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N-Phenethyl and *N*-naphthylmethyl isatins and analogues as in vitro cytotoxic agents

Lidia Matesic,[a,*] Julie M. Locke,[a] John B. Bremner,[a] Stephen G. Pyne,[a] Danielle Skropeta,[a] Marie Ranson[b] and Kara L. Vine[b]

[a]*School of Chemistry, University of Wollongong, Wollongong, NSW 2522, Australia*
[b]*School of Biological Sciences, University of Wollongong, Wollongong, NSW 2522, Australia*

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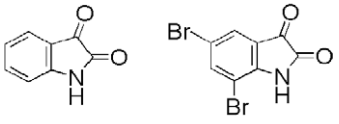
Abstract—A range of *N*-phenethyl, *N*-phenacyl, and *N*-(1- and 2-naphthylmethyl) derivatives of 5,7-dibromoisatin **2** were prepared by N-alkylation reactions. Their activity against human monocyte-like histiocytic lymphoma (U937), leukemia (Jurkat), and breast carcinoma (MDA-MB-231) cell lines was assessed. The results allowed further development of structure–activity relationships. The compound 5,7-dibromo-*N*-(1-naphthylmethyl)-1*H*-indole-2,3-dione **5a** was the most potent against U937 cells with an IC_{50} value of 0.19 μM.

1. Introduction

Isatin (1*H*-indole-2,3-dione) **1** is a synthetically versatile molecule which has led to an array of derivatives displaying a broad spectrum of biological properties including anti-cancer activities.[1–5] Recently, it has been reported that 5,7-dibromoisatin **2** is significantly more potent in vitro as a cytotoxin than the parent molecule against U937 (human monocyte-like histiocytic lymphoma) cells.[6] Other research within our laboratory has indicated that N-benzylation of 5,7-dibromoisatin **2** further increased the cytotoxicity toward these lymphoma cells and was potent against a range of human cancer cell lines including a metastatic breast adenocarcinoma cell line (MDA-MB-231).[7] In this context, it was of interest to further investigate the cytotoxicity of N-alkylated 5,7-dibromoisatin analogues by altering the chain length between *N*-1 and the aryl group, as well as increasing the hydrophobicity of the N-substituent through an extra aromatic ring fusion. Together with this, in vitro screening against various cancer cell lines was carried out in order to establish a more comprehensive structure–activity relationship (SAR). The results are described in this paper.



1 **2**

2. Results and discussion

2.1. Chemistry

In order to determine the structural requirements necessary for cytotoxicity, a simple modification to **2** was envisaged with a two-atom linker bridge between the nitrogen and a new aromatic ring, producing *N*-phenethyl derivatives **4a–e**. The phenethyl scaffold was also modified by introduction of another aromatic ring fusion which subsumed the benzylic methylene group in the new ring, to afford the 1-naphthylmethyl derivative **5a**. The 2-naphthylmethyl analogue **5b** was also synthesized for comparative purposes. With a view to assessing the hydrophobicity requirements, *N*-phenacyl derivatives **6a–e** of 5,7-dibromoisatin **2** were also investigated.

The procedure for the synthesis of the *N*-phenethyl derivatives **4a–e** was based on a combination of the literature methods.[8–10] Briefly, **2** was treated with a base such as NaH or K_2CO_3 which formed an intense purple colored anion (**A**) that was subsequently reacted with the appropriate aryl alkyl halide **3a–e** to give the *N*-phenethyl derivatives **4a–e** in moderate yields

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* Corresponding author. Tel.: +61 2 4221 4334; fax: +61 2 4221 4287; e-mail: lm66@uow.edu.au

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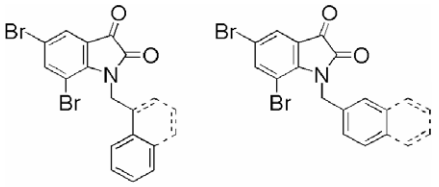
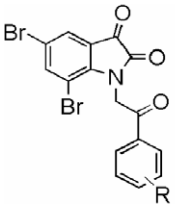
(Scheme 1). High temperatures are usually required in these syntheses to drive the reactions to completion because the reaction mixtures are prone to crystallization of the impure product at low temperatures.[8] *N*-Benzylisatin derivatives had been obtained previously by heating at 80 °C,[7] however a temperature of 50 °C was used to produce **4a**–**e** to reduce the risk of substituted styrenes forming as side products from the base catalyzed elimination of the phenethyl bromides **3a**–**e**. The isatin intermediate (**A**) is also an ambidentate anion which could undergo N- or O-alkylation,[11] however no evidence for O-alkylation was found through ^{1}H and ^{13}C NMR spectroscopic studies.



5a **5b**

The *N*-naphthylmethyl analogues **5a** and **5b** were prepared in a similar manner to the *N*-phenethyl series **4a**–**e**, however, the synthesis of the *N*-phenacyl derivatives **6a**–**e** proved difficult. Attempts to synthesize these compounds included various alkylation protocols and protecting group strategies. A possible competing reaction was a Darzens condensation involving the C3 carbonyl group of the isatin **2**. While the Darzens condensation occurs when a ketone or aldehyde reacts with a haloester to form an epoxy ester, it also proceeds with halogenomethylsulfones and halogenoketones such as phenacyl halides.[12] It has been reported that phenacyl halides preferentially yield a Darzens product rather than the corresponding phenacylisatin,[13] although in the current study this was not observed in the NMR spectra. The desired compounds **6a**–**e** were obtained in a pure form but in low yields, which were not optimized as the required compounds were obtained in sufficient quantity for cytotoxicity screening.



