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ARTICLE TYPE

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 aryl ring at C3 and C1 respectively were designed and synthesized. The key step for their preparation involves condensation of substituted *o*-phenylenediamines with 1-(substituted phenyl)-9H-pyrido[3,4-*b*]indole-3-carbaldehyde using $La(NO_3)_3.6H_2O$ as a catalyst and evaluated their cytotoxic potential. Conjugates **5a**, **5d**, **5h** and **5r** showed enhanced cytotoxic activity (GI_{50} values range from 0.3-7.1 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 by 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 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 mode of interaction with DNA. Moreover, an *in silico* study of these *β*-carboline-benzimidazole conjugates reveal 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 these *β*-carboline family are harmane, harmine and norharman. Recently there is an 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 intercalating 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 intercalaters due to the presence of polycyclic aromatic planar pharmacophore, which is capable of stacking between DNA base pairs.[21,10] For example, harmane and norharman have been reported to intercalate into DNA leading to alter DNA replication fidelity or to influence on enzymatic activities in DNA-repair processes apart from inhibiting DNA topoisomerase I.[22-24]

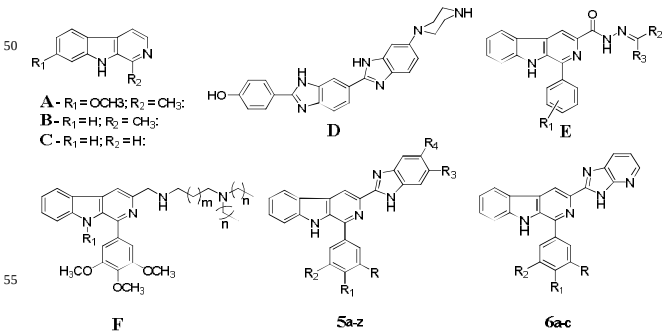


Fig. 1 Chemical Structures of Harmine (A), Harmane (B), Norharman (C), Hoechst 33258 (D), Carbohydrazide linked *β*-carboline derivatives (E), N^9-arylated alkyl substituted *β*-carbolines (F) and designed C3 substituted *β*-carboline-benzimidazole conjugates (**5a-z** & **6a-c**).

On the other hand, benzimidazole moiety is structurally related to purine bases and is found in a variety of natural products, such as vitamin B12. 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 activity and DNA binding affinity.[33] The well-known bisbenzimidazole derivative i.e., Hoechst 33258 is widely used as a 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, ortho esters) has been widely used for the synthesis of benzimidazole scaffold under harsh dehydrating conditions (170-180 ^{o}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, aromatisation reaction of *o*-phenylenediamines with aldehydes under oxidative conditions is considered as 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 $La(NO_3)_3.6H_2O$ as a catalyst.

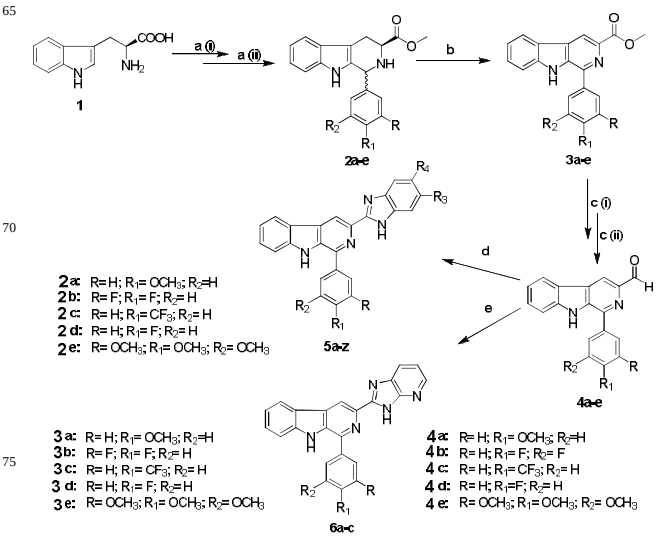
Previous reports on *β*-carboline derivatives revealed that the presence of various substituents at 1, 3 and 9 position was related to their cytotoxic activity against numerous transplanted animal tumours.[39-42] Chen and coworkers reported that 3-chlorobenzyl and 3-phenylpropyl substituents at position-9 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 was very important for the antitumor activity; (ii) the introduction of appropriate substituent's 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 0.3-7.1 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 for 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 & 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 the esterification of *L*-tryptophan using $SOCl_2$ and MeOH, with various benzaldehydes in the presence of 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 nitrogen atmosphere followed by oxidation with Dess Martin periodinane in CH_2Cl_2 provides the corresponding1-aryl substituted-9H-pyrido[3,4-*b*]indole-3-carbaldehydes **4a-e**. Finally, the 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**).



