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Synthesis and antiprotozoal activity of mono- and bis-uracil isatin conjugates against the human pathogen *Trichomonas vaginalis*

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ABSTRACT

A library of mono- and bis-uracil isatin conjugates were synthesized and subjected for the assessment of their in vitro activity against the protozoal pathogen *Trichomonas vaginalis*. The structure activity studies (SAR) revealed that the bis-uracil-isatin based conjugates were more effective than their corresponding mono conjugates in inhibiting the growth of *T. vaginalis* at approximately 10 μM with no visual effect on mammalian cells at the same concentration.

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1. Introduction

Human trichomoniasis is caused by *Trichomonas vaginalis*. This protozoal infection is the most common chronic non-viral infection and is responsible for approximately 248 million cases each year, being more prevalent than either chlamydia or gonorrheal infections.[1,2] Trichomoniasis has a ubiquitous distribution especially in under-developed countries with a combination of poor sanitation, hygiene and tropical climate. Malodorous vaginal discharge, vulval irritation, inflammation and cervical micro hemorrhages are the common symptoms which may lead to serious health complications such as infertility, preterm delivery, low birth weight and cervical cancer.[3,4] Despite its high prevalence, trichomoniasis is neglected compared to other sexually transmitted diseases and was long regarded as a self-clearing infection.[5,6] The importance of trichomoniasis has further increased dramatically because of its strong association with the increased risk of acquisition and transmission of human immunodeficiency virus (HIV).[7–10] Metronidazole has been the main-stay for the treatment of trichomoniasis for more than 40 years,[11] however recent disclosures of genotoxicity, gastric mucus irritation and developed of clinical resistant isolates have provided strong impetus for the development of novel, non-cytotoxic and efficient scaffolds against trichomoniasis.[12–16]

Isatin (1*H*-indole-2,3-dione) is a chemical scaffold present widely in both human and other mammalian tissues and is also found to be a common structural motif in a variety of dyes, agrochemicals and pharmacologically active compounds.[17] The synthetic versatility of isatin makes it an ideal platform for structural alteration and derivatization. This observation has been confirmed by its enormous biological potential with myriad activities such as anticancer,[18] antidepressant,[19] anticonvulsant,[20] antifungal,[21] anti-HIV,[22] and anti-angiogenic properties.[23] Further, it can function as a potent inhibitor of DNA gyrase, the enzyme which catalyzes the introduction of negative supercoils in a closed circular DNA.[24] Note-worthy examples of 2-oxoindole analogues include SU11248 (Sutent), a 5-fluoro-3-substituted isatin derivative, was approved by the FDA in 2006 for the treatment of advanced renal carcinoma and gastrointestinal stromal tumors.[25] C5- and C6-substituted isatin analogues were revealed to be selective inhibitors of monoamine oxidase B (MAOB) with 5-(4-phenylbutyl) isatin exhibiting the highest activity and being 18,500-fold more potent than isatin.[26]

Uracils are also considered important scaffolds in drug discovery with a wide range of biological activities, synthetic expediency and ability to confer drug like properties to the compound libraries appended on them at the N1, N3, C5 and C6 positions.[27] Apart from their antiviral and anti-tumor potential, uracil analogs also possess herbicidal, insecticidal and bactericidal activities.[28] Inhibition of a

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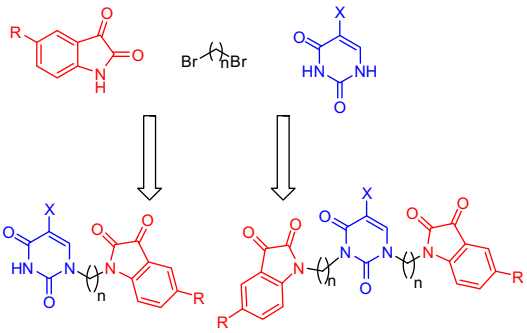


Figure 1. General structures assigned to the target hybrids.

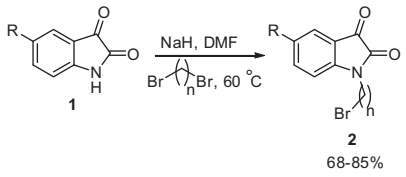
key step in viral replication pathways form the basis of their antiviral potential resulting in potent activities against HIV, hepatitis B and C (HBV and HCV), the herpes viruses (HSV-1 and -2), Epstein Barr virus (EBV), and human cytomegalovirus (HCMV).[29] 5-fluorouracil is widely used as an anti-metabolism drug in the treatment of malignancies including primarily colorectal, stomach and breast cancer. Its poor selectivity, however, limits its therapeutic relevance, resulting in high incidences of bone marrow, gastrointestinal tract and central nervous toxicity.[30] Numerous alterations of 5-FU structure have been accomplished to tackle these problems ensuing in the development of 5-FU derivatives viz. floxuridine and tegafur exhibiting better pharmacological and pharmacokinetic properties.[31] Molecular conjugate of 5-FU and Camptothecin (CPT), a topoisomerase cytotoxic alkaloid, is another approach to circumvent the problems associated with uracil analogues and have shown to exhibit comparable or superior cytotoxicity to irinotecan (semi-synthetic analogue of CPT and acts via topoisomerase 1 inhibition) with improved selectivity, efficacy and safety.

Recent studies have shown the synthesis of 1*H*-1,2,3-triazole-tethered isatin-β-lactam conjugates and preliminary analysis of their in vitro activity against *T. vaginalis*.[32] The methodology was further extended to the synthesis of β-amino alcohol tethered isatin-β-lactam as well as piperazine-linked isatin-7-chloroquinoline conjugates, along with their in vitro inhibitory activity against *T. vaginalis* and *Tritrichomonas foetus*.[33] In continuation of these findings and in view of the reported anti-parasitic potential of uracil, the present study expands the synthesis of mono- and bis-uracil-isatin conjugates as shown in Figure 1 and their in vitro activity against *T. vaginalis*. The chain length has been meticulously altered so as to carefully analyze the structure–activity relationship among the synthesized hybrids.

2. Results and discussion

C-5 substituted *N*-alkylbromo-isatins **2** (Scheme 1) were synthesized via our previously reported protocol involving NaH promoted reaction of isatin with dibromoalkanes at 60 °C. The precursors **2** were purified via column chromatography using 15:85 EtOH/Hexane as eluent and the % age yields of each substituent have been shown in Table 1. The *N*-alkylated precursors were then employed in the synthesis of desired mono- and bis-uracil-isatin hybrids **4** and **5**, respectively as shown in Scheme 2.[34] It was observed that the use of 1.0 mmol of uracil and 1.1 mmol of *N*-alkylbromo-isatins **2** resulted in the formation of mono- and bis-uracil-isatin hybrids **4** and **5** (8:2) respectively, while the use of 1.0 mmol of uracil and the 2.2 mmol of C-5 substituted *N*-alkylbromo-isatins exclusively resulted in the formation of bis-uracil-isatin conjugates **5** (Table 2).

The mono- and di-alkylation of uracil in the present case could be rationalized in terms of the acidity of the ionizable protons.[35]



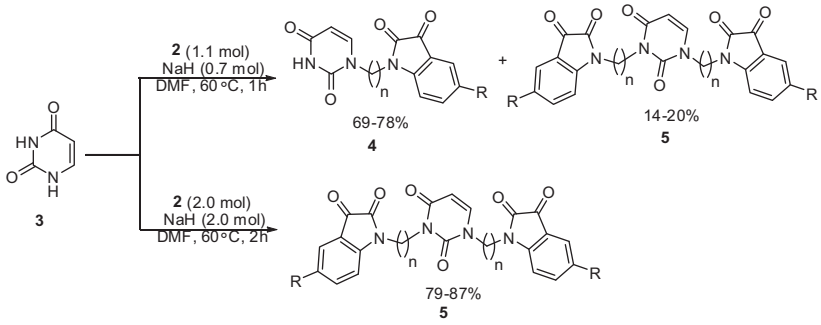
Scheme 1. Synthesis of *N*-alkylbromo-isatins.

