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A cascade synthetic route to new bioactive spiroindolinepyrido[1,2-a]indolediones from indirubin

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A Cascade Synthetic Route to New Bioactive Spiroindolinepyrido[1,2-*a*]indolediones from Indirubin

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Abstract

The allylation of indirubin produced the expected indolic *N*'-allylindirubin and *N*,*N*'-diallylindirubin derivatives in moderate yields, together with the corresponding *N*-substituted isatin products. At higher temperatures, the base-initiated reaction with allylic halides yielded spiroindolinepyrido[1,2-*a*]indolediones in a one-pot cascade reaction sequence with yields of up to 70%. These readily accessed, new spiro compounds represent the first reported examples of indirubin participating in cascade reactions. Preliminary *in vitro* biological testing of some of the products indicated promising activity against some cancer cell lines and against *Plasmodium falciparum* for two spiro derivatives. Computational methods were used to gain a greater understanding of the UV/Vis spectroscopic data for the *N*'-substituted and *N*,*N*'-disubstituted indirubin derivatives.

1. Introduction

The search for new molecular entities, especially easily accessible complex heterocycles, is of significant interest in the exploration of new bioactive compounds and in materials chemistry. Often, cascade reactions are used to realize such synthetic targets starting with small molecules and utilizing them in complex multistep reactions. Our recent contributions to this field have focused on a notable variation to this strategy in that we started with a larger, but abundant and cheap molecule, the dye indigo **1** (Figure 1). Indigo afforded a unique opportunity in providing an advanced starting material for the potential rapid synthesis of the diindolic system of natural product analogs. To this end, we recently reported the first cascade reactions of indigo *via* base-initiated allylation and propargylation, producing unexpected polyheterocyclic compounds (Figure 1) in multistep reactions. This surprising result revealed a new arena in the previously unreported chemistry of indigo, allowing access to unique, relatively complex heterocycles in one pot.[1-3]

The structural isomers of indigo **1**, indirubin **2** and isoindigo **3** (Figure 2), are known,[4] but knowledge of their chemistry is still limited. Indirubin is the most well studied due to the reported biological activity of derivatives, known to suppress the metastatic ability of human head and neck cancer cells[5] and to reduce the invasion of glioma cells both *in vitro* and *in vivo*.[6] Further examples include inhibition of cyclin-dependent kinases[7] and glycogen synthase kinase-3, the latter being linked to Alzheimer's disease.[8]

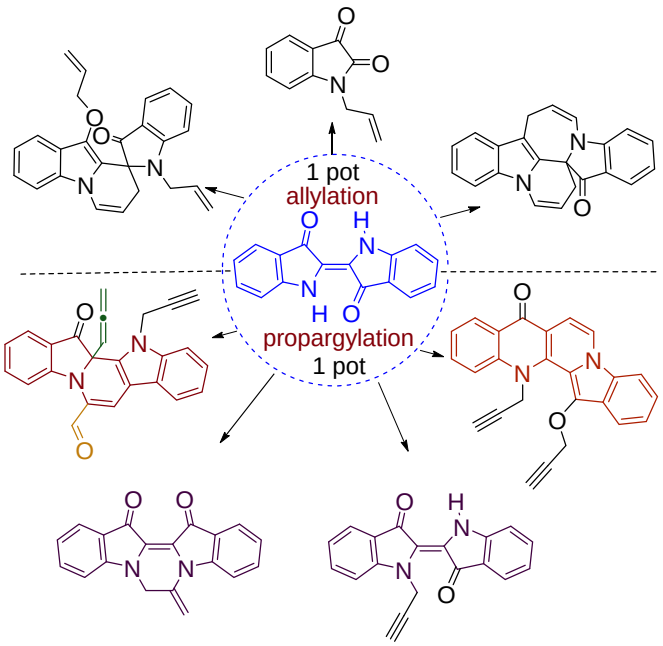
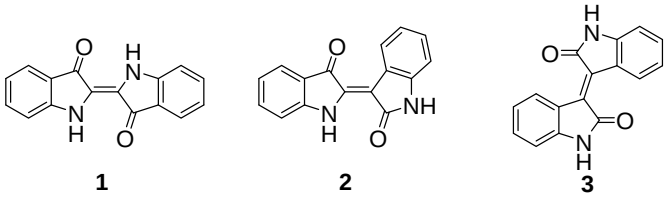


Figure 1: Selected examples of heterocycles produced *via* the cascade reactions of indigo using either allyl bromide or propargyl bromide and base.

Indirubin also inhibits intersegmental vessel growth and induces cellular apoptosis in an *in vivo* zebrafish model[9] while other indirubin derivatives or analogues,[10] including *N*-glycosides, show antiproliferative activity against a number of human cancer cell lines.[11,12] Other effects of indirubin or derivatives include anti-protozoal activity[13] as well as inhibition of lipoxygenase.[14] Isoindigo, its derivatives and analogues, also display a suite of useful biological effects including anti-cancer[15,16] and anti- protozoal activity.[17]



1 **2** **3**

Figure 2. Indigo **1** and its structural isomers indirubin **2** and isoindigo **3**.

Equally important is the basic chemistry of indirubin, including reaction at the carbonyl group, *e.g.* oxime formation,[18] which often leads to an increase in biological activity, attack by Grignard reagents[19] and substitution reactions at the amide functionality.[20] An isoindigo derivative has also been shown to provide an advanced starting template for total synthesis, *e.g.* the use of the 2,2'-double bond in *N*-tosylisoindigo as a dienophile in a key early Diels-Alder reaction to build up molecular complexity with stereochemical control in synthetic studies toward the anti-leukemic marine alkaloid communesin F.[21]

Therefore, given the recent understanding of cascade reactions of indigo **1**, we investigated the possibility of analogous cascade reactions of indirubin **2** and their potential to generate new heterocycles in one pot. These results are disclosed in this paper together with some initial *in vitro* antiplasmodial, antimycobacterial, and cell-based anticancer activity data.

2. Results and Discussion

2.1. Synthesis and Product Identification

Initial reactions with indirubin were performed as per the allylation of indigo.[1] In a typical reaction, a solution of indirubin in DMF with caesium carbonate was sonicated for 30 min to aid solubility and anion formation. The reaction was heated to 70 °C followed by the addition of the allyl bromide and heated for 16 hours. The outcomes of these reactions are summarized in Scheme 1. The use of the allyl bromide gave a 10% yield of the known[22] oxidative cleavage product *N*-allylisatin (Type **A**), together with the pink mono oxindolic *N*-allylated indirubin **9** (Type **B**) and the deep blue diallylindirubin **14** (Type **C**) in 24% and 19% yields, respectively. This initial reaction indicated that indirubin is significantly less reactive than the corresponding indigo. The use of substituted allyl bromides continued the trend with the pink mono allylated derivatives (Type **B**) being produced in yields from 20-49% in 16 hour reactions (entries 1-4), with an extended 20 hour reaction required to produce the *N*′-cinnamylindirubin **13** (21%). The diallylated indirubins (Type **C**) were also produced in the same reactions in yields of 3-30% although these ratios are somewhat misleading due to the variable stability of the Type **C** compounds under the reaction conditions, *e.g.* with cinnamyl bromide and a 4 hour reaction time the *N*,*N*′-dicinnamylindirubin **18** was isolated in an 11% yield (entry 6), but in a 20 hour reaction, the major outcome was the Type B *N*′-cinnamylindirubin **13**. The instability of Type **C** molecules presumably to arises from removal of the stabilizing H···OC H-bond once all NH functionality is substituted. Also, in contrast to the that observed with indigo,[1,2] no final *O*-allylated indirubin derivatives were observed. Further, no indirubin remained at the end of the reactions suggesting that compared to indigo, initial alkylation is easier (to the non-H-bonded N'H) but the resulting molecules are less reactive than indigo to subsequent cascade reactions.

	Calculated distances and angles				^{1}H NMR shifts (ppm)	
	Parameter	NH'···O=C **A** and α	H4'··O=C **B** and β	ΔE kJ/mol[c]	NH	ArH4'
	2		**9**		**14**	
2	Dist. (Å)	2.04	2.21	57.7	11.00[a^]	8.76[a]
	angle (º)	124.3	120.3			
9	Dist. (Å)	2.03	2.21	61.6	10.53[b]	8.89[b]
	angle (º)	124.2	120.1			
14	Dist.(Å)	-	2.15	36.1	-	8.63[b]
	angle (º)	-	117.7			

[a] in DMSO-d_6; [b] in $CDCl_3$; [c] ΔE: Energy difference between *E* and *Z* isomers; ^ both NH signals are assigned to the broad peak at 11.00 ppm

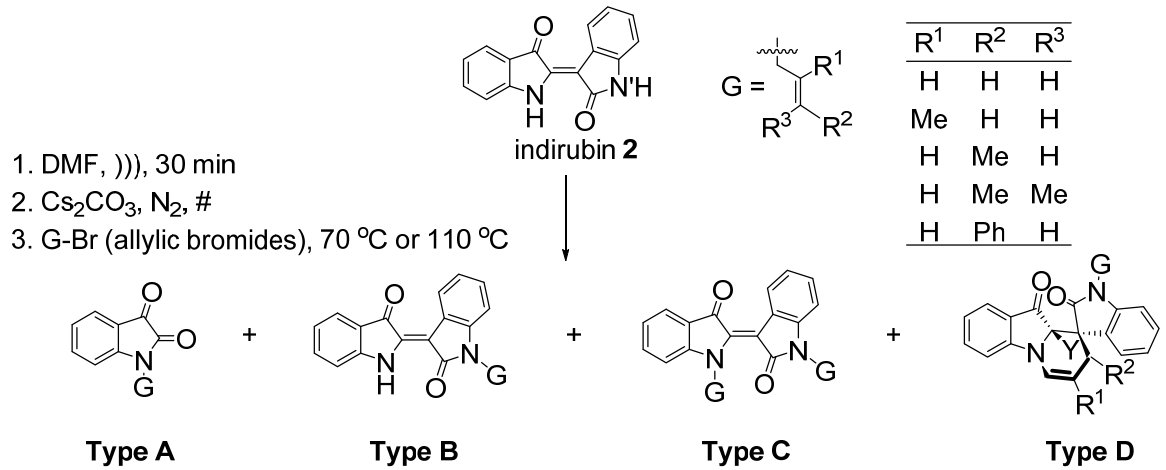
Figure 3. The *Z*-isomer of indirubin (**2**), *N*′-allylindirubin (**9**) and, *N*,*N*′-diallylindirubin (**14**): Illustrated on **9** – the calculated angles are indicated by dashed lines, distances are indicated by arrows. NH1--O=C2' distances and angles as well as ArH4'--O=C3 distances and angles and the corresponding ^{1}H NMR shifts of indirubin (**2**), *N*′-allylindirubin (**9**) and *N*,*N*′-diallylindirubin (**14**). Calculated data are obtained using Hartree-Fock theory after optimization at the 6-31G* level.

The ^{1}H NMR spectrum of **9** indicated peaks at 4.46 ppm (d, J = 5.5 Hz), 5.22 ppm (s), 5.25 ppm (d, J = 5.0 Hz) and 5.86-5.92 ppm (m), assigned to a single *N*-allyl substituent, as quantified by its relative integration to the indirubin aromatic protons. A single signal at 10.53 ppm was assigned to the NH, positioned downfield due to its H-bonding with the indirubin carbonyl. This also indicated that the allyl substituent was positioned on the N'-atom. Measurement of the HRMS (ESI) gave 303.1143, indicative of a molecular formula of $C_{19}H_{15}N_2O_2$, providing evidence for the presence of only a single allyl moiety. Analysis of the ^{1}H NMR spectra of **14** showed an absence of a peak in the ~10.5 ppm region suggesting the loss of an NH moiety. The integration of the peaks assigned to the allyl substituent (*e.g.* 4.43 ppm (2H) and 4.82 ppm (2H) relative to the total number of peaks assigned to the indirubin H-atoms indicated a ratio of indirubin:allyl substituents to be 1:2. Further, the measured HRMS (ESI) of **14** relayed a molecular formula of $C_{22}H_{19}N_2O_2$. In other base-induced N-alkylation reactions of indirubin by simple alkyl halides, preferential alkylation of the oxindolic nitrogen was also observed.[23]

In order to assess whether either the mono- or di-allylated products had undergone isomerization during the reaction process to form the 2,3'-*E* products (with a *syn* disposition of the carbonyl groups), and to support the product identifications, we undertook a series of computational experiments to characterize the monallylindirubin **9** and the diallylindirubin **14**.

Calculated distances (NH1···O=C2') and angles (N-H···O) between the amine group and the proximal carbonyl oxygen in indirubin (**2**) and its *N*'-allylated (**9**) and *N*,*N*'-diallylated (**14**) derivatives highlight the hydrogen bond character (Figure 3). Additionally, the distance (H4···O=C3) and angle (C-H···O) between the aromatic hydrogen H4' and the proximal carbonyl group are in accordance with the remarkable ^{1}H NMR downfield shift of the aromatic hydrogen H4' and indicate the strong interaction ("H-bond") of H4' with the proximal carbonyl group.