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

A number of synthetic methodologies [45] that are available in the literature for the synthesis of benzimidazoles most of these 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 demand 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 $La(NO_3)_3.6H_2O$ 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 oxidant in EtOH:H_2O (8:2) mixture heated at 70 ^{o}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 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 $La(NO_3)_3.6H_2O$ (10 mol%) was used in EtOH as a solvent produced the best results as shown in **Table 1**. It was observed that further increase in the amount of catalyst had no effect on the yield (**Table 1**, entry 7), whereas reduction in the amount of catalyst resulted in a significant decrease in the isolated yield of

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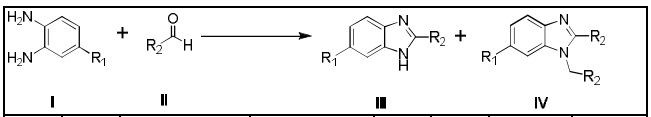
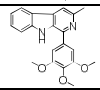
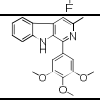
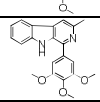
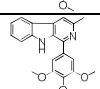
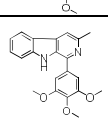
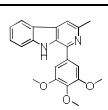
the product (**Table 1**, entry 6). However, there is no considerable increase in the yield even after prolonged reaction time (1 h). It was observed that there no formation of required product in the absence of catalyst (**Table 1** entry 9). Earlier reports also demonstrated the chemoselective property of $La(NO_3)_3.6H_2O$, that has allowed the selective deprotection of acetonides,[47] primary alcohols [48] and preparation of 1,5-benzodiazepines from ketones.[49]

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



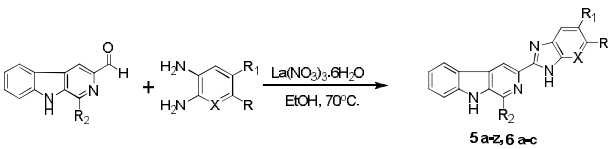
I II III IV

Entry	R_1	R_2	Catalyst/oxidant	Yields (%) III	Yields (%) IV	Solvent	Time
1	H		$Na_2S_2O_5$ (5eqvi)	65	35	EtOH: H_2O (8:2)	8 h
2	H		$Na_2S_2O_5$ (5eqvi)	70	30	EtOH: H_2O (8:2)	8 h
3	OCH_3		$Na_2S_2O_5$ (5eqvi)	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		$La(NO_3)_3.6H_2O$ (10 mol %)	69, 95	trace	EtOH	15 min, 30 min
6	H		$La(NO_3)_3.6H_2O$ (5 mol %)	75, 77	trace	EtOH	30 min, 1h
7	H		$La(NO_3)_3.6H_2O$ (20 mol %)	95	trace	EtOH	30 min
8	H		$La(NO_3)_3.6H_2O$ (10 mol %)	85	trace	DMF	1h
9	H		No catalyst	0	0	EtOH	30min

Cytotoxicity

Most of these conjugates were initially tested at a single dose higher concentration (10 μM) in the sixty-cell lines 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, is 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 result is expressed as the percent growth of treated cells relative to the control following 48 h 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 percentage of growth inhibition (GI_{50}) determined relative to that of untreated control cells.

Table 2 Synthesis of *β*-carboline-benzimidazole conjugates using $La(NO_3)_3.6H_2O$ as catalyst.



