPad Prism 5.00 (GraphPad Software Inc., San Diego, CA, USA) from at least two independent experiments performed in triplicate.

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References

1. (a) Vine, K. L.; Matesic, L.; Locke, J. M.; Ranson, M.; Skropeta, D. *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 397-414; (b) Vine, K. L.; Locke, J. M.; Ranson, M.; Benkendorff, K.; Pyne, S. G.; Bremner, J. B. *Bioorg. Med. Chem.* **2007**, *15*, 931-938; (c) Vine, K. L.; Locke, J. M.; Ranson, M.; Pyne, S. G.; Bremner, J. B. *J. Med. Chem.* **2007**, *50*, 5109-5117; (d) Matesic, L.; Locke, J. M.; Bremner, J. B.; Pyne, S. G.; Skropeta, D.; Ranson, M.; Vine, K. L. *Bioorg. Med. Chem.* **2008**, *16*, 3118-3124; (e) Penthala, N. R.; Yerramreddy, T. R.; Madadi, N. R.; Crooks, P. A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4468-4471; (f) Modi, N. R.; Shah, R. J.; Patel, M. J.; Suthar, M.; Chauhan, B. F.; Patel, L. J. *Med. Chem. Res.* **2011**, *20*, 615-625.
2. (a) Barrett, I.; Meegan, M. J.; Hughes, R. B.; Carr, M.; Knox, A. J. S.; Artemenko, N.; Golfis, G.; Zisterer, D. M.; Lloyd, D. G. *Bioorg. Med. Chem.* **2008**, *16*, 9554-9573; (b) Alhamadsheh, M. M.; Gupta, S.; Hudson, R. A.; Perera, L.; Tillekeratne, L. M. V. *Chem. Eur. J.* **2008**, *14*, 570-581; (c) Tron, G. C.; Pagliai, F.; Del Grosso, E.; Genazzani, A. A.; Sorba, G. *J. Med. Chem.* **2005**, *48*, 3260-3268.
3. Karali, N.; Terzioglu, N.; Gursoy, A. *Arch. Pharm.* **2002**, *335*, 374-380.
4. (a) Welstead Jr, W. J.; Moran, H. W.; Stauffer, H. F.; Turnbull, L. B.; Sancilio, L. F. *J. Med. Chem.* **1979**, *22*, 1074-1079; (b) Jumina; Kumar, N.; Black, D. S. *Tetrahedron* **2009**, *65*, 2591-2598; (c) Black, D. S.; Bowyer, M. C.; Catalano, M. M.; Ivory, A. J.; Keller, P. A.; Kumar, N.; Nugent, S. J. *Tetrahedron* **1994**, *50*, 10497-10508.
5. Black, D. S.; Keller, P. A.; Kumar, N. *Tetrahedron* **1992**, *48*, 7601-7608.
6. (a) Black, D. S.; Kumar, N. *Org. Prep. Proc. Int.* **1991**, *23*, 67-92; (b) Black, D. S.; Kumar, N.; Mitchell, P. S. R. *J. Org. Chem.* **2002**, *67*, 2464-2473.
7. Blurton, P.; Brickwood, A.; Dhanak, D. *Heterocycles* **1997**, *45*, 2395-2403.
8. Isaac, M.; Slassi, A.; O'Brien, A.; Edwards, L.; MacLean, N.; Bueschkens, D.; Lee, D. K. H.; McCallum, K.; de Lannoy, I.; Demchyshyn, L.; Kamboj, R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 919-921.
9. Wang, J.; Ali, S. M.; Kellerher, E.; Liu, Y.; Hill, J.; Ashwell, M. A. WO 2009/002806 A1, 2008.
10. (a) Hess, B. A.; Corbino, S. *J. Heterocycl. Chem.* **1971**, *8*, 161-162; (b) Shamsi, M.; Baradarani, M. M.; Afghan, A.; Joule, J. A. *Arkivoc* **2011**, 252-260.
11. Hutchings, R. H.; Meyers, A. I. *J. Org. Chem.* **1996**, *61*, 1004-1013.
12. Torres, J. C.; Pinto, A. C.; Garden, S. J. *Tetrahedron* **2004**, *60*, 9889-9900.
13. da Silva, J. F. M.; Garden, S. J.; Pinto, A. C. *J. Braz. Chem. Soc.* **2001**, *12*, 273-324.