The initial reaction product results at 70 °C clearly indicated a lower reactivity of indirubin to subsequent cyclisation. Therefore, in an attempt to force potential cascade reactions, the reactions were repeated at 110 °C (Scheme 1, entries 7-12). This gave rise to the new spiroindolinepyrido[1,2-*a*]indoledione heterocycles of the type **D** in up to 70% yield in the case of allyl bromide (entry 7), arising from the introduction of three allylic moieties; at 70 °C, **19** (see also Figure 4) was isolated in only 2% yield. The ^{1}H NMR of **19** showed a ddd centred at 2.06 ppm with J = 18.3, 5.3 and 1.8 Hz, assigned to the sp^2 alkenyl H8'$_a$ - the corresponding H8'$_b$ was assigned to the dt (J = 18.3, 1.8 Hz) at 3.00 ppm. The signals assigned to H6' and H7' are contained within multiplets at 7.00 - 7.06 and 5.02 - 5.41 ppm respectively. The signal in the ^{13}C NMR spectra at 48.0 ppm was assigned to the spiro C3, and the C2 amide carbonyl was assigned to the peak at 175.6 ppm whereas the peak at 199.1 ppm was attributed to the C10' carbonyl. Confirmation of the molecular formula of **19** was provided by analysis of the HR ESI mass spectrum which indicated an exact mass of 383.1751, assigned to the $[M + H]^+$ ion of $C_{25}H_{23}N_2O_2$.



Entry	Temp °C	Time h	R^1	R^2	R^3	**A**	**B**	**C**	**D**	Y
1	70	16	H	H	H	**4** (10%)	**9** (24%)	**14** (19%)	**19** (2%)	
2	70	16	Me	H	H	**5** (3%)	**10** (20%)	**15** (30%)		
3	70	16	H	Me	H	**6** (trace)	**11** (49%)	**16** (9%)		
4	70	16	H	Me	Me	**7** (trace)	**12** (29%)	**17** (3%)		
5	70	20	H	Ph	H		**13** (21%)			
6	70	4	H	Ph	H			**18** (11%)		
7	110	6	H	H	H				**19** (70%)	
8	110	6	Me	H	H		**10** (10%)		**20** (37%)	
9	110	6	H	Me	H				**21** (24%)†	
10	110	6	H	Me	Me		**12** (10%)			
11	110	6	H	Ph	H	**8** (9%)			**22** (8%)	
12	110	24	H	Ph	H				**22** (13%)	

Scheme 1. The products of the reactions of indirubin **2** with a series of allyl bromides: Type **A** - isatin derivatives (**4 - 8**), Type **B** - *N*'-substituted indirubin derivatives (**9 - 13**), Type **C** - *N*,*N*'-disubstituted indirubin derivatives (**14 - 18**) and Type **D** - spiroindolinepyrido[1,2-*a*]indoledione derivatives (**19 - 22**). † Note: *C*-allyl substituent is not crotyl but has rearranged – see Figure 4 for details of structure. Trace indicates detected by TLC analysis at the completion of the reaction. # 3 Å sieves were additionally added to procedure B reactions.

Analysis of the ^{1}H NMR spectra of **21** revealed a dd at 5.03 ppm, assigned to the H7' olefin and multiplets at 7.08-7.16 and 3.40-3.47 ppm which were assigned to H6' and H8' within these peaks respectively (Figure 4). The terminal methyl group of the *N*-1-butenyl substituent was assigned to the doublet at 1.72 ppm (J = 7.3 Hz) whereas the methyl substituent of the *N*-2-butenyl substituent was assigned to the doublet at 0.52 ppm (J = 8.0 Hz). The terminal olefinic methylene of this substituent was assigned to the doublet of doublets at 4.64 ppm (J = 10.0, 1.8 Hz) and 4.86 ppm (J = 17.0, 2.1 Hz) with the former in a *cis* orientation to the adjacent CH (multiplet at 5.72-5.89 ppm) and the latter in the relative *trans*

orientation. These assignments indicated the presence of the *C*-methylallyl substituent, possibly formed by a rearrangement process after *O*-alkylation as discussed in the mechanistic section.

The structures of the spiroindolinepyrido[1,2-*a*]indolediones **19**, **20** and **21** were confirmed by single crystal X-ray crystallographic analysis, corroborating the *C*-allylic substituents and relative stereochemistry with the *syn* disposition of the *C*-allylic substituent and the 3,8' C-C bond in each case (Figure 4).

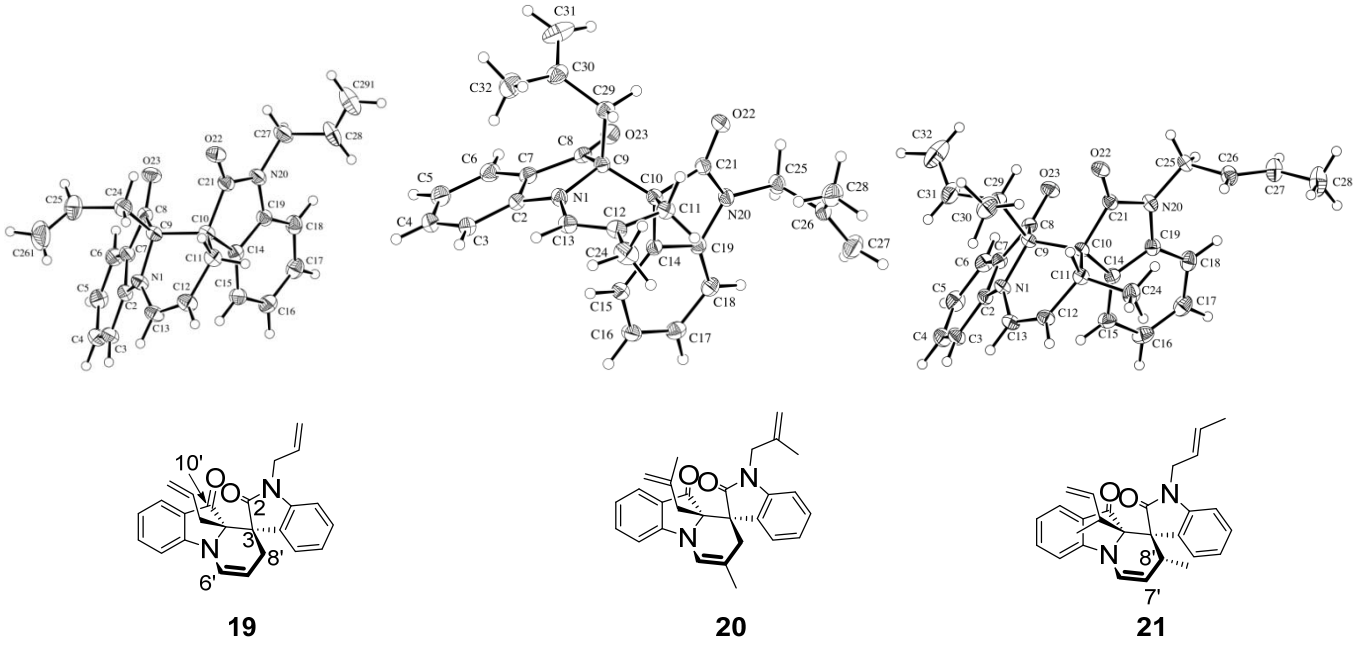


Figure 4. Crystal structures of the spiroindolinepyrido[1,2-*a*]indolediones **19**, **20** and **21**.

There are reports[24,25] of the synthesis of molecules with the same spiro heterocyclic skeleton to that in **19-22,** however these arose from reaction of skatole with 2-methylindole, followed by acylation in polar solvents – subsequent examination of the by-products revealed a spiro-type system, however, there was limited spectral evidence to support the proposed structures.

The yields for the spiroindolinepyrido[1,2-*a*]indoledione systems from indirubin decrease with substitution of the allyl bromide, *e.g.* the yield drops to 37% with the additional methyl substituent at C2 of the allyl moiety (entry 8) and down to 13% with the use of cinnamyl bromide (entry 12), this latter reaction requiring significantly longer reaction times (24 h) to achieve even this yield. With a very bulky terminal *gem*-dimethyl group in the allylic bromide, no spiro-cyclization was observed. This trend is likely to arise from the steric impedance of the orthogonal spiro scaffold blocking the approach of the substituted allyl units during the installation of the C-allyl substituent (see Section 2.2 Mechanistic Proposals). Although the spiroindolinepyrido[1,2-*a*]indoledione **19**, arising from addition of allyl bromide, was also observed in the reaction performed at 70 °C in a 2% yield (entry 1), no other examples were detected in reactions at this temperature (entries 2-6), probably indicative of the extra bulk of the substituted allyl moieties sterically impeding the spiro cyclisation. Further, in the 110 °C reactions (entries 7-12), there was no evidence of the presence of Type **C** products with Type **B** compounds

probably reacting further at the higher temperature to the Type **D** products. The *N*-allylisatins were only detected in the 110 °C reactions in the case of the cinnamyl moiety (9%) at 6 h (entry 11). It is likely that the bulky cinnamyl unit lead to a slower reaction, allowing time for the oxidative cleavage to occur. The longer reaction times at higher temperatures probably resulted in further degradation in the reaction mixture.

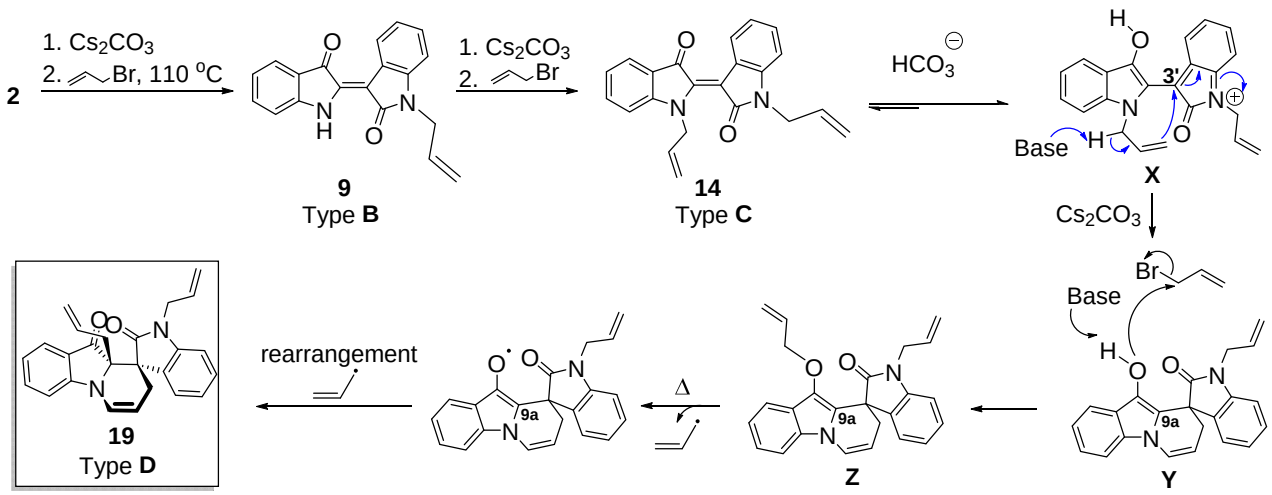
Along with these major products, minor fragmentation products were isolated including the anthranilate esters allyl 2-(allylamino)benzoate and 2-methylallyl 2-((2-methylallyl)amino)benzoate in very low yield from the respective allylation reactions at 110 °C and 70 °C. Structural elucidations of these esters arose from analysis of spectral data, with a diagnostic absorption band for the ester carbonyl group being apparent at 1673 cm^{-1} for the former and at 1682 cm^{-1} for the latter.

For comparison purposes with the indigo substrate,[3] reaction of propargyl bromide with indirubin in the presence of base at 70 °C for 16 hours only yielded *N*,*N*'-dipropargylindirubin **23** in low yield (13%) plus some (8%) of the isatin-derived anthranilate ester, prop-2-yn-1-yl 2-(prop-2-yn-1-ylamino)benzoate.