5 a-z, 6 a-c

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

The tested compounds showed GI_{50} values in the range of 0.3 to 63 μM. The conjugates which contain 4-methoxyphenyl ring at

C1 and 6-methoxy (**5a**), 6-fluoro (**5d**) substituted benzimidazole at C3; 3,4-difluorophenyl ring at C1, 6-methoxy (**5h**) benzimidazole at C3; 4-fluoro phenyl ring at C1, 6-chlorobenzimidazole (**5r**) at C3 possess significant cytotoxicity. In contrast 4-methoxyphenyl ring at C1 and 6-methyl benzimidazole (**5b**), 5,6-dimethyl benzimidazole (**5c**) at C3; 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, 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} value 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 **Table 4** corroborated with NCI-60 cell line screen, it has been observed that the conjugates which contain 4-methoxy phenyl ring at C1, electron donating groups like methoxy (**5a**), methyl (**5b**), 5,6-dimethyl (**5c**), unsubstituted (**5e**) and weak ring deactivating groups like fluoro (**5d**) on benzimidazole moiety at C3 of *β*-carboline ring possess significant to mederate cytotoxicity, whereas presence of electron withdrawing groups like trifluoromethyl (**5g**) on benzimidazole decreases the cytotoxicity. In addition 3,4-difluorophenyl ring at C1, 6-methoxy benzimidazole (**5h**) at C3; 4-fluorophenyl ring at C1, 6-chlorobenzimidazole (**5r**) at C3 also exhibited promising cytotoxicity.

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

Cancer panel/ Cell line	Growth inhibition : GI_{50} [μM][a]						
	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 578T	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).

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

Compound	[b]HeLa	[c]DU145	[d]A549	[e] BHK-21	[f] L929
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

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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 data represents mean + S.D. from three different experiments performed in triplicates. [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: Mice connective tissue fibroblast cells.

Based on the results that depict the cytotoxic potential it was considered of interest to understand the insights of interaction by these conjugates.

DNA binding studies

CD studies

Circular dichroism studies provide information on changes in DNA conformation upon small molecules interaction which in turn gives further insight in to the mode of DNA-ligand binding.

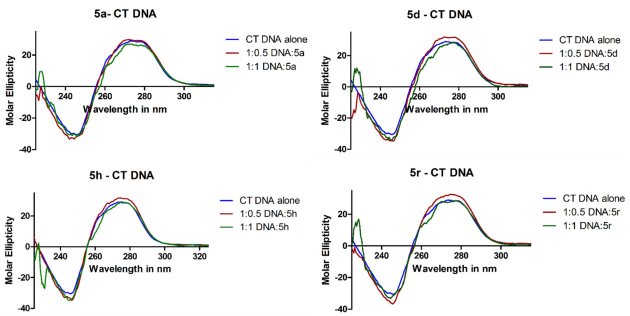

Fig. 2 CD spectra of CT DNA (15 μM) at two concentrations of ligands (**5a**, **5d**, **5h** and **5r**).

Among several biological macromolecules, DNA is known to play a crucial role in the cell proliferation and other biological activities. The CD spectrum of CT DNA exhibits a positive 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 at 1:0.5 DNA:ligand (5a) ratio, manifesting stabilization of the DNA structure on ligand interaction. The negative band intensities gradually reduced indicating 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. Similar type of interaction was also noticed in 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 produce 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 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 case of ligand **5a**, an isobestic point was observed at 325 nm indicating the existence of at least one **5a**-DNA complex with identical molar absorptive. In case of other ligands, a sharp isobestic point was observed at around 335 nm and it shows that their interaction with DNA occurs in a single step.