14. Hashiba, I.; Ando, Y.; Kawakami, I.; Sakota, R.; Nagano, K.; Mori, T. Japanese Patent 54073771, 1979.
15. Norman, M. H.; Navas III, F.; Thompson, J. B.; Rigdon, G. C. *J. Med. Chem.* **1996**, *39*, 4692-4703.
16. (a) Minter, D. E.; Stotter, P. L. *J. Org. Chem.* **1981**, *46*, 3965-3970; (b) Forrest, T. P.; Dauphinee, G. A.; Deraniyagala, S. A. *Can. J. Chem.* **1985**, *63*, 412-417.
17. (a) Gilchrist, T. L.; Kemmitt, P. D.; Germain, A. L. *Tetrahedron* **1997**, *53*, 4447-4456; (b) Beccalli, E. M.; Broggini, G.; Marchesini, A.; Rossi, E. *Tetrahedron* **2002**, *58*, 6673-6678; (c) Söderberg, B. C. G.; Hubbard, J. W.; Rector, S. R.; O'Neil, S. N. *Tetrahedron* **2005**, *61*, 3637-3649.
18. Lee, H. S.; Kim, S. H.; Gowrisankar, S.; Kim, J. N. *Tetrahedron* **2008**, *64*, 7183-7190.
19. Denny, W. A. *Curr. Med. Chem.* **2002**, *9*, 1655-1665.
20. Han, O.; Shih, Y.; Liu, L.; Liu, H. *J. Org. Chem.* **1988**, *53*, 2105-2108.
21. Uchimaru, T.; Narasaka, K.; Mukaiyama, T. *Chem. Lett.* **1981**, *10*, 1551-1554.
22. Garden, S. J.; Torres, J. C.; Pinto, A. C. *J. Braz. Chem. Soc.* **2000**, *11*, 441-446.
23. Kojima, E.; Fujimori, S.; Awano, K. European Patent 0 310 096 A2, 1989.
24. Bass, R. J.; Koch, R. C.; Richards, H. C.; Thorpe, J. E. British Patent 1 394 373, 1975.
25. Goldstein, S. W.; Dambek, P. J. *Synthesis* **1989**, 221-222.
26. Beauchard, A.; Laborie, H.; Rouillard, H.; Lozach, O.; Ferandin, Y.; Guével, R. L.; Guguen-Guillouzo, C.; Meijer, L.; Besson, T.; Thiéry, V. *Bioorg. Med. Chem.* **2009**, *17*, 6257-6263.
27. Kaila, N.; Janz, K.; DeBernardo, S.; Bedard, P. W.; Camphausen, R. T.; Tam, S.; Tsao, D. H. H.; Keith, J. C.; Nickerson-Nutter, C.; Shilling, A.; Young-Sciame, R.; Wang, Q. *J. Med. Chem.* **2007**, *50*, 21-39.
28. Srikrislina, A.; Reddy, T. J.; Viswajanani, R. *Tetrahedron* **1996**, *52*, 1631-1636.
29. Mulder, P.; Litwinienko, G.; Lin, S.; MacLean, P. D.; Barclay, L. R. C.; Ingold, K. U. *Chem. Res. Toxicol.* **2006**, *19*, 79-85.
30. McNab, H.; Monahan, L. C. *J. Chem. Soc.-Perkin Trans. 1* **1988**, 863-868.
31. Barluenga, J.; Fañanás, F. J.; Sanz, R.; Fernández, Y. *Chem. Eur. J.* **2002**, *8*, 2034-2046.
32. Lisowski, V.; Robba, M.; Rault, S. *J. Org. Chem.* **2000**, *65*, 4193-4194.
33. Kaila, N.; Janz, K.; Huang, A.; Moretto, A.; DeBernardo, S.; Bedard, P. W.; Tam, S.; Clerin, V.; Keith, J. C.; Tsao, D. H. H.; Sushkova, N.; Shaw, G. D.; Camphausen, R. T.; Schaub, R. G.; Wang, Q. *J. Med. Chem.* **2007**, *50*, 40-64.
34. Ribeiro, N. M.; Pinto, A. C.; da Silva, B. V.; Violante, F. A.; Dias, M. O. *Catal. Commun.* **2007**, *8*, 2130-2136.
35. Zysman-Colman, E.; Arias, K.; Siegel, J. S. *Can. J. Chem.* **2009**, *87*, 440-447.