2.2. Mechanistic Proposals

The proposed mechanism for the 70 °C reactions proceeds with *N*-allylation on the oxindole ring producing the Type **B** compounds with a simple second allylation affording the Type **C** compounds (Scheme 2). In the 110 °C reactions, the diallylindirubin (Type **C**) upon *O*-protonation (tentatively with the weakly acidic bicarbonate ion as the proton source) and subsequent electron redistribution yields the intermediate ion **X**, which under base-catalysed conditions, undergoes cyclisation to form the spiro moiety (intermediate **Y**), with the C3' position activated by the conjugated protonation nitrogen atom. Subsequent *O*-allylation would generate the intermediate **Z**, a molecule not yet isolated from this reaction, as it is presumably converted rapidly to the isolated Type **D** spiro compounds. A number of possibilities exist for the mechanism converting **Z** to Type **D** compounds: a 1,3-Claisen rearrangement would yield the observed outcome for spirocycles **19** and **20**, but not for **21** and **22.** Similarly, a route *via* enolate anion formation followed by a direct C9a allylation process, while satisfactory for most examples, does not explain outcome **21**. A possible unifying mechanism that explains all results is a thermally-induced homolytic cleavage radical cleavage of the allyloxy group in **Z** to produce two highly stabilised radical species, which could then react through attack at C9a to afford **21**.[2] With the comparable cinnamyl product **22** it is likely that steric factors mitigate against the cinnamyl radical at the carbon bearing the phenyl group.

The ^{13}C NMR spectra for these heterocycles showed no duplication in peaks, consistent with the formation of a single enantiomeric pair out of the four possible diastereomers. The proposed mechanism is in accordance with this observation with the initial cyclisation to form the spiro atom likely to produce both possible stereoisomers as approach from either face would be equal. The generation of the second



Scheme 2. Proposed mechanism towards the formation of the spiroindolinepyrido[1,2-*a*]indolediones **19** - **22**.

stereogenic atom occurs during the final rearrangement step and here, there is a clear facial discrimination with the approach of the allyl unit (probably an allylic radical species) from the less hindered carbonyl side. The higher temperature of 110 °C is required to drive the reaction through to the Type D spiro compounds, while at 70 °C, *N*-monoallylation and *N*,*N*'-diallyation products predominate. In a separate experiment, reaction of the *N*-monoallylated indirubin (**9**) with excess allyl bromide at 110 °C for 20 h, only produced a complex mixture with no indication of the spiroindolinepyridoindole heterocycle (**14**), presumably due to further reaction of this compound under the conditions. We observed that *N*,*N*'-disubstituted indirubins were not stable in solution at room temperature under light exposure. When *N*,*N*'-diallylindirubin was kept in dichloromethane for several days, its colour gradually changed from dark blue to reddish-yellow. It is known that indigo is stable against photoinduced *Z* -> *E* isomerisation due to its stabilizing NH hydrogen bonding, but substitutions on both nitrogens results in a loss of hydrogen bonding and leads to phototropic behaviour.[26] The same loss of H-bonding may be the reason for the low stability of the disubstituted indirubins (*c.f.* Fig. 3): the *Z* configuration of indirubin and its *N*'-substituted derivatives exhibit stabilizing NH-mediated hydrogen bonds and are therefore inert against *Z* to *E* isomerisation, while this is not the case for the disubstituted indirubins and photoinduced *Z* to *E* isomerization occurs.

The formation of the isatin based products can be rationalised in terms of oxidative cleavage with adventitious oxygen.[22] Subsequent nucleophilic attack by allyl alcohol (formed from the bromide in the presence of carbonate) on the isatin C2-carbonyl and then addition to the C3 carbonyl, followed by fragmentation with loss of carbon dioxide and propene would give the minor anthranilate ester observed.

2.3. Colour Properties

Indirubin (**2**) as well as its *N*'-substituted derivatives (**9**-**13**) are red compounds with UV/Vis absorption maxima of about 536 nm and 533 nm in methanol whereas the *N*,*N*'-disubstituted derivatives (**14**-**18**)

without an NH…OC hydrogen bond are blue and have a UV/Vis absorption maximum of about 575 nm in methanol. Selected data is given in Table 1.

Calculated UV/Vis absorption maxima differ significantly from the experimental values but the increase in λ_{max} from the *N*'-allylated to the *N*,*N*'-diallylated indirubin is predicted correctly with the Hartree Fock and DFT methodology. However, the calculated wavelengths using B3LYP theory are closer to the experimental wavelengths in comparison to Hartree-Fock theory. Taking into account the solvent effect, which leads to a bathochromic shift of the absorption maximum wavelengths the more polar the solvent used, the B3LYP results are acceptable as the calculated values are *in vacuo* (Table 2). In contrast to the indirubins, the spiroindolinepyrido[1,2-*a*]indoledione (**19**) is yellow, reflecting the absence of the 2,3'-double bond and the consequent decrease in conjugation. In order to examine this further, the calculated UV/Vis absorption spectra *in vacuo* and experimentally obtained UV/Vis spectra of **19** were compared (Table 1). Again, density functional theory (B3LYP) correctly predicted the observed large decrease in λ_{max} for **19** compared with **14**. The predictive power of DFT has been well established by Jacquemin *et al* for indirubin, isoindigo and derivatives[25,26] including the observation that better fits between theory and experiment occur when bulk solvent effects are included in the calculations. Further investigations are required to more fully examine this with compounds **9** and **14**.

The loss of a hydrogen bond should lead in theory to an increased energy difference between the ground state and the excited state and therefore to an UV/Vis absorption maximum at lower wavelengths.[29,30] However, the opposite is observed with the calculated values (**2** to **9**). This bathochromic shift can be explained by referring to the 'cross-conjugated' system ('H-chromophore'), which was found to be an important feature to explain (although other explanations have been proposed[31]), the colour of indigoid molecules.[32,33] It consists of a C=C double bond substituted with two electron donor groups (EDGs; in an *anti* configuration to each other) and two electron withdrawing groups (EWGs; in an *anti* configuration to each other). In indirubin and the *N*-allylated derivatives such a chromophore is present with the two carbonyl moieties as EWGs and an amino group and a fused aryl ring as electron donor groups. Calculations of the UV/Vis absorption maxima using B3LYP (after optimization at the 6-31G* level) on **2**, **9** and **14**, as well as cut down oxopyrrolidinylideneindolinone theoretical model systems lacking one fused benzene ring, supported the significance of the H-chromophore in determining the colour of these systems (see Supplementary Information for the structures of the model systems and the full results).

2.4. Biological Activity

As indirubin and its derivatives are known to be active against various cancer cell lines, we screened our derivatives against a small number of cancer cell lines, namely small cell lung cancer (NCI-H187), KB-oral cavity cancer and MCF-7 breast cancer, as well as testing for *in vitro* anti-TB (*Mycobacterium tuberculosis*, H37Ra strain) and anti-plasmodial (*Plasmodium falciparum*, K1 strain) activity. The results are summarized in Table 2.

Table 1. Summary of experimentally obtained UV/Vis absorption maxima (spectra are measured in methanol) and calculated UV/Vis absorption maxima (spectra are calculated *in vacuo*) using B3LYP and Hartree Fock theory after optimization at the 6-31G* level. The numbers in between rows are the differences between absorption maxima for the respective compound pairs.

	Colour	UV/Vis absorption maxima ($\lambda_{max,}$ nm)		
		Experimental	B3LYP	
Compound	(solid)	(CH_2Cl_2)		Hartree Fock
indirubin (**2**)	red	536	474	311
		-3	+16	+6
N'-allylindirubin (**9**)	red	533	490	317
		+44	+22	+18
N,*N*'-diallylindirubin (**14**)	blue	577	512	335
		-151	-127	0
spiroindolinepyrido[1,2-*a*]indoledione (**19**)	yellow	426	385	335

The isatin derivatives **4** and **5** showed modest, cytotoxic activity against the small cell lung cancer cell line but not against the other tested cancer cell lines, nor the mycobacterial or plasmodial strains. The *N*'-substituted and *N*,*N*'-disubstituted indirubins show activities against the lung cancer cell line with the most potent compound being the mono diamethylallyl substituted derivative **12,** with an IC_{50} value of 0.66 μg/mL (2.0 μM) comparable to the positive controls ellipticine (1.47 μg/mL; 5.97 μM) and doxorubicin (0.077 μg/mL; 0.147 μM). A little surprisingly, compound **12** was inactive against the other two cancer cell lines. The most promising compound in the *N*,*N*'-disubstituted indirubin series was the 2-methylallyl derivative **15**, with activity against all three cancer cell lines, and being somewhat better than the positive controls against the rather refractory MCF-7 cell line; modest anti-TB activity *in vitro* was also apparent with this compound, while the disubstituted indirubin **16** was active in the anti-plasmodial assay. Although indirubin derivatives have been shown[13] to inhibit the apicomplexan protozoal parasite *Toxoplasma gondii*, to the best of our knowledge anti-plasmodial activity of *N*,*N*'-disubstituted indirubins involving the apicomplexan *Plasmodium falciparum* has not been reported previously. However, derivatives with the isomeric isoindigo skeleton do show such inhibitory activity against *P. falciparum*.[17]

Of the four spiroindolinepyrido[1,2-*a*]indolediones tested, anti-proliferative activity was seen only with compounds **19** and **22**, although no clear SAR outcomes can be drawn from this limited series. Promising anti-plasmodial activity in the low micromolar range was also seen with **19** and **22**, and these compounds could serve as useful new leads for potential antimalarial drug development, with the incorporation of a spiro element in the framework of particular interest.[34,35]

3. Conclusions

We report here for the first time cascade reactions of indirubin through the reaction with allylic bromides in the presence of base. The initial reaction can be controlled to produce the pink-red *N*-monoallylated products, but the subsequent synthesis of the deep blue *N*,*N*'-diallylated indirubin was less successful due to the instability of the products. The cascade process begins when the reaction is heated to 110 °C – these are higher temperatures to induce the ongoing reaction process when compared to the reactions of

Table 2. Anti-cancer, anti-tubercular, and anti-plasmodial activity of compounds against three different cancer cell lines small cell lung cancer (NCI-H187), KB-oral cavity and MCF 7 breast cancer, all tested using a resazurin microplate assay (REMA), and an anti-TB (*Mycobacterium tuberculosis*, H37Ra strain), tested using a green fluorescent protein microplate assay (GFPMA); anti-plasmodial activity (*Plasmodium falciparum*, K1 strain), tested using a microculture radioisotope technique.

	Lung Cancer IC_{50} µg/mL (µM)	Oral Cancer IC_{50} µg/mL (µM)	Breast Cancer IC_{50} µg/mL (µM)	Anti-TB IC_{50} µM	Anti-Plasmodial IC_{50} µg/mL (µM)
4	9.37 (50.1)	-	-	-	-
5	2.9 (14.4)	-	-	-	-
9	5.86 (19.4)	-	-	-	-
10	12.50 (39.6)	-	-	-	-
11	7.52 (23.8)	-	-	-	-
12	0.66 (2.0)	-	-	-	-
13	33.78 (89.4)	43.37 (114.7)	-	-	-
15	6.46 (17.5)	12.37 (33.4)	3.94 (10.6)	50.00	-
16	20.69 (55.9)	-	-	-	4.82 (13.0)
17	20.72 (52.1)	15.19 (38.1)	-	-	-
23	33.15 (98.1)	29.98 (88.7)	-	-	-
19	9.82 (24.8)	18.94 (47.8)	-	-	3.69 (9.3)
20	-	-	-	-	-
21	-	-	-	-	-
22	7.94 (18.7)	11.07 (26.1)	-	-	3.54 (8.3)
Ellipticine	1.47	0.737			
Doxorubicin	0.077	0.504	7.97		
Tamoxifen			9.47		
Rifampicin				0.025	
Streptomycin				0.625	
Mefloquine					(0.0303)

- = inactive.

the related indigo where the cascade process completely consumes the starting material after 1 h at 88 °C.[2] Therefore, the indirubin molecule is more stable than indigo. The overall result of the cascade allylation of indirubin is the unexpected one-pot production of spiroindolinepyrido[1,2-*a*]indoledione heterocycles. This method provides simple access to these relatively complex structures with good diastereoselectivity. Importantly, this chemistry provides a facile route to the spirooxindole skeleton, a

motif that is becoming increasingly important with the emergence of numerous examples both from nature and with biological activity.[36-38] The results reported here highlight this importance with examples of derivatives which show significant activity as lead compounds against *P. falciparum* and micromolar activity against 3 different cancer cell lines. Therefore, this new chemistry of indirubin presents fresh possibilities to explore heterocyclic chemical space in a compact one-pot synthetic regime, which should be amenable to further generalisation.

3.1. Experimental section

3.2. General Methods

DMF for dry reactions was obtained from a solvent purification system. Dichloromethane for extractions and column chromatography was distilled in air prior to use or HPLC grade dichloromethane was used, while other solvents were purchased reagent grade and used without further purification. Indirubin (**2**) was synthesized according to a literature procedure from isatin and 3-iodo-(1*H*)-indole.[10] Reactions were carried out under nitrogen which was dried by passage through a 20 cm tube filled with $CaCl_2$ or silica based drying agent, unless otherwise stated. Solvents were removed *in vacuo* on a rotary evaporator and products dried under high vacuum (~1 mbar) at room temperature. Flash column chromatography was performed using Merck flash silica gel 60 (63-200 mesh), particle size 40-63 μm, under a positive pressure of air. Preparative TLC (PTLC) was performed using Merck silica gel F254 pre-coated glass plates (20 x 20 cm) with a layer thickness of 500, 1000, 1500 or 2000 μm.

