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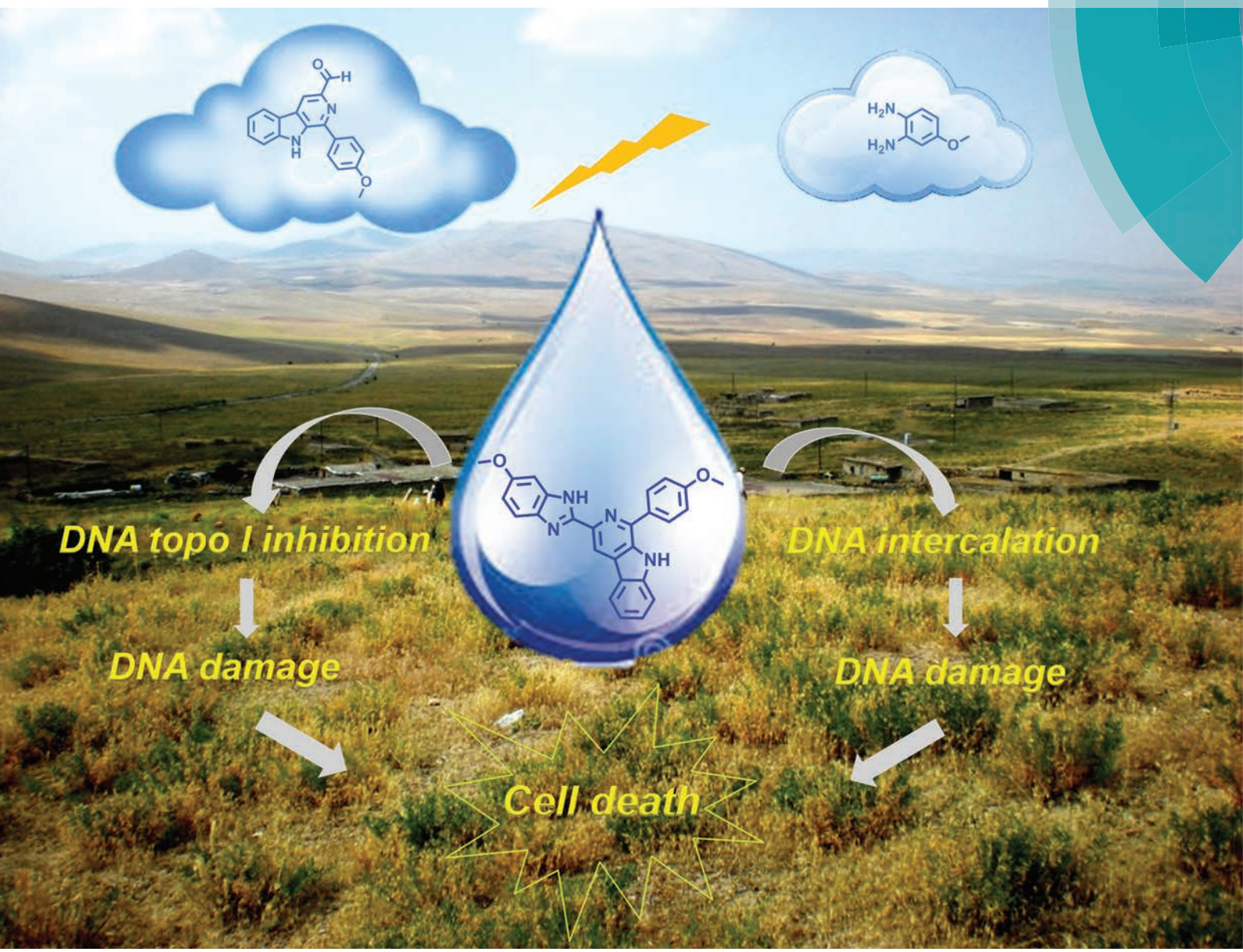

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Synthesis of β-carboline–benzimidazole conjugates using lanthanum nitrate as a catalyst and their biological evaluation†

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A series of β-carboline–benzimidazole conjugates bearing a substituted benzimidazole and an aryl ring at C3 and C1 respectively were designed and synthesized. The key step of their preparation was determined to involve condensation of substituted *o*-phenylenediamines with 1-(substituted phenyl)-9*H*-pyrido-[3,4-*b*]indole-3-carbaldehyde using $La(NO_3)_3 \cdot 6H_2O$ as a catalyst and their cytotoxic potential was evaluated. Conjugates **5a**, **5d**, **5h** and **5r** showed enhanced cytotoxic activity (GI_{50} values range from 0.3 to 7.1 μM in most of the human cancer cell lines) in comparison to some of the previously reported β-carboline derivatives. To substantiate the cytotoxic activity and to understand the nature of interaction of these conjugates with DNA, spectroscopy, DNA photocleavage and DNA topoisomerase I inhibition (topo-I) studies were performed. These conjugates (**5a**, **5d** and **5r**) effectively cleave pBR322 plasmid DNA in the presence of UV light. In addition, the effect of these conjugates on DNA Topo I inhibition was studied. The mode of binding of these new conjugates with DNA was also examined by using both biophysical as well as molecular docking studies, which supported their multiple modes of interaction with DNA. Moreover, an *in silico* study of these β-carboline–benzimidazole conjugates reveals that they possess drug-like properties.

Introduction

The β-carboline alkaloids are a large group of natural and synthetic indole alkaloids that possess a common tricyclic pyrido-[3,4-*b*]indole ring structure. The β-carboline alkaloids were originally isolated from the seeds of *Peganum harmala* (Zygophillaceae, Syrian Rue) that has been traditionally used for hundreds of years to treat the alimentary tract cancers and malaria in Northwest China.[1] Some of these alkaloids are widely found in nature, including various plants, foodstuffs, marine creatures, insects, mammalian as well as human tissues and body fluids.[2–5] The well-known members of this β-carboline family are harmane, harmine and norharman. Recently there has been increased interest in β-carboline derivatives due to their potential biological activities. In particular a large number of natural and synthetic β-carboline derivatives have been reported as potential anticancer agents.[6–13] These compounds exhibit their anticancer activity through multiple mechanisms, such as intercalation into DNA,[7,14] inhibiting topoisomerase I and II,[9,10] CDK,[15] MK-2,[16,17] and kinesin Eg5.[18] Among these, intercalation is of particular importance in the clinical oncology as some of them are valuable drugs currently used for the treatment of various cancers.[19,20] These have been characterized as DNA intercalators due to the presence of polycyclic aromatic planar pharmacophore, which is capable of stacking between DNA base pairs.[10,21] For example, harmane and norharman have been reported to intercalate into DNA leading to altered DNA replication fidelity or leading to an influence on enzymatic activities in DNA-repair processes apart from inhibiting DNA topoisomerase I[22–24] (Fig. 1).

On the other hand, the benzimidazole moiety is structurally related to purine bases and is found in a variety of natural products, such as vitamin B_{12}. In addition, the benzimidazole derivatives exhibited potential antitumor/anticancer activity,[25–28] antibacterial,[29] anti-fungal,[30] antiviral including anti-HIV[31] and antioxidant[32] activities. A series of 2-substituted benzimidazole-4-carboxamide derivatives have been synthesized and evaluated for *in vitro* and *in vivo* anticancer activities and DNA binding affinity.[33] The well-known bis-benzimidazole derivative Hoechst 33258 is widely used as a

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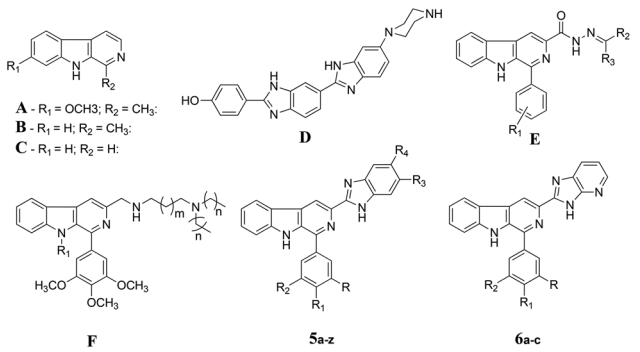


Fig. 1 Chemical structures of harmine (A), harmane (B), norharman (C), Hoechst 33258 (D), carbohydrazide linked β-carboline derivatives (E), N^9-arylated alkyl substituted β-carbolines (F) and designed C3 substituted β-carboline–benzimidazole conjugates (**5a–z** & **6a–c**).

fluorescent dye to stain DNA; it has undergone Phase I clinical evaluation and shows its activity by inhibiting DNA topoisomerase I and helicase.[34,35] There are currently a number of synthetic methodologies available for the synthesis of benzimidazoles. Generally, the condensation of *o*-phenylenediamines and carboxylic acids (or their derivatives such as nitriles, imidates, and *ortho* esters) has been widely used for the synthesis of the benzimidazole scaffold under harsh dehydrating conditions (170–180 °C).[36] Alternative approaches such as palladium or rhodium catalyzed reactions and solid-phase supported synthesis,[37] *etc.*, have also been developed to prepare functionalized benzimidazoles. However, directly employing the condensation and aromatisation reaction of *o*-phenylenediamines with aldehydes under oxidative conditions is considered to be a facile and effective method to prepare 2-substituted benzimidazole.[38] Herein, we describe a new and efficient synthetic methodology for the preparation of C3 substituted β-carboline–benzimidazole conjugates using $La(NO_3)_3{\cdot}6H_2O$ as a catalyst.

Previous reports on β-carboline derivatives revealed that the presence of various substituents at 1, 3 and 9 positions was related to their cytotoxic activity against numerous transplanted animal tumours.[39–42] Chen and coworkers reported that 3-chlorobenzyl and 3-phenylpropyl substituents at position 9 of β-carbolines showed significant antitumor activity.[41] Ikeda and coworkers recently reported 3-benzylamino-β-carboline derivatives as potential antitumor agents.[39] Their SAR analysis revealed that (i) the common β-carboline moiety is very important for the antitumor activity; (ii) the introduction of appropriate substituents at positions 1, 3 and 9 of the β-carboline nucleus enhanced the antitumor activity. Our earlier efforts toward the discovery of new synthetic molecules led to the development of a number of hybrid/conjugate based different heterocyclic scaffolds as potent antitumor agents.[43,44] In continuation of these efforts, we have designed and synthesized a series of β-carboline C3 linked benzimidazole conjugates with aryl substitution at C1 position as potential cytotoxic agents. Gratifyingly, among these, **5a**, **5d**, **5h** and **5r** showed significantly enhanced antitumor activity in comparison to some of the previously reported β-carboline derivatives[39–42] with GI_{50} values ranging from 0.3 to 7.1 μM in most of the cancer-cell lines of the NCI panel.

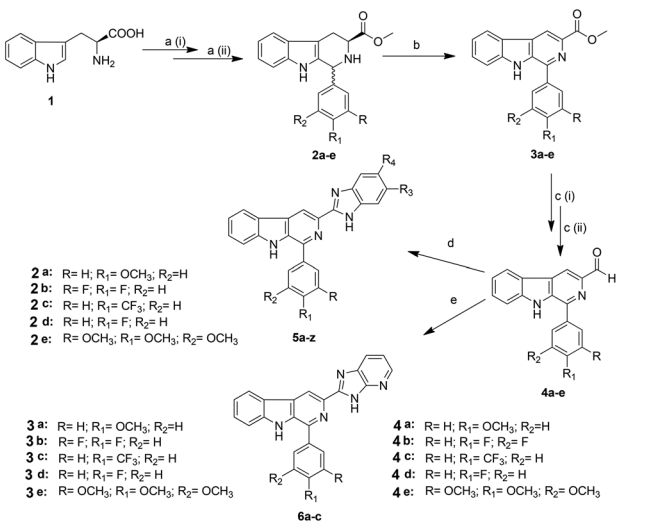
All these β-carboline–benzimidazole conjugates were evaluated for their cytotoxic activity, DNA intercalation, DNA topoisomerase I inhibition and photocleavage studies. The *in silico* study of the ADME properties of these conjugates was carried out by investigating Lipinski's parameters, topological polar surface area (TPSA) and percentage of absorption (% ABS).

Results and discussion

Chemistry

These β-carboline–benzimidazole conjugates (**5a–z**, **6a–c**) were prepared as shown in Scheme 1. The Pictet–Spengler condensation reaction of L-tryptophan methyl ester was obtained by esterification of L-tryptophan using $SOCl_2$ and MeOH, with various benzaldehydes in the presence of a catalytic amount of PTSA to yield the corresponding methyl tetrahydro-β-carboline-3-carboxylates (**2a–e**). Dehydrogenation of **2a–e** with sulfur in refluxing xylene affords the fully unsaturated methyl-β-carboline-3-carboxylates **3a–e**. Then reduction of **3a–e** with $LiAlH_4$ in dry THF under a nitrogen atmosphere followed by oxidation with Dess Martin periodinane in CH_2Cl_2 provides the corresponding 1-aryl substituted-9*H*-pyrido[3,4-*b*]indole-3-carbaldehydes **4a–e**. Finally, condensations of subsequent β-carboline aldehydes **4a–e** with various *o*-phenylenediamines afford the required substituted benzimidazole linked β-carboline conjugates (**5a–z**, **6a–c**).