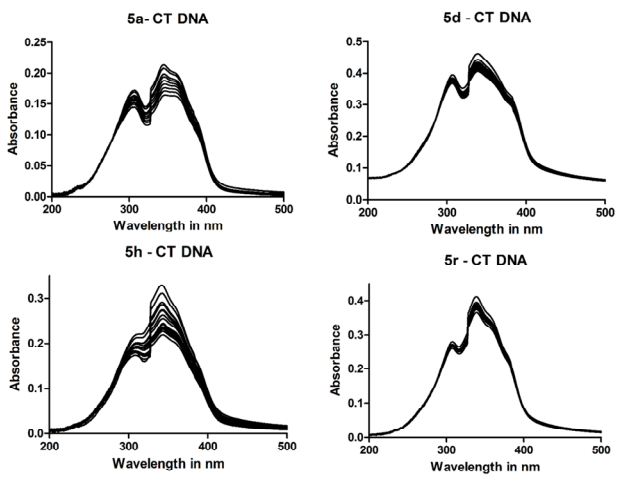

Fig. 3 The UV-Visible absorption changes with the titration of ligands (**5a**, **5d**, **5h** and **5r**) by CT DNA.

DNA intercalation assay

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

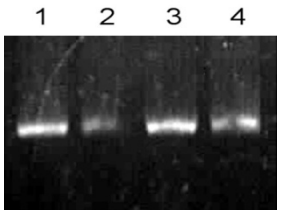

Fig. 4 Agarose gel picture showing the intercalation of **5a**, **5h**, **5r** and **5d** (2 μM) with DNA (5 μM) in Tris buffer (pH 7.0). Lane 1, (**5a** + DNA); lane 2, (**5h** + DNA); lane 3, (**5r** + DNA); and 4 (**5d** + DNA).

After running the DNA-ligand complex for 1h, 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 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 inter system crossing generate an excited triplet state of the hybrid which inturn activate 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 is 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 they were subjected to electrophoresis. The gel electrophoresis separation of pBR322 DNA after UV light irradiation in presence of two different concentrations (100 μM and 200 μM) of **5a**, **5r** and **5d** ligands was shown in **Figure 5 A**. 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, **Figure 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 case 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) and 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 involve 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 –NH group to DNA. From the DNA binding studies it was also observed that **5a**, **5r** and **5d** ligands intercalate with DNA. The above results suggest that these ligands intercalate with DNA and generate the free radicals on exposing to UV light.

With a view to understand 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 band corresponding to Form I diminish gradually, whereas that of Form II enhances upon increasing the irradiation time. After 2 h, the complete disappearance of supercoiled DNA was observed. This indicates that **5a** has higher photocleavage capacity and it increases with irradiation time. (**Fig. 5 B**).

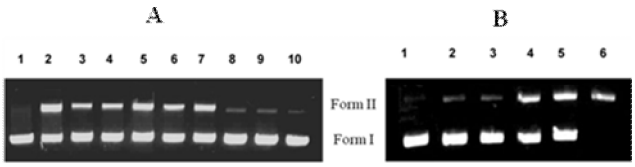

Fig. 5 Lane 1 (A & B) DNA solution irradiated in the absence of compounds; (A) lane 2-4 conjugates **5a**, **5r** and **5d** (100 μM) + pBR 322 plasmid DNA; lane 5-7 conjugates **5a**, **5r** and **5d** (200 μM)+ pBR 322 plasmid DNA; lane 8-10 DNA + conjugates in the absence of light. (B) Effect of **5a** as a function of the irradiation time (0.15, 0.30, 1, 1.5, 2 h; lane 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 transform it to a relaxed form. Topo I inhibitors has gained importance in cancer chemotherapy treatment as they inhibit Topo I and leaves the single stranded breaks in DNA, thereby damaging the genome integrity. Topo I has been identified as the potential target for several anticancer drugs that are clinically under 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 occur by 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 inhibits Topo I at 150 μM.[55]

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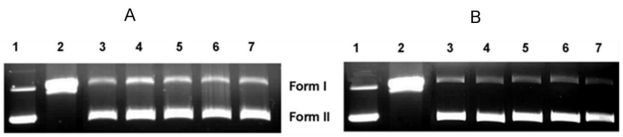

Fig. 6 Effects of **5a**, **5r**, **5d** and **5h** on the activity of DNA topoisomerase I in a cell free system. Lane 1, DNA alone; lane 2, DNA + Topo I; lane 3, DNA + Topo I + Camptothecin (100 μM); lane 4-7, DNA + Topo I + Compound **5a**, **5h**, **5r** and **5d** (100 μM) **A** and same in **B**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