Compounds were visualized under UV light (254 and 365 nm) using Merck silica gel F254 precoated aluminium plates. Melting points (Mp) were determined using a Gallenkamp (Griffin) melting point apparatus, are uncorrected and stated in degrees Celsius (°C). Infrared (IR) spectra were recorded with neat samples using a Nicolet Avatar 360 FT-IR spectrometer fitted with a Smart Omni-Sampler germanium crystal accessory. IR data is recorded in nanometers (nm) with peak intensity assigned as weak (w), medium (m) or strong (s). Low resolution mass spectra were obtained by electrospray ionisation (ESI) mass spectroscopy on a Micromass Platform LCZ spectrometer by injecting the samples as a solution in methanol. In some cases 1% HCOOH was added to suppress dimerization and/or aid in protonation. Alternatively, electron impact (EI) mass spectra were performed using a Shimadzu QP-5050 spectrometer. High resolution mass spectrometry (HRMS) was performed using electrospray ionisation technique on a Waters QTOF Xevo spectrometer or electron impact technique on a Fison/VG Autospec-TOF spectrometer at 70 eV with a source temperature of 250 °C. Ion mass charge (*m/z*) values of molecular ions (M), fragment peaks and adducts are stated with their relative abundances in parentheses. For compounds with more than one major isotope all significant isotopic peaks are reported. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded at 500 and 125 MHz respectively on a Varian Inova 500 MHz spectrometer or a VNMRS PS54 500 MHz spectrometer. Alternatively, ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz respectively on a Varian

Mercury 300 MHz spectrometer. All NMR shifts are reported in parts per million (ppm) and the δ values reported relative to a standardised value (standard in brackets). NMR data was acquired in $CDCl_3$ (TMS, ^1H: δ = 0 ppm; $CDCl_3$, ^{13}C: δ = 77.0 ppm), $(CD_3)_2SO$ (DMSO, ^1H: δ = 2.50 ppm; $(CD_3)_2SO$, ^{13}C: δ = 39.5 ppm), CD_3OD (methanol, ^1H: δ = 3.31 ppm; CD_3OD, ^{13}C: δ = 49.0 ppm) and $(CD_3)_2CO$ (acetone, ^1H: δ = 2.05 ppm; $(CD_3)_2CO$, ^{13}C: δ = 29.8 ppm). Coupling constants (*J*) are reported in Hertz (Hz) and refer to coupling between hydrogen nuclei over three bonds if one coupling constant is reported. If two coupling constants are reported, the smaller of the two refers to coupling between hydrogen nuclei over four bonds. Multiplicities are reported as singlet (s), broad singlet (bs), doublet (d), doublet of doublet (dd), triplet (t), doublet of triplet (dt), quartet (q), pentet (p), sextet, septet and multiplet (m). Assignment of carbons and protons for all compounds are based on the 1D and 2D NMR spectroscopic experiments APT, gCOSY, gHSQC, gHMBC and NOESY unless otherwise stated.

UV/Vis spectra were obtained on dichloromethane solutions using a double beam UV-Vis-NIR spectrophotometer (Cary 500) operating between 300 – 1400 nm. All solutions were appropriately diluted with dichloromethane prior to analysis to fit within the absorbance limits of the detector, and placed into 1 cm pathlength quartz cuvettes. All spectra were collected at room temperature. Sample sizes were between 1.000-2.000 mg, weighed with an accuracy of 6 decimal places using the seven figure balance CAHN C-35. Monosubstituted indirubin derivatives were diluted to 50.0 mL and all other indirubin derivatives to 25.0 mL The ε values reported have the units $M^{-1}cm^{-1}$.

X-ray Structure Determination – images were measured on a Nonius Kappa CCD diffractometer (MoKα, graphite monochromator, λ = 0.71073 Å) and data extracted using the DENZO package.[39] Structure solution was by direct methods (SIR92).[40] The structures were refined using the CRYSTALS program package.[41] Atomic coordinates, bond lengths and angles, and displacement parameters for compounds **19**, **20** and **21** have been deposited at the Cambridge Crystallographic Data Centre (CCDC 1048189-1048191). These data can be obtained free-of-charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Computational Methods

Geometry Optimisations. The calculations were performed *in vacuo* throughout using the Spartan package 2010 (V1.1.0) as the calculation tool and the geometry of the molecule in the ground state was optimized using Hartree-Fock (HF) theory and the 6-31G* basis set.

UV/Vis spectra. The Spartan package 2010 (V1.1.0) was used as the tool to calculate the spectra of the molecules in the ground state using either density functional theory and the hybrid functional B3LYP or HF theory after geometry optimisation with the 6-31G* basis set.

3.3. Synthesis

Synthetic Note: All potentially chiral compounds in this work were isolated or used as racemates.

3.3.1. General Procedure A.

A suspension of indirubin (1.0 eq.) in anhydrous DMF (20 mL) was sonicated for 5 min. To the stirred solution was added caesium carbonate (2.4 eq.) and the reaction mixture was heated to 70 °C. Allylic bromide (or derivative) (2.9 eq.) was added and the reaction mixture was stirred for the specified time at 70 °C. The mixture was filtered while hot, washed with a little DMF and the filtrate concentrated under reduced pressure. The crude residue was subjected to flash silica gel column chromatography (100% petroleum spirit to CH_2Cl_2/petroleum spirit 4:1).

*3.3.1.1. N'-Allylindirubin (**9**); N,N'-diallylindirubin (**14**); N-allylisatin (**4**).*

Following the general procedure A, a suspension of indirubin (202 mg, 771 μmol) in DMF (20 mL) was heated with caesium carbonate (603 mg, 1.85 mmol) and allyl bromide (193 μL, 2.24 mmol) for 16 h to obtain *N'*-allylindirubin (**9**) (56.1 mg, 24%) as a red solid. Mp 177-179 °C. UV/Vis: λ_{max} (ε) 535 (10184) nm. IR [cm^{-1}]: ν 3306 (NH, w), 1690 (w), 1654 (s), 1610 (s), 1463 (s), 1321(s), 1174 (s), 1103 (m), 1036 (m). ^{1}H NMR [500 MHz]: δ 4.46 (2H, d, *J* = 5.5 Hz, H3''), 5.22 (1H, s, H1''$_a$), 5.25 (1H, d, *J* = 5.0 Hz, H1''$_b$), 5.86-5.92 (1H, m, H2''), 6.86 (1H, d, *J* = 8.0 Hz, H7'), 6.96 (1H, d, *J* = 8.0 Hz, H7), 7.00 (1H, t, *J* = 8.0 Hz, H5), 7.12 (1H, t, *J* = 8.0 Hz, H5'), 7.28 (1H, t, *J* = 8.0 Hz, H6'), 7.49 (1H, t, *J* = 7.5 Hz, H6), 7.73 (1H, d, *J* = 7.5 Hz, H4), 8.89 (1H, d, *J* = 8.0 Hz, H4'), 10.53 (1H, s, NH). ^{13}C NMR [125 MHz]: δ 42.4 (C3''), 106.6 (C3'), 108.8 (C7'), 112.1 (C7), 117.7 (C1''), 120.3 (C3a), 121.4 (C2), 121.8 (C5), 122.9 (C5'), 125.5 (C4), 125.8 (C4'), 129.4 (C6'), 131.7 (C2''), 137.1 (C6), 139.6 (C3'a), 141.4 (C7'a), 151.9 (C7a), 170.6 (C2'), 188.5 (C3). MS (EI): *m/z* 302 (M^+, 100%). HRMS (ESI): calculated for $C_{19}H_{15}N_2O_2$: 303.1134, found 303.1143.

Further elution of the SiO_2 flash column provided *N,N'*-diallylindirubin (**14**) (50.1 mg, 19%) as a blue solid. Mp 82-84 °C. UV/Vis: λ_{max} (ε) 577 (4660^{42}) nm. IR [cm^{-1}]: ν 1670 (s), 1603 (s), 1461 (s), 1328 (s), 1173(s), 748.3 (s). ^{1}H NMR [500 MHz]: δ 4.43 (2H, d, *J* = 5.0 Hz, H3''), 4.82 (2H, d, *J* = 6.0 Hz, H3'''), 5.19-5.23 (3H, m, H1''$_a$, H1''$_b$, H1'''$_a$), 5.28 (1H, d, *J* = 10.0 Hz, H1'''$_b$), 5.84-5.89 (1H, m, H2''), 5.94-5.97 (1H, m, H2'''), 6.82 (1H, d, *J* = 8.0 Hz, H7'), 7.02-7.09 (2H, m, H5, H5'), 7.12 (1H, d, *J* = 8.5 Hz, H7), 7.25 (1H, t, *J* = 7.5 Hz, H6'), 7.53 (1H, t, *J* = 7.5 Hz, H6), 7.72 (1H, d, *J* = 7.5 Hz, H4), 8.63 (1H, d, *J* = 7.5 Hz, H4'). ^{13}C NMR [125 MHz]: δ 42.5 (C3''), 52.5 (C3'''), 108.7 (C7'), 110.9 (C3'), 112.5 (C7), 117.4 (C1''), 118.6 (C1'''), 121.8 (C3'a), 122.0 (C5'), 122.4 (C3a), 122.6 (C5), 125.0 (C4), 125.6 (C4'), 129.7 (C5'), 131.9 (C2''), 133.8 (C2'''), 136.6 (C6), 142.0 (C7'a), 142.4 (C2), 154.4 (C7a), 166.8 (C2'), 188.5 (C3). MS (EI): *m/z* 342 (M^+, 100%), 301 (M^+-C_3H_5, 52). MS (ESI): 343 ($M+H^+$, 100%). HRMS (ESI): calculated for $C_{22}H_{19}N_2O_2$: 343.1447, found 343.1425.

Further elution of the SiO_2 flash column provided *N*-allylisatin (**4**) (29.0 mg, 10%) as an orange solid. Mp 87-88 ºC (Lit.[1] 89-91 °C). UV/Vis: λ_{max} (ε) 410 (435) nm. IR [cm^{-1}]: ν 2959 (w), 2919 (w), 2847 (w), 1722 (s), 1601 (s), 1464 (s), 1348 (m), 1183 (w), 1085 (w), 925 (w), 760 (s). ^{1}H NMR [500 MHz]: δ 4.37 (2H, d, *J* = 5.5 Hz, H3'), 5.29-5.35 (2H, m, H1'), 5.82-5.88 (1H, m, H2'), 6.90 (1H, d, *J* = 8.0 Hz, H7), 7.13 (1H, t, *J* = 8.0 Hz, H5), 7.57 (1H, t, *J* = 8.0 Hz, H6), 7.62 (1H, d, *J* = 7.5 Hz, H4). ^{13}C NMR [125 MHz]: δ 42.7 (C3'), 111.1 (C7), 117.8 (C3a), 118.9 (C1'), 124.0 (C5), 125.6 (C4), 130.6 (C2'), 138.5 (C6), 151.1 (C7a), 158.1 (C2), 183.5 (C3). MS (ESI): *m/z* 188 ($M+H^+$, 100%).

*3.3.1.2. 2-Methylallyl 2-((2-methylallyl)amino)benzoate; (Z)-1'-(2-Methylallyl)-[2,3'-biindolinylidene]-2',3-dione (**10**); (Z)-1,1'-bis(2-methylallyl)-[2,3'-biindolinylidene]-2',3-dione (**15**); 1-allylindoline-2,3-dione (**5**).*

Following the general procedure A, a suspension of indirubin (201 mg, 767 μmol) in DMF (20 mL) was heated with caesium carbonate (600 mg, 1.84 mmol) and 3-bromo-2-methylpropene (224 μL, 2.22 mmol) for 16 h to obtain 2-methylallyl 2-((2-methylallyl)amino)benzoate as a colourless oil (12.8 mg, 7%). IR [cm^{-1}]: ν 3371 (w), 2919 (m), 2845 (w), 1682 (s), 1222 (s). ^{1}H NMR [500 MHz]: δ 1.79 (3H, s, H1''), 1.84 (3H, s, H1'''), 3.78 (2H, s, H3), 4.70 (s, 2H, H3'), 4.89 (1H, s, $H1_a$), 4.98 (2H, s, $H1_a$', $H1_b$), 5.07 (1H, s, $H1_b$'), 6.59 (1H, t, *J* = 8.0 Hz, ArH3), 6.63 (1H, d, *J* = 8.5 Hz, ArH5), 7.33 (1H, t, *J* = 8.0 Hz, ArH4), 7.97 (1H, d, *J* = 8.0 Hz, ArH2). ^{13}C NMR [125 MHz]: δ 19.8 (C1''), 20.6 (C1'''), 49.0 (C3), 67.6 (C3'), 110.0 (ArC1), 111.2 (C1''), 111.9 (ArC5), 112.8 (C1'''), 114.8 (ArC3), 131.7 (ArC2), 134.8 (ArC4), 140.5 (C2''), 142.0 (C2'''), 151.6 (ArC6), 166.2 (C=O). MS (EI) *m/z*: 245 (M^+, 63), 172 (100%). HRMS (EI) *m/z*: calculated for $C_{15}H_{19}NO_2$: 245.1416; found 245.1415.