Table 1
% age yields of synthesized *N*-alkylbromo-isatins **2**

Compound	*n*	R	Yield (%)
2a	2	H	71
2b	3	H	77
2c	4	H	73
2d	5	H	71
2e	6	H	80
2f	8	H	83
2g	2	F	75
2h	3	F	70
2i	4	F	68
2j	5	F	78
2k	6	F	81
2l	8	F	79
2m	2	Cl	67
2n	3	Cl	74
2o	4	Cl	72
2p	5	Cl	78
2q	6	Cl	82
2r	8	Cl	85

This chemical library of mono- and bis-uracil-isatin conjugates was evaluated in a general inhibitory screen against the protozoal pathogen *Trichomonas vaginalis* at 50 μM; and the results are summarized in Table 3. As observed, the mono uracil-isatin conjugates viz. **4a**–**r** were not efficient in inhibiting the growth of *T. vaginalis* and exhibited percentage growth inhibition in the range of 1.76–48.55%. The observed activity of the conjugates were found to be independent of the nature of substituent at C-5 position of isatin as well as the length of alkyl chain linker. Further, a closer inspection of Table 3 revealed that bis-uracil-isatin conjugates (**5a**–**r**) exhibited better percentage inhibition compared to mono-uracil-isatin conjugates. Analyzing the effect of substituent at C-5 position of isatin ring among bis-conjugates revealed that the presence of electron withdrawing substituent viz. chloro and flouro substantially improved the activity profiles compared to the unsubstituted isatins (R = H). Further, an increase in alkyl chain length, introduced as a linker, improved the activity profile regardless to the nature of substituent at C-5 position of isatin ring as evident by conjugate **5f** (R = H, *n* = 8), exhibiting 92.93% inhibition. The scaffolds **5k** (R = F, *n* = 6); **5l** (R = F, *n* = 8); **5q** (R = Cl, *n* = 6) and **5r** (R = Cl, *n* = 8) exhibited >90% inhibition confirming the role of electron withdrawing halogen substituents and longer chain length in improving the activity profiles of the synthesized conjugates. These conjugates were then serially diluted and re-tested on parasite cultures.

Five potent synthesized conjugates viz. **5f**, **5j**, **5k**, **5m** and **5q** were further evaluated for their IC_{50} (μM) and compared with the standard drug metronidazole, the only and current FDA approved drug used for the treatment of *Trichomonas*; shown in Table 4. The synthesized conjugates were not as active as the standard drug metronidazole. However, **5f** and **5m** represented the most potent inhibitory activity in serial dilution. The IC_{50} values of **5f** and **5m** were determined to be 9.86 and 9.79 μM respectively. These $IC_{50's}$ were then re-confirmed using the same assays and standard error was determined to be within 10%. Mammalian cells



Scheme 2. Synthesis of mono- and bis-uracil-isatin conjugates **4** and **5**.

Table 2
% age yields of mono- and bis-uracil-isatin conjugates **4** and **5**

Compound	*n*	R	Yield (%)	Compound	*n*	R	Yield (%)
4a	2	H	73	**5a**	2	H	80
4b	3	H	78	**5b**	3	H	79
4c	4	H	71	**5c**	4	H	86
4d	5	H	75	**5d**	5	H	84
4e	6	H	69	**5e**	6	H	81
4f	8	H	72	**5f**	8	H	79
4g	2	F	72	**5g**	2	F	84
4h	3	F	76	**5h**	3	F	87
4i	4	F	75	**5i**	4	F	83
4j	5	F	74	**5j**	5	F	86
4k	6	F	78	**5k**	6	F	87
4l	8	F	73	**5l**	8	F	80
4m	2	Cl	71	**5m**	2	Cl	82
4n	3	Cl	73	**5n**	3	Cl	85
4o	4	Cl	75	**5o**	4	Cl	81
4p	5	Cl	75	**5p**	5	Cl	84
4q	6	Cl	74	**5q**	6	Cl	80
4r	8	Cl	76	**5r**	8	Cl	82

Table 3
Antiprotozoal inhibition of **4a**–**r** and **5a**–**r** against *T. vaginalis* at 50 μM

Compound	Percentage inhibition	Compound	Percentage inhibition
4a	37.77 ± 11.04	**5a**	10.46 ± 1.37
4b	30.13 ± 16.27	**5b**	0.54 ± 0.54
4c	41.48 ± 17.72	**5c**	18.28 ± 18.28
4d	26.56 ± 18.64	**5d**	4.30 ± 4.30
4e	19.58 ± 15.62	**5e**	67.45 ± 8.30
4f	32.23 ± 17.37	**5f**	92.93 ± 7.08
4g	32.38 ± 7.62	**5g**	47.23 ± 5.29
4h	26.68 ± 2.92	**5h**	44.54 ± 7.98
4i	20.95 ± 11.05	**5i**	37.00 ± 0.64
4j	35.80 ± 3.80	**5j**	86.69 ± 9.27
4k	37.01 ± 18.19	**5k**	100
4l	22.05 ± 13.15	**5l**	97.47 ± 2.53
4m	14.04 ± 14.03	**5m**	82.46 ± 0.40
4n	4.39 ± 4.38	**5n**	78.07 ± 0.70
4o	48.55 ± 0.57	**5o**	57.89 ± 2.72
4p	1.76 ± 1.75	**5p**	74.56 ± 1.45
4q	25.38 ± 23.74	**5q**	90.35 ± 0.18
4r	41.84 ± 23.95	**5r**	96.49 ± 0.92

Table 4
Antiprotozoal inhibition of **4a**–**r** and **5a**–**r** against *T. vaginalis*

Compound	IC_{50} value (mM)
5f	9.86
5j	10.41
5k	19.50
5m	9.79
5q	10.26
Metronidazole	0.72

were then tested with **5f** and **5m** at the same concentration and no visual effects on morphology or cell count was observed.

3. Conclusion

A series of mono- and bis-uracil-isatin conjugates were synthesized and assessed for their in vitro activity against the protozoal pathogen *T. vaginalis*. SAR studies revealed that the bis-uracil-isatin conjugates viz. **5f**, **5k**, **5l**, **5q** and **5r** were more effective than their corresponding mono-uracil-isatin counterparts with more than 90% inhibition at 50 μM. The most potent and non-cytotoxic conjugates of this group, **5f** and **5m**, had an optimum combination of longer alkyl chain length (*n* = 2 for **5f** and, 6 for **5m**) and no or electron withdrawing-substituent at C-5 position of isatin, exhibited an IC_{50} values of 9.86 and 9.79 μM respectively. These conjugates may represent a novel scaffold for development of chemotherapy for resistant cases of human trichomoniasis, and may represent an effective strategy for new anti-protozoals against related anaerobic pathogens.

4. Material and methods

4.1. Biological evaluation

4.1.1. In vitro protozoal parasite susceptibility assay

Protozoal parasites were cultured for 24 h at 37 °C. To perform the initial susceptibility screens on *T. vaginalis*, compounds were suspended in DMSO to obtain concentrations of 100 μM; 5 μL aliquots of these suspensions were diluted in 5 mL of TYM diamond's media to obtain a final concentration of 100 μM. After 24 h, cells were counted using a hemacytometer. Cell counts were normalized to the DMSO controls, in order to allow direct comparison and averaging of the various trials. These data sets were then transformed using Prism Software, GraphPad, by taking the log of the drug concentrations for the trials, and inputting this transform into a log (inhibitor) versus response-variable slope regression option. Within this non-linear regression, constraints were set to force the maximum value (top) to 1 and the minimum value (bottom) to 0. The slope was left variable, and then determined through which regression was performed. The sample size consists of 4 independent trials carried out on four different days (to account for possible variation in parasite culture). The assays were performed in 15 mL culture tubes, with both wildtype parasites and 0.1% DMSO-only treated parasites serving as control tubes to normalize for the effects of the solvent and in vitro conditions. After 24 h, cells were counted using a hemacytometer. The IC_{50} value for compound **5q** was determined by running assays of increasing drug concentrations, 5–40 μM, and performing a regression analysis using Prism software from GraphPad. Calculated IC_{50} value of

5q was then re-confirmed by testing again using the same assay described above.