Interestingly, the present *β*-carboline-benzimidazole scaffolds have also shown significant Topo I inhibition at 100 as well as 200 μM concentration (**Figure 5**). 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 in 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 PM3 method using Gaussian 09 package.[56] Coordinates of the protein were obtained from the Protein Data Bank (PDB ID: 1SC7)[57] and necessary correction to the protein structure was carried out using Protein Preparation Wizard in Schrodinger package. The docking studies were performed by using GOLD docking software, docking poses showed that these conjugates intercalate with DNA at the binding site of ternary complex (**Fig 7**). Docking pose for **5a** shows that there is a hydrogen-bonding interaction between the N2 of *β*-carboline ring and guanidine group of Arg364 similar to the cocrystal ligand; besides this it also possess an additional hydrogen bonding interaction between the methoxy group of 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, *β*-carboline ring shows the π- π* interactions with the C10 and G11 DNA base pairs, whereas C1 substitution extends outwards the minor groove. In addition to this, **5a** possess hydrophobic interactions with amino acids near to binding site, in that the C1 substitution shows interaction with the Asp533 and Ile535, *β*-carboline ring with Asn722 and Arg364 and benzimidazole ring with Glu365 and Lys425. As compare to the cocrystal ligand, these conjugates exhibit high gold score. These studies indicate that the C1 and C3 substituted *β*-carbolines are capable to fit properly in the binding site of the DNA topoisomerase I.

The biophysical studies already showed that these conjugates possess DNA intercalation property. Therefore, docking studies were performed to obtain a better insight into the binding mode of these conjugates to the DNA. Coordinates of the DNA were obtained from the Protein Data Bank (PDB ID 1NAB).[58] Necessary corrections to the crystal structure was carried out using protein preparation wizard in Schrodinger. Docking studies showed that all conjugates (**5a**, **5h**, **5r** and **5d**) bind to the DNA through intercalation. Docking pose of **5a** shows that *β*-carboline ring lies at the central part of the DNA intercalation cavity and 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 benzimidazole substitution towards the major groove (**Fig 8**).

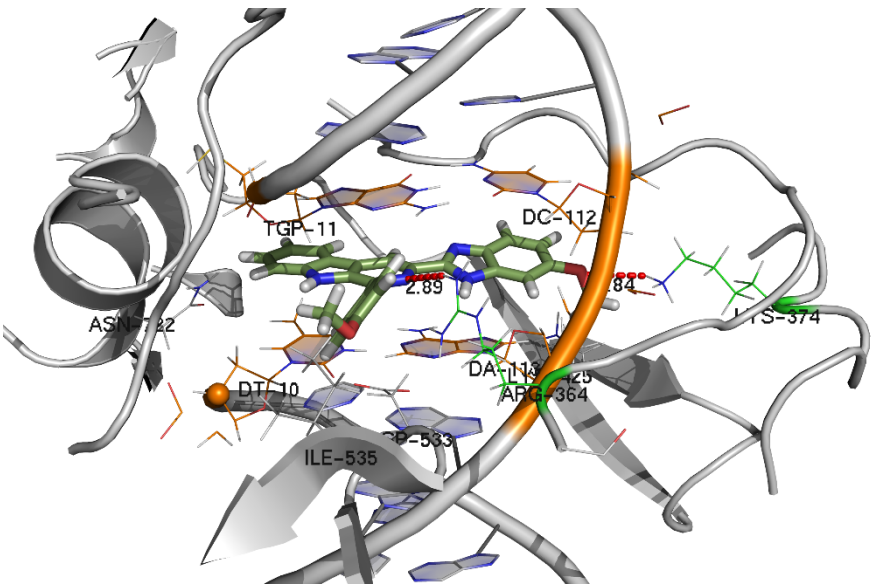

Fig. 7 Docking pose for **5a** in DNA Topo I; Hydrogen bonds are shown in red dotted lines, and hydrogen bonding residues are shown in green colour, nucleic acid residues, which are showing π- π stacking, are shown in brown colour, amino acids having hydrophobic interactions are shown in gray colour.