Further elution of the SiO_2 flash column provided (*Z*)-1'-(2-methylallyl)-[2,3'-biindolinylidene]-2',3-dione (**10**) (48.5 mg, 20%) as a red solid. Mp 154-156 °C. UV/Vis: λ_{max} (ε) 536 (10339) nm. IR [cm^{-1}]: ν 3314 (w), 2915 (w), 2845 (w), 1645 (s), 1604 (s), 1464 (s), 1316 (s), 1168 (s), 1082 (s). ^{1}H NMR [500 MHz]: δ 1.76 (3H, s, H1'''), 4.38 (2H, s, H3''), 4.87 (1H, s, $H1''_a$), 4.95 (1H, s, $H1''_b$), 6.86 (1H, d, *J* = 8.0 Hz, H7'), 6.96 (1H, d, *J* = 8.0 Hz, H7), 7.00 (1H, t, *J* = 7.0 Hz, H5), 7.12 (t, *J* = 8.0 Hz, H5'), 7.26 (1H, t, *J* = 8.0 Hz, H6'), 7.49 (1H, t, *J* = 7.5 Hz, H6), 7.72 (1H, d, *J* = 7.0 Hz, H4), 8.89 (1H, d, *J* = 7.5 Hz, H4'), 10.54 (1H, s, NH). ^{13}C NMR [125 MHz]: δ 20.2 (C1'''), 46.0 (C3''), 106.6 (C3'), 109.0 (C7'), 112.1 (C7), 112.8 (C1''), 120.3 (C3a), 121.3 (C3'a), 121.8 (C5), 122.9 (C5'), 125.5 (C4), 125.7 (C4'), 129.4 (C6), 134.4 (C2), 137.1 (C6), 139.5 (C2''), 141.6 (C7'a), 151.9 (C7a), 170.8 (C2'), 188.5 (C3). MS (EI): *m/z* 316 (M^+, 100%). HRMS (EI): calculated for $C_{20}H_{16}N_2O_2$: 316.1212, found 316.1218.

Further elution of the SiO_2 flash column provided (*Z*)-1,1'-bis(2-methylallyl)-[2,3'-biindolinylidene]-2',3-dione (**15**) (85.1 mg, 30%) as a blue solid. Mp 88-90 °C. UV/Vis: λ_{max} (ε) 580 (4957) nm. IR [cm^{-1}]: ν 1666 (s), 1596 (s), 1464 (s), 1324 (s), 1168 (s), 1078 (s), 741 (s). ^{1}H NMR [500 MHz]: δ 1.50 (3H, s, H1''''), 1.75 (3H, s, H1'''''), 4.35 (2H, s, H3'''), 4.81-4.91 (4H, m, $H1''_a$, $H1''_b$, $H1'''_a$, $H1'''_b$), 4.91 (2H, s, H3''), 6.80 (1H, d, *J* = 7.5 Hz, H7'), 7.03-7.06 (2H, m, H5, H5'), 7.08 (1H, d, *J* = 8.5 Hz, H7), 7.23 (1H, t,

J = 7.5 Hz, H6'), 7.51 (1H, t, *J* = 8.0 Hz, H6), 7.72 (1H, d, *J* = 7.5 Hz, H4), 8.66 (1H, d, *J* = 8.0 Hz, H4'). ^{13}C NMR [125 MHz]: δ 19.9 (C1''''), 20.2 (C1''''), 46.0 (C3''), 54.5 (C3'''), 108.8 (C7'), 111.0 (C3'), 112.3 (C1''), 112.5 (C7), 114.9 (C1'''), 121.5 (C3'a), 121.9 (C5'), 122.3 (C3a), 122.5 (C5), 125.1 (C4), 125.6 (C4'), 129.8 (C6'), 136.6 (C6), 139.1 (C2'''), 139.7 (C2''), 142.0 (C2), 142.3 (C7'a), 154.5 (C7a), 167.0 (C2'), 188.6 (C3). MS (EI): *m/z* 370 (M^+, 100%). HRMS (EI): calculated for $C_{24}H_{22}N_2O_2$: 370.1681, found 370.1686.

Further elution of the SiO_2 flash column provided 1-(2-methylallyl)indoline-2,3-dione (**5**) (8.0 mg, 3%) as an orange solid. Mp 87-88 ºC (Lit.[3b] Mp 87-88 °C). ^{1}H NMR [500 MHz]: δ 1.77 (3H, s, H1''), 4.30 (2H, s, H3'), 4.98 (2H, d, *J* = 19.5 Hz, H1'), 6.89 (1H, d, *J* = 8.0 Hz, H7), 7.12 (1H, t, *J* = 7.5 Hz, H5), 7.56 (1H, t, *J* = 7.5 Hz, H6), 7.62 (1H, d, *J* = 7.5 Hz, H4). ^{13}C NMR [125 MHz]: δ 20.1 (C1''), 46.3 (C3'), 111.3 (C7), 113.7 (C1'), 117.7 (C3a), 124.0 (C5), 125.5 (C4), 138.3 (C2'), 138.6 (C6), 151.3 (C7a), 158.3 (C2), 183.4 (C3). MS (EI): *m/z* 201 (M^+, 100%).

*3.3.1.3. (Z)-1'-(But-2-en-1-yl)-[2,3'-biindolinylidene]-2',3-dione (**11**); (Z)-1,1'-di(but-2-en-1-yl)-[2,3'-biindolinylidene]-2',3-dione (**16**).*

Following the general procedure A, a suspension of indirubin (201 mg, 767 μmol) in DMF (20 mL) was heated with caesium carbonate (600 mg, 1.84 mmol) and crotyl bromide (229 μL, 2.22 mmol) for 16 h to obtain (*Z*)-1'-(but-2-en-1-yl)-[2,3'-biindolinylidene]-2',3-dione (**11**) with a 2.3:1.0 ratio mixture of E:Z in the *N*'-side chain (119 mg, 49 %) as a red solid. Mp 190-192 °C. UV/Vis: λ_{max} (ε) 535 (10711) nm. IR [cm^{-1}]: ν 3306 (w), 2923 (w), 1646 (s), 1601 (s), 1459 (s), 1317 (s), 1165 (s), 1103 (s), 1072 (s), 743 (s). ^{1}H NMR [300 MHz]: δ 1.69 (3H, d, *J* = 6.0 Hz, H1''), 4.39 (2H, d, 5.5 Hz, H4''), 5.40-5.55 (1H, m, H3''), 5.69-5.77 (1H, m, H2''), 6.89 (1H, d, *J* = 7.8 Hz, H7'), 6.96-7.03 (2H, m, H5, H7), 7.13 (1H, t, *J* = 7.5 Hz, H5'), 7.29 (1H, t, *J* = 8.0 Hz, H6'), 7.50 (1H, t, 7.4 Hz, H6), 7.73 (1H, d, *J* = 7.5 Hz, H4), 8.89 (1H, d, *J* = 8.1 Hz, H4'), 10.56 (1H, s, NH). ^{13}C NMR [75 MHz]: δ 17.9 (C1''), 41.8 (C4''), 108.6 (C3), 108.9 (C7'), 112.2 (C7), 120.4 (C3'a), 121.5 (C3a), 121.9 (C5), 122.9 (C5'), 124.6 (C3''), 125.5 (C4), 125.8 (C4'), 128.7 (C2''), 129.4 (C6'), 137.2 (C6), 139.6 (C5), 141.6 (C3'a), 151.6 (C3a), 170.6 (C2'), 188.6 (C3). MS (EI): *m/z* 316 (M^+, 100%), 262 (M^+-C_4H_6, 40). HRMS (ESI): calculated for $C_{20}H_{17}N_2O_2$: 317. 1290, found 317.1303.

Further elution of the SiO_2 flash column provided (*Z*)-1,1'-di(but-2-en-1-yl)-[2,3'-biindolinylidene]-2',3-dione (**16**) with a 5.5:1.0 ratio mixture of *E*:*Z* in the *N*-side chain and a 2.0:1.0 ratio mixture of E:Z in the *N*'-side chain (25.5 mg, 9%) as a blue solid. Mp 88-90 °C. IR [cm^{-1}]: ν 2952 (w), 2914 (m), 2844 (w), 1705 (s), 1606 (s), 1467 (s), 1356 (m), 1176 (m), 1081 (m), 752 (s). ^{1}H NMR [300 MHz]: δ 1.63-1.66 (6H, m, H1'', H1'''), 4.36 (2H, d, *J* = 5.7 Hz, H4''), 4.76 (2H, d, *J* = 6.0 Hz, H4'''), 5.41-5.74 (4H, m, H3'', H3''', H2'', H2'''), 6.82 (1H, d, *J* = 8.7 Hz, H7'), 6.70-7.08 (2H, m, H5, H5'), 7.16 (1H, d, *J* = 8.1 Hz, H7), 7.25 (1H, t, *J* = 7.8 Hz, H6'), 7.52 (1H, t, *J* = 8.1 Hz, H6), 7.70 (1H, d, *J* = 7.5 Hz, H4), 8.62 (1H, d, *J* = 7.5 Hz, H4'). ^{13}C NMR [75 MHz]: δ 17.9 (H1'''), 18.1 (H1''), 41.9 (H4'''), 52.0 (H4''), 108.7 (C7'), 110.9

(C3), 112.7 (C7), 121.8 (C5'), 122.0 (C3a), 122.48 (C5'), 122.53 (C3'a), 124.8 (C4), 125.0 (C3''), 125.7 (H4'), 126.3 (C3'''), 126.4 (C2'''), 129.7 (C6'), 130.4 (C2''), 136.6 (C6), 142.2 (C7'a), 142.5 (C2), 154.6 (C7a), 166.8 (C2'), 188.7 (C3'). MS (EI): *m/z* 370 (M^+, 100%), 315 (M+-C_4H_7, 56). HRMS (ESI): calculated for $C_{24}H_{23}N_2O_2$: 371.1760, found 371.1744.

*3.3.1.4. (Z)-1'-(3-Methylbut-2-en-1-yl)-[2,3'-biindolinylidene]-2',3-dione (**12**); (Z)-1,1'-bis(3-methylbut-2-en-1-yl)-[2,3'-biindolinylidene]-2',3-dione (**17**).*

Following the general procedure A, a suspension of indirubin (209 mg, 798 µmol) in DMF (20 mL) was heated with caesium carbonate (624 mg, 1.91 mmol) and 3,3-dimethylallyl bromide (267 µL, 2.31 mmol) for 16 h to obtain (*Z*)-1'-(3-methylbut-2-en-1-yl)-[2,3'-biindolinylidene]-2',3-dione (**12**) as a red solid (76.4 mg, 29%). Mp 184-186 °C. UV/Vis: λ_{max} (ε) 535 (10777) nm. IR [cm^{-1}]: ν 3316 (w), 2920 (w), 1641 (s), 1600 (s), 1464 (s), 1350 (s), 1318 (s), 1166 (s), 1068 (s), 1036 (s), 742 (s). ^{1}H NMR [300 MHz]: δ 1.73 (3H, s, H1''), 1.86 (3H, s, H1'''), 4.41 (2H, d, *J* = 6.3 Hz, H4''), 5.22 (1H, t, *J* = 6.6 Hz, H3''), 6.83 (1H, d, *J* = 8.1 Hz, H7'), 6.93-7.00 (2H, m, H5, H7), 7.10 (1H, t, *J* = 7.5 Hz, H5'), 7.47 (1H, t, *J* = 7.2 Hz, H6), 7.71 (1H, d, *J* = 7.5 Hz, H4), 8.86 (1H, d, *J* = 7.5 Hz, H4'), 10.53 (1H, s, NH). ^{13}C NMR [75 MHz]: δ 18.5 (C1''), 26.0 (C1'''), 38.2 (C4''), 107.1 (C3'), 108.7 (C7'), 112.2 (C7), 118.8 (C2''), 120.4 (C3a), 121.6 (C3'a), 121.8 (C5), 122.8 (C5'), 125.5 (C4), 125.9 (C4'), 129.4 (C6'), 137.0 (C2''), 137.2 (C6), 139.6 (C2), 141.7 (C7'a), 152.0 (C7a), 170.5 (C2'), 188.6 (C3). MS (EI): *m/z* 330 (M^+, 80), 262 (M^+-C_5H_8, 100%). HRMS (ESI): calculated for $C_{21}H_{19}N_2O_2$: 331.1447, found 331.1425.