6a R = H
6b R = *m*-Br
6c R = *p*-Br
6d R = *m*-OCH_3
6e R = *p*-OCH_3

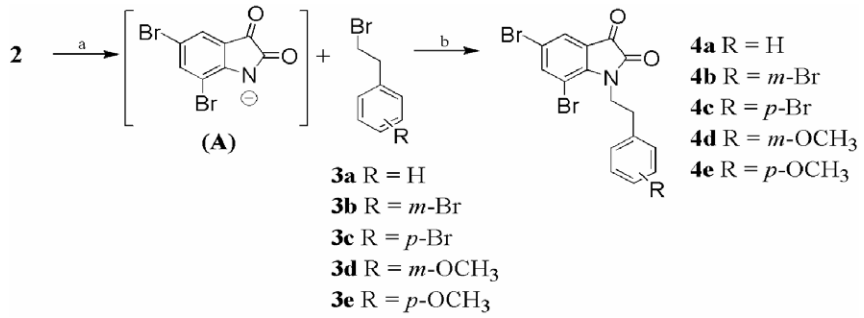
Table 1. Initial cytotoxicity screening (IC_{50} μM) of novel *N*-substituted isatin derivatives **4a**–**e**, **5a**–**b**, and **6a**–**e**[a]

Compound	U937[b]	Jurkat[c]	MDA-MB-231[d]
2	10.5[e]	14.3[e]	42.3[e]
4a	0.78	1.52	4.35
4b	0.78	1.21	2.72
4c	0.88	1.15	2.01
4d	1.07	2.00	5.24
4e	2.35	2.66	4.51
5a	0.19	0.91	2.49
5b	0.74	0.41	2.56
6a	9.97	nt[f]	nt
6b	6.36	nt	nt
6c	9.18	nt	nt
6d	4.70	nt	nt
6e	5.32	nt	nt
Vinblastine	6.88[g]	nt	nt

[a] Values are means of triplicates of at least two independent experiments.
[b] Human monocyte-like histiocytic lymphoma cell line.
[c] Human leukemic T-cell line.
[d] Human metastatic mammary gland adenocarcinoma cell line.
[e] Vine et al.[6]
[f] Not tested.
[g] Vine et al.[7]

2.2. Cytotoxic activity and SAR

The *N*-phenethyl **4a**–**e** and *N*-naphthylmethyl derivatives **5a**–**b** were initially tested for cytotoxicity using the MTS cell proliferation assay against three human cancer cell lines including a lymphoma (U937), leukemia (Jurkat), and metastatic breast adenocarcinoma cell line (MDA-MB-231). The results showed that the cytotoxicity of the parent molecule **2** significantly increased through N-alkylation (Table 1), as reported previously for the 5,7-dibromo-*N*-benzylisatin derivatives.[7] Compounds **4a**–**e**, which contain a two-carbon linker at the nitrogen, typically increased the anti-proliferative activity 10- to 15-fold against the three tumor cell lines compared to that of the parent brominated isatin **2**. Three of the *N*-phenethyl derivatives, **4a**–**c**, displayed IC_{50} values in the sub-micromolar range against U937 cells (e.g., **4a**, IC_{50} = 0.78 μM, Table 1). Introduction of a hydrophobic bromo substituent in the *meta* (**4b**) or *para* (**4c**) position yielded the most active compounds in this series, while analogous compounds containing methoxy substituents **4d**–**e** were less cytotoxic. The introduction of a substituent in the *meta* position (**4b** and **4d**) enhanced



(A)

3a R = H
3b R = *m*-Br
3c R = *p*-Br
3d R = *m*-OCH_3
3e R = *p*-OCH_3

4a R = H
4b R = *m*-Br
4c R = *p*-Br
4d R = *m*-OCH_3
4e R = *p*-OCH_3

Scheme 1. Reagents and conditions: (a) NaH or K_2CO_3, DMF, 4 °C–rt, 20 min to 3 h; (b) KI, 50 °C, 18 h (32–62%).

Table 2. Further cytotoxicity screening (IC_{50} μM) of derivatives **4b–c** and **5a–b** against a panel of human adherent tumor cell lines[a]

Compound	HCT-116[b]	PC-3[c]	MCF-7[d]	A375[e]
4b	1.41	2.09	4.04	3.44
4c	1.62	2.21	6.02	4.34
5a	1.15	1.44	7.71	3.95
5b	1.51	2.29	3.50	6.56

[a] Values are means of triplicates of at least two independent experiments.
[b] Human colorectal carcinoma cell line.
[c] Human prostate adenocarcinoma cell line.
[d] Human non-metastatic mammary gland adenocarcinoma cell line.
[e] Human malignant melanoma cell line.

cytotoxicity against the U937 and Jurkat cell lines compared to the *para* position (**4c** and **4e**) although this trend was not observed against the MDA-MB-231 cell line. Overall, the U937 lymphoma cell line appeared to be the most susceptible to treatment with the *N*-phenethylisatins **4a–e**, while the metastatic MDA-MB-231 cell line was the least susceptible.

The *N*-naphthylmethylisatins **5a** and **5b** demonstrated enhanced cytotoxic activity against the lymphoma (U937) and leukemia (Jurkat) cell lines compared to the *N*-phenethyl derivatives **4a–e**. Both **5a** and **5b** exhibited sub-micromolar IC_{50} values against these two cell lines, and were the only compounds to do so against Jurkat cells. Compound **5a** was the most cytotoxic of all those tested, with an IC_{50} value of 0.19 μM against U937 cells. This compound was four times more potent against U937 cells than the lead *N*-phenethyl compounds **4a** and **4b**.

The most active analogue in the *N*-phenacyl series was **6d**, which exhibited an IC_{50} value of 4.70 μM against U937 cells. The parent phenacyl derivative **6a** was the least active of the series with an IC_{50} value of 9.97 μM (Table 1). This suggests that the presence of the polar carbonyl group is detrimental to activity. Since the *N*-phenacylisatins **6a–e** were less active than the *N*-phenethyl **4a–e** and *N*-naphthylmethyl **5a–b** series, they were not tested against any other cell lines.

The four most potent derivatives, **4b**, **4c**, **5a**, and **5b**, (Table 1) were selected for further cytotoxic screening against a panel of other adherent human tumor cell lines: colorectal (HCT-116), prostate (PC-3), non-metastatic breast (MCF-7), and melanoma (A375). The results in Table 2 indicate that activity against the panel of adherent cell lines varied but all compounds tested had IC_{50} values in the low micromolar range. The lower sensitivity in these adherent cell lines compared to the non-adherent cell lines may be due to reduced cell surface areas leading to decreased drug uptake as well as slower proliferation rates in the former.