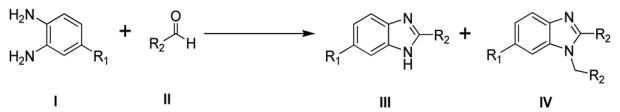
A number of synthetic methodologies[45] are available in the literature for the synthesis of benzimidazoles; most of these

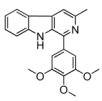
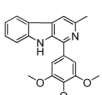


Scheme 1 Synthesis of β-carboline–benzimidazole conjugates. Reagents and conditions: (a-i) $SOCl_2$, MeOH, rt, 6–8 h; (a-ii) Ar-CHO, toluene, cat. PTSA, reflux, 10–12 h; (b) sulfur, xylene, reflux, 10–12 h; (c-i) LAH, dry THF, 0 °C to rt, 4 h; (c-ii) DMP, CH_2Cl_2, rt, 2 h; (d) respective *o*-phenylenediamine, EtOH, catalyst ($La(NO_3)_3{\cdot}6H_2O$), 60 °C, 30–40 min; (e) pyridine-2,3-diamine, EtOH, catalyst ($La(NO_3)_3{\cdot}6H_2O$), 60 °C, 30 min.

require longer reaction times and higher temperatures. In addition, these methods produce toxic as well as inseparable by-products that often require laborious workup and purification processes,[46] resulting in poor isolated yields of the desired products. Therefore, there is still a need for the introduction of milder and efficient methods to overcome the drawbacks of existing procedures. In this investigation, we report an efficient method for the synthesis of benzimidazoles using $La(NO_3)_3{\cdot}6H_2O$ as a catalyst under aerobic conditions. This reaction proceeds *via* condensation followed by aerobic oxidation. Initially, a reaction of *o*-phenylenediamine (1 mmol) with **4a** (1.2 mmol) was performed using $Na_2S_2O_5$ (4–5 mmol) as an oxidant in an EtOH–H_2O (8 : 2) mixture heated at 70 °C for about 4–6 h. Both the required as well as *N*-benzylated products were obtained in the ratio of 65 : 35. In order to improve the selectivity of the reaction, we have studied the reaction conditions by screening various catalysts as well as solvents. Among the conditions screened, the one in which $La(NO_3)_3{\cdot}6H_2O$ (10 mol%) was used in EtOH as a solvent produced the best results as shown in Table 1. It was observed that a further increase in the amount of catalyst had no effect on the yield (Table 1, entry 7), whereas a reduction in the amount of catalyst resulted in a significant decrease in the isolated yield of the product (Table 1, entry 6). However, there was no considerable increase in the yield even after prolonged reaction time (1 h). It was observed that there was no formation of the required product in the absence of catalyst (Table 1, entry 9). Earlier reports have also demonstrated the

Table 1 Optimization of reaction conditions for the chemoselective formation of 2-aryl benzimidazole derivatives



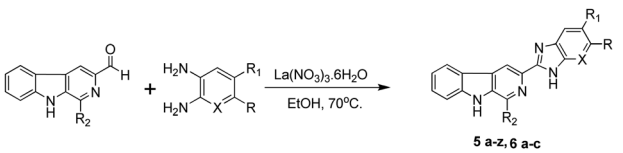
Entry	R_1	R_2	Catalyst/oxidant	Yields (%) III	Yields (%) IV	Solvent	Time
1	H		$Na_2S_2O_5$ (5 equiv.)	65	35	EtOH–H_2O (8 : 2)	8 h
2	H		$Na_2S_2O_5$ (5 equiv.)	70	30	EtOH–H_2O (8 : 2)	8 h
3	OCH_3		$Na_2S_2O_5$ (5 equiv.)	71	29	EtOH–H_2O (8 : 2)	8 h
4	H		$K_4[Fe(CN)_6]$ (20 mol%)	71	29	Neat	20 min
5	H		$La(NO_3)_3{\cdot}6H_2O$ (10 mol%)	69, 95	Trace	EtOH	15 min, 30 min
6	H		$La(NO_3)_3{\cdot}6H_2O$ (5 mol%)	75, 77	Trace	EtOH	30 min, 1 h
7	H		$La(NO_3)_3{\cdot}6H_2O$ (20 mol%)	95	Trace	EtOH	30 min
8	H		$La(NO_3)_3{\cdot}6H_2O$ (10 mol%)	85	Trace	DMF	1 h
9	H		No catalyst	0	0	EtOH	30 min

chemoselective properties of $La(NO_3)_3{\cdot}6H_2O$, which have allowed the selective deprotection of acetonides,[47] primary alcohols[48] and preparation of 1,5-benzodiazepines from ketones.[49]

Cytotoxicity

Most of these conjugates were initially tested at a single dose at higher concentration (10 μM) in the sixty-cell line panel of NCI (One-Dose Screen). This panel is organized into subpanels representing leukemia, melanoma, cancers of lung, colon, kidney, ovary, breast, prostate, and central nervous system. The compounds that satisfy predetermined threshold inhibition criteria in a minimum number of cell lines are taken up for the five-dose assay. The threshold inhibition criteria for progression to the five-concentration screen were selected to efficiently capture compounds with antiproliferative activity based on the analysis of historical DTP screening data. The results are expressed as the percent growth of treated cells relative to the control following 48 h of incubation. Amongst these conjugates, **5a–d**, **5h**, **5r** and **5w** were active in the preliminary test and progressed to the five-concentration (0.01, 0.1, 1.0, 10 and 100 μM) assay. Table 3 summarizes the results obtained as

Table 2 Synthesis of β-carboline–benzimidazole conjugates using $La(NO_3)_3{\cdot}6H_2O$ as a catalyst



Compound	R	R_1	R_2	X	Time (min)	Yield (%)
5a	OCH_3	H	4-$OCH_3C_6H_4$	—	40	80
5b	CH_3	H	4-$OCH_3C_6H_4$	—	40	82
5c	CH_3	CH_3	4-$OCH_3C_6H_4$	—	30	85
5d	F	H	4-$OCH_3C_6H_4$	—	40	85
5e	H	H	4-$OCH_3C_6H_4$	—	30	87
5f	COC_6H_4	H	4-$OCH_3C_6H_4$	—	40	85
5g	CF_3	H	4-$OCH_3C_6H_4$	—	40	80
5h	OCH_3	H	3,4-$F_2C_6H_3$	—	40	90
5i	COC_6H_4	H	3,4-$F_2C_6H_3$	—	40	90
5j	CH_3	CH_3	3,4-$F_2C_6H_3$	—	30	95
5k	Br	H	3,4-$F_2C_6H_3$	—	30	90
5l	CH_3	H	3,4-$F_2C_6H_3$	—	30	96
5m	Cl	H	3,4-$F_2C_6H_3$	—	30	88
5n	H	H	3,4-$F_2C_6H_3$	—	30	95
5o	F	H	4-$CF_3C_6H_4$	—	40	83
5p	H	H	4-$CF_3C_6H_4$	—	30	85
5q	Cl	H	4-$CF_3C_6H_4$	—	30	82
5r	Cl	H	4-FC_6H_4	—	30	86
5s	H	H	4-FC_6H_4	—	30	89
5t	CH_3	H	4-FC_6H_4	—	30	88
5u	Cl	Cl	4-FC_6H_4	—	30	85
5v	COC_6H_4	H	3,4,5-$(OCH_3)_3C_6H_2$	—	40	92
5w	H	H	3,4,5-$(OCH_3)_3C_6H_2$	—	30	93
5x	CH_3	CH_3	3,4,5-$(OCH_3)_3C_6H_2$	—	30	94
5y	Cl	H	3,4,5-$(OCH_3)_3C_6H_2$	—	30	91
5z	OCH_3	H	3,4,5-$(OCH_3)_3C_6H_2$	—	30	89
6a	H	H	4-$OCH_3C_6H_4$	N	40	90
6b	H	H	3,4-$F_2C_6H_3$	N	40	89
6c	H	H	4-$CF_3C_6H_4$	N	40	85

Table 3 Cytotoxicity of β-carboline–benzimidazole conjugates (**5a**, **5b**, **5c**, **5d**, **5h**, **5r** and **5w**) in 60 human cancer cell lines

	Growth inhibition : GI_{50}[a] [μM]						
Cancer panel/cell line	**5a**[b]	**5b**[c]	**5c**[d]	**5d**[e]	**5h**[f]	**5r**[g]	**5w**[h]
Leukemia							
CCRF-CEM	0.88	2.94	2.51	2.29	2.36	3.37	3.96
HL-60(TB)	4.08	4.11	2.59	2.83	2.78	4.32	4.37
K-562	1.52	3.09	3.36	2.79	3.35	3.32	4.33
MOLT-4	1.98	2.31	2.37	1.56	2.03	2.48	3.93
RPMI-8226	0.36	1.85	1.77	1.16	1.55	1.84	2.15
SR	4.29	2.68	2.33	3.34	1.97	3.71	9.50
Non-small cell lung cancer							
A549/ATCC	1.92	3.43	3.94	2.21	4.05	2.89	3.20
HOP-62	4.24	5.78	7.63	3.22	10.9	3.87	5.87
NCI-H226	4.37	2.17	3.32	3.12	8.92	3.34	0.52
NCI-H23	5.80	4.81	11.0	3.01	6.56	3.66	7.19
NCI-H322M	3.85	5.36	8.11	2.77	4.93	3.54	4.40
NCI-H460	2.05	2.99	3.49	1.98	2.97	2.97	44.1
NCI-H522	2.70	4.39	3.00	2.00	10.0	2.74	3.96
Colon cancer							
COLO 205	6.76	2.83	1.79	2.10	1.88	1.89	4.58
HCC-2998	6.57	7.61	3.88	3.71	8.96	5.56	7.32
HCT-116	2.48	3.55	3.22	2.69	2.05	3.29	3.95
HCT-15	2.88	3.20	2.68	2.82	3.41	3.63	4.14
HT29	3.46	5.29	3.76	2.96	3.74	4.31	6.87
KM12	2.57	3.26	3.50	2.78	2.93	3.46	3.66
SW-620	4.83	6.23	7.54	3.73	4.82	4.01	7.14
CNS cancer							
SF-268	4.90	6.80	12.7	3.24	5.80	3.66	4.81
SF-539	9.29	3.92	1.72	2.80	15.5	3.55	23.1
SNB-19	3.37	7.28	68.9	2.94	8.57	4.28	9.45
U251	2.89	3.42	3.04	2.42	2.77	3.31	4.76
Melanoma							
LOX IMVI	3.14	3.34	3.08	2.22	2.86	2.41	4.72
MALME-3M	27.6	5.10	4.60	2.32	16.3	4.54	4.31
M14	3.34	3.09	4.16	2.54	4.02	3.05	4.18
MDA-MB-435	4.31	3.94	3.97	2.58	3.69	3.59	3.65
SK-MEL-2	3.31	3.11	3.28	2.11	12.1	2.76	3.58
SK-MEL-28	100	5.00	2.89	3.40	12.7	4.25	6.89
SK-MEL-5	2.18	3.12	2.90	1.80	4.91	3.52	2.99
UACC-257	4.34	3.94	3.47	2.01	5.68	2.41	3.57
UACC-62	2.75	3.34	2.25	1.86	2.76	2.28	3.38
Ovarian cancer							
IGROV1	2.83	3.58	9.99	2.49	4.62	2.89	2.94
OVCAR-3	3.51	3.59	2.25	2.23	1.94	2.74	6.20
OVCAR-4	>100	4.09	6.98	5.10	6.30	4.81	47.4
OVCAR-5	>100	6.83	4.01	4.46	17.8	6.13	>100
OVCAR-8	1.04	3.02	3.46	1.78	3.08	2.88	2.79
NCI/ADR-RES	2.10	3.72	6.37	2.77	4.22	3.42	3.59
SK-OV-3	>100	6.10	13.0	4.00	17.1	7.43	>100
Renal cancer							
786-0	4.62	1.93	2.60	3.33	10.2	5.71	50.7
A498	3.34	2.36	14.4	1.92	9.85	2.01	2.43
ACHN	2.33	3.05	2.60	2.87	3.20	3.06	4.86
CAKI-1	6.07	3.28	2.44	3.87	2.41	3.22	7.03
RXF 393	2.77	3.82	1.72	1.72	3.46	1.62	3.25
SN12C	3.49	3.96	5.54	2.97	3.97	3.04	6.27
TK-10	3.14	5.45	7.85	2.20	4.29	3.35	4.70
UO-31	1.66	2.99	2.53	2.54	1.46	2.40	3.62
Prostate cancer							
PC-3	1.52	2.74	2.77	1.69	3.25	2.09	2.64
DU-145	2.06	5.02	8.53	2.99	4.13	3.41	6.27
Breast cancer							
MCF-7	2.55	3.06	3.22	2.53	3.23	3.27	4.11
MDA-MB 231/ATCC	8.51	6.88	2.54	2.30	10.4	2.88	8.38
HS 578 T	2.86	4.94	4.60	2.82	8.68	3.38	4.44
BT-549	7.10	6.88	2.96	3.18	6.30	4.62	5.48
T-47D	2.27	3.67	3.09	2.56	3.76	2.68	2.92
MDA-MB-468	3.16	2.69	1.99	2.08	1.70	2.43	3.32

[a] Compound concentration required to decrease cell growth to half that of untreated cells. [b] **5a** (NSC765800). [c] **5b** (NSC764568). [d] **5c** (NSC764567). [e] **5d** (NSC765814). [f] **5h** (NSC764570). [g] **5r** (NSC765810). [h] **5w** (NSC765815).

percentage of growth inhibition (GI_{50}) determined relative to that of untreated control cells.

The tested compounds showed GI_{50} values in the range of 0.3 to 63 μM. The conjugates which contain a 4-methoxyphenyl ring at C1 and 6-methoxy (**5a**), 6-fluoro (**5d**) substituted benzimidazole at C3; a 3,4-difluorophenyl ring at C1, 6-methoxy (**5h**) benzimidazole at C3; and a 4-fluorophenyl ring at C1, 6-chlorobenzimidazole (**5r**) at C3 possess significant cytotoxicity. In contrast, a 4-methoxyphenyl ring at C1 and 6-methyl benzimidazole (**5b**), 5,6-dimethyl benzimidazole (**5c**) at C3; and a 3,4,5-trimethoxyphenyl group at C1 and unsubstituted benzimidazole (**5w**) at C3 displayed moderate activity. The other conjugates **5g**, **5k**, **5l**, **5m**, **5q**, **5u**, **5v**, **5y**, **5z**, **6a** and **6c** displayed weak activity. The conjugate **5a** showed promising cytotoxic activity with GI_{50} values of 0.3 and 0.8 μM against RPMI-8226 and CCRF-CEM cancer cell lines (leukemia). Conjugates **5d**, **5r** and **5h** also showed significant cytotoxic activity against most of the tested human cancer cell lines with mean GI_{50} values of 2.4, 3.1 and 5.3 μM.

Further, to understand the cytotoxicity potential of the conjugates that were not evaluated in the sixty cell line panel of NCI screening, an MTT assay was performed for all conjugates **5a–z**, **6a–c** against three human cancer cell lines HeLa, DU145 and A549. Taken together, results from our cytotoxicity assays in Table 4 corroborated the NCI-60 cell line screen; it has been observed that the conjugates which contain a 4-methoxy phenyl ring at C1, electron donating groups like methoxy (**5a**), methyl (**5b**), 5,6-dimethyl (**5c**), and unsubstituted (**5e**) and weak ring deactivating groups like fluoro (**5d**) on the benzimidazole moiety at C3 of a β-carboline ring possess significant to moderate cytotoxicity, whereas the presence of electron withdrawing groups like trifluoromethyl (**5g**) on benzimidazole decreases the cytotoxicity. In addition a 3,4-difluorophenyl ring at C1, 6-methoxy benzimidazole (**5h**) at C3; and a 4-fluorophenyl ring at C1, 6-chlorobenzimidazole (**5r**) at C3 also exhibited promising cytotoxicity.