Further elution of the SiO_2 flash column provided (*Z*)-1,1'-bis(3-methylbut-2-en-1-yl)-[2,3'-biindolinylidene]-2',3-dione (**17**) (9.53 mg, 3%) as a blue solid. Mp: 92-94 °C. UV/Vis: λ_{max} (ε) 578 (5892) nm. IR [cm^{-1}]: ν 2958 (w), 2917 (m), 2875 (w), 1673 (s), 1603 (s), 1460 (s), 1325 (s), 1163 (s), 1071 (s), 742 (s). ^{1}H NMR [500 MHz]: δ 1.66 (3H, s, H1'''), 1.70 (3H, s, H1''''), 1.71 (3H, s, H1''), 1.84 (3H, s, H1''''), 4.41 (2H, d, *J* = 7.0 Hz, H4''), 4.82 (2H, d, *J* = 6.0 Hz, H4'''), 5.18-5.24 (2H, m, H3'', H3'''), 6.90 (1H, d, *J* = 7.5 Hz, H7'), 7.02-7.06 (3H, m, H5, H5', H7), 7.25 (1H, t, *J* = 7.5 Hz, H6'), 7.51 (1H, t, *J* = 7.0 Hz, H6), 7.70 (1H, d, *J* = 7.0 Hz, H4), 8.61 (1H, d, *J* = 7.5 Hz, H4'). ^{13}C NMR [125 MHz]: δ 18.4 (C1'''), 18.5 (CH_3'''), 25.8 (C1''), 25.9 (CH_3''), 38.3 (C4'''), 48.1 (C4''), 108.5 (C7'), 108.7 (C3'), 112.5 (C7), 119.1 (C3''), 120.2 (C3'''), 121.8 (C5'), 122.0 (C3'a), 122.4 (C5), 122.6 (C3a), 124.9 (C4), 125.6 (C4'), 129.6 (C6'), 136.4 (C2'''), 136.5 (C6), 136.6 (C2''), 142.1 (C7'a), 142.6 (C2), 154.5 (C7a), 166.6 (C2'), 188.9 (C3). MS (EI): *m/z* 398 (M^+, 90), 329 (M+-C_5H_9, 57), 262 (M^+-$C_{10}H_{16}$, 100%). HRMS (ESI): calculated for $C_{26}H_{27}N_2O_2$: 399.2073, found 399.2060.

*3.3.1.5. (Z)-1'-Cinnamyl-[2,3'-biindolinylidene]-2',3-dione (**13**).*

Following the general procedure A, a suspension of indirubin (204 mg, 779 µmol) in DMF (20 mL) was heated with caesium carbonate (609 mg, 1.87 mmol) and cinnamyl bromide (450 mg, 2.26 mmol) for 16 h to obtain (*Z*)-1'-cinnamyl-[2,3'-biindolinylidene]-2',3-dione (**13**) (61.8 mg, 21%) as a red solid. Mp 180-182 °C. UV/Vis: λ_{max} (ε) 535 (10159) nm. IR [cm^{-1}]: ν 3303 (w), 2920 (w), 2847 (w), 1638 (s), 1600 (s),

1464 (s), 1356 (s), 1315 (s), 1169 (s), 1074 (s), 742 (s). ^{1}H NMR [300 MHz]: δ 4.58 (2H, d, *J* = 5.7 Hz, H3''), 6.22 (1H, dt, *J* = 15.9 Hz, *J* = 5.7 Hz, H2''), 6.59 (1H, d, *J* = 15.9 Hz, H1''), 6.87-6.99 (3H, m, H5, H7, H7'), 7.09 (1H, t, *J* = 7.8 Hz, H5'), 7.20-7.32 (6H, m, ArH2, ArH3, ArH4, ArH5, ArH6, H6'), 7.45 (1H, t, *J* = 7.5 Hz, H6), 7.69 (1H, d, *J* = 7.2 Hz, H7'), 8.86 (1H, d, *J* = 7.5 Hz, H4'), 10.51 (1H, s, NH). ^{13}C NMR [75 MHz]: δ 42.1 (H3''), 106.8 (C3'), 108.9 (C7), 112.2 (C7'), 120.4 (C3a), 121.5 (C3'a), 121.9 (C5), 123.0 (C2''), 123.3 (C5'), 125.6 (C4), 125.9 (C4'), 126.8 (ArC2, ArC6), 128.2 (C6'), 128.9 (ArC3, ArC5), 129.5 (Ar4), 133.1 (C1''), 136.5 (ArC1), 137.2 (C6), 139.7 (C2), 141.4 (C7'a), 151.9 (C7a), 170.7 (C2'), 188.5 (C3). MS (EI): *m/z* 378 (M^+, 86), 263 (M^+-C_9H_7, 100%). HRMS (ESI): calculated for $C_{25}H_{19}N_2O_2$: 379.1447, found 379.1454.

*3.3.1.6. (Z)-1,1'-Dicinnamyl-[2,3'-biindolinylidene]-2',3-dione (**18**).*

Following the general procedure A, a suspension of indirubin (201 mg, 767 μmol) in DMF (20 mL) was heated with caesium carbonate (600 mg, 1.84 mmol) and cinnamyl bromide (443 mg, 2.22 mmol) for 4 h to obtain **(***Z***)**-1,1'-dicinnamyl-[2,3'-biindolinylidene]-2',3-dione (**18**) with a 1.0:1.1 ratio mixture of *E*:*Z* in the *N*-side chain and a 1.0:1.5 ratio mixture of *E*:*Z* in the *N*'-side chain (41.7 mg, 11%) as a blue solid. Mp 89-93 °C. IR [cm^{-1}]: ν 2917 (m), 1673 (s), 1603 (s), 1464 (s), 1325 (m), 1173 (s), 1084 (s), 739 (s). ^{1}H NMR [300 MHz]: δ 2.81 (2H, d, *J* = 7.2 Hz, H3'') 4.61 (2H, d, *J* = 6.0 Hz, H3'''), 6.21 (1H, dt, *J* = 15.9 Hz, 5.7 Hz, H2''), 6.36 (1H, dt, *J* = 15.9 Hz, 5.7 Hz, H2'''), 6.59 (1H, d, *J* = 15.9 Hz, H1''), 6.63 (1H, d, *J* = 16.2 Hz, H1'''), 6.88 (1H, d, *J* = 7.8 Hz, H7'), 7.04 (1H, t, *J* = 8.1 Hz, H5'), 7.10-7.32 (12H, m, ArH2, ArH2', ArH3, ArH3', ArH4, ArH4', ArH5, ArH5', ArH6, ArH6', H6', H7), 7.49 (1H, t, *J* = 7.8 Hz, H6), 7.73 (1H, d, *J* = 7.8 Hz, H4), 8.65 (1H, d, *J* = 8.1 Hz, H4'). ^{13}C NMR [75 MHz]: δ 40.8 (C3''), 42.2 (C3'''), 108.9 (C7'), 111.0 (ArC), 112.7 (ArC), 121.9 (ArC), 122.1 (C5), 122.5 (ArC), 122.8 (C5'), 123.5 (C2'''), 123.7 (C2''), 124.1 (ArC), 125.1 (C4), 125.8 (C4''), 126.5 (2 x ArC), 126.6 (ArC), 126.76 (2 x ArC), 127.5 (ArC), 128.3 (ArC), 128.8 (2 x ArC), 129.9 (ArC), 131.4 (ArC), 132.8 (ArC), 134.1 (ArC), 136.8 (C6), 142.1 (ArC), 143.1 (C7'a), 154.5 (C7a), 166.9 (C2), 178.8 (C2'), 188.6 (C3). MS (EI): *m/z* 494 (M^+, 40), 377 (M+-C_9H_9, 33), 362 (M+-$C_{10}H_{12}$, 100%). HRMS (ESI): calculated for $C_{34}H_{27}N_2O_2$: 495.2073, found 495.2080.

3.3.1.7. Prop-2-yn-1-yl 2-(prop-2-yn-1-ylamino)benzoate; (Z)-1,1'-di(prop-2-yn-1-yl)-[2,3'-biindolinylidene]-2',3-dione.

Following the general procedure A, a suspension of indirubin (205 mg, 767 μmol) in DMF (20 mL) was heated with caesium carbonate (600 mg, 1.84 mmol) and propargyl bromide (443 mg, 2.22 mmol) for 16 h to obtain prop-2-yn-1-yl 2-(prop-2-yn-1-ylamino)benzoate (13.2 mg, 8%) as a colourless solid. Mp 53-54 ºC (Lit.[5a] 55-56 °C). ^{1}H NMR [300 MHz]: δ 2.24 (1H, t, *J* = 2.7 Hz, H1''), 2.50 (1H, t, *J* = 2.7 Hz, H1'), 4.04 (2H, dd, *J* = 5.9 Hz, 2.4 Hz, H3''), 4.87 (2H, d, *J* = 2.4 Hz, H3'), 6.69 (1H, td, *J* = 7.5 Hz, 0.6 Hz, H5), 6.80 (1H, d, *J* = 8.4 Hz, H3), 7.43 (1H, td, *J* = 8.0 Hz, 1.5 Hz, H4), 7.85 (1H, bs, NH), 7.97 (1H, dd, *J* = 8.1 Hz, 1.8 Hz, H6). ^{13}C NMR [75 MHz]: δ 32.7 (C3''), 52.2 (C3'), 71.6 (C1''), 75.0 (C1'), 78.2

(C2'), 79.8 (C2''), 110.4 (C1), 111.9 (C3), 116.1 (C5), 132.1 (C6), 135.3 (C4), 150.4 (C2), 167.8 (C=O). MS (ESI), *m/z* 214 ($M+H^+$, 100%). HRMS (ESI): calculated for $C_{13}H_{12}NO_2$: 214.0868, found 214.0860.

Further elution of the SiO_2 flash column provided (*Z*)-1,1'-di(prop-2-yn-1-yl)-[2,3'-biindolinylidene]-2',3-dione (33.8 mg, 13%) as a blue solid. Mp 183-185 °C. UV/Vis: λ_{max} (ε) 559 (4292) nm. IR [cm^{-1}]: ν 3261 (w), 3234 (w), 1650 (s), 1606 (s), 1464 (s), 1330 (s), 1165 (s), 1076 (s), 1019 (m), 930 (m), 756 (s). ^1H NMR [500 MHz]: δ 2.24 (1H, t, *J* = 2.0 Hz, H1''), 2.27 (1H, t, *J* = 2.0 Hz, H1'''), 4.62 (2H, d, *J* = 2.5 Hz, H3''), 5.02 (2H, d, *J* = 2.5 Hz, H3'''), 7.05 (1H, d, *J* = 8.0 Hz, H7'), 7.09-7.15 (2H, m, H5, H5'), 7.27 (1H, d, *J* = 8.0 Hz, H7), 7.34 (1H, t, *J* = 7.5 Hz, H6'), 7.61 (1H, t, *J* = 8.0 Hz, H6), 7.75 (1H, d, *J* = 7.0 Hz, H4), 8.70 (1H, d, *J* = 8.0 Hz, H4'). ^{13}C NMR [125 MHz]: δ 29.5 (C3''), 39.5 (C3'''), 72.3 (C1''), 74.0 (C1'''), 77.2 (C2''), 77.3 (C2'''), 108.9 (C7'),111.5 (C7'a), 112.6 (C7), 122.05 (C3'), 122.07 (C3'a), 122.6 (C5), 123.1 (C5'), 125.1 (C4), 126.3 (C4'), 130.3 (C6'), 136.8 (C6), 141.2 (C7'a), 142.2 (C2), 153.7 (C7a), 166.3 (C2'), 188.0 (C3). MS (EI): *m/z* 338 (M^+, 100%), 299 (M^+-C_3H_3, 78). HRMS (ESI): calculated for $C_{22}H_{15}N_2O_2$: 339.1134, found 339.1127.

3.3.2. General Procedure B.

A suspension of indirubin (1.0 eq.) in dry DMF (20 mL for 763 μmol) was sonicated for 5 min and then 3 Å molecular sieves (40.0 g for 763 μmol) were added. To the stirred solution was added caesium carbonate (2.4 eq.) and the reaction mixture was flushed with N_2 and heated to 70 °C. The allyl bromide or an analogous derivative (4.7 eq.) was added and the reaction mixture was heated to 110 °C and stirred at this temperature for 6-24 h under an atmosphere of N_2. The mixture was filtered while hot, washed with a little DMF and the filtrate concentrated under reduced pressure. The crude residue was subjected to flash silica gel column chromatography (gradient to CH_2Cl_2/petroleum spirit 4:1).