4.1.2. Mammalian cell cytotoxicity studies

HeLa cells were cultured in DMEM/penicillin streptomycin/10% FBS, 5%CO_2, 37 °C, and 60% confluency. Cells were treated with **5q** to a final concentration of 10 μM. DMSO was also used as a negative control. Cells were then incubated for 24 h, trypsinized with 0.25% Trypsin-EDTA, then counted using light microscopy and a corpuscle counting chamber. Controls included untreated cells and cells treated with 0.1% DMSO.

4.2. General

Melting points were determined by open capillary using Veego Precision Digital Melting Point apparatus (MP-D) and are uncorrected. ^{1}H NMR spectra were recorded in DMSO-d_6 with BRUKER AVANCE II (500 MHz) spectrometer using TMS as internal standard. Chemical shift values are expressed as parts per million downfield from TMS and J values are in hertz. Splitting patterns are indicated as s: singlet, d: doublet, t: triplet, m: multiplet, dd: double doublet, ddd: doublet of a doublet of a doublet, and br: broad peak. ^{13}C NMR spectra were recorded in DMSO-d_6 with BRUKER AVANCE II (125 MHz) using TMS as internal standard. Mass spectra were recorded on a BRUCKER high resolution mass spectrometer (micrOTOF-QII). Elemental analyses were performed on Heraus CHN-O-Rapid Elemental Analyzer. Column chromatography was performed on a silica gel (60–120 mesh) using ethyl acetate–hexane mixture as eluent.

4.2.1. Typical procedure for the synthesis of hybrids 4a–r

To a stirred suspension of NaH (0.7 mmol) in dry DMF (5 mL) at 0 °C was added uracil **3** (1.0 mmol) and the mixture was allowed to stir for 5 min. followed by the dropwise addition of a solution of C-5 substituted *N*-alkylbromo-isatins **2** (1.1 mmol) in dry DMF. The reaction mixture was stirred at 60 °C for 1 h and the progress was monitored by using TLC. On completion of the reaction, the solvent was evaporated under vacco followed by the extraction with ethyl acetate (2 × 25 ml) and water. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified via column chromatography using silica gel (60–120 mesh) yielding **4** in good yields.

4.2.1.1. 1-[2-(2,4-Dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-ethyl]-1*H*-indole-2,3-dione (4a). Yield 73%; orange solid; mp 168–169 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.84 (t, J = 6.3 Hz, 2H, –NCH_2), 3.95 (t, J = 6.3 Hz, 2H, –NCH_2), 5.59 (d, J = 7.4 Hz, 1H, olefinic H), 7.12–7.16 (m, 1H, –ArH), 7.20 (d, J = 8.0 Hz, 1H, –ArH), 7.63 (d, J = 7.4 Hz, 1H, olefinic H), 7.69–7.72 (m, 2H, –ArH), 11.23 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 43.1, 45.5, 101.4, 111.2, 118.0, 123.3, 124.7, 138.1, 146.3, 150.5, 151.2, 158.4, 164.1, 183.5; HRMS: Calcd for $C_{14}H_{11}N_3O_4$ $[M]^+$ 285.0750, found 285.0742; Anal. Calcd (%) for: C, 58.95; H, 3.89; N, 14.73, found: C, 58.86; H, 3.94; N, 14.66.

4.2.1.2. 1-[3-(2,4-Dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-propyl]-1*H*-indole-2,3-dione (4b). Yield 78%; orange solid; mp 164–165 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.97 (t, J = 7.0 Hz, 2H, –CH_2), 3.72–3.78 (m, 4H, 2×NCH_2), 5.57 (d, J = 7.8 Hz, 1H, olefinic H), 7.15–7.18 (m, 1H, –ArH), 7.23 (d, J = 7.8 Hz, 1H, –ArH), 7.59 (d, J = 7.2 Hz, 1H, olefinic H), 7.67–7.70 (m, 2H, –ArH), 11.25 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 26.7, 37.3, 45.9, 101.3, 111.0, 118.1, 123.6, 124.8, 138.5, 146.0, 150.8, 151.3, 158.7, 164.2, 183.8; HRMS: Calcd for $C_{15}H_{13}N_3O_4$ $[M]^+$ 299.0906, found 299.0901; Anal. Calcd (%) for: C, 60.20; H, 4.38; N, 14.04, found: C, 60.12; H, 4.30; N, 14.16.

4.2.1.3. 1-[4-(2,4-Dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-butyl]-1*H*-indole-2,3-dione (4c). Yield 71%; orange solid; mp 171–172 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.73–1.79 (m, 4H, 2×CH_2), 3.89–3.92 (m, 2H, –NCH_2), 3.96–3.98 (m, 2H, –NCH_2), 5.58 (d, J = 7.4 Hz, 1H, olefinic H), 7.11–7.15 (m, 1H, –ArH), 7.26 (d, J = 8.1 Hz, 1H, –ArH), 7.63 (d, J = 7.4 Hz, 1H, olefinic H), 7.66–7.69 (m, 2H, –ArH), 11.21 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.5, 26.1, 37.8, 44.2, 101.0, 111.3, 118.1, 123.3, 124.6, 138.3, 146.1, 150.2, 151.5, 158.2, 164.6, 183.7; HRMS: Calcd for $C_{16}H_{15}N_3O_4$ $[M]^+$ 313.1063, found 313.1054; Anal. Calcd (%) for: C, 61.34; H, 4.83; N, 13.41, found: C, 61.39; H, 4.88; N, 13.32.

4.2.1.4. 1-[5-(2,4-Dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-pentyl]-1*H*-indole-2,3-dione (4d). Yield 75%; orange solid; mp 166–167 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.33–1.37 (m, 2H, –CH_2), 1.81–1.87 (m, 4H, 2×CH_2), 3.82–3.84 (m, 2H, –NCH_2), 3.89–3.91 (m, 2H, –NCH_2), 5.62 (d, J = 7.2 Hz, 1H, olefinic H), 7.12–7.17 (m, 1H, –ArH), 7.22 (d, J = 7.8 Hz, 1H, –ArH), 7.60 (d, J = 7.2 Hz, 1H, olefinic H), 7.64–7.66 (m, 2H, –ArH), 11.25 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 24.7, 25.1, 26.0, 37.7, 44.1, 101.4, 111.5, 118.5, 123.2, 124.4, 138.6, 146.0, 150.3, 151.2, 158.7, 164.4, 183.8; HRMS: Calcd for $C_{17}H_{17}N_3O_4$ $[M]^+$ 327.1219, found 327.1208; Anal. Calcd (%) for: C, 62.38; H, 5.23; N, 12.84, found: C, 62.49; H, 5.36; N, 12.71.

4.2.1.5. 1-[6-(2,4-Dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-hexyl]-1*H*-indole-2,3-dione (4e). Yield 69%; orange solid; mp 173–174 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.33–137 (m, 4H, 2×CH_2), 1.55–1.61 (m, 4H, 2×CH_2), 3.69–3.71 (m, 4H, 2×NCH_2), 5.58 (d, J = 7.4 Hz, 1H, olefinic H), 7.13–7.17 (m, 1H, –ArH), 7.25 (d, J = 7.7 Hz, 1H, –ArH), 7.64 (d, J = 7.4 Hz, 1H, olefinic H), 7.68–7.73 (m, 2H, –ArH), 11.20 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.8, 26.4, 26.8, 28.9, 47.5, 101.2, 111.4, 118.7, 123.1, 124.5, 138.8, 146.4, 150.6, 151.8, 158.4, 164.3, 183.3; HRMS: Calcd for $C_{18}H_{19}N_3O_4$ $[M]^+$ 341.1376, found 341.1367; Anal. Calcd (%) for: C, 63.33; H, 5.61; N, 12.31, found: C, 63.24; H, 5.52; N, 12.43.