2.3. Mode of action studies

While performing in vitro cytotoxicity testing, it was observed that the isatin derivatives **4a–e**, **5a–b**, and **6a–e** caused the U937 and, to a lesser extent, the Jurkat cells to undergo elongation. The elongated morphology was more pronounced when the cells were treated with *N*-naphthylmethyl derivatives **5a** and **5b** (Fig. 1B). This morphological change was also observed in U937 cells treated with vinblastine (Fig. 1C), a microtubule destabilizer. This suggested that these isatin analogues interfere with microtubule dynamics in a similar fashion to previously reported 5,7-dibromo-*N*-benzylisatin derivatives.[7]

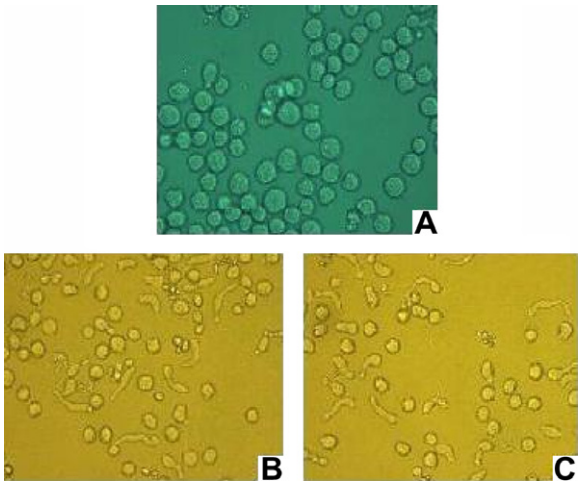


Figure 1. Elongated U937 cell morphology after treatment with various isatin derivatives. Cells were incubated for 24 h with (A) DMSO vehicle control, (B) 5,7-dibromo-*N*-(2-naphthylmethyl)isatin **5b** (0.39 μg/mL), and (C) vinblastine sulfate (0.39 μg/mL). Magnification: 1000×.

To further investigate the effects of the aforementioned analogues on microtubule formation, a cell-free in vitro tubulin polymerization assay was performed (Fig. 2). The two naphthyl derivatives **5a** and **5b** were chosen due to their in vitro potency against U937 and Jurkat cells, as well as two representative phenethyl compounds, **4a** and **4c**. Vinblastine sulfate and paclitaxel were used as a known microtubule destabilizer and stabilizer, respectively. Consistent with the literature reports,[14,15] at 10 μM paclitaxel stabilized microtubules, while vinblastine sulfate was a potent microtubule destabilizer (Fig. 2). The test compounds, in particular

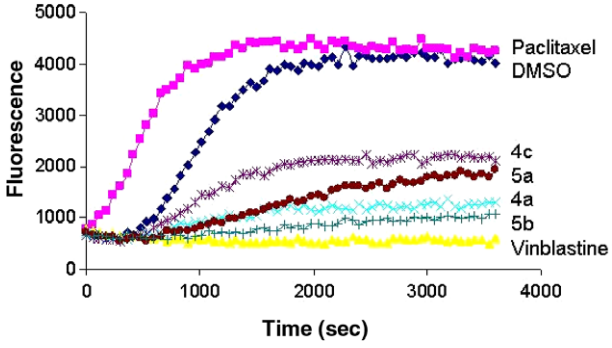


Figure 2. Effect of compounds **4a**,**4c**, **5a**, **5b**, paclitaxel, and vinblastine sulfate at 10 μM on the assembly of purified bovine neuronal tubulin. DMSO was used as a control.

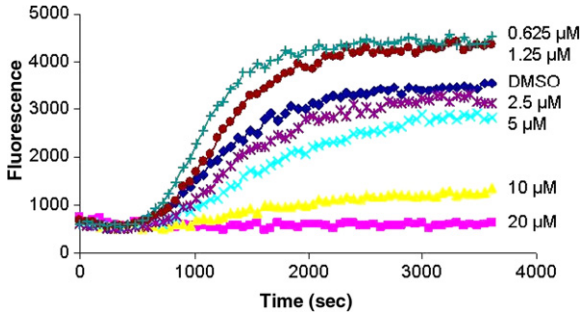


Figure 3. Effect of varying concentrations of the 2-naphthylmethyl derivative **5b** on the assembly of purified bovine neuronal tubulin. DMSO was used as a control.

Table 3. Inhibition of tubulin polymerization (IC_{50} μM) of compounds **4a**, **5a**, **5b** and vinblastine at varying time points[a]

Compound	20 min	60 min
4a	4.04	6.60
5a	4.71	12.0
5b	2.87	6.47
Vinblastine	0.95	1.66

[a] Values are means of duplicates in one experiment.

the 2-naphthyl derivative **5b**, appeared to be potent microtubule destabilizers at 10 μM (Fig. 2), as observed by the shift of the curve to the right of the control, indicating a decrease in the rate of tubulin polymerization. These results are consistent with reports that structurally similar indole[16–18] and indolinone[7,15,19] compounds inhibit tubulin polymerization.

The three most potent destabilizers, **4a**, **5a**, and **5b**, were chosen for further studies on tubulin polymerization. All of these compounds inhibited the rate of tubulin polymerization in a dose-dependent manner (representative result shown in Fig. 3) and the IC_{50} values are reported in Table 3. Vinblastine displayed similar inhibition in this assay to that reported previously.[20]

It was of interest to note that in Figure 3, the test compound **5b** displayed potent destabilizing effects at higher concentrations (as seen by the shift in the curves to the right of the DMSO control) and stabilized microtubules at lower concentrations (as seen by the shift in the curves to the left of the DMSO control). This phenomenon has also been reported in the case of vinblastine[14] and suggests that these compounds may bind to more than one binding site on tubulin with different affinities.