Based on the results that depict the cytotoxic potential, it was considered of interest to gain insights into the interaction of these conjugates.

DNA binding studies

CD studies. Circular dichroism studies provide information on changes in DNA conformation upon small molecule interaction which in turn gives further insight into the mode of DNA–ligand binding (Fig. 2).

Among several biological macromolecules, DNA is known to play a crucial role in cell proliferation and other biological activities. The CD spectrum of CT DNA exhibits a positive

Table 4 IC_{50} [a] (μM) for the 48 h of action of the investigated compounds and (std) on the HeLa, DU145, A549, BHK-21 and L929 cells determined by the MTT assay

Compound	HeLa[b]	DU145[c]	A549[d]	BHK-21[e]	L929[f]
5a	1.8 ± 2.3	2.4 ± 1.8	2.0 ± 0.4	>100 ± 1.2	>100 ± 1.5
5b	3.1 ± 1.5	5.5. ± 2.6	3.7 ± 2.2	>100 ± 1.2	>100 ± 1.3
5c	4.3 ± 1.9	7.8 ± 1.1	3.1 ± 2.6	>100 ± 2.0	>100 ± 1.3
5d	1.9 ± 1.1	3.0 ± 3.7	2.4 ± 2.9	85 ± 1.4	>100 ± 1.4
5e	4.3 ± 1.9	6.3 ± 1.8	2.0 ± 1.0	>100 ± 2.6	>100 ± 1.2
5f	22.9 ± 2.5	28.3 ± 2.2	19.4 ± 1.0	>100 ± 1.5	>100 ± 2.0
5g	12.6 ± 1.9	13.0 ± 2.4	23.7 ± 3.8	63 ± 1.1	>100 ± 1.5
5h	1.9 ± 1.1	3.6 ± 1.7	4.3 ± 1.9	>100 ± 1.8	>100 ± 1.7
5i	8.5 ± 4.2	10.7 ± 1.1	6.4 ± 1.4	>100 ± 0.7	>100 ± 2.7
5j	18.5 ± 3.2	19.8 ± 1.3	8.3 ± 1.7	>100 ± 2.0	>100 ± 1.1
5k	14.3 ± 2.5	15.5 ± 2.2	15.4 ± 2.8	>100 ± 1.1	>100 ± 1.2
5l	12.6 ± 2.2	11.5 ± 4.8	13.7 ± 3.7	>100 ± 1.5	>100 ± 2.1
5m	15.9 ± 5.1	13.7 ± 7.4	15.8 ± 3.3	>100 ± 1.7	>100 ± 1.8
5n	7.8 ± 1.2	7.2 ± 1.1	10.7 ± 3.1	>100 ± 1.2	>100 ± 1.3
5o	8.5 ± 4.1	11.1 ± 6.0	4.4 ± 2.0	>100 ± 3.7	>100 ± 1.6
5p	6.4 ± 2.7	6.7 ± 2.4	6.0 ± 3.6	>100 ± 2.1	>100 ± 2.8
5q	8.3 ± 1.9	20.6 ± 4.3	18.2 ± 6.6	>100 ± 2.0	>100 ± 1.6
5r	2.6 ± 0.4	3.2 ± 1.1	2.4 ± 1.2	>100 ± 2.7	>100 ± 1.1
5s	9.4 ± 1.8	22.4 ± 1.2	18.4 ± 0.4	>100 ± 2.0	>100 ± 1.1
5t	4.0 ± 3.8	3.4 ± 1.2	2.5 ± 3.3	>100 ± 2.5	>100 ± 1.3
5u	13.5 ± 3.2	16.1 ± 1.5	9.8 ± 1.7	>100 ± 2.2	>100 ± 1.8
5v	24.6 ± 1.8	37.6 ± 1.4	16.4 ± 2.5	>100 ± 1.8	>100 ± 2.0
5w	3.6 ± 2.1	6.5 ± 2.4	3.6 ± 1.5	>100 ± 0.7	>100 ± 1.8
5x	11.7 ± 3.3	3.5 ± 6.4	17.0 ± 1.9	49 ± 1.2	>100 ± 1.6
5y	16.4 ± 4.7	5.6 ± 1.5	13.4 ± 0.9	>100 ± 1.5	>100 ± 3.3
5z	15.5 ± 3.4	21.9 ± 5.3	19.5 ± 2.2	>100 ± 1.9	>100 ± 2.9
6a	13.9 ± 4.1	14.1 ± 2.4	12.0 ± 2.0	>100 ± 1.2	>100 ± 2.0
6b	16.9 ± 2.6	27.4 ± 3.9	23.3 ± 4.2	>100 ± 1.8	>100 ± 1.5
6c	21.0 ± 3.6	21.5 ± 1.7	15.5 ± 3.2	>100 ± 0.8	>100 ± 1.7
Harmine	16.0 ± 1.1	12.5 ± 1.7	6.5 ± 2.0	>100 ± 1.2	>100 ± 1.5

[a] Each datum represents mean + S.D. from three different experiments performed in triplicate. [b] HeLa: human cervix cancer cell line. [c] DU145: human prostate cancer cell line. [d] A549: human lung adenocarcinoma epithelial cell line. [e] BHK-21: Hamster kidney cells. [f] L929: mouse connective tissue fibroblast cells.

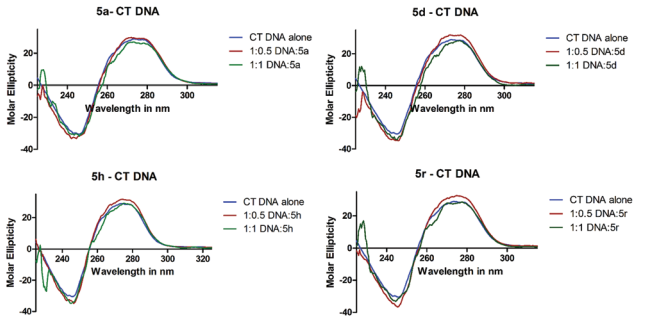


Fig. 2 CD spectra of CT DNA (15 μM) at two concentrations of ligands (**5a**, **5d**, **5h** and **5r**).

band at 275 nm and a negative band at 245 nm due to π–π base stacking and right-hand helicity which is the characteristic profile of B form DNA. In the present study, the positive band exhibited hyperchromicity on addition of **5a** to CT DNA in a 1 : 0.5 DNA : ligand (**5a**) ratio, manifesting stabilization of the DNA structure on ligand interaction. The negative band intensities gradually reduced, indicating a decrease in the DNA helicity on complex interaction with DNA. On further increasing the concentration of the ligand, the positive band at 275 nm exhibited hypochromicity, indicating unfolding of the DNA structure. A similar type of interaction was also noticed in the case of ligands **5h**, **5r** and **5d**. The results indicate that these ligands stabilize the DNA at lower concentration and, upon increasing the ligand concentration, they start unfolding of the CT DNA structure.[50]

UV-visible spectral studies. UV-visible spectroscopic titration studies were carried out to obtain preliminary information about the mode of DNA–ligand interactions. The UV-visible spectra of ligand **5a** demonstrate a prominent absorption band at 285 nm and 342 nm. On addition of equal increments of 2 μM CT DNA to 25 μM ligand solution, the ligand absorption band intensities were reduced continuously. The hypochromicity of the ligand Soret band with the addition of DNA is a characteristic feature of ligand intercalation with DNA. The reduction in the intensities of the ligand Soret band is usually attributed to the interaction between the electronic states of the compound and those of the DNA bases.[51] Moreover the extent of hypochromism of the Soret band generally indicates the intercalative binding strength.[52] The interaction of ligands **5h**, **5r** and **5d** was also similar to **5a**, manifesting their intercalative mode of binding with DNA.

In the case of ligand **5a**, an isosbestic point was observed at 325 nm indicating the existence of at least one **5a**-DNA complex with identical molar absorptivity. In the case of other ligands, a sharp isosbestic point was observed at around 335 nm and this shows that their interaction with DNA occurs in a single step (Fig. 3).

DNA intercalation assay

It is known that the unfolding of DNA takes place due to small molecule intercalation between the bases.[53] From the spectroscopic studies it is evident that these ligands unfold the DNA

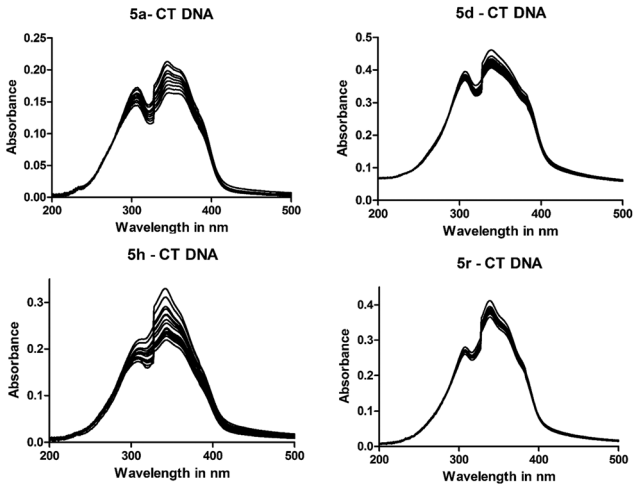


Fig. 3 The UV-visible absorption changes with the titration of ligands (**5a**, **5d**, **5h** and **5r**) by CT DNA.

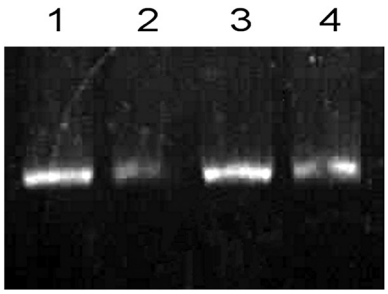


Fig. 4 Agarose gel picture showing the intercalation of **5a**, **5h**, **5r** and **5d** (2 μM) with DNA (5 μM) in Tris buffer (pH 7.0). Lane 1, (**5a** + DNA); lane 2, (**5 h** + DNA); lane 3, (**5r** + DNA); and lane 4 (**5d** + DNA).

maybe due to the intercalation. The intercalation nature of these ligands was further confirmed by performing the DNA intercalation assay. The pBR322 plasmid DNA was incubated with these ligands for 1 h at 37 °C and then loaded on a 0.8% agarose gel (Fig. 4).

After running the DNA–ligand complex for 1 h, DNA bands were seen in the agarose gel indicating that **5a**, **5h**, **5r** and **5d** ligands exhibit intercalative mode of DNA binding.

Photocleavage studies

DNA photocleavage reaction was carried out in the presence of **5a**, **5d** and **5r** to find the efficiency of these conjugates in generating free radicals in the system. Generally, photocleavage reactions proceed through the generation of molecular oxygen or hydroxyl radical species. These reactions involving the triplet oxygen state ($^3O_2$) are known to proceed by two major mechanistic pathways. In the first one, the singlet excited electronic state of the hybrid through intersystem crossing generates an excited triplet state of the hybrid which in turn activates the molecular oxygen in its stable triplet oxygen state ($^3O_2$) to a more reactive singlet oxygen state ($^1O_2$).[54] In the second pathway, the excited state molecule could reduce the

molecular oxygen to generate the highly reactive hydroxyl radical.

When pBR322 plasmid DNA in the presence of ligands was subjected to electrophoresis, relatively fast migration was observed for the supercoiled form (Form I). If scission occurs on one strand, the supercoiled form will relax to generate a relaxed circular form (Form II). In the present study, the pBR322 plasmid DNA was irradiated with UV light (365 nm) in the presence and absence of ligands and subjected to electrophoresis. The gel electrophoresis separation of pBR322 DNA after UV light irradiation in the presence of two different concentrations (100 μM and 200 μM) of the **5a**, **5r** and **5d** ligands is shown in Fig. 5A. It is evident that the control samples as well as DNA + ligands (100 μM) incubated in the absence of light did not show any considerable cleavage (lanes 8–10, Fig. 5A). However, the pBR322 DNA samples irradiated along with ligands exhibited remarkable photocleavage as indicated by the decrease in the band intensity of supercoiled DNA. The photocleavage studies show that all the three ligands are efficient in cleaving pBR322 plasmid DNA; however, the effect of photocleavage is prominent in the cases of **5a** followed by **5r** and **5d**.

Moreover, the photocleavage of pBR322 DNA was enhanced on increasing the concentration (200 μM) of the ligand. On the other hand, **5a** is considerably active even at lower concentration (100 μM) demonstrating its higher ability to generate free radicals for the effective cleavage of the DNA. The photocleavage activity of these hybrids may be due to the presence of –NH and polyaromatic rings, which could be involved in n–π* and π–π* transitions upon their intercalation with DNA. The photocleavage efficiency of these ligands depends on their relative orientation upon interaction with DNA and the proximity of the –NH group to DNA. From the DNA binding studies it was also found that the **5a**, **5r** and **5d** ligands intercalate with DNA. The above results suggest that these ligands intercalate with DNA and generate the free radicals on exposure to UV light.

With a view to understanding the effect of DNA photocleavage as a function of irradiation time, 100 μM of **5a** was irradiated with UV light at different time intervals (15 min, 30 min, 60 min, 90 min and 120 min). It was observed that the intensity of the band corresponding to Form I diminishes gradually, whereas that of Form II increases upon increasing the irradiation time. After 2 h, complete disappearance of supercoiled DNA was observed. This indicates that **5a** has a higher photocleavage capacity, which increases with irradiation time (Fig. 5B).

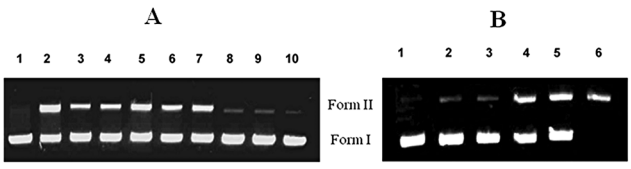


Fig. 5 Lane 1 (A & B), DNA solution irradiated in the absence of compounds; (A) lanes 2–4, conjugates **5a**, **5r** and **5d** (100 μM) + pBR 322 plasmid DNA; lanes 5–7, conjugates **5a**, **5r** and **5d** (200 μM) + pBR 322 plasmid DNA; lanes 8–10, DNA + conjugates in the absence of light. (B) Effect of **5a** as a function of the irradiation time (0.15, 0.30, 1, 1.5, 2 h; lanes 2–6). Form II, relaxed circular DNA; Form I, supercoiled DNA.