4.2.1.6. 1-[8-(2,4-Dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-octyl]-1*H*-indole-2,3-dione (4f). Yield 72%; orange solid; mp 163–164 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.28–133 (m, 8H, 4×CH_2), 1.56–1.60 (m, 4H, 2×CH_2), 3.68–3.71 (m, 4H, 2×NCH_2), 5.57 (d, J = 7.8 Hz, 1H, olefinic H), 7.14–7.19 (m, 1H, –ArH), 7.29 (d, J = 7.7 Hz, 1H, –ArH), 7.61 (d, J = 7.8 Hz, 1H, olefinic H), 7.64–7.68 (m, 2H, –ArH), 11.24 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 26.0, 26.2, 27.3, 28.6, 28.8, 29.3, 47.5, 101.4, 111.1, 118.6, 123.3, 124.8, 138.2, 146.2, 150.1, 151.2, 158.6, 164.7, 183.8; HRMS: Calcd for $C_{20}H_{23}N_3O_4$ $[M]^+$ 369.1689, found 389.1680; Anal. Calcd (%) for: C, 65.03; H, 6.28; N, 11.37, found: C, 65.15; H, 6.36; N, 11.29.

4.2.1.7. 1-[2-(2,4-Dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-ethyl]-5-fluoro-1*H*-indole-2,3-dione (4g). Yield 72%; orange solid; mp 158–159 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.81 (t, J = 6.1 Hz, 2H, –NCH_2), 3.97 (t, J = 6.1 Hz, 2H, –NCH_2), 5.57 (d, J = 7.4 Hz, 1H, olefinic H), 7.22 (d, J = 7.8 Hz, 1H, –ArH), 7.44 (d, J = 7.8 Hz, 1H, –ArH), 7.61 (s, 1H, –ArH), 7.67 (d, J = 7.4 Hz, 1H, olefinic H), 11.20 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 42.8, 44.6, 101.6, 112.1, 118.3, 124.3,

124.9, 146.7, 147.8, 151.6, 157.3, 158.8, 164.4, 183.2; HRMS: Calcd for $C_{14}H_{10}FN_3O_4$ $[M]^+$ 303.0655, found 303.0647; Anal. Calcd (%) for: C, 55.45; H, 3.32; N, 13.86, found: C, 55.41; H, 3.26; N, 13.93.

4.2.1.8. 1-[3-(2,4-Dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-propyl]-5-fluoro-1*H*-indole-2,3-dione (4h). Yield 76%; orange solid; mp 152–153 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.95 (t, J = 6.9 Hz, 2H, –CH_2), 3.71–3.75 (m, 4H, 2×NCH_2), 5.58 (d, J = 7.2 Hz, 1H, olefinic H), 7.25 (d, J = 7.7 Hz, 1H, –ArH), 7.48 (d, J = 7.7 Hz, 1H, –ArH), 7.63 (s, 1H, –ArH), 7.68 (d, J = 7.2 Hz, 1H, olefinic H), 11.22 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 26.6, 37.1, 45.8, 101.1, 112.4, 118.0, 124.3, 124.7, 146.8, 147.6, 151.4, 157.2, 158.3, 164.0, 183.7; HRMS: Calcd for $C_{15}H_{12}FN_3O_4$ $[M]^+$ 317.0812, found 318.0803; Anal. Calcd (%) for: C, 56.78; H, 3.81; N, 13.24, found: C, 56.87; H, 3.89; N, 13.15.

4.2.1.9. 1-[4-(2,4-Dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-butyl]-5-fluoro-1*H*-indole-2,3-dione (4i). Yield 75%; orange solid; mp 154–155 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.70–1.74 (m, 4H, 2×CH_2), 3.88–3.93 (m, 2H, –NCH_2), 3.94–3.99 (m, 2H, –NCH_2), 5.60 (d, J = 7.4 Hz, 1H, olefinic H), 7.29 (d, J = 7.8 Hz, 1H, –ArH), 7.49 (d, J = 7.8 Hz, 1H, –ArH), 7.60 (s, 1H, –ArH), 7.66 (d, J = 7.4 Hz, 1H, olefinic H), 11.21 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.2, 26.8, 37.0, 44.4, 101.0, 112.2, 118.2, 124.5, 124.6, 146.4, 147.5, 151.7, 157.3, 158.2, 164.1, 183.4; HRMS: Calcd for $C_{16}H_{14}FN_3O_4$ $[M]^+$ 331.0968, found 331.0959; Anal. Calcd (%) for: C, 58.01; H, 4.26; N, 12.68, found: C, 57.92; H, 4.17; N, 12.79.

4.2.1.10. 1-[5-(2,4-Dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-pentyl]-5-fluoro-1*H*-indole-2,3-dione (4j). Yield 74%; orange solid; mp 151–152 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.31–1.34 (m, 2H, –CH_2), 1.77–1.81 (m, 4H, 2×CH_2), 3.80–3.85 (m, 2H, –NCH_2), 3.87–3.90 (m, 2H, –NCH_2), 5.57 (d, J = 7.7 Hz, 1H, olefinic H), 7.24 (d, J = 8.0 Hz, 1H, –ArH), 7.42 (d, J = 8.0 Hz, 1H, –ArH), 7.62 (s, 1H, –ArH), 7.63 (d, J = 7.7 Hz, 1H, olefinic H), 11.27 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 24.7, 25.7, 26.3, 37.5, 44.2, 101.7, 112.1, 118.3, 124.3, 124.8, 146.1, 147.8, 151.6, 157.4, 158.5, 164.4, 183.6; HRMS: Calcd for $C_{17}H_{16}FN_3O_4$ $[M]^+$ 345.1125, found 345.1114; Anal. Calcd (%) for: C, 59.13; H, 4.67; N, 12.17, found: C, 59.19; H, 4.63; N, 12.22.

4.2.1.11. 1-[6-(2,4-Dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-hexyl]-5-fluoro-1*H*-indole-2,3-dione (4k). Yield 78%; orange solid; mp 157–158 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.31–138 (m, 4H, 2×CH_2), 1.54–1.62 (m, 4H, 2×CH_2), 3.64–3.70 (m, 4H, 2×NCH_2), 5.58 (d, J = 7.4 Hz, 1H, olefinic H), 7.21 (d, J = 7.8 Hz, 1H, –ArH), 7.43 (d, J = 7.8 Hz, 1H, –ArH), 7.60 (s, 1H, –ArH), 7.65 (d, J = 7.4 Hz, 1H, olefinic H), 11.20 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 26.1, 26.4, 27.3, 28.6, 28.9, 30.3, 46.7, 101.3, 112.2, 118.1, 124.5, 124.9, 146.0, 147.6, 151.5, 157.7, 158.2, 164.6, 183.5; HRMS: Calcd for $C_{18}H_{18}FN_3O_4$ $[M]^+$ 359.1281, found 359.1294; Anal. Calcd (%) for: C, 60.16; H, 5.05; N, 11.69, found: C, 60.10; H, 4.99; N, 11.76.

4.2.1.12. 1-[8-(2,4-Dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-octyl]-5-fluoro-1*H*-indole-2,3-dione (4l). Yield 73%; orange solid; mp 153–154 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.31 (s, 8H, 4×CH_2), 1.58–1.61 (m, 4H, 2×CH_2), 3.66–3.67 (m, 4H, 2×NCH_2), 5.56 (d, J = 7.2 Hz, 1H, olefinic H), 7.22 (d, J = 7.7 Hz, 1H, –ArH), 7.43 (d, J = 7.7 Hz, 1H, –ArH), 7.61 (s, 1H, –ArH), 7.66 (d, J = 7.4 Hz, 1H, olefinic H), 11.23 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 26.1, 26.5, 27.0, 28.8, 28.9, 29.0, 47.8, 101.1, 112.5, 118.8, 124.3, 124.5, 146.1, 147.4, 151.3, 157.9, 158.5, 164.1, 183.4; HRMS: Calcd for $C_{20}H_{22}FN_3O_4$ $[M]^+$ 387.1594, found 387.1585; Anal. Calcd (%) for: C, 62.01; H, 5.72; N, 10.85, found: C, 61.97; H, 5.65; N, 10.94.