3. Conclusions

A number of *N*-phenethyl, *N*-phenacyl, and *N*-naphthylmethyl derivatives of 5,7-dibromoisatin **2** were synthesized and tested against a range of human cancer cell lines. These compounds displayed greater cytotoxicity against non-adherent U937 and Jurkat cells compared to adherent human breast (MDA-MB-231 and MCF-7), colon (HCT-116), prostate (PC-3), and melanoma (A375) tumor cell lines. Cytotoxicity testing showed that 5,7-dibromo-*N*-(1-naphthylmethyl)isatin **5a** was the most active compound against all of the cell lines tested and exhibited an IC_{50} value of 0.19 μM against U937 cells. The *N*-phenethyl derivatives **4a**–**c** also showed potent sub-micromolar activity against U937 cells. Additionally, the tumor cells displayed elongated cell morphology upon treatment with these compounds, suggesting that these analogues interfere with microtubule dynamics. The results described indicate that these compounds could serve as the basis for the development of a new group of cancer chemotherapeutics.

4. Experimental

4.1. General

4.1.1. Chemistry. All solvents were of AR grade except dichloromethane (DCM) which was of LR grade and distilled before use. The term petroleum spirit (PS) refers to petroleum spirit with a boiling range of 40–60 °C. Solvent removal was performed (in vacuo) using temperatures not greater than 60 °C. Organic halides were purchased from Sigma–Aldrich Chemical Co. and used as supplied. Sodium hydride was supplied as a 60% dispersion in mineral oil and masses used were calculated appropriately. Melting points were obtained using a Reichert melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) on aluminum backed sheets of Merck Silica Gel 60 F_{254} plates was employed to monitor the progress of chemical reactions. Preparative TLC was performed on 20×20 cm plates. Generally, the isatins were highly colored and visible on the TLC plate; colorless compounds were detected by exposure to UV light at λ 254 nm. Column chromatography was performed under 'flash' conditions[21] on silica gel 60 (230–400 mesh). The solvent used in individual chromatographic experiments is indicated and all solvent proportions are given as vol/vol ratios. NMR spectra were acquired on a Varian Unity 300 MHz spectrometer, where proton (^{1}H) and carbon (^{13}C) spectra were obtained at 300 MHz and 75 MHz, respectively, or on a Varian Inova 500 spectrometer, where the ^{1}H and ^{13}C were obtained at 500 MHz and 126 MHz, respectively. All spectra were obtained with a probe temperature of 298 K. Spectra were recorded in $CDCl_3$ (unless otherwise indicated) and were referenced to the residual non-deuterated solvent signal or TMS. Hydrogen and carbon assignments were also made using gradient correlation spectroscopy (gCOSY), gradient heteronuclear single quantum correlation (gHSQC), and gradient heteronuclear multiple bond correlation (gHMBC) spectroscopic techniques. The superscript letter [a] denotes coincident peaks. Low resolution electron ionization mass spectra (LREI-MS) were obtained using a Shimadzu QP5050 spectrometer. High resolution electron ionization mass spectra (HREI-MS) were obtained using a Fisons/VG Autospec spectrometer operating with an electron beam of 70 eV, with a source temperature of 250 °C and perfluorokerosene (PFK) as the internal standard. Compounds for testing were >95% pure on the basis of TLC and ^{1}H NMR analysis.

4.1.2. Cell biology. All cancer cell lines 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 (FCS) (at 37 °C 95% humidified atmosphere and 5% CO_2). Adherent cells were detached with sterile trypsin–EDTA, washed with culture media, and reseeded following centrifugation for 5 min at 1600 rpm. The number of viable cells was counted with the aid of a hemocytometer and Trypan blue staining. Cytotoxicity of the isatin derivatives was determined using the CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay, utilizing [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt] (MTS) (Promega Co., Madison, WI, USA) as described previously.[6,7] The inhibitory 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.). Cell images were obtained by brightfield microscopy on an inverted light microscope using a Leica DC500 12-megapixel high-performance FireWire camera system. The in vitro tubulin polymerization assay was obtained from Cytoskeleton Inc. (Jomar Diagnostics, Australia) and the assay was conducted with reagents as described by the manufacturer. Briefly, 5 μL of vehicle control, paclitaxel, vinblastine sulfate, and compounds **4a**, **4c**, **5a**, and **5b** at the desired concentrations were incubated with 50 μL of purified bovine neuronal tubulin reaction mix. The rate of polymerization was monitored kinetically for 1 h at 37 °C using an excitation wavelength of 360 ± 10 nm and fluorescence emission at 440 ± 10 nm.

4.2. Chemistry

4.2.1. 5,7-Dibromoisatin (2)[6]. Yield: 2.90 g, (28%) as bright red/orange needles, mp 251–253 °C (lit.[22] 248–250 °C), R_f 0.63 (silica, DCM/MeOH, 9:1). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.66 (d, J = 1.5 Hz, 1 H, H4), 8.02 (d, J = 1.5 Hz, 1H, H6), 11.43 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6, 126 MHz): δ 105.7, 114.5, 121.3, 125.9, 141.1, 148.6, 159.4, 182.5. LREI-MS: *m*/*z* 303; 305; 307 [M^+]^{79}Br^{79}Br; ^{79}Br^{81}Br; ^{81}Br^{81}Br.