DNA topoisomerase I inhibition

It is well known that DNA topoisomerase I (Topo I) binds to double stranded supercoiled DNA and cuts the single stranded portion, which releases the superhelical tension in DNA and transforms it to a relaxed form. Topo I inhibitors have gained importance in cancer chemotherapy treatment as they inhibit Topo I and leave single stranded breaks in DNA, thereby damaging the genome integrity. Topo I has been identified as a potential target for several anticancer drugs that are clinically in use even today. Since Topo I is involved in the replication and proliferation process, overproduction of Topo I was observed in cancer cells compared to normal cells. The Topo I inhibition occurs in two ways. The inhibitors may bind topoisomerase directly or they may bind to DNA and alter its structure, so that it cannot be recognised by topoisomerases.

Cao and coworkers reported that camptothecin inhibits Topo I at 100 μM concentration whereas harmine derivatives inhibit Topo I at 150 μM[55].

Interestingly, the present β-carboline–benzimidazole scaffolds have also shown significant Topo I inhibition at 100 as well as 200 μM concentrations (Fig. 6). The observed low IC_{50} values for the conjugates **5a**, **5d**, **5h** and **5r** may be the result of effective Topo I inhibition as well as better DNA intercalation.

Molecular docking studies

Docking studies were performed to obtain an insight into the mode of binding of the C1 and C3 substituted β-carboline conjugates to the protein and the DNA ternary complex. Geometries of all the conjugates (**5a**, **5h**, **5r** and **5d**) were optimized by the PM3 method using the Gaussian 09 package.[56] The coordinates of the protein were obtained from the Protein Data Bank (PDB ID: 1SC7)[57] and necessary corrections to the protein structure were carried out using the Protein Preparation Wizard in Schrodinger package. The docking studies were performed using GOLD docking software; docking poses showed that these conjugates intercalate with DNA at the binding site of the ternary complex (Fig. 7). The docking pose for **5a** shows that there is a hydrogen-bonding interaction between the N2

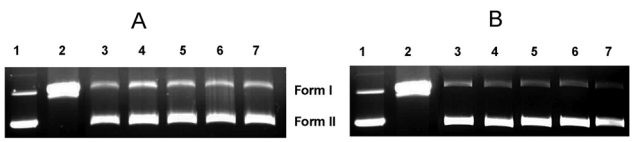


Fig. 6 Effects of **5a**, **5r**, **5d** and **5h** on the activity of DNA topoisomerase I in a cell free system. Lane 1, DNA alone; lane 2, DNA + Topo I; lane 3, DNA + Topo I + camptothecin (100 μM); lanes 4–7, DNA + Topo I + compounds **5a**, **5h**, **5r** and **5d** (100 μM) (A) and the same in (B) (200 μM); Form II, relaxed circular DNA; Form I, supercoiled DNA.

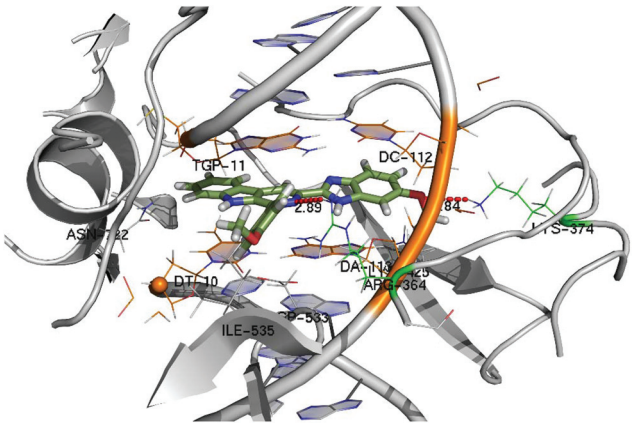


Fig. 7 Docking pose for **5a** in DNA Topo I; hydrogen bonds are shown in red dotted lines, and hydrogen bonding residues are shown in green colour; nucleic acid residues which are showing π–π stacking are shown in brown colour; amino acids having hydrophobic interactions are shown in gray colour.

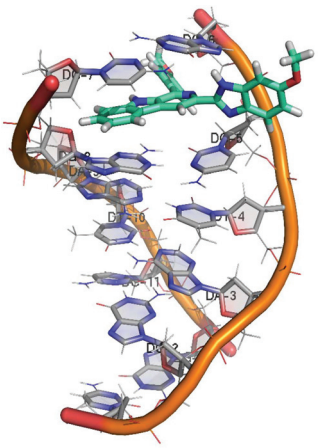


Fig. 8 Docking pose for **5a** in DNA showing the intercalation-binding mode.

of a β-carboline ring and the guanidine group of Arg364 similar to the cocrystal ligand; besides this, it also possesses an additional hydrogen bonding interaction between the methoxy group of the benzimidazole ring and the amino group of the side chain of Lys374. The benzimidazole ring shows π–π* interactions with the C112 and A113 DNA base pairs; on the other hand, the β-carboline ring shows π–π* interactions with the C10 and G11 DNA base pairs, whereas C1 substitution extends outwards to the minor groove. In addition to this, **5a** possesses hydrophobic interactions with amino acids close to the binding site, in which the C1 substitution shows interaction with the Asp533 and Ile535, the β-carboline ring with Asn722 and Arg364 and the benzimidazole ring with Glu365 and Lys425. As compared to the cocrystal ligand, these conjugates exhibit a high gold score. These studies indicate that the C1 and C3 substituted β-carbolines are capable of fitting properly in the binding site of the DNA topoisomerase I.

The biophysical studies have already shown that these conjugates possess DNA intercalation properties. Therefore, docking studies were performed to obtain a better insight into the binding mode of these conjugates to the DNA. The coordinates of the DNA were obtained from the Protein Data Bank (PDB ID 1NAB).[58] Necessary corrections to the crystal structure were carried out using the protein preparation wizard in Schrodinger. Docking studies showed that all conjugates (**5a**, **5h**, **5r** and **5d**) bind to the DNA through intercalation. The docking pose of **5a** shows that the β-carboline ring lies in the central part of the DNA intercalation cavity and is stacked between the C5, G6 of chain A and C7, G8 of chain B, while C1 and C3 substitution extended outwards. The phenyl substitution extended towards the minor groove and the benzimidazole substitution towards the major groove (Fig. 8).

Docking studies on DNA topoisomerase-I and CT DNA showed that these conjugates have the potential to bind to the DNA Topo-I and also intercalate with DNA. Images for docking poses were generated using Pymol visualization software.[59]

In silico computational studies

An *in silico* computational study of the representative C-3 substituted β-carboline–benzimidazole conjugates (**5a–d**, **5h**, **5r**, **5w**) was performed for determining the Lipinski's parameters, topological polar surface area (TPSA) and percentage of absorption (% ABS).[60–62] Calculations were performed using the Molinspiration online Property Calculation Toolkit (http://www.molinspiration.com)[61] and OSIRIS Property Explorer (http://www.organic-chemistry.org/prog/peo).[62] The percentage of absorption was estimated using the equation: % ABS = 109 − 0.345 × TPSA[60] and the data generated are shown in Table 5.

In vivo absorption of the new synthesized derivatives was tentatively assessed by means of theoretical calculations following Lipinski's rule of five, which establishes that the absorption or permeation of an orally administered compound is more likely to be good if the molecule satisfies the following criteria: (a) hydrogen bond donors ≤5 (OH and NH groups); (b) hydrogen bond acceptors ≤10 (N and O atoms); (c) molecular weight < 500; (d) calculated log P < 5.[60–62] Compounds that violate more than one of these parameters could have problems related to bioavailability.

Conclusion

In summary, we have synthesized a series of β-carboline–benzimidazole conjugates bearing the substituted benzimidazole moiety at C3 and the substituted aryl group at C1. The final key step of the benzimidazole ring formation was carried out using $La(NO_3)_3{\cdot}6H_2O$ as a catalyst that resulted in higher yields with chemoselectivity. In general, this method may be highly useful for the chemoselective formation of various 2-sustituted benzimidazole derivatives. The SAR analysis reveals that amongst the conjugates synthesized, the ones having 4-methoxy phenyl substitution at C1 (**5a–e**) exhibit significant activity. Surprisingly, some conjugates with 3,4-difluoro phenyl and 4-fluoro phenyl rings at C1 (**5h** and **5r**) are also equally

Table 5 Lipinski's parameters and % ABS, TPSA, log *S* for compounds **5a–d**, **5h**, **5r** and **5w**

			Lipinsk's parameters					
Compound	% ABS	TPSA[a] (A^2)	*n*HBA (ON)	*n*HBD (OHNH)	Log P^b	M_W	*n* violations	Log S^b
5a	82.84	75.8	6	2	5.27	420	1	−6.71
5b	86.02	66.6	5	2	5.69	404	1	−7.06
5c	86.02	66.6	5	2	6.01	418	1	−7.38
5d	86.02	66.6	5	2	5.44	408	1	−7.01
5h	86.02	66.6	5	2	5.51	426	1	−7.32
5r	89.21	57.3	4	2	6.16	412	1	−7.72
5w	79.65	85.0	7	2	5.17	450	1	−6.73

[a] http://www.molinspiration.com; [b] http://www.organic-chemistry.org/prog/peo; % ABS = 109 − 0.345 × TPSA; number of hydrogen bond acceptors (NO) = *n*HBA ≤ 10; number of hydrogen bond donors (OHNH) = *n*HBD ≤ 5; $M_W \leq 500$; octanol–water partition coefficient = log $P < 5$; solubility = log $S > -4$.

active irrespective of the SAR analysis. The representative conjugates (**5a**, **5d**, **5h** and **5r**) showed potential cytotoxic activity with GI_{50} values ranging from 0.36 to 7.1 μM in most of the human cancer cell lines panel of the NCI. These conjugates also showed promising DNA topoisomerase-I inhibition activity. Further, biophysical studies speculated that these conjugates could intercalate into the DNA, which is supported by molecular docking studies. The DNA photocleavage activity of these conjugates was measured using pBR322 plasmid DNA in the presence of UV light. This study suggests that **5a** has the potential to generate singlet oxygen species which cleaves DNA by converting the supercoiled form to a relaxed/circular form and can be used in photodynamic therapy. Based on the above results it is evident that these β-carboline–benzimidazole conjugates (particularly **5a**) have the potential to be developed as a new class of cancer therapeutics. Detailed cellular and molecular biology as well as *in vivo* studies are actively in progress in our laboratory.

Materials and methods

Synthesis data

Chemistry. All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA), or Spectrochem Pvt. Ltd (Mumbai, India) and were used without further purification. TLC was performed on silica gel glass plates containing 60 GF-254 and visualization was achieved by UV light or iodine indicator monitored reactions. Column chromatography was performed with Merck 60–120 mesh silica gel. ^1H and ^{13}C spectra were recorded on Bruker UXNMR/XWIN-NMR (300 MHz) or Inova Varian-VXR-unity (400, 500 MHz) instruments. Chemical shifts (δ) are reported in ppm downfield from a TMS internal standard. ESI spectra were recorded on a Micromass Quattro LC using ESI+ software with a capillary voltage of 3.98 kV and an ESI mode positive ion trap detector. High-resolution mass spectra (HRMS) were recorded on a QSTAR XL Hybrid MS-MS mass spectrometer. Melting points were determined with an electrothermal melting point apparatus, and are uncorrected. ^1H NMR and ^{13}C NMR spectra of the final compounds **5a–z**, **6a–c** are provided in the ESI.†

General procedure for the preparation of compounds (2a–e)

To a stirred solution of L-tryptophan (0.1 mol) in methanol (50 mL), 8.02 mL (0.11 mol) of thionyl chloride was added dropwise at 0 °C and stirring was continued for 6.0 h at room temperature. Then, an excess amount of solvent was removed under vacuum and the crude product was co-distilled with toluene (2 × 10 mL) to obtain a solid. Then, the resulting solid was dissolved in CH_2Cl_2, washed with saturated $NaHCO_3$ solution, and extracted with an excess amount of CH_2Cl_2. Then, the combined organic layers were dried over anhydrous sodium sulphate and concentrated under vacuum to obtain a white solid product. To a mixture of tryptophan ester (0.023 mol) and substituted benzaldehyde (0.023 mol) in toluene (50 mL), a catalytic amount of PTSA was added and the mixture was refluxed for 24 h. After completion of the reaction, the solvent was removed under vacuum. The crude mixture was extracted with ethyl acetate and the combined organic layers were dried over anhydrous sodium sulphate and concentrated under vacuum. Then, the resulting diastereomeric mixture (**2a–e**) obtained was used for the next step without further purification.

General procedure for the preparation of compounds (3a–e)

The suspension of the above compounds (**2a–e**, **5** gr) and sulphur (0.075 mol) in xylene (100 mL) was refluxed for 12 h. Then, the mixture was cooled to room temperature and kept at 4 °C for 3 h to obtain a precipitate. Then, the precipitate was filtered, washed with petroleum ether and dried. The obtained solid was recrystallized using ethyl acetate to afford products (**3a–e**) with high purity.

Methyl 1-(4-methoxyphenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (3a). White solid; 80% yield; mp: 228–230 °C; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.18 (bs, 1H), 8.80 (s, 1H), 8.17 (d, J = 7.7 Hz, 1H), 7.78 (d, J = 8.8 Hz, 2H), 7.57–7.55 (m, 2H), 7.36–7.33 (m, 1H), 6.88 (d, J = 8.6 Hz, 2H), 4.03 (s, 3H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 165.8, 159.6,

141.8, 141.2, 136.2, 134.2, 129.8, 129.7, 129.1, 128.7, 128.0, 121.2, 120.9, 119.9, 115.7, 113.7, 113.1, 112.4, 54.9, 51.6; MS (ESI): *m*/*z* 333 $[M + H]^+$.

Methyl 1-(3,4-difluorophenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (3b). White solid; 71% yield; mp: 236–239 °C; ^{1}H NMR (300 MHz, DMSO[d_6]) δ (ppm): 12.05 (bs, 1H), 8.97 (s, 1H), 8.46 (d, *J* = 7.9 Hz, 1H), 7.60–7.78 (m, 4H), 7.45 (t, *J* = 9.4 Hz, 1H), 7.35 (t, *J* = 7.7 Hz, 1H), 3.94 (s, 3H); ^{13}C NMR (75 MHz, DMSO[d_6]) δ (ppm): 163.6, 143.0, 141.5, 135.2, 134.3, 131.4, 130.9, 130.2, 129.1, 126.1, 122.1, 121.1, 120.7, 114.4, 112.8, 111.9, 111.5, 52.1; MS (ESI): *m*/*z* 339 $[M + H]^+$.