4.2.1.13. 5-Chloro-1-[2-(2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-ethyl]-1*H*-indole-2,3-dione (4m). Yield 71%; orange solid; mp 156–157 °C;; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.81 (t, J = 6.1 Hz, 2H, –NCH_2), 3.94 (t, J = 6.1 Hz, 2H, –NCH_2), 5.59 (d, J = 7.7 Hz, 1H, olefinic H), 7.22 (d, J = 7.2 Hz, 1H, –ArH), 7.42–7.68 (m, 3H, 2ArH+1olefinic H), 11.23 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 43.8, 45.4, 101.0, 112.5, 119.8, 124.2, 127.5, 137.3, 146.4, 149.2, 151.8, 158.0, 164.6, 182.3; HRMS: Calcd for $C_{14}H_{10}ClN_3O_4$ $[M]^+$ 319.0360, found 319.0369; Anal. Calcd (%) for: C, 52.60; H, 3.15; N, 13.14, found: C, 52.69; H, 3.20; N, 13.03.

4.2.1.14. 5-Chloro-1-[3-(2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-propyl]-1*H*-indole-2,3-dione (4n). Yield 73%; orange solid; mp 150–151 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.91 (t, J = 6.6 Hz, 2H, –CH_2), 3.72–3.78 (m, 4H, 2×NCH_2), 5.58 (d, J = 7.8 Hz, 1H, olefinic H), 7.20 (d, J = 7.5 Hz, 1H, –ArH), 7.46–7.64 (m, 3H, 2ArH+1olefinic H), 11.26 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 26.9, 38.2, 45.1, 101.6, 112.4, 119.7, 124.5, 127.7, 137.2, 146.6, 149.0, 151.7, 158.3, 164.2, 182.7; HRMS: Calcd for $C_{15}H_{12}ClN_3O_4$ $[M]^+$ 333.0516, found 333.0508; Anal. Calcd (%) for: C, 53.98; H, 3.62; N, 12.59, found: C, 54.07; H, 3.68; N, 12.54.

4.2.1.15. 5-Chloro-1-[4-(2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-butyl]-1*H*-indole-2,3-dione (4o). Yield 75%; orange solid; mp 155–156 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.75–1.78 (m, 4H, 2×CH_2), 3.83–3.91 (m, 2H, –NCH_2), 3.95–3.98 (m, 2H, –NCH_2), 5.61 (d, J = 7.4 Hz, 1H, olefinic H), 7.25 (d, J = 7.8 Hz, 1H, –ArH), 7.42–7.61 (m, 3H, 2ArH+1olefinic H), 11.24 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.1, 26.2, 37.6, 44.9, 101.4, 112.6, 119.5, 124.8, 127.9, 137.0, 146.3, 149.1, 151.3, 158.5, 164.3, 182.5; HRMS: Calcd for $C_{16}H_{14}ClN_3O_4$ $[M]^+$ 347.0673, found 347.0677; Anal. Calcd (%) for: C, 55.26; H, 4.06; N, 12.08, found: C, 55.35; H, 4.11; N, 11.98.

4.2.1.16. 5-Chloro-1-[5-(2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-pentyl]-1*H*-indole-2,3-dione (4p). Yield 75%; orange solid; mp 152–153 °C;; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.37–1.42 (m, 2H, –CH_2), 1.76–1.82 (m, 4H, 2×CH_2), 3.83–3.86 (m, 2H, –NCH_2), 3.89–3.94 (m, 2H, –NCH_2), 5.58 (d, J = 7.7 Hz, 1H, olefinic H), 7.23 (d, J = 7.2 Hz, 1H, –ArH), 7.47–7.68 (m, 3H, 2ArH+1 olefinic H), 11.23 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 24.9, 25.0, 26.6, 37.3, 44.7, 101.5, 112.5, 119.7, 124.7, 127.3, 137.2, 146.4, 149.4, 151.0, 158.6, 164.5, 182.2; HRMS: Calcd for $C_{17}H_{16}ClN_3O_4$ $[M]^+$ 361.0829, found 361.0820; Anal. Calcd (%) for: C, 56.44; H, 4.46; N, 11.61, found: C, 56.38; H, 4.37; N, 11.66.

4.2.1.17. 5-Chloro-1-[6-(2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-hexyl]-1*H*-indole-2,3-dione (4q). Yield 74%; orange solid; mp 158–159 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.31–138 (m, 4H, 2×CH_2), 1.59–1.62 (m, 4H, 2×CH_2), 3.67–3.68 (m, 4H, 2×NCH_2), 5.57 (d, J = 7.4 Hz, 1H, olefinic H), 7.25 (d, J = 7.7 Hz, 1H, –ArH), 7.48–7.74 (m, 3H, 2ArH+1olefinic H), 11.24 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.9, 26.1, 26.9, 28.7, 47.8, 101.2, 112.8, 119.3, 124.3, 127.7, 137.4, 146.1, 149.6, 151.3, 158.3, 164.1, 182.8; HRMS: Calcd for $C_{18}H_{18}ClN_3O_4$ $[M]^+$ 375.0986, found 375.0979; Anal. Calcd (%) for: C, 57.53; H, 4.83; N, 11.18, found: C, 57.60; H, 4.91; N, 11.08.

4.2.1.18. 5-Chloro-1-[8-(2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-octyl]-1*H*-indole-2,3-dione (4r). Yield 76%; orange solid; mp 152–153 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.35 (s, 8H,

4×CH_2), 1.54–1.60 (m, 4H, 2×CH_2), 3.64–3.68 (m, 4H, 2×NCH_2), 5.58 (d, J = 7.2 Hz, 1H, olefinic H), 7.20 (d, J = 7.8 Hz, 1H, –ArH), 7.43–7.71 (m, 3H, 2ArH+1olefinic H), 11.26 (s, 1H, –NH–, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 26.0, 26.5, 27.4, 28.2, 28.7, 29.8, 47.3, 101.0, 112.3, 119.1, 124.7, 127.4, 137.5, 146.6, 149.3, 151.1, 158.0, 164.4, 182.7; HRMS: Calcd for $C_{20}H_{22}ClN_3O_4$ $[M]^+$ 403.1299, found 403.1291; Anal. Calcd (%) for: C, 59.48; H, 5.49; N, 10.40, found: C, 59.57; H, 5.56; N, 10.28.

4.2.2. Typical procedure for the synthesis of hybrids 5a–r

To a stirred suspension of NaH (2.0 mmol) in dry DMF (5 mL) at 0 °C was added uracil **3** (1.0 mmol) and the mixture was allowed to stir for 10 min. followed by the dropwise addition of a solution of C-5 substituted *N*-alkylbromo-isatins **2** (2.0 mmol) in dry DMF. The reaction mixture was stirred at 60 °C for 2 h and the progress was monitored by using TLC. On completion of the reaction, the solvent was evaporated under vacco followed by the extraction with ethyl acetate (2×25 ml) and water. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified via column chromatography using silica gel (60–120 mesh) yielding **5** in good yields.