4.2.2. 5,7-Dibromo-1-phenethyl-1*H*-indole-2,3-dione (4a). A mixture of 5,7-dibromoisatin **2** (200 mg, 0.66 mmol) and K_2CO_3 (128 mg, 0.92 mmol) or NaH (36.8 mg, 0.92 mmol) was dissolved in anhydrous DMF (4 mL) and stirred under nitrogen at 4 °C for 3 h (or 20 min at rt for NaH) before the addition of KI (22.0 mg, 0.13 mmol) and (2-bromoethyl)benzene **3a** (270 mg, 0.2 mL, 1.46 mmol). The reaction mixture was heated at 50 °C and stirred at this temperature for 18 h. To the resulting solution were added water (80 mL) and 1 M HCl (2 mL) to acidify to pH 1. The suspension was filtered and the precipitate washed with water. The resulting solid was purified by flash chromatography on silica gel ($CHCl_3$) to yield **4a** (86.3 mg, 32%) as bright red crystals, mp 165–168 °C, R_f 0.71 (silica, DCM). ^{1}H NMR (500 MHz): δ 3.03 (t, J = 8 Hz, 2H, H2′), 4.39 (t, J = 8 Hz, 2H, H1′), 7.30 (m, 5H, H2″–H6″), 7.69 (d, J = 1.5 Hz, 1H, H4), 7.89 (d, J = 1.5 Hz, 1H, H6). ^{13}C NMR (126 MHz): δ 35.0, 42.4, 104.5, 116.6, 121.1, 126.7, 127.2, 128.4[a], 128.6[a], 136.7, 144.8, 146.4, 157.5, 181.1. HREI-MS: *m*/*z* calcd for $C_{16}H_{11}NO_2{}^{79}Br^{79}Br$ [M^+]: 406.9157; found: 406.9166.

4.2.3. 5,7-Dibromo-1-[2-(3-bromophenyl)ethyl]-1*H*-indole-2,3-dione (4b). The compound was prepared according to the method for **4a** using 5,7-dibromoisatin **2** (100 mg, 0.33 mmol) and 3-bromophenethyl bromide **3b** (193 mg, 0.11 mL, 0.73 mmol) as starting materials. The resulting solid was purified by flash chromatography on silica gel (DCM) to yield **4b** (67.1 mg, 42%) as bright red/orange crystals, mp 188–190 °C, R_f 0.54 (silica, DCM). ^{1}H NMR (acetone-d_6, 500 MHz): δ 3.09 (t, J = 8 Hz, 2H, H2′), 4.38 (t, J = 8 Hz, 2H, H1′), 7.27 (t, J = 8 Hz, 1H, H5″), 7.34 (d, J = 8 Hz, 1H, H6″), 7.42 (d, J = 8 Hz, 1H, H4″), 7.51 (s, 1H, H2″), 7.72 (d, J = 1.5 Hz, 1H, H4), 8.06 (d, J = 1.5 Hz, 1H, H6). ^{13}C NMR (acetone-d_6, 126 MHz): δ 34.9, 42.4, 104.7, 110.0, 116.0, 122.3, 126.8, 128.2, 130.0, 130.8, 132.0, 140.9, 144.4, 147.5, 158.4, 181.6. HREI-MS: *m*/*z* calcd for $C_{16}H_{10}NO_2{}^{79}Br^{81}Br^{81}Br$ [M^+]: 488.8221; found: 488.8207.

4.2.4. 5,7-Dibromo-1-[2-(4-bromophenyl)ethyl]-1*H*-indole-2,3-dione (4c). The compound was prepared according to the method for **4a** using 5,7-dibromoisatin **2** (100 mg, 0.33 mmol) and 4-bromophenethyl bromide **3c** (193 mg, 0.11 mL, 0.73 mmol) as starting materials. The resulting solid was purified by flash chromatography on silica gel ($CHCl_3$) to yield **4c** (81.1 mg, 50%) as bright red crystals, mp 189–190 °C, R_f 0.48 (silica, DCM). ^{1}H NMR (300 MHz): δ 2.99 (t, J = 8.4 Hz, 2H, H2′), 4.35 (t, J = 8.4 Hz, 2H, H1′), 7.13 (d, J = 8.7 Hz, 2H, H2″, H6″), 7.42 (d, J = 8.7 Hz, 2H, H3″, H5″), 7.69 (d, J = 1.8 Hz, 1H, H4), 7.89 (d, J = 1.8 Hz, 1H, H6). ^{13}C NMR (126 MHz): δ 35.1, 42.6, 104.9, 117.3, 121.2, 121.6, 127.9, 130.8[a], 132.1[a], 136.2, 145.4, 146.8, 158.0, 181.4. HREI-MS: *m*/*z* calcd for $C_{16}H_{10}NO_2{}^{79}Br^{81}Br^{81}Br$ [M^+]: 488.8221; found: 488.8214.

4.2.5. 5,7-Dibromo-1-[2-(3-methoxyphenyl)ethyl]-1*H*-indole-2,3-dione (4d). The compound was prepared according to the method for **4a** using 5,7-dibromoisatin **2** (100 mg, 0.33 mmol) and 3-methoxyphenethyl bromide **3d** (157 mg, 0.11 mL, 0.73 mmol) as starting materials. The resulting solid was purified by flash chromatography on silica gel (DCM) to yield **4d** (65.0 mg, 45%) as bright red/orange crystals, mp 179–180 °C, R_f 0.56 (silica, DCM). ^{1}H NMR (500 MHz): δ 3.00 (t, J = 8 Hz, 2H, H2′), 3.79 (s, 3H, OCH_3), 4.38 (t, J = 8 Hz, 2H, H1′), 6.76 (d, J = 7.5 Hz, 1H, H4″), 6.80 (s, 1H, H2″), 6.84 (d, J = 7.5 Hz, 1H, H6″), 7.22 (t, J = 7.5 Hz, 1H, H5″), 7.69 (d, J = 2 Hz, 1H, H4), 7.89 (d, J = 2 Hz, 1H, H6). ^{13}C NMR (126 MHz): δ 35.3, 42.6, 55.2, 104.8, 112.2, 114.8, 116.9, 121.2, 121.4, 127.5, 129.8, 138.5, 145.1, 146.7, 157.8, 159.8, 181.3. HREI-MS: *m*/*z* calcd for $C_{17}H_{13}NO_3{}^{79}Br^{81}Br$ [M^+]: 438.9242; found: 438.9244.