Methyl1-(4-(trifluoromethyl)phenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (3c). Pale yellow solid; 72% yield; mp: 252–254 °C; ^{1}H NMR (300 MHz, DMSO[d_6]) δ (ppm): 11.99 (bs, 1H), 8.93 (s, 1H), 8.36 (d, *J* = 7.7 Hz, 1H), 8.23 (d, *J* = 7.7 Hz, 2H), 7.92 (d, *J* = 8.1 Hz, 2H), 7.68 (d, *J* = 8.1 Hz, 1H), 7.59 (t, *J* = 7.9 Hz, 1H), 7.32 (t, *J* = 7.5 Hz, 1H), 3.94 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 163.9, 139.7, 138.4, 134.9, 132.8, 127.7, 127.5, 127.1, 126.8, 124.1, 123.6, 119.9, 118.5, 115.2, 110.8, 50.1; MS (ESI): *m*/*z* 371 $[M + H]^+$.

Methyl 1-(4-fluorophenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (3d). White solid: 76% yield; mp: 197–198 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.68–8.76 (bs, 1H), 8.84–8.90 (s, 1H), 7.88 (d, *J* = 5.4 Hz, 2H), 7.60–7.68(t, *J* = 5.4 Hz, 1H), 7.52–7.58 (d, *J* = 7.7 Hz, 1H), 7.36–7.44 (t, *J* = 7.9 Hz, 1H), 7.26 (d, *J* = 8.6 Hz, 2H), 4.04 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 165.7, 164.0, 160.7, 141.3, 136.4, 134.3, 133.8, 130.6, 130.5, 128.2, 121.5, 120.9, 120.1, 116.3, 115.4, 115.1, 112.4, 51.7; MS (ESI): *m*/*z* 291 $[M + H]^+$.

Methyl1-(3,4,5-trimethoxyphenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (3e). Yellow solid: 76% yield; mp: 229–230 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.38 (bs, 1H), 8.75 (s, 1H), 8.21(d, *J* = 7.5 Hz, 1H), 7.60 (d, *J* = 7.5 Hz, 1H), 7.56–7.61 (m, 1H), 7.32–7.38 (m, 1H), 6.94 (s, 2H), 4.03 (s, 3H), 3.84 (s, 3H), 3.77 (s, 6H); ^{13}C NMR (75 MHz, DMSO[d_6]) δ (ppm): 166.1, 159.8, 141.9, 141.3, 136.5, 134.3, 129.9, 128.9, 128.4, 121.8, 121.1, 120.2, 116.1, 114.0, 112.7, 55.2, 51.9; MS (ESI): *m*/*z* 363 $[M + H]^+$.

General procedure for the preparation of compounds (4a–e)

The compounds (**3a–e**, 0.019 mol) taken in dry THF (100 mL) were cooled to −5 °C to 0 °C in an ice–salt (NaCl) bath. To the reaction mixture, LAH (0.076 mol) was added slowly portion-wise and stirring was continued at room temperature for about 4 h. After completion of the reaction, further the reaction mixture was cooled to 0 °C and the excess of LAH was quenched with Na_2SO_4 paste, filtered on a Buckner funnel washed with MeOH (2 × 20 mL). The filtrate was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude mixture obtained (0.014 mol) was taken in dry CH_2Cl_2 (100 mL). To that, DMP (0.021 mol) was added, and stirred at room temperature for about 2 h. After the completion of the reaction the reaction mixture was washed with water (2 × 100 mL), and the organic layer was dried over anhydrous Na_2SO_4, and concentrated under vacuum. Then, the resulting crude obtained was purified by column chromatography using EtOAc–*n*-hexane (1 : 1) to afford products (**4a–e**) with high purity.

1-(4-Methoxyphenyl)-9*H*-pyrido[3,4-*b*]indole-3-carbaldehyde (4a). Pale yellow solid; 80% yield; mp: 218–220 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 10.62 (s, 1H), 9.18 (bs, 1H), 8.80 (s. 1H), 8.17 (d, *J* = 7.7 Hz, 1H), 7.78 (d, *J* = 8.8 Hz, 2H), 7.57–7.55(m, 2H), 7.36–7.33 (m, 1H), 6.88 (d, *J* = 8.6 Hz, 2H), 4.03 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 192.5, 159.7, 142.9, 142.2, 141.3, 135.0, 131.6, 130.7, 129.6, 128.8, 128.2, 128.1, 121.3, 120.1, 113.7, 112.6, 54.9; MS (ESI): *m*/*z* 303 $[M + H]^+$.

1-(3,4-Difluorophenyl)-9*H*-pyrido[3,4-*b*]indole-3-carbaldehyde (4b). Pale yellow solid: 84% yield; mp: 225–228 °C; ^{1}H NMR (300 MHz, DMSO[d_6]) δ (ppm): 10.16 (s, 1H), 8.88 (s, 1H), 8.48 (d, *J* = 7.9 Hz, 1H), 7.92–8.03 (m, 2H), 7.62–7.80 (m, 2H), 7.44–7.49 (m, 1H), 7.37 (t, *J* = 7.9 Hz, 1H); ^{13}C NMR (75 MHz, DMSO[d_6]) δ (ppm): 192.6, 143.0, 141.5, 135.2, 134.3, 132.2, 130.9, 130.2, 129.1, 126.1, 122.1, 120.7, 114.4, 112.8, 111.9, 111.5; MS (ESI): *m*/*z*: 309 $[M + H]^+$.

1-(4-(Trifluoromethyl)phenyl)-9*H*-pyrido[3,4-*b*]indole-3-carbaldehyde (4c). Pale yellow solid: 84% yield; mp 280–283 °C; ^{1}H NMR (300 MHz, DMSO[d_6]) δ (ppm): 12.15 (s, 1H), 10.16 (s, 1H), 8.86 (s, 1H), 8.44 (d, *J* = 7.9 Hz, 1H), 8.28 (t, *J* = 8.1 Hz, 2H), 8.01 (d, *J* = 8.1 Hz, 2H), 7.61–7.71 (m, 2H), 7.36 (t, *J* = 7.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 192.4, 143.1, 141.5, 140.9, 135.4, 129.8, 129.5, 129.1, 128.7, 125.7, 125.3, 122.1, 121.8, 121.1, 120.4, 113.9, 112.6, 125.7; MS (ESI): *m*/*z*: 341 $[M + H]^+$.

1-(4-Fluorophenyl)-9*H*-pyrido[3,4-*b*]indole-3-carbaldehyde (4d). White solid of 90% yield; mp: 189–190 °C; ^{1}H NMR (300 MHz, DMSO[d_6]) δ (ppm): 11.64 (bs, 1H), 10.24 (s, 1H), 8.68 (s, 1H), 8.23 (d, *J* = 7.7 Hz, 1H), 8.04–8.16 (m, 2H), 7.68 (d, *J* = 7.7 Hz, 1H), 7.56–7.62 (m, 1H), 7.37 (d, *J* = 7.7 Hz, 2H), MS (ESI): *m*/*z* 291 $[M + H]^+$.

1-(3,4,5-Trimethoxyphenyl)-9*H*-pyrido[3,4-*b*]indole-3-carbaldehyde (4e). Pale yellow solid: 80% yield; mp: 213–214 °C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.29 (s, 1H), 9.18 (bs, 1H), 8.72 (s, 1H), 8.23 (d, *J* = 7.9 Hz, 1H), 7.66–7.61 (m, 2H), 7.42–7.39 (m, 1H), 7.15 (s, 2H), 3.95 (s, 6H), 3.93 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 193.5, 153.7, 144.1, 143.4, 141.5, 140.9, 138.6, 136.0, 133.1, 131.5, 129.7, 129.1, 122.2, 122.0, 121.3, 113.7, 112.2, 105.4, 60.8, 56.2; MS (ESI): *m*/*z* 363 $[M + H]^+$.

General procedure for the preparation of compounds (5a–z and 6a–c)

To a solution of **4a–e** (1 equiv.) and the respective *o*-phenylenediamine (1 equiv.) in ethanol (20 mL), 10 mol% of catalyst ($La(NO_3)_3 \cdot 6H_2O$) was added. The reaction mixture was heated at 60 °C for a stipulated time mentioned in Table 2. After the completion of the reaction, ethanol was removed under vacuum. The crude reaction was dissolved in ethyl acetate (50 mL) and washed with water (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. Then the crude product was purified by silica gel column chromatography using EtOAc–*n*-hexane (4 : 6) as the

eluent to afford the final products (**5a–z** and **6a–c**) with high purity.

3-(6-Methoxy-1*H*-benzo[*d*]imidazol-2-yl)-1-(4-methoxyphenyl)-9*H*-pyrido[3,4-*b*]indole (5a). This compound was prepared according to the general procedure, employing **4a** (200 mg, 0.66 mmol) and 4-methoxybenzene-1,2-diamine (91 mg, 0.66 mmol) to obtain pure product **5a** as a pale yellow solid. Yield: 222 mg (80%); mp: 166–168 °C; IR (KBr): λ_{max}/cm^{-1} = 3420, 2929, 2832, 1663, 1624, 1452, 1401, 1244, 1026, 834, 744, 578, 507, 435; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 10.49 (bs, 1H), 8.98 (s, 1H), 8.74 (s, 1H), 8.14 (d, J = 8.3 Hz, 1H), 7.98–7.94 (m, 2H), 7.58–7.49 (m, 2H), 7.35–7.29 (m, 2H), 7.12–7.09 (m, 2H), 6.91 (dd, J = 2.26, 8.30 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 160.2, 141.9, 140.7, 138.3, 133.7, 130.5, 130.3, 129.4, 128.6, 122.1, 122.0, 120.6, 114.4, 111.6, 55.7, 55.3; MS (ESI, m/z): 421 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{26}H_{21}O_2N_4$: 421.16590, found: 421.16486 $[M + 1]^+$.

1-(4-Methoxyphenyl)-3-(6-methyl-1*H*-benzo[*d*]imidazol-2-yl)-9*H*-pyrido[3,4-*b*]indole (5b). This compound was prepared according to the general procedure, employing **4a** (200 mg, 0.66 mmol) and 4-methylbenzene-1,2-diamine (80 mg, 0.66 mmol) to obtain pure product **5b** as a pale yellow solid. Yield: 219 mg (82%); mp: 188–190 °C; IR (KBr): λ_{max}/cm^{-1} = 3420, 3054, 2920, 1719, 1607, 1564, 1510, 1454, 1401, 1316, 1243, 1174, 1109, 875, 802, 743, 576, 434; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO [d_6]) δ (ppm): 11.09 (bs, 1H), 9.00 (s, 1H), 8.13 (d, J = 8.6 Hz, 3H), 7.66–7.46 (m, 4H), 7.27 (t, J = 7.3 Hz, 1H), 7.10 (d, J = 8.6 Hz, 2H), 7.05 (d, J = 8.1 Hz, 1H), 3.90 (s, 3H), 2.49 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 159.6, 152.1, 141.5, 141.2, 137.5, 133.4, 131.5, 130.4, 130.0, 129.7, 127.9, 123.3, 121.3, 121.1, 119.6, 113.6, 111.9, 111.1, 54.9, 21.2; MS (ESI, m/z): 405 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{26}H_{21}ON_4$: 405.17099, found: 405.17072 $[M + 1]^+$.

3-(5,6-Dimethyl-1*H*-benzo[*d*]imidazol-2-yl)-1-(4-methoxyphenyl)-9*H*-pyrido[3,4-*b*]indole (5c). This compound was prepared according to the general procedure, employing **4a** (200 mg, 0.66 mmol) and 4,5-dimethylbenzene-1,2-diamine (90 mg, 0.66 mmol) to obtain pure product **5c** as a pale yellow solid. Yield: 235 mg (85%); mp: 246–248 °C; IR (KBr): λ_{max}/cm^{-1} = 3461, 3058, 2920, 2852, 1723, 1607, 1510, 1455, 1399, 1315, 1241, 1174, 1109, 1024, 836, 746, 578, 435; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 10.49 (bs, 1H), 9.02 (s, 1H), 8.65 (s, 1H), 8.16 (d, J = 7.5 Hz, 1H), 7.97 (d, J = 9.0 Hz, 2H), 7.59–7.50 (m, 3H), 7.33 (t, J = 6.7 Hz, 2H), 7.16 (d, J = 8.3 Hz, 2H), 3.92 (s, 3H), 2.40 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 160.3, 151.6, 141.9, 140.7, 138.6, 133.7, 132.4, 130.6, 130.5, 129.4, 128.7, 122.2, 122.1, 120.7, 114.6, 111.6, 55.4, 29.6, 20.4; MS (ESI, m/z): 419 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{27}H_{23}ON_4$: 419.18446, found: 419.18588 $[M + 1]^+$.

3-(6-Fluoro-1*H*-benzo[*d*]imidazol-2-yl)-1-(4-methoxyphenyl)-9*H*-pyrido[3,4-*b*]indole (5d). This compound was prepared according to the general procedure, employing **4a** (200 mg, 0.66 mmol) and 4-fluorobenzene-1,2-diamine (83 mg, 0.66 mmol) to obtain pure product **5d** as a pale yellow solid. Yield: 229 mg (85%); mp: 232–234 °C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.59 (bs, 1H), 9.00 (s, 1H), 8.68 (s, 1H), 8.15 (d, J = 7.5 Hz, 1H), 7.97 (d, J = 8.3 Hz, 2H), 7.61–7.51 (m, 3H), 7.34 (t, J = 6.7 Hz, 1H), 7.11 (d, J = 9.0 Hz, 2H), 7.02 (dt, J = 6.7, 2.2 Hz, 1H), 3.91 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$ + DMSO) δ (ppm): 159.6, 158.4 (d, J = 235.2 Hz, ArC-F), 153.6, 141.5, 141.4, 137.2, 133.2, 130.1, 129.9, 129.7, 121.0, 121.1, 119.6, 113.6, 112.3, 111.1, 109.5, 54.9; MS (ESI, m/z): 409 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{25}H_{18}ON_4F$: 409.14592, found: 409.14500 $[M + 1]^+$.