4.2.2.1. 1,1′-(2,2′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(ethane-2,1-diyl))diindoline-2,3-dione (5a). Yield 80%; orange solid; mp 195–196 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.81 (t, J = 6.3 Hz, 2H, –NCH_2), 3.93 (t, J = 6.3 Hz, 2H, –NCH_2), 3.97–4.01 (m, 4H, 2×NCH_2), 5.63 (d, J = 7.8 Hz, 1H, olefinic H), 7.14–7.20 (m, 4H, –ArH), 7.56–7.57 (m, 2H, –ArH), 7.66–7.72 (m, 3H, 2ArH+1olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 37.4, 38.0, 38.7, 46.9, 100.8, 110.7, 110.8, 117.8, 117.9, 123.7, 123.8, 125.0, 125.1, 138.6, 138.7, 145.0, 150.7, 151.0, 151.9, 158.7, 158.9, 162.8, 183.4, 183.6; HRMS: Calcd for $C_{24}H_{18}N_4O_6$ $[M]^+$ 458.1226, found 458.1221; Anal. Calcd (%) for: C, 62.88; H, 3.96; N, 12.22, found: C, 62.94; H, 4.04; N, 12.16.

4.2.2.2. 1,1′-(3,3′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(propane-3,1-diyl))diindoline-2,3-dione (5b). Yield 79%; orange solid; mp 197–198 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.91 (t, J = 7.0 Hz, 2H, –CH_2), 1.99 (t, J = 6.9 Hz, 2H, –CH_2), 3.70–3.74 (m, 4H, 2×NCH_2), 3.80 (t, J = 6.9 Hz, 2H, –NCH_2), 3.87 (t, J = 7.0 Hz, 2H, –NCH_2), 5.69 (d, J = 7.8 Hz, 1H, olefinic H), 7.14–7.21 (m, 4H, –ArH), 7.58 (d, J = 7.4 Hz, 2H, –ArH), 7.68–7.71 (m, 3H, 2ArH+1olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.6, 26.5, 36.4, 37.5, 38.3, 47.0, 100.6, 111.0, 111.1, 117.9, 118.0, 123.5, 123.6, 124.8, 124.9, 138.5, 138.6, 144.5, 150.8, 150.9, 151.4, 158.5, 158.7, 162.8, 183.7, 183.8; HRMS: Calcd for $C_{26}H_{22}N_4O_6$ $[M]^+$ 486.1539, found 486.1534; Anal. Calcd (%) for: C, 64.19; H, 4.56; N, 11.52, found: C, 64.11; H, 4.44; N, 11.61.

4.2.2.3. 1,1′-(4,4′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(butane-4,1-diyl))diindoline-2,3-dione (5c). Yield 86%; orange solid; mp 190–191 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.61–165 (m, 6H, 3×CH_2), 1.67–1.71 (m, 2H, –CH_2), 3.65–3.72 (m, 4H, 2×NCH_2), 3.79–3.83 (m, 2H, –NCH_2), 3.87 (s, 2H, –NCH_2), 5.69 (d, J = 7.7 Hz, 1H, olefinic H), 7.12–7.20 (m, 4H, –ArH), 7.55 (d, J = 7.4 Hz, 2H, –ArH), 7.63–7.70 (m, 3H, 2ArH+1olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 24.3, 24.7, 25.4, 25.8, 36.5, 37.5, 45.4, 47.2, 100.2, 111.5, 111.8, 117.5, 118.6, 123.8, 123.9, 124.3, 124.6, 138.2, 138.8, 144.3, 150.4, 150.7, 151.1, 158.0, 158.3, 162.5, 183.5, 183.7; HRMS: Calcd for $C_{28}H_{26}N_4O_6$ $[M]^+$ 514.1852, found 514.1845; Anal. Calcd (%) for: C, 65.36; H, 5.09; N, 10.89, found: C, 65.33; H, 5.03; N, 10.95.

4.2.2.4. 1,1′-(5,5′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(pentane-5,1-diyl))diindoline-2,3-dione (5d). Yield 84%; orange solid; mp 194–195 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.30–1.36 (m, 4H, 2×CH_2), 1.83–1.89 (m, 8H, 4×CH_2), 3.85–3.89 (m, 4H, 2×NCH_2), 3.92–3.96 (m, 4H, 2×NCH_2), 5.65 (d, J = 7.4 Hz, 1H, olefinic H), 7.17–7.22 (m, 4H, –ArH), 7.58 (d, J = 7.7 Hz, 2H, –ArH), 7.61–7.69 (m, 3H, 2ArH+1olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.6, 25.9, 26.4, 27.1, 27.7, 28.6, 36.8, 37.9, 44.2, 47.4, 100.7, 111.1, 111.4, 117.4, 118.7, 123.3, 123.5, 124.5, 124.7, 138.1, 138.6, 144.1, 150.2, 150.4, 151.2, 158.7, 158.9, 162.2, 183.6, 183.8; HRMS: Calcd for $C_{30}H_{30}N_4O_6$ $[M]^+$ 542.2165, found 542.2161; Anal. Calcd (%) for: C, 66.41; H, 5.57; N, 10.33, found: C, 66.49; H, 5.61; N, 10.26.

4.2.2.5. 1,1′-(6,6′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(hexane-6,1-diyl))diindoline-2,3-dione (5e). Yield 81%; orange solid; mp 198–199 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.29–1.37 (m, 8H, 4×CH_2), 1.55–1.63 (m, 8H, 4×CH_2), 3.61–3.68 (m, 4H, 2×NCH_2), 3.75–3.79 (m, 4H, 2×NCH_2), 5.69 (d, J = 7.2 Hz, 1H, olefinic H), 7.19–7.27 (m, 4H, –ArH), 7.59 (d, J = 7.7 Hz, 2H, –ArH), 7.64–7.69 (m, 3H, 2ArH+1olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.3, 25.5, 26.4, 27.0, 27.3, 28.8, 28.9, 29.3, 37.8, 38.1, 44.1, 47.3, 100.2, 111.4, 111.6, 117.3, 118.5, 123.1, 123.2, 124.7, 124.9, 138.4, 138.8, 144.3, 150.0, 150.5, 151.6, 158.5, 158.7, 162.4, 183.3, 183.5; HRMS: Calcd for $C_{32}H_{34}N_4O_6$ $[M]^+$ 570.2478, found 570.2469; Anal. Calcd (%) for: C, 67.35; H, 6.01; N, 9.82, found: C, 67.30; H, 5.94; N, 9.92.

4.2.2.6. 1,1′-(8,8′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(octane-8,1-diyl))diindoline-2,3-dione (5f). Yield 79%; orange solid; mp 193–194 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.21–1.34 (m, 16H, 8×CH_2), 1.64–1.69 (m, 8H, 4×CH_2), 3.63–3.68 (m, 6H, 3×NCH_2), 3.89–3.93 (m, 2H, –NCH_2), 5.60 (d, J = 7.7 Hz, 1H, olefinic H), 7.23–7.29 (m, 4H, –ArH), 7.55 (d, J = 7.8 Hz, 2H, –ArH), 7.62–7.68 (m, 3H, 2ArH+1olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 26.3, 26.6, 27.6, 27.7, 27.9, 28.1, 28.4, 28.5, 28.7, 28.8, 29.0, 29.2, 37.8, 37.9, 48.0, 48.4, 100.4, 111.1, 111.2, 117.2, 118.6, 123.0, 123.4, 124.1, 124.4, 138.7, 138.9, 144.2, 150.4, 150.7, 151.3, 158.3, 158.5, 162.9, 183.8, 183.9; HRMS: Calcd for $C_{36}H_{42}N_4O_6$ $[M]^+$ 626.3104, found 626.3113; Anal. Calcd (%) for: C, 68.99; H, 6.75; N, 8.94, found: C, 69.04; H, 6.83; N, 8.88.