4.2.6. 5,7-Dibromo-1-[2-(4-methoxyphenyl)ethyl]-1*H*-indole-2,3-dione (4e). The compound was prepared according to the method for **4a** using 5,7-dibromoisatin **2** (100 mg, 0.33 mmol) and 4-methoxyphenethyl bromide **3e**

(157 mg, 0.11 mL, 0.73 mmol) as starting materials. The resulting solid was purified by flash chromatography on silica gel ($CHCl_3$) to yield **4e** (89.5 mg, 62%) as bright red/orange crystals, mp 176–178 °C, R_f 0.53 (silica, DCM). ^{1}H NMR (500 MHz): δ 2.96 (t, J = 8 Hz, 2H, H2′), 3.78 (s, 3H, OCH_3), 4.34 (t, J = 8 Hz, 2H, H1′), 6.83 (d, J = 8.5 Hz, 2H, H3″, H5″), 7.16 (d, J = 8.5 Hz, 2H, H2″, H6″), 7.68 (d, J = 2 Hz, 1H, H4), 7.89 (d, J = 2 Hz, 1H, H6). ^{13}C NMR (126 MHz): δ 34.7, 43.1, 55.5, 105.0, 114.4[a], 117.1, 121.6, 127.8, 129.2, 130.1[a], 145.3, 147.0, 158.1, 158.8, 181.6. HREI-MS: *m*/*z* calcd for $C_{17}H_{13}NO_3$ $^{79}Br^{81}Br$ [M^+]: 438.9242; found: 438.9241.

4.2.7. 5,7-Dibromo-1-(1-naphthylmethyl)-1*H*-indole-2,3-dione (5a). A mixture of 5,7-dibromoisatin **2** (101 mg, 0.33 mmol) and NaH (18.0 mg, 0.46 mmol) was dissolved in anhydrous DMF (2.5 mL) and stirred under nitrogen at rt for 20 min before the addition of KI (11.0 mg, 0.066 mmol) and 1-chloromethylnaphthalene (128 mg, 0.11 mL, 0.73 mmol). The reaction mixture was heated at 60 °C and stirred at this temperature for 19 h. After cooling, ethyl acetate (50 mL) was added and the resulting solution was extracted with 0.5 M HCl (50 mL) followed by brine (50 mL). The orange organic layer was dried over $MgSO_4$ and the solvent was removed to yield a sticky red/orange residue. The resulting solid was purified by flash chromatography on silica gel [DCM/PS (3:2)] to yield **5a** (90.8 mg, 62%) as dark red crystals, mp 218–219 °C, R_f 0.35 (silica, DCM). ^{1}H NMR (500 MHz): δ 5.81 (s, 2H, H1′), 7.06 (d, J = 7 Hz, 1H, H2″), 7.34 (t, J = 7.5 Hz, 1H, H3″), 7.53 (t, J = 7 Hz, 1H, H6″), 7.58 (t, J = 7 Hz, 1H, H7″), 7.76 (m, 3H, H4, H6, H4″) 7.88 (d, J = 8.5 Hz, 1H, H5″), 7.93 (d, J = 8.5 Hz, 1H, H8″). ^{13}C NMR (126 MHz): δ 42.8, 105.5, 117.1, 121.3, 121.4, 122.1, 125.3, 126.0, 126.6, 127.5, 128.0, 129.0, 129.8, 130.7, 133.8, 145.3, 146.8, 158.1, 181.2. HREI-MS: *m*/*z* calcd for $C_{19}H_{11}NO_2{}^{79}Br^{81}Br$ [M^+]: 444.9136; found: 444.9131.

4.2.8. 5,7-Dibromo-1-(2-naphthylmethyl)-1*H*-indole-2,3-dione (5b). The compound was prepared according to the method for **5a** using 5,7-dibromoisatin **2** (50.5 mg, 0.16 mmol) and 2-bromomethylnaphthalene (80.1 mg, 0.36 mmol) as starting materials. The resulting red solid was purified by flash chromatography on silica gel [DCM/PS (3:2)] to yield **5b** (46.6 mg, 64%) as dark red crystals, mp 140–142 °C, R_f 0.58 (silica, DCM). ^{1}H NMR (500 MHz): δ 5.56 (s, 2H, H1′), 7.36 (dd, J = 2 Hz, 8 Hz, 1H, H3″), 7.47 (m, 2H, ArH × 2), 7.63 (s, 1H, H1″), 7.74 (d, J = 2 Hz, 1H, H4), 7.76 (m, 1H, ArH), 7.79 (d, J = 2 Hz, 1H, H6), 7.81 (m, 2H, ArH × 2). ^{13}C NMR (126 MHz): δ 45.1, 105.5, 117.5, 121.7, 124.6, 125.3, 126.4, 126.7, 127.8, 128.0, 128.0, 129.1, 133.0, 133.3, 133.5, 145.6, 147.0, 158.6, 181.5. HRMS: *m*/*z* calcd for $C_{19}H_{11}NO_2{}^{79}Br^{81}Br$ [M^+]: 444.9136; found: 444.9135.

4.2.9. 5,7-Dibromo-1-(2-oxo-2-phenylethyl)-1*H*-indole-2,3-dione (6a). A mixture of KI (113 mg, 0.68 mmol) and phenacyl bromide (68.0 mg, 0.34 mmol) was dissolved in anhydrous DMF (0.5 mL) and stirred at −5 °C under nitrogen for 5 h, followed by cooling in a freezer at −18 °C for 20 h.[23] A mixture of the 5,7-dibromoisatin **2** (103 mg, 0.34 mmol) and K_2CO_3 (47.0 mg, 0.34 mmol) or NaH (13.7 mg, 0.34 mmol) was dissolved in anhydrous DMF (5 mL) and stirred under nitrogen at 4 °C for 3 h. This anion solution was added in portions (0.5 mL) to the phenacyl iodide maintained at −2 °C such that each portion had reacted before the addition of the next portion (monitored by TLC). The yellow/brown reaction mixture was stirred at rt for 25 h but no change in color intensity was observed after 2 h. To the resulting solution were added water (60 mL) and 1 M HCl (2 mL) to acidify to pH 1. The suspension was filtered and the precipitate washed with water. The resulting solid was purified by flash chromatography on silica gel (DCM) to yield **6a** (13.5 mg, 9%) as bright red/orange crystals, mp 173–175 °C, R_f 0.58 (silica, DCM). ^{1}H NMR (500 MHz): δ 5.64 (s, 2H, H1′), 7.54 (t, J = 7.5 Hz, 2H, H3″, H5″), 7.67 (t, J = 7.5 Hz, 1H, H4″), 7.75 (d, J = 2 Hz, 1H, H4), 7.80 (d, J = 2 Hz, 1H, H6), 8.00 (d, J = 7.5 Hz, 2H, H2″, H6″). ^{13}C NMR (126 MHz): δ 48.2, 105.4, 117.1, 121.3, 126.5, 127.6, 128.3[a], 129.1[a], 134.4, 144.8, 146.9, 158.2, 180.8, 191.3. HREI-MS: *m*/*z* calcd for $C_{16}H_9NO_3$ $^{79}Br^{81}Br$ [M^+]: 422.8929; found: 422.8928.