*3.3.2.1. Allyl 2-(allylamino)benzoate; 1,9a'-Diallyl-8'H-spiro[indoline-3,9'-pyrido[1,2-a]indole]-2,10'(9a'H)-dione (**19**).*

Following the general procedure B, a suspension of indirubin (206 mg, 786 μmol) in DMF (20 mL) was heated with caesium carbonate (615 mg, 1.89 mmol) and allyl bromide (319 μL, 3.69 mmol) for 6 h to obtain allyl 2-(allylamino)benzoate as a colourless oil (11.6 mg, 7%). IR [cm^{-1}]: ν 3364 (w), 3266 (m), 2914 (w), 2848 (w), 1673 (m), 1232 (s), 1103 (s). ^1H NMR [500 MHz]: δ 3.87 (2H, t, *J* = 5.5 Hz, H3), 4.78 (2H, d, *J* = 6.0 Hz, H3'), 5.18 (1H, d, *J* = 10.5 Hz, $H1_a$), 5.27 (1H, d, *J* = 9.0 Hz, $H1_b$), 5.29 (1H, d, *J* = 15.3 Hz, $H1_a'$), 5.40 (1H, d, *J* = 17.5 Hz, $H1_b'$), 5.91-5.99 (1H, m, H2), 5.99-6.06 (1H, m, H2'), 6.59 (1H, t, *J* = 7.5 Hz, ArH3), 6.66 (1H, d, *J* = 8.5 Hz, ArH5), 7.34 (1H, t, *J* = 7.8 Hz, ArH4), 7.86 (1H, bs, NH), 7.96 (1H, d, *J* = 8.0 Hz, ArH2). ^{13}C NMR [125 MHz]: δ 45.5 (C3), 65.1 (C3'), 111.8 (ArC1), 114.9 (ArC5), 116.3 (C1), 118.0 (C1'), 131.9 (ArC5), 132.8 (C2'), 134.5 (C2), 134.8 (ArC4), 151.3 (ArC6), 168.5 (C=O). MS (ESI) *m/z*: 217 ($M+H^+$, 100%). HRMS (ESI) *m/z*: calculated for $C_{13}H_{16}NO_2$: 218.1181; found 218.1181.

Further elution of the silica gel flash column provided 1,9a'-diallyl-8'*H*-spiro[indoline-3,9'-pyrido[1,2-*a*]indole]-2,10'(9a'*H*)-dione (**19**) (210 mg, 70%) as a yellow solid. Mp 189-191 °C. UV/Vis: λ_{max} (ε) 414 (2014). IR [cm^{-1}]: ν 1699 (s), 1605 (s), 1472 (m), 1361 (w), 1197 (w), 752 (s). ^{1}H NMR [300 MHz]: δ 2.06 (1H, ddd, *J* = 18.3 Hz, 5.3 Hz, 1.8 Hz, H8'$_a$), 2.74 (1H, dd, *J* = 13.7 Hz, 7.2 Hz, H3'''$_a$), 3.00 (1H, dt, *J* = 18.3 Hz, 1.8 Hz, H8'$_b$), 3.33 (1H, dd, *J* = 14.1 Hz, 6.9 Hz, H3'''$_b$), 4.33 (1H, ddt, *J* = 15.9 Hz, 5.4 Hz, 3.6 Hz, H3''$_a$), 4.54-4.61 (1H, m, H3''$_b$), 4.83 (1H, dd, *J* = 9.9 Hz, 2.4 Hz, H1'''$_a$), 5.05 (1H, dd, *J* = 16.8 Hz, 1.8 Hz, H1'''$_b$), 5.02-5.41 (4H, m, H1''a, H1''b, H2''', H7'), 5.88-6.11 (1H, m, H2''), 6.57 (1H, t, *J* = 7.5 Hz, H5), 6.68 (1H, t, *J* = 8.3 Hz, H2'), 6.74 (1H, d, *J* = 7.5 Hz, H7), 6.83 (1H, d, *J* = 7.2 Hz, H4), 7.00-7.06 (3H, m, H6, H6', H4'), 7.24 (1H, d, *J* = 8.1 Hz, H1'), 7.45 (1H, t, *J* = 8.4 Hz, H3'). ^{13}C NMR [75 MHz]: δ 30.1 (C8'), 35.0 (C3'''), 42.8 (C3''), 48.0 (C3), 70.4 (C9'a), 103.3 (C1''), 107.8 (C4'), 108.9 (C7), 117.5 (C2'''), 119.2 (C2'), 119.4 (C1'''), 121.8 (C5), 122.0 (C10'a), 122.5 (C6'), 124.3 (C1'), 124.7 (C4), 128.2 (C6), 128.9 (C3a), 131.0 (C7'), 131.6 (C2''), 137.3 (C3'), 142.1 (C7a), 156.1 (C4'a), 175.6 (C2), 199.1 (C10'). MS (EI): *m/z* 382 (M^+, 74), 341 (M^+-C_3H_5, 100%), 300 (M^+-C_6H_{10}, 51). HRMS (ESI): calculated for $C_{25}H_{23}N_2O_2$: 383.1760, found 383.1751.

*3.3.2.2. 7'-Methyl-1,9a'-bis(2-methylallyl)-8'H-spiro[indoline-3,9'-pyrido[1,2-a]indole]-2,10'(9a'H)-dione (**20**).*

Following the general procedure B, a suspension of indirubin (205 mg, 782 μmol) in DMF (20 mL) was heated with caesium carbonate (612 mg, 1.88 mmol) and 3-bromo-2-methylpropene (370 μL, 3.68 mmol) for 6 h to obtain 7'-methyl-1,9a'-bis(2-methylallyl)-8'*H*-spiro[indoline-3,9'-pyrido[1,2-*a*]indole]-2,10'(9a'*H*)-dione (**20**) (123 mg, 37%) as a yellow solid. Mp 212-214 °C. UV/Vis: λ_{max} (ε) 435 (2274) nm. IR [cm^{-1}]: ν 2969 (w), 2923 (w), 1690 (s), 1600 (s), 1476 (s), 1349 (m), 745 (s). ^{1}H NMR [500 MHz]: δ 1.51 (3H, s, H1''''''), 1.85 (3H, s, H1''''), 1.89 (3H, s, H1''''), 1.89 (1H, d, *J* = 18.0 Hz, H8'$_a$), 2.68 (1H, d, *J* = 13.5 Hz, H3'''$_a$), 2.98 (1H, d, *J* = 18.0 Hz, H8'$_b$), 3.29 (1H, d, *J* = 14.0 Hz, H3'''$_b$), 4.32 (1H, d, *J* = 16.0 Hz, H3''$_a$), 4.41 (1H, d, *J* = 16.0 Hz, H3''$_b$), 4.59 (1H, s, H1'''$_a$), 4.61 (1H, s, H1'''$_b$), 4.97 (1H, s, H1''$_a$), 5.02 (1H, s, H1''$_b$), 6.54 (1H, t, *J* = 7.5 Hz, H5), 6.60 (1H, t, *J* = 7.5 Hz, H2'), 6.74 (1H, d, *J* = 8.0 Hz, H7), 6.78 (1H, d, *J* = 8.0 Hz, H4), 6.79 (1H, s, H6'), 6.95 (1H, d, *J* = 8.5 Hz, H4'), 7.01 (1H, t, *J* = 8.0 Hz, H6), 7.20 (d, *J* = 7.5 Hz, H1'), 7.41 (1H, t, *J* = 7.0 Hz, H3'). ^{13}C NMR [125 MHz]: δ 20.3 (C1''''), 20.4 (C1''''), 24.3 (C1''''''), 36.0 (C8'), 38.4 (C3'''), 46.8 (C3''), 49.0 (C3), 70.5 (C9'a), 107.8 (C4'), 109.3 (C7), 112.8 (C1''), 113.2 (C7'), 116.2 (C1'''), 117.8 (C4), 118.8 (C2'), 122.1 (C5), 122.7 (C10'a), 124.5 (C1'), 124.9 (C6'), 128.4 (C6), 129.3 (C3a), 137.4 (C3'), 139.4 (C2''), 139.8 (C2'''), 142.7 (C7a), 156.7 (C4'a), 176.1 (C2), 199.6 (C10'). MS (EI): *m/z* 424 (M^+, 30), 369 (M^+-C_4H_7, 100%). HRMS (EI): calculated for $C_{28}H_{28}N_2O_2$: 424.2151, found 424.2150.

*3.3.2.3. (E)-1-(But-2-en-1-yl)-9a'-(but-3-en-2-yl)-8'-methyl-8'H-spiro[indoline-3,9'-pyrido[1,2-a]indole]-2,10'(9a'H)-dione (**21**).*

Following the general procedure B, a suspension of indirubin (202 mg, 771 μmol) in DMF (20 mL) was heated with caesium carbonate (603 mg, 1.85 mmol) and crotyl bromide (373 μL, 3.62 mmol) for 6 h to obtain a fraction that was further purified by PTLC providing a mixture of at least two stereoisomers (arising from the *cis/trans* mixture in the double bond of the crotyl bromide starting material) of 1-(but-2-en-1-yl)-9a'-(but-3-en-2-yl)-8'-methyl-8'*H*-spiro[indoline-3,9'-pyrido[1,2-*a*]indole]-2,10'(9a'*H*)-dione (**21**) (78.5 mg, 24%) as a yellow solid. Mp 154-160 °C. UV/Vis: λ_{max} (ε) 411 (2211) nm. IR [cm^{-1}]: ν 1691 (s), 1606 (s), 1470 (m), 1363 (w), 1337 (w), 748 (s). ^{1}H NMR [300 MHz]: δ 0.52 (3H, d, *J* = 8.0 Hz, H1''''), 1.53 (3H, d, *J* = 7.3 Hz, H1''''), 1.72 (3H, d, *J* = 7.3 Hz, H1''), 3.40-3.47 (2H, m, H8', H3'''), 4.33-4.46 (2H, m, H4''), 4.64 (1H, dd, *J* = 10.0 Hz, 1.8 Hz, H1'''$_a$), 4.86 (1H, dd, *J* = 17.0 Hz, 2.1 Hz, H1'''$_b$), 5.03 (1H, dd, *J* = 8.9 Hz, 2.3 Hz, H7'), 5.35-5.48 (1H, m, H2'''), 5.51-5.57 (1H, m, H3''), 5.72-5.89 (1H, m, H2''), 6.48 (1H, t, *J* = 8.3 Hz, H5), 6.57 (1H, d, *J* = 7.8 Hz, H4), 6.67-6.76 (2H, m, H2', H4'), 7.01 (1H, t, *J* = 8.3 Hz, H6), 7.08-7.16 (3H, m, H1', H6', H7), 7.45 (1H, t, *J* = 7.8 Hz, H3'). ^{13}C NMR [75 MHz]: δ 15.6 (C1'''), 17.9 (C1''''), 18.0 (C1''), 34.6 (C8'), 42.6 (C4''), 43.3 (C3'''), 53.7 (C3), 73.6 (C9'a), 109.07 (C6'), 109.12 (C4'), 110.9 (C7'), 116.0 (C1'''), 120.1 (C2'), 121.5 (C5), 123.9 (C10'a), 124.5 (C1'), 124.9 (C3''), 125.2 (C7), 125.7 (C4), 127.2 (C3'a), 128.2 (C6), 129.3 (C2''), 136.8 (C3'), 139.6 (C2'''), 143.6 (C7a), 157.5 (C4'a), 175.9 (C2), 200.0 (ArC10'). MS (EI): *m/z* 424 (M^+, 26), 369 (M^+-C_4H_7, 75), 315 (M^+-C_4H_6, 100%). HRMS (ESI): calculated for $C_{28}H_{29}N_2O_2$: 425.2229, found 425.2237.