4.2.2.7. 1,1′-(2,2′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(ethane-2,1-diyl))bis(5-fluoroindoline-2,3 dione) (5g). Yield 84%; orange solid; mp 183–184 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.83 (t, J = 6.1 Hz, 2H, –NCH_2), 3.92–3.95 (m, 2H, –NCH_2), 3.99–4.04 (m, 4H, 2×NCH_2), 5.65 (d, J = 7.4 Hz, 1H, olefinic H), 7.23–7.29 (m, 2H, –ArH), 7.44–7.49 (m, 2H, –ArH), 7.50–7.55 (m, 2H, –ArH), 7.65 (d, J = 7.4 Hz, 1H, olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 37.9, 38.2, 38.5, 48.4, 100.3, 111.3, 112.2, 112.3, 112.7, 112.9, 118.5, 118.8, 124.2, 124.6, 144.4, 147.6, 147.9, 151.4, 157.6, 158.2, 158.5, 159.6, 162.5, 183.7; HRMS: Calcd for $C_{24}H_{16}F_2N_4O_6$ $[M]^+$ 494.1038, found 494.1030; Anal. Calcd (%) for: C, 58.30; H, 3.26; N, 11.33, found: C, 58.23; H, 3.21; N, 11.40.

4.2.2.8. 1,1′-(3,3′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(propane-3,1-diyl))bis(5-fluoroindoline-2,3-dione) (5h). Yield 87%; orange solid; mp 180–181 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.94 (t, J = 6.8 Hz, 2H, –CH_2), 1.97 (t, J = 6.9 Hz, 2H, –CH_2), 3.73–3.78 (m, 4H, 2×NCH_2), 3.82 (t, J = 6.9 Hz, 2H, –NCH_2), 3.88 (t, J = 6.9 Hz, 2H, –NCH_2), 5.67 (d, J = 7.8 Hz, 1H, olefinic H), 7.21–7.27 (m, 2H, –ArH), 7.43–7.47 (m, 2H, –ArH), 7.56–7.59 (m, 2H, –ArH), 7.62 (d, J = 7.8 Hz, 1H, olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.2, 26.6, 36.3, 37.7, 38.1, 47.4, 100.4, 111.1, 112.1, 112.2, 112.5, 112.6, 118.1, 118.5, 124.6, 124.9, 144.8, 147.3, 147.5, 151.6, 157.3, 158.8, 158.9, 159.4, 162.3, 183.5; HRMS:

Calcd for $C_{26}H_{20}F_2N_4O_6$ $[M]^+$ 522.1351, found 522.1343; Anal. Calcd (%) for: C, 59.77; H, 3.86; N, 10.72, found: C, 59.69; H, 3.75; N, 10.78.

4.2.2.9. 1,1′-(4,4′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(butane-4,1-diyl))bis(5-fluoroindoline-2,3-dione) (5i). Yield 83%; orange solid; mp 187–188 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.61 (s, 6H, 3xCH_2), 1.68–1.69 (m, 2H, CH_2), 3.70–3.72 (m, 4H, 2×NCH_2), 3.75–3.77 (m, 2H, –NCH_2), 3.83 (s, 2H, –NCH_2), 5.69 (d, J = 7.7 Hz, 1H, olefinic H), 7.24–7.28 (m, 2H, –ArH), 7.46–7.47 (m, 2H, –ArH), 7.53–7.56 (m, 2H, –ArH), 7.69 (d, J = 7.7 Hz, 1H, olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 24.0, 24.5, 24.9, 26.1, 48.4, 100.6, 111.8, 112.0, 112.4, 112.5, 112.6, 118.8, 118.9, 124.3, 124.4, 144.5, 147.2, 147.3, 151.5, 157.9, 158.6, 158.7, 159.8, 162.8, 183.2; HRMS: Calcd for $C_{28}H_{24}F_2N_4O_6$ $[M]^+$ 550.1664, found 550.1669; Anal. Calcd (%) for: C, 61.09; H, 4.39; N, 10.18, found: C, 60.97; H, 4.34; N, 10.23.

4.2.2.10. 1,1′-(5,5′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(pentane-5,1-diyl))bis(5-fluoroindoline-2,3-dione) (5j). Yield 86%; orange solid; mp 182–183 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.32–1.35 (m, 4H, 2×CH_2), 1.80–1.88 (m, 8H, 4×CH_2), 3.83–3.87 (m, 4H, 2×NCH_2), 3.94–3.98 (m, 4H, 2×CH_2), 5.64 (d, J = 7.4 Hz, 1H, olefinic H), 7.23–7.29 (m, 2H, –ArH), 7.44–7.49 (m, 2H, –ArH), 7.52–7.59 (m, 2H, –ArH), 7.68 (d, J = 7.4 Hz, 1H, olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.2, 25.4, 26.3, 27.5, 27.8, 28.3, 36.5, 37.7, 44.5, 47.7, 100.8, 111.5, 112.1, 112.3, 112.6, 112.9, 118.5, 118.6, 124.6, 124.9, 144.4, 147.6, 147.8, 151.3, 157.8, 158.4, 158.7, 159.5, 162.6, 183.7; HRMS: Calcd for $C_{30}H_{28}F_2N_4O_6$ $[M]^+$ 578.1977, found 578.1971; Anal. Calcd (%) for: C, 62.28; H, 4.88; N, 9.68, found: C, 62.44; H, 4.91; N, 9.76.

4.2.2.11. 1,1′-(6,6′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(hexane-6,1-diyl))bis(5-fluoroindoline-2,3-dione) (5k). Yield 87%; orange solid; mp 186–187 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.25–1.31 (m, 8H, 4×CH_2), 1.53–1.61 (m, 8H, 4×CH_2), 3.63–3.66 (m, 4H, 2×NCH_2), 3.74–3.77 (m, 4H, 2×NCH_2), 5.67 (d, J = 7.8 Hz, 1H, olefinic H), 7.20–7.28 (m, 2H, –ArH), 7.43–7.47 (m, 2H, –ArH), 7.51–7.56 (m, 2H, –ArH), 7.64 (d, J = 7.8 Hz, 1H, olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.1, 25.3, 26.2, 27.5, 27.6, 28.4, 28.7, 29.6, 37.4, 38.3, 44.0, 47.2,100.4, 111.4, 112.0, 112.5, 112.8, 112.9, 118.2, 118.7, 124.6, 124.8, 144.2, 147.5, 147.9, 151.3, 157.6, 158.2, 158.5, 159.8, 162.8, 183.4; HRMS: Calcd for $C_{32}H_{32}F_2N_4O_6$ $[M]^+$ 606.2290, found 606.2298; Anal. Calcd (%) for: C, 63.36; H, 5.32; N, 9.24, found: C, 63.27; H, 5.27; N, 9.33.

4.2.2.12. 1,1′-(8,8′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(octane-8,1-diyl))bis(5-fluoroindoline-2,3-dione) (5l). Yield 80%; orange solid; mp 184–185 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.26–1.33 (m, 16H, 8×CH_2), 1.62–1.67 (m, 8H, 4×CH_2), 3.66–3.69 (m, 6H, 3×NCH_2), 3.84–3.90 (m, 2H, –NCH_2), 5.68 (d, J = 7.7 Hz, 1H, olefinic H), 7.22–7.25 (m, 2H, –ArH), 7.41–7.46 (m, 2H, –ArH), 7.55–7.58 (m, 2H, –ArH), 7.67 (d, J = 7.7 Hz, 1H, olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 26.2, 26.7, 27.2, 27.5, 27.8, 28.0, 28.2, 28.7, 28.8, 28.9, 29.3, 29.6, 37.5, 37.7, 48.4, 48.6, 100.1, 111.3, 112.4, 112.5, 112.7, 112.9, 118.6, 118.8, 124.4, 124.6, 144.1, 147.4, 147.6, 151.2, 157.7, 158.7, 158.9, 159.4, 162.6, 183.3; HRMS: Calcd for $C_{36}H_{40}F_2N_4O_6$ $[M]^+$ 662.2916, found 662.2907; Anal. Calcd (%) for: C, 65.24; H, 6.08; N, 8.45, found: C, 65.16; H, 5.96; N, 8.57.