4.2.10. 5,7-Dibromo-1-[2-(3-bromophenyl)-2-oxo-ethyl]-1*H*-indole-2,3-dione (6b). This compound was prepared according to the method for **6a** using 5,7-dibromoisatin **2** (305 mg, 1.00 mmol) and 3-bromophenacyl bromide (278 mg, 1.00 mmol) as starting materials. The reaction mixture was stirred at 100 °C for 16 h.[24] The resulting solid was purified by flash chromatography on silica gel (DCM) and subsequent preparative TLC (silica, DCM) to yield **6b** (10.6 mg, 2%) as bright red/orange crystals, mp 160–162 °C, R_f 0.54 (silica, DCM). ^{1}H NMR (500 MHz): δ 5.60 (s, 2H, H1′), 7.44 (t, J = 8 Hz, 1H, H5″), 7.77 (d, J = 2 Hz, 1H, H4), 7.80 (d, J = 8.5 Hz, 1H, H4″), 7.81 (d, J = 2 Hz, 1H, H6), 7.93 (d, J = 8.5 Hz, 1H, H6″), 8.13 (s, 1H, H2″). ^{13}C NMR (126 MHz): δ 48.0, 105.3, 117.3, 121.3, 123.5, 126.6, 127.7, 130.7, 131.2, 135.4, 137.3, 144.8, 146.6, 158.0, 180.6, 190.2. HREI-MS: *m*/*z* calcd for $C_{16}H_8NO_3$ $^{79}Br^{79}Br^{81}Br$ [M^+]: 500.8034; found: 500.8037.

4.2.11. 5,7-Dibromo-1-[2-(4-bromophenyl)-2-oxo-ethyl]-1*H*-indole-2,3-dione (6c). 5,7-Dibromoisatin **2**(153 mg, 0.5 mmol) and NaH (20.0 mg, 0.5 mmol) were dissolved in anhydrous DMF (1.25 mL) and stirred at rt under nitrogen for 20 min before the addition of freshly distilled trimethylsilyl chloride (81.0 mg, 0.095 mL, 1.5 mmol).[25] The reaction mixture was heated at 50 °C and stirred at this temperature for 1 h before the addition of 4-bromophenacyl bromide (139 mg, 0.5 mmol) and further heating at 100 °C for 1.5 h. Upon cooling, water (15 mL) was added, the suspension was filtered, and the precipitate washed with hot water (90 °C) to yield a rust colored compound. The product was recrystallized from glacial AcOH, filtered, and washed with ice cold water to yield **6c** (11.2 mg, 5%) as a light yellow powder, mp 184–186 °C, R_f 0.65 (silica, DCM). ^{1}H NMR (500 MHz): δ 5.60 (s, 2H, H1′), 7.70 (d, J = 8.5 Hz, 2H, H3″, H5″), 7.76 (d, J = 2 Hz, 1H, H4), 7.80 (d, J = 2 Hz, 1H, H6), 7.87 (d, J = 8.5 Hz, 2H, H2″, H6″). ^{13}C NMR

(126 MHz): δ 47.9, 105.3, 117.2, 121.3, 127.6, 129.6[a], 129.8, 132.5[a], 132.5, 144.7, 146.7, 158.1, 180.7, 190.4. HREI-MS: *m/z* calcd for $C_{16}H_8NO_3$ $^{79}Br^{81}Br^{81}Br$ [M^+]: 502.8013; found: 502.8007.

4.2.12. 5,7-Dibromo-1-[2-(3-methoxyphenyl)-2-oxo-ethyl]-1*H*-indole 2,3-dione (6d). This compound was prepared according to the method for **6a** using 5,7-dibromoisatin **2** (306 mg, 1.00 mmol) and 3-methoxyphenacyl bromide (230 mg, 1.00 mmol) as starting materials.[26] The resulting solid was purified by flash chromatography on silica gel (DCM) to yield **6d** (43.5 mg, 10%) as bright orange crystals, m.p. 155–157 °C, R_f 0.50 (silica, DCM). ^{1}H NMR (500 MHz): δ 3.88 (s, 3H, OCH_3), 5.62 (s, 2H, H1′), 7.20 (d, J = 8 Hz, 1H, H4″), 7.45 (t, J = 8 Hz, 1H, H5″), 7.51 (s, 1H, H2″), 7.58 (d, J = 8 Hz, 1H, H6″), 7.75 (d, J = 2 Hz, 1H, H4), 7.81 (d, J = 2 Hz, 1H, H6). ^{13}C NMR (75 MHz): δ 48.1, 55.5, 105.4, 112.5, 117.1, 120.5, 120.7, 121.3, 127.5, 130.1, 135.0, 144.7, 146.9, 158.1, 160.1, 180.8, 191.2. HREI-MS: *m/z* calcd for $C_{17}H_{11}NO_4$ $^{79}Br^{79}Br$ [M^+]: 450.9055; found: 450.9048.