3-(1*H*-Benzo[*d*]imidazol-2-yl)-1-(4-methoxyphenyl)-9*H*-pyrido-[3,4-*b*]indole (5e). This compound was prepared according to the general procedure, employing **4a** (200 mg, 0.66 mmol) and benzene-1,2-diamine (71 mg, 0.66 mmol) to obtain pure product **5e** as a pale yellow solid. Yield: 224 mg (87%); mp: 201–202 °C; IR (KBr): λ_{max}/cm^{-1} = 3435, 3061, 2929, 1624, 1608, 1563, 1513, 1497, 1468, 1453, 1427, 1407, 1321, 1298, 1244, 1176, 1111, 1030, 838, 742, 613, 585; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 10.57 (bs, 1H), 9.05 (s, 1H), 8.74 (s, 1H), 8.15 (d, J = 7.5 Hz, 1H), 7.96 (d, J = 9.0 Hz, 2H), 7.60–7.52 (m, 3H), 7.35–7.26 (m, 3H), 7.11 (d, J = 8.3 Hz, 2H), 3.91 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 159.1, 151.9, 141.1, 140.1, 137.0, 132.9, 129.8, 129.3, 127.3, 121.1, 120.7, 120.5, 119.1, 113.1, 111.7, 110.6, 54.4; MS (ESI, m/z): 391 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{25}H_{19}ON_4$: 391.15534, found: 391.15518 $[M + 1]^+$.

(2-(1-(4-Methoxyphenyl)-9*H*-pyrido[3,4-*b*]indol-3-yl)-1*H*-benzo[*d*]imidazol-6-yl)(phenyl)methanone (5f). This compound was prepared according to the general procedure, employing **4a** (200 mg, 0.66 mmol) and (3,4-diaminophenyl)-(phenyl)methanone (140 mg, 0.66 mmol) to obtain pure product **5f** as a pale yellow solid. Yield: 278 mg (85%); mp: 186–188 °C; IR (KBr): λ_{max}/cm^{-1} = 3366, 2924, 2356, 1644, 1610, 1573, 1513, 1494, 1464, 1447, 1426, 1403, 1320, 1247, 1176, 1112, 1028, 893, 741, 718, 587, 456; ^{1}H NMR (500 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.80 (bs, 1H), 9.08 (d, J = 18.3 Hz, 1H), 8.76 (s, 1H), 8.18 (s, 1H), 7.98 (d, J = 8.3 Hz, 2H), 7.89–7.79 (m, 3H), 7.60–7.53 (m, 4H), 7.48 (t, J = 7.7 Hz, 2H), 7.35 (t, J = 7.3 Hz, 1H), 7.12 (d, J = 8.0 Hz, 2H), 3.91 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 194.8, 158.7, 140.8, 140.5, 137.2, 135.9, 132.6, 130.4, 129.8, 129.2, 129.0, 128.8, 128.2, 127.0, 126.7, 120.1, 120.0, 118.8, 112.6, 111.4, 110.7, 54.0; MS (ESI, m/z): 495 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{32}H_{23}O_2N_4$: 495.18155, found: 495.18005 $[M + 1]^+$.

1-(4-Methoxyphenyl)-3-(6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)-9*H*-pyrido[3,4-*b*]indole (5g). This compound was prepared according to the general procedure, employing **4a** (200 mg, 0.66 mmol) and 4-(trifluoromethyl)benzene-1,2-diamine (116 mg, 0.66 mmol) to obtain pure product **5g** as a pale brown colour solid. Yield: 242 mg (80%); mp: 296–298 °C; IR (KBr): λ_{max}/cm^{-1} = 3440, 3086, 2930, 2835, 1626, 1608, 1566, 1549, 1513, 1501, 1471, 1455, 1406, 1371, 1328, 1244, 1217, 1161, 1114, 1051, 1029, 975, 932, 819, 666, 611, 589; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 11.33 (bs, 1H), 9.03 (d, J = 6.6 Hz, 1H), 8.23–8.15 (m, 3H), 7.96 (s, 1H), 7.73–7.21 (m, 5H), 7.18–7.05 (m, 2H), 3.92 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$ + DMSO) δ (ppm): 159.6, 154.8, 146.2, 143.3, 141.6,

136.8, 134.1, 133.4, 130.0, 129.9, 129.6, 128.0, 124.7 (q, J = 271.4 Hz, ArC-CF_3), 122.7, 121.1, 120.9, 119.7, 118.1(m), 115.4, 113.6, 112.3, 111.8, 108.9, 54.9; MS (ESI, m/z): 459 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{26}H_{18}ON_4F_3$: 459.14032, found: 459.14099 $[M + 1]^+$.

1-(3,4-Difluorophenyl)-3-(6-methoxy-1*H*-benzo[*d*]imidazol-2-yl)-9*H*-pyrido[3,4-*b*]indole (5h). This compound was prepared according to the general procedure, employing **4b** (200 mg, 0.64 mmol) and 4-methoxybenzene-1,2-diamine (89 mg, 0.64 mmol) to obtain pure product **5h** as a pale yellow solid. Yield: 248 mg (90%); mp: 173–175 °C; IR (KBr): λ_{max}/cm^{-1} = 3452, 3059, 2930, 1719, 1625, 1515, 1492, 1451, 1403, 1341, 1272, 1200, 1153, 1025, 745, 603; ^{1}H NMR (500 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.42 (bs, 1H), 9.00 (s, 1H), 8.85 (s, 1H), 8.10 (d, J = 7.7 Hz, 1H), 7.88–7.84 (m, 1H), 7.77–7.74 (m, 1H), 7.59–7.53 (m, 2H), 7.39–7.30 (m, 3H), 6.94 (dd, J = 8.6, 2.4 Hz, 1H), 3.89 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 155.3, 151.3, 141.1, 138.3, 137.2, 134.5, 132.7, 130.1, 127.6, 124.3, 120.5, 119.3, 118.6, 117.3, 117.1, 116.4, 116.1, 111.6, 111.2, 110.8, 54.7; MS (ESI, m/z): 427 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{25}H_{17}ON_4F_2$: 427.13649, found: 427.13549 $[M + 1]^+$.

(2-(1-(3,4-Difluorophenyl)-9*H*-pyrido[3,4-*b*]indol-3-yl)-1*H*-benzo[*d*]imidazol-6-yl)(phenyl)methanone (5i). This compound was prepared according to the general procedure, employing **4b** (200 mg, 0.64 mmol) and (3,4-diaminophenyl)(phenyl)methanone (137 mg, 0.64 mmol) to obtain pure product **5i** as a pale brown colour solid. Yield: 292 mg (90%); mp: 182–184 °C; IR (KBr): λ_{max}/cm^{-1} = 3269, 3059, 1721, 1643, 1614, 1574, 1542, 1517, 1495, 1467, 1446, 1404, 1340, 1242, 1114, 1043, 936, 891, 737, 717, 642, 606, 509, 936; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 12.88 (bs, 1H), 11.59 (s, 1H), 9.06 (s, 1H), 8.33–8.25 (m, 1H), 8.15 (d, J = 7.9 Hz, 1H), 8.11–8.08 (bs, 1H), 7.83–7.77 (m, 4H), 7.67 (d, J = 8.3 Hz, 1H), 7.64–7.38 (m, 5H), 7.35–7.27 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 195.2, 140.9, 140.8, 140.0, 138.4, 137.4, 136.3, 134.1, 133.2, 133.0, 132.8, 130.7, 130.3, 129.6, 129.9, 129.4, 128.6, 127.6, 127.4, 127.0, 124.2, 123.4, 120.3, 119.3, 119.1, 117.2, 116.9, 116.2, 116.0, 114.5, 114.2, 111.9, 111.7; MS (ESI, m/z): 501$[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{31}H_{19}ON_4F_2$: 501.15214, found: 501.15126 $[M + 1]^+$.

1-(3,4-Difluorophenyl)-3-(5,6-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)-9*H*-pyrido[3,4-*b*]indole (5j). This compound was prepared according to the general procedure, employing **4b** (200 mg, 0.64 mmol) and 4,5-dimethylbenzene-1,2-diamine (88 mg, 0.64 mmol) to obtain pure product **5j** as a pale yellow solid. Yield: 261 mg (95%); mp: 186–184 °C; IR (KBr): λ_{max}/cm^{-1} = 3420, 3059, 2921, 1724, 1607, 1563, 1510, 1457, 1430, 1407, 1399, 1315, 1242, 1176, 1109, 1026, 838, 742, 613, 580; ^{1}H NMR (500 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.38 (bs, 1H), 9.08 (s, 1H), 8.85 (s, 1H), 8.18 (d, J = 7.9 Hz, 1H), 7.63 (bs, 1H), 7.60–7.54 (m, 2H), 7.34 (dt, J = 6.8, 1.0 Hz, 1H), 7.27 (s, 1H), 7.16 (s, 2H), 2.40 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 151.0, 147.9, 145.9, 141.1, 140.7, 138.8, 138.0, 134.9, 132.5, 131.7, 131.4, 130.7, 130.4, 127.9, 127.5, 120.9, 120.7, 120.3, 119.5, 118.7, 117.1, 116.8, 111.7, 111.5, 30.1, 19.87; MS (ESI, m/z): 425 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{26}H_{19}N_4F_2$: 425.15723, found: 425.15678 $[M + 1]^+$.

3-(6-Bromo-1*H*-benzo[*d*]imidazol-2-yl)-1-(3,4-difluorophenyl)-9*H*-pyrido[3,4-*b*]indole (5k). This compound was prepared according to the general procedure, employing **4b** (200 mg, 0.64 mmol) and 4-bromobenzene-1,2-diamine (121 mg, 0.64 mmol) to obtain pure product **5k** as a pale brown colour solid. Yield: 277 mg (90%); mp: 156–158 °C; IR (KBr): λ_{max}/cm^{-1} = 3420, 3059, 2921, 1724, 1607, 1563, 1510, 1457, 1430, 1407, 1399, 1315, 1242, 1176, 1109, 1026, 838, 742, 613, 580; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.06 (bs, 1H), 7.78 (s, 1H), 7.02–6.82 (m, 2H), 6.78 (s, 1H), 6.57 (s, 1H), 6.43–6.22 (m, 4H), 6.09–6.04 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 152.3, 140.8, 138.2, 136.3, 134.1, 132.6, 129.7, 129.3, 127.4, 127.3, 124.1, 123.8, 120.2, 119.1, 117.1, 116.8, 116.1, 115.9, 114.4, 114.1, 113.5, 111.4, 111.1; MS (ESI, m/z): 475 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{24}H_{14}N_4F_2Br$: 475.03644, found: 475.03595 $[M + 1]^+$.

1-(3,4-Difluorophenyl)-3-(6-methyl-1*H*-benzo[*d*]imidazol-2-yl)-9*H*-pyrido[3,4-*b*]indole (5l). This compound was prepared according to the general procedure, employing **4b** (200 mg, 0.64 mmol) and 4-methylbenzene-1,2-diamine (79 mg, 0.64 mmol) to obtain pure product **5l** as a yellow colour solid. Yield: 255 mg (96%); mp: 286–288 °C; IR (KBr): λ_{max}/cm^{-1} = 3451, 3061, 2921, 1625, 1606, 1565, 1517, 1497, 1468, 1453, 1436, 1404, 1319, 1274, 1240, 1201, 1155, 1144, 936, 880, 804, 746, 604, 570, 516, 435; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.39 (bs, 1H), 9.03 (s, 1H), 8.91 (s, 1H), 8.09 (d, J = 8.3 Hz, 1H), 7.89–7.82 (m, 1H), 7.76–7.74 (s, 1H), 7.62–7.50 (m, 2H), 7.39–7.29 (m, 3H), 7.11 (d, J = 8.3 Hz, 1H), 2.51 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 151.4, 141.1, 138.5, 137.4, 132.8, 130.9, 130.2, 127.7, 124.4, 122.9, 120.6, 119.4, 117.4, 117.2, 116.4, 116.2, 114.7, 111.7, 111.4, 111.0, 20.9; MS (ESI, m/z): 411 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{25}H_{17}N_4F_2$: 411.14158, found: 411.14059 $[M + 1]^+$.

3-(6-Chloro-1*H*-benzo[*d*]imidazol-2-yl)-1-(3,4-difluorophenyl)-9*H*-pyrido[3,4-*b*]indole (5m). This compound was prepared according to the general procedure, employing **4b** (200 mg, 0.64 mmol) and 4-chlorobenzene-1,2-diamine (92 mg, 0.64 mmol) to obtain pure product **5m** as a pale orange colour solid. Yield: 246 mg (88%); mp: 180–182 °C; IR (KBr): λ_{max}/cm^{-1} = 3436, 3062, 1721, 1624, 1606, 1563, 1517, 1495, 1467, 1452, 1401, 1318, 1272, 1239, 1201, 1108, 1056, 923, 885, 845, 804, 721, 746, 606, 569; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 11.44 (bs, 1H), 9.04 (s, 1H), 8.33–8.14 (m, 2H), 8.08 (s, 1H), 7.74–7.51 (m, 4H), 7.48–7.40 (m, 1H), 7.35–7.25 (m, 1H), 7.20 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 141.2, 141.2, 138.7, 136.9, 133.2, 133.1, 130.2, 130.1, 127.9, 127.7, 124.4, 121.7, 120.7, 119.5, 119.4, 117.5, 117.3, 116.5, 116.3, 114.8, 114.5, 111.9, 111.8; MS (ESI, m/z): 431$[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{24}H_{14}N_4F_2Cl$: 431.08696, found: 431.08591 $[M + 1]^+$.

3-(1*H*-Benzo[*d*]imidazol-2-yl)-1-(3,4-difluorophenyl)-9*H*-pyrido[3,4-*b*]indole (5n). This compound was prepared according to the general procedure, employing **4b** (200 mg, 0.64 mmol) and benzene-1,2-diamine (70 mg, 0.64 mmol) to

obtain pure product **5n** as a pale orange colour solid. Yield: 244 mg (95%); mp: 178–179 °C; IR (KBr): λ_{max}/cm^{-1} = 3436, 3062, 1721, 1624, 1606, 1563, 1517, 1495, 1467, 1452, 1401, 1318, 1272, 1239, 1201, 1108, 1056, 923, 885, 845, 804, 721, 746, 606, 569; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.77 (bs, 1H), 9.10 (s, 1H), 8.21–8.05 (m, 3H), 7.95 (s, 1H), 7.66–7.48 (m, 4H), 7.32–7.22 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 151.7, 143.4, 141.1, 137.2, 132.8, 130.1, 129.7, 127.7, 127.5, 124.3, 121.6, 120.9, 120.6, 119.3, 118.0, 117.4, 117.2, 116.4, 116.1, 111.6, 111.5, 110.7; MS (ESI, *m*/*z*): 397 $[M + 1]^+$; HRMS (ESI, *m*/*z*) calculated for $C_{24}H_{15}N_4F_2$: 397.12593, found: 397.12534 $[M + 1]^+$.