4.2.2.13. 1,1′-(2,2′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(ethane-2,1-diyl))bis(5-chloroindoline-2,3-dione) (5m). Yield 82%; orange solid; mp 180–181 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.80 (t, J = 6.3 Hz, 2H, –NCH_2), 3.96–3.99 (m, 2H, –NCH_2), 4.01–4.05 (m, 4H, 2×NCH_2), 5.67 (d, J = 7.4 Hz, 1H, olefinic H), 7.29–7.33 (m, 2H, –ArH), 7.35–7.40 (m, 2H, –ArH), 7.45–7.49 (m, 2H, –ArH), 7.64 (d, J = 7.4 Hz, 1H, olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 37.3, 38.1, 38.3, 48.8, 100.7, 111.8, 112.3, 112.5, 112.8, 118.3, 118.5, 124.2, 124.4, 124.8, 144.7, 146.4, 146.6, 148.5, 157.2, 158.6, 158.8, 159.7, 162.2, 183.6; HRMS: Calcd for $C_{24}H_{16}Cl_2N_4O_6$ $[M]^+$ 526.0447, found 526.0443; Anal. Calcd (%) for: C, 54.67; H, 3.06; N, 10.62, found: C, 54.60; H, 2.98; N, 10.58.

4.2.2.14. 1,1′-(3,3′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(propane-3,1-diyl))bis(5-chloroindoline-2,3-dione) (5n). Yield 85%; orange solid; mp 186–187 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.93 (t, J = 6.7 Hz, 2H, –CH_2), 1.95 (t, J = 6.7 Hz, 2H, –CH_2), 3.70–3.77 (m, 4H, 2×NCH_2), 3.85 (t, J = 6.6 Hz, 2H, –NCH_2), 3.88 (t, J = 6.6 Hz, 2H, –NCH_2), 5.69 (d, J = 7.8 Hz, 1H, olefinic H), 7.24–7.29 (m, 2H, –ArH), 7.31–7.37 (m, 2H, –ArH), 7.47–7.50 (m, 2H, –ArH), 7.61 (d, J = 7.8 Hz, 1H, olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.0, 26.3, 36.7, 37.8, 38.4, 47.6, 100.2, 111.5, 112.5, 112.8, 112.9, 118.2, 118.3, 124.5, 124.6, 124.9, 144.4, 146.6, 146.8, 148.1, 157.8, 158.5, 158.6, 159.4, 162.7, 183.3; HRMS: Calcd for $C_{26}H_{20}Cl_2N_4O_6$ $[M]^+$ 554.0760, found 554.0769; Anal. Calcd (%) for: C, 56.23; H, 3.63; N, 10.09, found: C, 56.28; H, 3.68; N, 9.98.

4.2.2.15. 1,1′-(4,4′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(butane-4,1-diyl))bis(5-chloroindoline-2,3-dione) (5o). Yield 81%; orange solid; mp 184–185 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.60 (s, 6H, 3xCH_2), 1.63–1.68 (m, 2H, –CH_2), 3.71–3.75 (m, 4H, 2×NCH_2), 3.77–3.81 (m, 2H, –NCH_2), 3.87 (s, 2H, –NCH_2), 5.65 (d, J = 7.2 Hz, 1H, olefinic H), 7.29–7.33 (m, 2H, –ArH), 7.35–7.38 (m, 2H, –ArH), 7.44–7.46 (m, 2H, –ArH), 7.66 (d, J = 7.2 Hz, 1H, olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 24.3, 24.6, 24.8, 26.0, 48.1, 100.1, 111.4, 112.2, 112.7, 112.8, 118.4, 118.6, 124.2, 124.7, 124.8, 144.6, 146.7, 146.8, 148.0, 157.3, 158.6, 158.7, 159.5, 162.5, 183.2; HRMS: Calcd for $C_{28}H_{24}Cl_2N_4O_6$ $[M]^+$ 582.1073, found 582.1068; Anal. Calcd (%) for: C, 57.64; H, 4.15; N, 9.60, found: C, 57.58; H, 4.08; N, 9.67.

4.2.2.16. 1,1′-(5,5′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(pentane-5,1-diyl))bis(5-chloroindoline-2,3-dione) (5p). Yield 84%; orange solid; mp 180–181 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.34–1.38 (m, 4H, 2×CH_2), 1.82–1.85 (m, 8H, 4×CH_2), 3.81–3.84 (m, 4H, 2×NCH_2), 3.92–3.97 (m, 4H, 2×NCH_2), 5.61 (d, J = 7.4 Hz, 1H, olefinic H), 7.26–7.31 (m, 2H, –ArH), 7.33–7.37 (m, 2H, –ArH), 7.41–7.44 (m, 2H, –ArH), 7.62 (d, J = 7.4 Hz, 1H, olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.4, 25.7, 26.2, 27.4, 27.6, 28.5, 36.7, 37.9, 44.3, 47.5, 100.4, 111.6, 112.0, 112.3, 112.7, 118.5, 118.8, 124.0, 124.2, 124.5, 144.1, 146.3, 146.6, 148.4, 157.1, 158.5, 158.8, 159.9, 162.4, 183.8; HRMS: Calcd for $C_{30}H_{28}Cl_2N_4O_6$ $[M]^+$ 610.1386, found 610.1379; Anal. Calcd (%) for: C, 58.93; H, 4.62; N, 9.16, found: C, 58.86; H, 4.54; N, 9.27.

4.2.2.17. 1,1′-(6,6′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(hexane-6,1-diyl))bis(5-chloroindoline-2,3-dione) (5q). Yield 80%; orange solid; mp 186–187 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.21–1.28 (m, 8H, 4×CH_2), 1.51–1.57 (m, 8H, 4×CH_2), 3.65–3.68 (m, 4H, 2×NCH_2), 3.72–3.78 (m, 4H, 2×NCH_2), 5.65 (d, J = 7.8 Hz, 1H, olefinic H), 7.28–7.33 (m, 2H, –ArH), 7.35–7.40 (m, 2H, –ArH), 7.43–7.46 (m, 2H, –ArH), 7.60 (d, J = 7.8 Hz, 1H, olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.3, 25.6, 26.5, 27.7, 27.8, 28.2, 28.5, 29.4, 37.7, 38.2, 44.3, 47.5, 100.2, 111.4, 112.2, 112.4, 112.8, 118.2, 118.4, 124.4, 124.5, 124.7, 144.6, 146.7, 146.8, 148.1, 157.3, 158.7, 158.9, 159.5, 162.7, 183.4; HRMS: Calcd for $C_{32}H_{32}Cl_2N_4O_6$ $[M]^+$ 638.1699, found

638.1691; Anal. Calcd (%) for: C, 60.10; H, 5.04; N, 8.76, found: C, 59.98; H, 4.96; N, 8.82.

4.2.2.18. 1,1′-(8,8′-(2,4-Dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(octane-8,1-diyl))bis(5-chloroindoline-2,3-dione) (5r). Yield 82%; orange solid; mp 188–189 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.22–1.31 (m, 16H, 8×CH_2), 1.64–1.69 (m, 8H, 4×CH_2), 3.63–3.68 (m, 6H, 3xNCH_2), 3.82–3.88 (m, 2H, –NCH_2), 5.61 (d, J = 7.4 Hz, 1H, olefinic H), 7.24–7.31 (m, 2H, –ArH), 7.34–7.39 (m, 2H, –ArH), 7.45–7.48 (m, 2H, –ArH), 7.63 (d, J = 7.4 Hz, 1H, olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): 26.4, 26.8, 27.5, 27.6, 27.9, 28.3, 28.5, 28.6, 28.8, 28.9, 29.2, 29.5, 37.7, 37.9, 48.1, 48.5, 100.3, 111.3, 112.5, 112.6, 112.8, 118.6, 118.7, 124.2, 124.4, 124.8, 144.4, 146.8, 146.9, 148.0, 157.0, 158.5, 158.7, 159.8, 162.8, 183.8; HRMS: Calcd for $C_{36}H_{40}Cl_2N_4O_6$ $[M]^+$ 694.2325, found 694.2321; Anal. Calcd (%) for: C, 62.16; H, 5.80; N, 8.05, found: C, 62.24; H, 5.86; N, 7.97.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.04.075.

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