4.2.13. 5,7-Dibromo-1-[2-(4-methoxyphenyl)-2-oxo-ethyl]-1*H*-indole-2,3-dione (6e). This compound was prepared according to the method for **6d** using 5,7-dibromoisatin **2** (111 mg, 0.36 mmol) and 4-methoxyphenacyl bromide (82 mg, 0.36 mmol) as starting materials. The reaction mixture was then stirred at rt for 51 h. The suspension was filtered and the precipitate washed with water. The resulting solid was purified by flash chromatography on silica gel (DCM) to yield **6e** (18.2 mg, 11%) as bright orange crystals, mp 205–207 °C, R_f 0.50 (silica, DCM). ^{1}H NMR (500 MHz): δ 3.91 (s, 3H, OCH_3), 5.59 (s, 2H, H1′), 7.00 (d, J = 8 Hz, 2H, H3″, H5″), 7.74 (d, J = 2 Hz, 1H, H4), 7.80 (d, J = 2 Hz, 1H, H6), 7.98 (d, J = 8 Hz, 2H, H2″, H6″). ^{13}C NMR (126 MHz): δ 47.9, 55.9, 105.7, 114.6[a], 117.2, 121.6, 126.9, 127.7, 130.7[a], 145.0, 147.3, 158.5, 164.7, 181.2, 186.9. HREI-MS: *m/z* calcd for $C_{17}H_{11}NO_4$ $^{79}Br^{81}Br$ [M^+]: 452.9034; found: 452.9048.

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References and notes

1. Pandeya, S. N.; Smitha, S.; Jyoti, M.; Sridhar, S. K. *Acta Pharm.* **2005**, *55*, 27–46.
2. Sharma, V. M.; Prasanna, P.; Seshu, V. A.; Renuka, B.; Rao, V. L.; Kumar, G. S.; Narasimhulu, C. P.; Babu, P. A.; Puranik, R. C.; Subramanyam, D.; Venkateswarlu, A.; Rajagopal, S.; Kumar, K. B. S.; Rao, C. S.; Mamidi, N. V. S. R.; Deevi, D. S.; Ajaykumar, R.; Rajagopalan, R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2303–2307.
3. Moon, M. J.; Lee, S. K.; Lee, J.-W.; Song, W. K.; Kim, S. W.; Kim, J. I.; Cho, C.; Choi, S. J.; Kim, Y.-C. *Bioorg. Med. Chem.* **2006**, *14*, 237–246.
4. Abadi, A. H.; Abou-Seri, S. M.; Abdel-Rahman, D. E.; Klein, C.; Lozach, O.; Meijer, L. *Eur. J. Med. Chem.* **2006**, *41*, 296–305.
5. Gursoy, A.; Karali, N. *Eur. J. Med. Chem.* **2003**, *38*, 633–643.
6. Vine, K. L.; Locke, J. M.; Ranson, M.; Benkendorff, K.; Pyne, S. G.; Bremner, J. B. *Bioorg. Med. Chem.* **2007**, *15*, 931–938.
7. Vine, K. L.; Locke, J. M.; Ranson, M.; Pyne, S. G.; Bremner, J. B. *J. Med. Chem.* **2007**, *50*, 5109–5117.
8. Garden, S. J.; Torres, J. C.; da Silva, J. F. M.; Pinto, A. C. *Synth. Commun.* **1998**, *28*, 1679–1689.
9. Bouhfid, R.; Joly, N.; Massoui, M.; Cecchelli, R.; Lequart, V.; Martin, P.; Essassi, E.-M. *Heterocycles* **2005**, *65*, 2949–2955.
10. Maynard, G. D.; Cheng, H. C.; Kane, J. M.; Staeger, M. A. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 753–756.
11. Khuseinov, K. *Doklady Akademii Nauk Tadzhikskoi.* **1976**, *19*, 30–32, Chem. Abstr. 85, 159815.
12. Ballester, M. *Chem. Rev.* **1955**, *55*, 283–300.
13. Black, D. C.; Wong, L. C. H. *J. Chem Soc., Chem. Commun.* **1980**, 200.
14. Jordan, M. A. *Curr. Med. Chem.—Anti-Cancer Agents.* **2002**, *2*, 1–17.
15. Li, P.-K.; Xiao, Z.; Hu, Z.; Pandit, B.; Sun, Y.; Sackett, D. L.; Werbovetz, K.; Lewis, A.; Johnsamuel, J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5382–5385.
16. Bacher, G.; Nickel, B.; Emig, P.; Vanhoefer, U.; Seeber, S.; Shandra, A.; Klenner, T.; Beckers, T. *Cancer Res.* **2001**, *61*, 392–399.
17. Brancale, A.; Silvestri, R. *Med. Res. Rev.* **2007**, *27*, 209–238.
18. Kaufmann, D.; Pojarova, M.; Vogel, S.; Liebl, R.; Gastpar, R.; Gross, D.; Nishino, T.; Pfaller, T.; von Angerer, E. *Bioorg. Med. Chem.* **2007**, *15*, 5122–5136.
19. Chen, Z.; Merta, P. J.; Lin, N.-H.; Tahir, S. K.; Kovar, P.; Sham, H. L.; Zhang, H. *Mol. Cancer Ther.* **2005**, *4*, 562–568.
20. Goldbrunner, M.; Loidl, G.; Polossek, T.; Mannschreck, A.; von Angerer, E. *J. Med. Chem.* **1997**, *40*, 3524–3533.
21. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.
22. Lindwall, H. G.; Bandes, J.; Weinberg, I. *J. Am. Chem. Soc.* **1931**, *53*, 317–319.
23. Rekhter, M. A. *Chem. Het. Compounds* **2005**, *41*, 1119–1120.
24. Jones, A. W.; Purwono, B.; Bowyer, P. K.; Mitchell, P. S. R.; Kumar, N.; Nugent, S. J.; Joliffe, K. A.; Black, D. C. *Tetrahedron* **2004**, *60*, 10779–10786.
25. Rekhter, M. A. *Chem. Het. Compounds* **1999**, *35*, 1165–1166.
26. Garden, S. J.; da Silva, R. B.; Pinto, A. C. *Tetrahedron* **2002**, *58*, 8399–8412.