3-(6-Fluoro-1*H*-benzo[*d*]imidazol-2-yl)-1-(4-(trifluoromethyl)-phenyl)-9*H*-pyrido[3,4-*b*]indole (5o). This compound was prepared according to the general procedure, employing **4c** (200 mg, 0.58 mmol) and 4-fluorobenzene-1,2-diamine (74 mg, 0.58 mmol) to obtain pure product **5o** as a pale yellow solid. Yield: 217 mg (83%); mp: 302–304 °C; IR (KBr): λ_{max}/cm^{-1} = 3456, 3067, 1623, 1598, 1566, 1549, 1999, 1455, 1396, 1321, 1251, 1202, 1164, 1109, 1064, 844, 750, 607, 573; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.47 (bs, 1H), 9.13 (s, 1H), 8.61 (s, 1H), 8.23 (d, *J* = 8.1 Hz, 1H), 8.16 (d, *J* = 8.1 Hz, 2H), 7.94 (d, *J* = 8.1 Hz, 2H), 7.66–7.52 (m, 3H), 7.40 (t, *J* = 7.9 Hz, 1H), 7.06 (dt, *J* = 9.4, 2.2 Hz, 1H); ^{13}C NMR (175 MHz, CDCl3 + DMSO) δ (ppm): 158.5 (dd, *J* = 244.9 Hz, 54.6 Hz, ArC-CF), 153.6, 152.6, 144.2, 141.6, 141.3, 140.4, 139.6, 137.6, 133.5, 130.5, 129.3 (q, *J* = 32.4 Hz, CF3Ar-C), 129.2, 128.3, 125.0, 123.8 (q, *J* = 272.1 Hz, ArC-CF3), 121.3, 120.8, 119.8, 119.0, 112.2 (m), 109.6 (m), 103.6, 97.8; MS (ESI, *m*/*z*): 447 $[M + 1]^+$; HRMS (ESI, *m*/*z*) calculated for $C_{25}H_{15}N_4F_4$: 447.12274, found: 447.12133 $[M + 1]^+$.

3-(1*H*-Benzo[*d*]imidazol-2-yl)-1-(4-(trifluoromethyl)phenyl)-9*H*-pyrido[3,4-*b*]indole (5p). This compound was prepared according to the general procedure, employing **4c** (200 mg, 0.58 mmol) and benzene-1,2-diamine (63 mg, 0.58 mmol) to obtain pure product **5p** as a pale yellow solid. Yield: 214 mg (85%); mp: 299–301 °C; IR (KBr): λ_{max}/cm^{-1} = 3458, 3067, 2925, 2358, 1622, 1566, 1548, 1498, 1471, 1455, 1414, 1321, 1168, 1149, 1106, 1064, 1017, 849, 750, 737, 622, 583, 561; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.52 (bs, 1H), 9.14 (s, 1H), 8.77 (s, 1H), 8.21 (s, 1H), 8.15 (d, *J* = 8.1 Hz, 2H), 7.90 (d, *J* = 7.9 Hz, 3H), 7.65–7.51 (m, 3H), 7.40–7.30 (m, 2H); ^{13}C NMR (175 MHz, CDCl3 + DMSO) δ (ppm): 152.0, 141.6, 141.4, 139.6, 137.9, 133.5, 130.4, 129.2, 129.1 (q, *J* = 31.7 Hz, CF3Ar-C), 128.5, 128.3, 124.9, 123.8 (q, *J* = 272.1 Hz, ArC-CF3), 112.3, 112.2; MS (ESI, *m*/*z*): 429 $[M + 1]^+$; HRMS (ESI, *m*/*z*) calculated for $C_{25}H_{16}N_4F_3$: 429.13216, found: 429.13076 $[M + 1]^+$.

3-(6-Chloro-1*H*-benzo[*d*]imidazol-2-yl)-1-(4-(trifluoromethyl)-phenyl)-9*H*-pyrido[3,4-*b*]indole (5q). This compound was prepared according to the general procedure, employing **4c** (200 mg, 0.58 mmol) and 4-chlorobenzene-1,2-diamine (83 mg, 0.58 mmol) to obtain pure product **5q** as a pale yellow solid. Yield: 223 mg (82%); mp: 326–328 °C; IR (KBr): λ_{max}/cm^{-1} = 3453, 3065, 2923, 1722, 1625, 1565, 1547, 1466, 1454, 1396, 1321, 1248, 1165, 1108, 1064, 924, 882, 850, 747, 732, 596, 576; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 11.36 (bs, 1H), 9.08 (s, 1H), 8.36 (d, *J* = 7.5 Hz, 2H), 8.18 (d, *J* = 7.5 Hz, 1H), 7.87 (d, *J* = 7.5 Hz, 2H), 7.67–7.52 (m, 4H), 7.32 (t, *J* = 7.5 Hz, 1H), 7.19 (dd, *J* = 8.3, 2.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 152.6, 140.9, 140.7, 139.1, 136.7, 133.1, 129.8, 129.0, 128.5, 128.1, 127.6, 124.2, 121.3, 120.4, 120.2, 119.2, 111.8, 111.5; MS (ESI, *m*/*z*): 463 $[M + 1]^+$; HRMS (ESI, *m*/*z*) calculated for $C_{25}H_{14}N_4F_3Cl$: 463.13216, found: 463.13076 $[M + 1]^+$.

3-(6-Chloro-1*H*-benzo[*d*]imidazol-2-yl)-1-(4-fluorophenyl)-9*H*-pyrido[3,4-*b*]indole (5r). This compound was prepared according to the general procedure, employing **4d** (200 mg, 0.68 mmol) and 4-chlorobenzene-1,2-diamine (97 mg, 0.68 mmol) to obtain pure product **5r** as a pale yellow solid. Yield: 244 mg (86%); mp: 157–159 °C; IR (KBr): λ_{max}/cm^{-1} = 3429, 3061, 2924, 1717, 1622, 1603, 1506, 1450, 1397, 1318, 1224, 1154, 1094, 1052, 973, 922, 840, 801, 744, 700, 590, 565, 504, 434; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.51 (bs, 1H), 9.07 (s, 1H), 8.62 (s, 1H), 8.20 (d, *J* = 7.9 Hz, 1H), 8.03–8.00 (m, 2H), 7.75 (d, *J* = 8.3 Hz, 1H), 7.62–7.59 (m, 1H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 8.3 Hz, 1H), 7.39–7.32 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 162.7, 161.5, 159.4, 152.1, 140.2, 139.2, 135.9, 132.5, 132.1, 129.4, 129.3, 128.7, 126.8, 125.1, 120.5, 119.8, 119.6, 118.5, 113.9, 113.6, 111.0, 110.6; MS (ESI, *m*/*z*): 413$[M + 1]^+$; HRMS (ESI, *m*/*z*) calculated for $C_{24}H_{15}N_4FCl$: 413.09638, found: 413.09602 $[M + 1]^+$.

3-(1*H*-Benzo[*d*]imidazol-2-yl)-1-(4-fluorophenyl)-9*H*-pyrido-[3,4-*b*]indole (5s). This compound was prepared according to the general procedure, employing **4d** (200 mg, 0.68 mmol) and benzene-1,2-diamine (74 mg, 0.68 mmol) to obtain pure product **5s** as a pale yellow solid. Yield: 232 mg (89%); mp: 178–180 °C; IR (KBr): λ_{max}/cm^{-1} = 3456, 3291, 3058, 2918, 1625, 1554, 1510, 1498, 1469, 1454, 1425, 1408, 1275, 1241, 1217, 1158, 1112, 1092, 837, 797, 730, 665, 577, 501; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 10.51 (bs, 1H), 9.10 (s, 1H), 8.65 (s, 1H), 8.21 (d, *J* = 7.5 Hz, 1H), 8.06–7.99 (m, 2H), 7.87 (s, 1H), 7.62–7.50 (m, 3H), 7.40–7.29 (m, 5H); ^{13}C NMR (175 MHz, $CDCl_3$ + DMSO) δ (ppm): 162.9 (d, *J* = 247.5 Hz, ArC-F), 152.8, 142.1, 141.0, 138.2, 134.5, 133.9, 131.3, 131.2, 130.7, 128.7, 122.6, 121.7, 121.5, 120.3, 115.7, 115.6, 112.9, 112.2; MS (ESI, *m*/*z*): 379 $[M + 1]^+$; HRMS (ESI, *m*/*z*) calculated for $C_{24}H_{16}N_4F$: 379.13535, found: 379.13529 $[M + 1]^+$.

1-(4-Fluorophenyl)-3-(6-methyl-1*H*-benzo[*d*]imidazol-2-yl)-9*H*-pyrido[3,4-*b*]indole (5t). This compound was prepared according to the general procedure, employing **4d** (200 mg, 0.68 mmol) and 4-methylbenzene-1,2-diamine (84 mg, 0.68 mmol) to obtain pure product **5t** as a pale yellow solid. Yield: 237 mg (88%); mp: 236–238 °C; IR (KBr): λ_{max}/cm^{-1} = 3454, 3252, 3053, 2920, 1626, 1604, 1509, 1455, 1470, 1425, 1404, 1320, 1278, 1218, 1157, 844, 806, 745, 608, 572, 504; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.37 (bs, 1H), 9.08 (s, 1H), 8.63 (s, 1H), 8.19 (d, *J* = 7.5 Hz, 1H), 8.05–8.00 (m, 2H), 7.62–7.50 (m, 2H), 7.41–7.28 (m, 4H), 7.11 (d, *J* = 7.5 Hz, 1H), 2.52 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$ + DMSO) δ (ppm): 162.3 (d, *J* = 247.9 Hz, ArC-F), 151.9, 141.4, 140.3, 137.7, 133.9, 133.2, 131.0, 130.6 (d, *J* = 8.2 Hz), 130.0, 128.0, 123.1, 120.9

(d, J = 8.2 Hz), 119.6, 115.0, 114.9, 112.2, 111.3, 21.2; MS (ESI, m/z): 393 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{25}H_{18}N_4F$: 393.15100, found: 393.15062 $[M + 1]^+$.

3-(5,6-Dichloro-1*H*-benzo[*d*]imidazol-2-yl)-1-(4-fluorophenyl)-9*H*-pyrido[3,4-*b*]indole (5u). This compound was prepared according to the general procedure, employing **4d** (200 mg, 0.68 mmol) and 4,5-dichlorobenzene-1,2-diamine (121 mg, 0.68 mmol) to obtain pure product **5u** as a pale yellow solid. Yield: 262 mg (85%); mp: 158–160 °C; IR (KBr): λ_{max}/cm^{-1} = 3417, 3324, 2921, 2356, 1625, 1607, 1562, 1540, 1510, 1499, 1468, 1448, 1392, 1319, 1228, 1183, 1156, 1097, 874, 835, 735, 659, 573, 548, 507; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.33 (bs, 1H), 8.15 (s, 1H), 7.34 (s, 3H), 6.92 (s, 1H), 6.85–6.55 (m, 4H), 6.53–6.39 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 154.1, 141.1, 140.4, 136.5, 133.6, 133.3, 130.2, 130.1, 129.7, 127.8, 124.6, 120.7, 119.5, 114.9, 114.6, 111.8; MS (ESI, m/z): 447 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{24}H_{14}N_4Cl_2F$: 447.05741, found: 447.05731 $[M + 1]^+$.

Phenyl(2-(1-(3,4,5-trimethoxyphenyl)-9*H*-pyrido[3,4-*b*]indol-3-yl)-1*H*-benzo[*d*]imidazol-6-yl)methanone (5v). This compound was prepared according to the general procedure, employing **4e** (200 mg, 0.55 mmol) and (3,4-diaminophenyl)(phenyl)methanone (117 mg, 0.55 mmol) to obtain pure product **5v** as a pale yellow solid. Yield: 281 mg (92%); mp: 208–210 °C; IR (KBr): λ_{max}/cm^{-1} = 3323, 3060, 2926, 1644, 1615, 1585, 1542, 1497, 1468, 1446, 1405, 1347, 1320, 1296, 1235, 1178, 1126, 1002, 892, 829, 791, 719, 642, 434; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.88 (bs, 1H), 9.12 (d, J = 10.5 Hz, 1H), 8.85 (d, J = 8.3 Hz, 1H), 8.22 (t, J = 6.7 Hz, 1H), 7.91–7.77 (m, 4H), 7.61–7.51 (m, 4H), 7.51–7.45 (m, 2H), 7.40–7.33 (m, 1H), 7.16 (s, 2H), 3.95 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 159.0, 154.2, 141.2, 140.8, 136.2, 133.0, 129.6, 129.3, 127.3, 120.5, 120.4, 119.1, 117.8, 113.0, 111.6, 111.1, 54.3; MS (ESI, m/z): 555 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{34}H_{27}N_4O_4$: 555.20268, found: 555.20184 $[M + 1]^+$.

3-(1*H*-Benzo[*d*]imidazol-2-yl)-1-(3,4,5-trimethoxyphenyl)-9*H*-pyrido[3,4-*b*]indole (5w). This compound was prepared according to the general procedure, employing **4e** (200 mg, 0.55 mmol) and benzene-1,2-diamine (59 mg, 0.55 mmol) to obtain pure product **5w** as a pale yellow solid. Yield: 231 mg (93%); mp: 224–226 °C; IR (KBr): λ_{max}/cm^{-1} = 3407, 2937, 1623, 1587, 1505, 1452, 1410, 1384, 1325, 1292, 1178, 1127, 740, 688; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 10.55 (bs, 1H), 9.13 (s, 1H), 8.22 (s, 1H), 7.88 (s, 1H), 7.62–7.47 (m, 3H), 7.38–7.30 (m, 1H), 7.30–7.26 (s, 2H), 7.17 (d, J = 8.3 Hz, 2H), 3.97 (s, 6H), 3.94 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 152.9, 152.3, 141.8, 141.4, 137.9, 137.3, 133.6, 133.3, 130.1, 128.0, 121.8, 121.3, 121.2, 119.7 112.0, 111.8, 105.7, 60.3, 55.8; MS (ESI, m/z): 451 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{27}H_{23}O_3N_4$: 451.17647, found: 451.17549 $[M + 1]^+$.