3.3.2.4. *1,9a'-Dicinnamyl-8'-phenyl-8'H-spiro[indoline-3,9'-pyrido[1,2-a]indole]-2,10'(9a'H)-dione (**22**); 1-cinnamylindoline-2,3-dione (**8**).*

Following the general procedure B, a suspension of indirubin (205 mg, 782 μmol) in DMF (20 mL) was heated with caesium carbonate (612 mg, 1.88 mmol) and cinnamyl bromide (731 mg, 3.68 mmol) for 24 h to obtain a fraction that was further purified by PLC providing two products, the first of which was 1,9a'-dicinnamyl-8'-phenyl-8'*H*-spiro[indoline-3,9'-pyrido[1,2-*a*]indole]-2,10'(9a'*H*)-dione (**22**) (39.2 mg, 8%), obtained as a yellow solid. Mp 118-120 °C. UV/Vis: λ_{max} (ε) 414 (1659[42]) nm. IR [cm^{-1}]: ν 1701(s), 1606 (s), 1470 (s), 1340 (w), 962 (w), 745 (s) ^{1}H NMR [300 MHz]: δ 3.17 (1H, ddd, *J* = 14.1 Hz, 7.8 Hz, 1.2 Hz, H3'''$_a$), 3.71 (1H, ddd, *J* = 13.5 Hz, 7.2 Hz, 1.2 Hz, H3'''$_b$), 4.23 (1H, ddd, *J* = 16.4, 5.7 Hz, 1.8 Hz, H3''$_a$), 4.52-4.61 (2H, m, H3''$_b$, H8'), 5.30 (1H, dd, *J* = 8.0 Hz, 2.1 Hz, H7'), 5.74-5.85 (2H, m, H2''', H2''), 6.23 (1H, d, *J* = 16.2 Hz, H1'''), 6.45-6.54 (1H, m, H1''), 6.68 (1H, t, *J* = 7.2 Hz, H2'), 6.87 (1H, t, *J* = 6.6 Hz, H5), 6.9-7.30 (21H, m, ArH2, ArH3, ArH4, ArH5, ArH6, ArH2', ArH3', ArH4', ArH5', ArH6', ArH2'', ArH3'', ArH4'', ArH5'', ArH6'', H1', H4', H6', H4, H6, H7), 7.46 (1H, t, *J* = 7.7 Hz, H3'). ^{13}C NMR [75 MHz]: δ 34.8 (C3'''), 42.3 (C3''), 45.2 (C8'), 53.7 (C3), 72.3 (C9'a), 107.8 (C7'), 108.3 (C1''), 109.1 (C1'''), 119.9 (C5), 121.6 (C1''), 123.0 (ArC), 123.3 (C2'''), 123.4 (C2''), 123.5 (ArC), 124.9 (ArC), 125.7 (ArC), 126.39 (2 x ArC), 126.44 (ArC), 126.7 (ArC), 126.8 (2 x ArC), 127.3 (ArC), 127.7 (2 x ArC), 127.8 (ArC), 128.5 (ArC), 128.6 (2 x ArC), 128.7 (2 x ArC), 128.8 (ArC), 129.9 (2 x ArC), 132.3 (ArC), 134.7 (ArC), 136.8 (ArC), 137.7 (ArC), 138.0 (ArC1), 142.9 (C7a), 156.1 (C4a), 175.2 (C2), 199.4

(C10'). MS (EI): *m/z* 610 (M^+, 90%), 493 (M^+-C_9H_9, 100%), 375 (M^+-$C_{18}H_{19}$, 40%). MS (ESI): *m/z* 611 ($M+H^+$, 100%). HRMS (ESI): calculated for $C_{43}H_{35}N_2O_2$: 611.2699, found 611.2681.

The second product separated from the above PTLC procedure was 1-cinnamylindoline-2,3-dione (**8**) (17.7 mg, 9%), obtained as an orange solid. Mp 108-110 °C. UV/Vis: λ_{max} (ε) 424 (569) nm. IR [cm^{-1}]: ν 1726 (s), 1601 (s), 1464 (m), 1366 (m), 1343 (m), 1165 (m), 762 (s). ^{1}H NMR [300 MHz]: δ 4.53 (2H, d, *J* = 5.7 Hz, H3'), 6.18 (1H, dt, *J* = 5.7 Hz, *J* = 16.2 Hz, H2'), 6.68 (1H, d, *J* = 16.2 Hz, H1'), 6.96 (1H, d, *J* = 7.8 Hz, H7), 7.12 (1H, t, *J* = 7.8 Hz, H5), 7.25-7.37 (5H, m, ArH2, ArH3, ArH4, ArH5, ArH6), 7.56 (1H, t, *J* = 7.8 Hz, H6), 7.62 (1H, d, *J* = 7.5 Hz, H4). ^{13}C NMR [75 MHz]: δ 42.5 (C3'), 111.2 (C7), 118.0 (C3a), 121.8 (C2'), 124.1 (C5), 125.7 (C7), 126.8 (ArC3, ArC5), 128.5 (ArC4), 129.0 (ArC2, ArC6), 134.4 (C1'), 136.0 (ArC1), 138.7 (C6), 151.1 (C7a), 158.2 (C2), 183.6 (C3). MS (EI): *m/z* 263 (M^+, 100%). HRMS (ESI): calculated for $C_{17}H_{14}NO_2$: 264.1025, found 264.1012.

3.3.2.5. Attempted allylation starting from N1'-allylindirubin.

Following general procedure **B**, *N1'*-indirubin (**9**) (97.1 mg, 321 μmol) in DMF (8 mL) was heated with caesium carbonate (126 mg, 385 μmol) and allyl bromide (74.3 μL, 770 μmol) was added before being stirred for 20 h. Workup of the reaction produced an indefinable mixture of compounds with only the corresponding *N*-allylisatin detected by TLC analysis.

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Supporting information

Analytical and spectral characterization data for all new compounds, computational details, selected UV/Vis spectra and biological testing procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

1. Abdel-Hamid, M. K.; Bremner, J. B.; Coates, J.; Keller, P. A.; Miländer, C.; Torkamani,Y. S.; Skelton, B. W; White, A. H.; Willis, A. C. *Tetrahedron Lett.* **2009**, *50*, 6947-6950.
2. Shakoori, A.; Bremner, J. B.; Abdel-Hamid, M. K.; Willis , A. C.; Haritakun, R.; Keller, P. A. *Beil. J. Org. Chem.* **2015**, *11*, 481-492.
3. Shakoori, A.; Bremner, J. B.; Willis, A. C.; Haritakun, R.; Keller, P. A., *J. Org. Chem.* **2013**, *78*, 7639-7647.
4. Zollinger, H. Color Chemistry: Syntheses, Properties and Applications of Organic Dyes and Pigments, 3rd ed. (ISBN 3-906390-23-3); Wiley-VCH, Helvetica Chimica Acta: Zürich (Switzerland), 2003. a) pp 39-42, b) p 43-46, c) p 43, d) pp 44-45.
5. Kim, S.-A.; Kwond, S.-M.; Kim, J.-A.; Kang, K. W.; Yoon, Y.-H.; Ahn, S.-G. *Cancer Lett.* **2011**, *2*, 197-204.
6. Williams, S.; Nowicki, M. O.; Liu, F.; Press, R.; Godlewski, J.; Abdel-Rasoul, M.; Kaur, B.; Fernandez, S. A.; E. Chiocca, A.; Lawler, S. E. *Cancer Res.* **2011**, *71*, 5374-5380.

7. a) Choi, S.; Lee, J.; Jeong, S.; Im, I.; Lee, S.; Lee, E.; Lee, S. K.; Kwon, S.; Ahn, S.; Yoon, J.; Han, S.; Kim, J.; Kim, Y. *J. Med. Chem.*, **2010**, *53*, 3696-3706. b) Libnow, S.; Methling, K.; Hein, M.; Michalik, D.; Harms, M.; Wende, K.; Flemming, A.; Köcherling, M., Reinke, H., Bednarski, P.J.; Lalk, M.; Langer, P. *Bioorg. Med. Chem.* **2008**, *16*, 5570-5583.
8. Martin, L.; Magnaudeix, A.; Wilson, C. M.; Yardin, C.; Terro, F. J. *Neurosci. Res.* **2011**, *89*, 1802-1811.
9. Alex, D.; Lam, I. K.; Lin, Z. X.; Lee, S. M. Y. *J. Ethnopharmacol.* **2011**, *131*, 242-247.
10. Erben, F.; Michalik, D.; Feist, H.; Kleeblatt, D.; Hein, M.; Matin, A.; Iqbal, J.; Langer, P. *RSC Adv.* **2014**, *I*, 10879-10893.
11. Saito, H.; Tabata, K.; Hanada, S.; Kanda, Y.; Suzuki, T.; Miyairi, S. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5370-5373.
12. Karapetyan, G.; Chakrabarty, K.; Hein, M.; Langer, P. *ChemMedChem* **2011**, *6*, 25–37.
13. Krivogorsky, B.; Grundt, P.; Yolken, R.; Jones-Brando, L. *Antimicrob. Agents Chemother.* **2008**, *52*, 4466-4469.
14. Pergola, C.; Gaboriaud-Kolar, N; Jestädt, N.; König, S.; Kritsanida, M.; Schaible,A. M.; Li, H.; Garscha, U.; Weinigel, C.; Barz, D.; Albring, K. F.; Huber, O.; Skaltsounis, A. L.; Werz, O. *J. Med. Chem.* **2014**, *57*, 3715-3723.
15. Bouchikhi, F.; Anizon, F.; Moreau, P. *Eur. J. Med. Chem.* **2008**, *43*, 755-762.
16. Wee, X. K.; Yeo, W. K.; Zhang, B.; Tan, V. B. C.; Kian Meng Lim, K. M.; Tay, T. E.; Go, M.-L. *Bioorg. Medchem.* **2009**, *17*, 7562-7571.
17. Bouchikhi, F.; Anizon, F.; Brun, R.; Moreau, P. *Bioorg. Med.Chem. Lett.* **2011**, *21*, 6319-6321.
18. Li, C.; Go, Y.; Mao, Z.; Koyano, K.; Kai, Y.; Kanehisa, N.; Zhu, Q.; Zhou, Z.; Wu, S. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1621-1627.
19. Jautelat, R.; Brumby, T.; Schäfer, M.; Briem, H.; Eisenbrand, G.; Schwahn, S.; Krüger, M.; Lücking, U.; Prien, O.; Siemeister, G. *ChemBioChem* **2005**, *6*, 531-540.
20. Cuong, N. M.; Tai, B. H.; Hoan, D. H. Nat. Prod. Res., 2010, 24, 99-105.
21. Danielsson, J.; Somfai, P. *Org. Lett.* **2014**, *16*, 784.
22. Nikokavouras, J.; Vassilopoulos, G. *Monatsh. Chem.*, **1981**, *112*, 1239-1244.
23. Wang, Z., Wang, Y., Feng, M., Tan, X., Cheng, J, Hua, W., and Yao, Q., *Chin. J. Org. Chem.*, **2009**, *29*, 1606-1610.
24. Nolan, W. E.; Hammer, C. F. *J. Org. Chem.* **1960**, *25*, 1525-1535.
25. Kochetkov, N. K.; Likhosherstov, A. M.; Budovskii, E. I. *Z. Obs. Khim*. **1960**, *30*, 2077-2082
26. Grimme, G.; Grimme, S.; Jones, P. G.; Boldt, P. *Chem. Ber.* **1993**, *126*, 1015-1021.
27. Perpète, E. A.; Preat, J.; André, J.-M.; Jacquemin, D. *J. Phys. Chem. A 2006*, **110**, 5629-5635.
28. Perpète, E. A.; Jacquemin, D. *J. Mol. Struct.:THEOCHEM.* **2009**, *914*, 100-105.
29. Bouhfid, R.; Jolym, N.; Essassi, E. M.; Lequart, V.; Massoui, M.; Martin, P. *Synth. Commun.* **2011**, *41*, 2096-2102.
30. Hsieh, C.; Chou, P.; Shih, C.; Chuang, W.; Chung, M.; Lee, J.; Joo, T. *J. Am. Chem. Soc.* **2011**, *133*, 2932-2943.
31. Christie, R. M. *Biotech. Histochem.* **2007**, *82*, 51-56.
32. Meijer, L.; Guyard, N., Skaltsounis, L.; Eisenbrand, G. Indirubin, the red shade of indigo (IBSN 2-9518029-0-0); Station Biologique Roscoff, Roscoff (France), **2006**; pp 103-108.
33. Serrano-Andrés, L.; Roos, B. O. *Chem. Eur. J.* **1997**, *3*, 717-725.
34. Zheng, Y; Tice, C. M.; Singh, S. B., *Bioorg. Med.Chem. Lett.*, **2014**, *24*, 3673-3682.
35. Rottmann, M.; McNamara, C.; Yeung, B. K. S.; Lee, M. C. S.; Zou, B.; Russell, B.; Seitz, P.; Plouffe, D. M.; Dharia, N. V.; Tan, J.; Cohen, S. B.; Spencer, K. R.; González-Páez, G. E.; Lakshminarayana, S. B.; Goh, A.; Suwanarusk, R.; Jegla, T.; Schmitt, E. K.; Beck, H.-P.; Brun, R.; Nosten, F.; Renia, L.; Dartois, V.; Keller, T. H.; Fidock, D. A.; Winzeler, E. A.; Diagana, T. T. *Science*, **2010**, *329*, 1175-1180.
36. Xia, M.; Ma, R.-Z. *J. Heterocyclic. Chem.* **2014**, *51*, 539-554.
37. Narayan, R.; Potowski, M.; Jia, Z.-J.; Antonchick, A. P.; Waldmann, H. *Acc. Chem. Res.* **2014**, *47*, 1296-1310.
38. Santos, M. M. M. *Tetrahedron* **2014**, *70*, 9735-9757.
39. S1. DENZO–SMN. Otwinowski, Z.; Minor, W. Processing of X-ray diffraction data collected in oscillation mode. in Methods in Enzymology, vol. 276: Macromolecular Crystallography, Part A; ed. C. W. Carter Jr., R. M. Sweet, Academic Press: New York, 1997; pp. 307-326.
40. Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Camalli, M. *J. Appl. Crystallogr.* **1994**, *27*, 435-436.
41. Betteridge, P. W.; Carruthers, J. R.; Cooper, R. I.; Prout, K.; Watkin, D. J. *J. Appl. Crystallogr*. **2003**, *36*, 1487.
42. This absorption coefficient is only an approximate value due to the very small amount of compound available for the UV/Vis spectrum measurement.