3-(5,6-Dimethyl-1*H*-benzo[*d*]imidazol-2-yl)-1-(3,4,5-trimethoxyphenyl)-9*H*-pyrido[3,4-*b*]indole (5x). This compound was prepared according to the general procedure, employing **4e** (200 mg, 0.55 mmol) and 4,5-dimethylbenzene-1,2-diamine (75 mg, 0.55 mmol) to obtain pure product **5x** as a pale brown colour solid. Yield: 248 mg (94%); mp: 284–286 °C; IR (KBr): λ_{max}/cm^{-1} = 3444, 3201, 2925, 2852, 1625, 1585, 1505, 1454, 1467, 1402, 1309, 1234, 1127, 1003, 845, 743, 692, 630, 558; ^{1}H NMR (500 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.41 (bs, 1H), 9.07 (s, 1H), 8.95 (s, 1H), 8.18 (d, J = 7.6 Hz, 1H), 7.65–7.54 (m, 3H), 7.35–7.31 (m, 1H), 7.15 (s, 2H), 3.92 (s, 9H), 2.40 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 152.3, 151.0, 141.1, 141.0, 137.3, 137.2, 132.9, 129.9, 129.5, 127.4, 120.7, 120.5, 119.0, 111.7, 110.7, 105.4, 59.7, 55.3, 19.4; MS (ESI, m/z): 479 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{29}H_{26}O_3N_4$: 479.20647, found: 479.20536 $[M + 1]^+$.

3-(6-Chloro-1*H*-benzo[*d*]imidazol-2-yl)-1-(3,4,5-trimethoxyphenyl)-9*H*-pyrido[3,4-*b*]indole (5y). This compound was prepared according to the general procedure, employing **4e** (200 mg, 0.55 mmol) and 4-chlorobenzene-1,2-diamine (78 mg, 0.55 mmol) to obtain pure product **5y** as a dark brown colour solid. Yield: 243 mg (91%); mp: 232–234 °C; IR (KBr): λ_{max}/cm^{-1} = 3389, 3149, 2931, 1624, 1587, 1562, 1504, 1468, 1452, 1404, 1272, 1236, 1181, 1130, 1058, 1005, 924, 849, 804, 743, 690; ^{1}H NMR (500 MHz, DMSO [d_6]) δ (ppm): 10.48 (bs, 1H), 8.19 (s, 1H), 7.29 (d, J = 7.3 Hz, 1H), 6.84–6.61 (m, 5H), 6.48 (s, 2H), 6.40 (t, J = 7.5 Hz, 1H), 6.31 (d, J = 8.4 Hz, 1H), 3.14 (s, 6H), 3.04 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO [d_6]) δ (ppm): 152.7, 152.1, 141.2, 140.7, 137.2, 135.9, 132.9, 132.4, 129.1, 127.3, 126.1, 121.4, 120.3, 119.0, 116.6, 115.6, 111.3, 105.3, 59.8, 59.5, 55.1; MS (ESI, m/z): 485 $[M + 1]^+$; HRS (ESI, m/z) calculated for $C_{27}H_{22}O_3N_4Cl$: 485.13749, found: 485.13742 $[M + 1]^+$.

3-(6-Methoxy-1*H*-benzo[*d*]imidazol-2-yl)-1-(3,4,5-trimethoxyphenyl)-9*H*-pyrido[3,4-*b*]indole (5z). This compound was prepared according to the general procedure, employing **4e** (200 mg, 0.55 mmol) and 4-methoxybenzene-1,2-diamine (76 mg, 0.55 mmol) to obtain pure product **5z** as a pale brown colour solid. Yield: 236 mg (89%); mp: 276–278 °C; IR (KBr): λ_{max}/cm^{-1} = 3425, 3062, 2933, 2830, 1626, 1546, 1504, 1468, 1453, 1408, 1326, 1289, 1235, 1154, 1127, 1012, 941, 847, 747, 629; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 10.60 (bs, 1H), 8.98 (d, J = 21.1 Hz, 2H), 8.13 (d, J = 8.3 Hz, 1H), 7.56 (t, J = 6.7 Hz, 2H), 7.35–7.30 (m, 2H), 7.14 (s, 2H), 6.91 (dd, J = 9.0, 2.2 Hz, 1H), 3.91 (s, 3H), 3.89 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 155.0, 152.0, 151.4, 140.9, 140.8, 136.9, 132.6, 129.2, 127.2, 120.4, 120.3, 118.8, 111.5, 110.6, 110.5, 105.1, 59.5, 55.0, 54.4; MS (ESI, m/z): 481 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{28}H_{25}O_4N_4$: 481.18703, found: 481.18573 $[M + 1]^+$.

3-(3*H*-Imidazo[4,5-*b*]pyridin-2-yl)-1-(4-methoxyphenyl)-9*H*-pyrido[3,4-*b*]indole (6a). This compound was prepared according to the general procedure, employing **4a** (200 mg, 0.66 mmol) and pyridine-2,3-diamine (72 mg, 0.66 mmol) to obtain pure product **6a** as a yellow solid. Yield: 233 mg (90%); mp: 298–300 °C; IR (KBr): λ_{max}/cm^{-1} = 3074, 2928, 1624, 1608, 1512, 1494, 1464, 1452, 1423, 1409, 1386, 1319, 1280, 1263, 1247, 1172, 1114, 1031, 832, 767, 755; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 11.47 (bs, 1H), 9.02 (s, 1H), 8.32

(d, J = 4.1 Hz, 1H), 8.23 (t, J = 8.8 Hz, 3H), 7.93 (d, J = 6.9 Hz, 1H), 7.83 (s, 1H), 7.64 (d, J = 8.3 Hz, 1H), 7.51 (t, J = 7.7 Hz, 1H), 7.27 (t, J = 7.5 Hz, 1H), 7.18–7.12 (m, 3H), 3.93 (s, 3H); ^{13}C NMR (75 MHz, DMSO[d_6]) δ (ppm): 159.9, 156.7, 154.0, 153.7, 149.2, 143.7, 143.4, 141.5, 137.0, 133.4, 130.3, 130.1, 129.9, 128.5, 127.2, 125.8, 121.9, 121.1, 120.0, 119.3, 117.7, 114.0, 112.6, 55.3; MS (ESI, m/z): 392 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{24}H_{18}ON_5$: 392.15059, found: 392.15003 $[M + 1]^+$.

1-(3,4-Difluorophenyl)-3-(3*H*-imidazo[4,5-*b*]pyridin-2-yl)-9*H*-pyrido[3,4-*b*]indole (6b). This compound was prepared according to the general procedure, employing **4b** (200 mg, 0.64 mmol) and pyridine-2,3-diamine (70 mg, 0.64 mmol) to obtain pure product **6b** as a pale brown colour solid. Yield: 229 mg (89%); mp: 320–322 °C; IR (KBr): λ_{max}/cm^{-1} = 3288, 3061, 2919, 2850, 2360, 1677, 1624, 1518, 1496, 1464, 1452, 1438, 1422, 1409, 1388, 1317, 1274, 1204, 1138, 1107, 885, 828, 797, 772, 730, 640, 607, 524; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 11.42 (bs, 1H), 9.11 (s, 1H), 8.39 (s, 1H), 8.18 (d, J = 8.3 Hz, 1H), 8.11–7.97 (m, 2H), 7.67 (d, J = 7.5 Hz, 1H), 7.57 (m, 2H), 7.42 (q, J = 18.1 Hz, 1H), 7.32 (t, J = 7.5 Hz, 1H), 7.21 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 153.0, 142.8, 140.9, 138.5, 136.4, 132.9, 129.9, 127.6, 124.3, 120.4, 120.3, 119.3, 117.4, 117.2, 116.8, 116.2, 116.0, 112.0, 111.6; MS (ESI, m/z): 398 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{23}H_{14}N_5F_2$: 398.10153, found: 398.10091 $[M + 1]^+$.

3-(3*H*-Imidazo[4,5-*b*]pyridin-2-yl)-1-(4-(trifluoromethyl)-phenyl)-9*H*-pyrido[3,4-*b*]indole (6c). This compound was prepared according to the general procedure, employing **4c** (200 mg, 0.58 mmol) and pyridine-2,3-diamine (64 mg, 0.58 mmol) to obtain pure product **6c** as a pale yellow solid. Yield: 214 mg (85%); mp: 338–340 °C; IR (KBr): λ_{max}/cm^{-1} = 3438, 3152, 1624, 1592, 1567, 1496, 1466, 1454, 1425, 1405, 1322, 1267, 1213, 1165, 1125, 1066, 1018, 848, 798, 773, 751, 623; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO[d_6]) δ (ppm): 10.91 (bs, 1H), 9.96 (s, 1H), 7.87 (s, 1H), 7.17–7.05 (m, 2H), 6.96 (d, J = 8.1 Hz, 1H), 6.82 (d, J = 7.7 Hz, 1H), 6.62 (d, J = 7.7 Hz, 2H), 6.44–6.28 (m, 2H), 6.13–6.04 (m, 1H), 5.95 (s, 1H); MS (ESI, m/z): 430 $[M + 1]^+$; HRMS (ESI, m/z) calculated for $C_{24}H_{15}F_3N_5$: 430.12741, found: 430.12640 $[M + 1]^+$.

In vitro cytotoxicity data

Cell culture and reagents. All the cell lines used in this study were obtained from the American Type Culture Collection (ATCC). DU145 (human prostate carcinoma epithelial) and BHK-21 (Hamster kidney cells) cells were cultured in Eagle's minimal essential medium (MEM) containing non-essential amino acids, 1 mM sodium pyruvate, and 10% FBS. HeLa (human epithelial cervical cancer), A549 (human lung carcinoma epithelial) and L929 (mouse connective tissue fibroblast cells) were grown in Dulbecco's modified Eagle's medium (DMEM) containing non-essential amino acids and 10% FBS. All the cells were maintained under a humidified atmosphere of 5% CO_2 at 37 °C. Cells were trypsinized when subconfluent from T75 flasks/90 mm dishes and seeded onto 96 well test plates at a concentration of 1×10^4 cells mL^{-1} in complete medium, treated with compounds at desired concentrations and harvested as required.[63]

Cytotoxicity

Cell proliferation and viability was determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. The pale yellow coloured tetrazolium salt (MTT) reduces to a dark blue water-insoluble formazan by metabolically active cells and this is measured quantitatively after soluble in DMSO. The absorbance of the soluble formazan is directly proportional to the number of viable cells. Cells were seeded at a density of 1×10^4 cells in 200 μL of medium per well of a 96-well plate. The 96-well microliter plates were incubated for 24 h prior to addition of the experimental compounds. Cells were treated with vehicle alone (0.4% DMSO) or compounds (drugs were dissolved in DMSO previously) at different concentrations (1, 10 and 25 μM) of test compounds for 48 hours. The assay was completed with the addition of MTT (5%, 10 μL) and incubated for 60 min at 37 °C. The supernatant was aspirated and plates were air dried and the MTT–formazon crystals dissolved in 100 μL of DMSO. The optical density was measured at 560 nm using a TECAN multimode reader. The growth percentage of each treated well of a 96-well plate has been calculated based on test wells relative to control wells. The cell growth inhibition was calculated by generating dose–response curves as a plot of the percentage of surviving cells *versus* drug concentration. Antiproliferative activity of the cancer cells to the test compounds was expressed in terms of IC_{50} value, which is defined as the concentration of the compound that produced 50% absorbance reduction relative to the control.[64]

CD studies

Circular dichroism experiments were carried out using a JASCO 815 CD spectropolarimeter (Jasco, Tokyo, Japan). All the CD titrations were performed in 100 mM KBPES buffer (pH 7.0) at 25 °C. CD spectra were recorded from 200 to 350 nm and for each experiment, 15×10^{-6} M of CT DNA was used initially. Further, for the characterization of ligand–DNA interaction, CD spectra were recorded in 1 : 0.5 and 1 : 1 molar ratios of CT DNA and ligand respectively. Each spectrum was recorded three times and the average of three scans was taken.

UV-visible titration studies

UV-visible spectroscopic titrations were performed using an ABI Lambda 40 UV-Vis spectrophotometer (Foster City, USA) at 25 °C using a 1 cm path length quartz cuvette. Stock solutions of 10 μM of ligand solution and 25 μM CT DNA were prepared in 100 mM KBPES buffer (pH 7.0). Complex stock solution of the ligand was prepared in a 1 : 1 water–methanol mixture and diluted to the required concentration in suitable buffer solutions. The quartz cells were thoroughly cleaned with distilled water followed by nitric acid (~0.1 N) after each experiment. UV-visible absorption titrations were done by adding CT DNA stock solution in 100 mM KBPES buffer (pH 7.0) to the quartz cuvette containing approximately 10 μM ligand solution

prepared in the same buffer. Preparation of CT DNA and ligands was done on the same day of performing the experiment. Titrations were carried out until the ligand Soret band remains at a fixed wavelength upon successive additions of CT DNA.

DNA intercalation

To verify the mode of ligand interaction with DNA, 2 μM of each ligand was taken in Tris buffer (pH 7.0) and 5 μM of pBR 322 plasmid DNA was added. The mixture was incubated at 37 °C for 1 h. After incubation, the ligand–DNA mixture was resolved using 0.8% agarose gel. The sample treated with 2 μM ethidium bromide was considered as the control.

DNA photocleavage

This experiment was carried out according to the protocol reported by Toshima and coworkers.[65] 0.45 μg of pBR322 plasmid DNA was taken in Tris–HCl buffer (50 mM, pH 7.5) and 100 and 200 μM of each ligand were added and the total volume was maintained at 20 μL. The DNA samples with complexes were taken in a TPP tissue culture test plate and irradiated with UV light (8 W, 365 nm, 4 cm distance). After irradiation, the samples were collected and mixed with 2 μL of loading dye (50% sucrose and 0.25% bromophenol blue). For the dark reaction, 100 μM ligand and 0.45 μg pBR322 plasmid DNA were taken in a PCR tube and the samples were wrapped with aluminium foil and placed in the dark. Then, samples were analysed by gel electrophoresis on a 0.8% agarose horizontal slab gel containing 0.5 μg mL^{-1} ethidium bromide in Tris–EDTA buffer (40 mM Tris, 20 mM acetic acid and 1 mM EDTA, pH 8.0) at 10 V cm^{-1}. Gels were photographed under UV light with a Bio-Rad digital camera and analysed with Gel-Pro software.

DNA topoisomerase I inhibition

The DNA Topo I inhibition study was carried out as described in the previous paper.[66] 0.5 μg of pBR322 DNA was incubated with 1 unit of Topo I (Invitrogen) in 1× NEB buffer (50 mM potassium acetate, 20 mM Tris acetate buffer, 10 mM magnesium acetate, 1 mM DTT). The ligands to be studied were added to the Topo I-DNA complex and incubated at 37 °C for 30 min, allowing the formation of ternary enzyme–DNA–ligand complexes. Then, the enzyme was inactivated by increasing the temperature to 65 °C. Next, the samples were resolved using 0.8% agarose gel electrophoresis which enables the visualisation of cleavage products. 100 μM camptothecin (CPT) treated DNA was considered as the positive control.

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