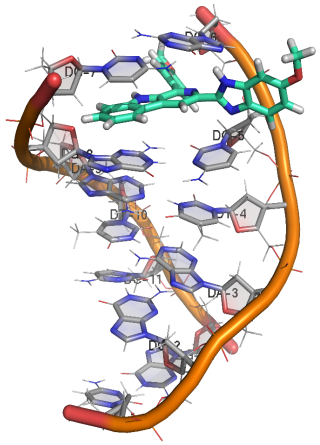

Fig. 8 Docking pose for **5a** in DNA showing intercalation-binding mode.

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

In silico computational studies

An *in silico* computational study for 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 (www.molinspiration.com)[61] and OSIRIS Property Explorer (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 is 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 logP < 5.[60-62] Compounds that violate more than one of these parameters could have problems relating to bioavailability.

Table 5 Lipinsk's parameters and % ABS, TPSA, LogS for compounds **5a-d**, **5h**, **5r** and **5w**.

			Lipinsk's parameters					
compound	**%ABS**	**TPSA[a] (A^2)**	**nHBA (ON)**	**nHBD (OHNH)**	**logP[b]**	**MW**	**n violations**	**logS[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]www.molinspiration.com; [b]www.organic-chemistry.org/prog/peo; %ABS = 109 − 0.345 × TPSA; Number hydrogen bond acceptor (NO)= nHBA ≤ 10; Number hydrogen bond donors (OHNH) = nHBD ≤ 5; MW ≤ 500; Octanol-water partition coefficient = LogP < 5; Solubility = LogS > −4.

Conclusion

In summary, we have synthesized a series of *β*-carboline-benzimidazole conjugates bearing substituted benzimidazole moiety at C3 and substituted aryl group at C1. The final key step of the benzimidazole ring formation was carried out using $La(NO_3)_3.6H_2O$ 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 active irrespective of the SAR analysis. The representative conjugates (**5a**, **5d**, **5h** and **5r**) showed potential cytotoxic activity with GI_{50} values ranging from 0.36-7.1 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 carried out by using pBR322 plasmid DNA in presence of UV light. This study suggests that **5a** has the potential to generate singlet oxygen species which cleaves DNA by converting supercoiled form to 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 therapeutic. Detailed cellular and molecular biology as well as *in vivo* studies are actively under 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 performed on silica gel glass plates containing60 GF-254 and visualization was achieved by UV light or iodine indicator monitored reactions. Column chromatography performed with Merck 60–120 mesh silica gel.^{1}H and ^{13}C spectra 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 an internal TMS standard. ESI spectra were recorded on a Micro-mass Quattro LC using ESI+ software with capillary voltage 3.98 kVand 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. ^{1}H NMR and ^{13}C NMR spectra of final compounds **5a-z**, **6a-c** is provided in the Supporting information.

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 drop wise at 0 ^{o}C and continued stirring for 6.0 h at room temperature. Then, the excess amount of solvent was removed under vacuum and the crude product was codistilled with toluene (2×10 mL) to obtain solid. Then, the resulting solid was dissolved in CH_2Cl_2, washed with saturated $NaHCO_3$ solution, extracted with excess amount of CH_2Cl_2. Then, the combined organic layers were dried over anhydrous sodium sulphate and concentrated under vacuum to obtain white solid product. To a mixture of tryptophan ester (0.023 mol) and substituted benzaldehyde (0.023 mol) in toluene (50 mL), catalytic amount of PTSA was added and the mixture was refluxed for 24 h. After completion of the reaction, solvent was removed under vacuum. The crude 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 used for the next step without further purification.

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

The suspension of 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 stood at 4 ^{o}C for 3 h to obtain precipitate. Then, the precipitate was filtered, washed with petroleum ether and dried. The obtained solid was recrystallized by using ethyl acetate to afford products (**3a-e**) with high purity.

*Methyl 1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxylate (**3a**).*White solid; 80% yield; mp: 228-230 ^{o}C; ^{1}H 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)-9H-pyrido[3,4-b]indole-3-carboxylate (**3b**).*White solid; 71% yield; mp: 236-239 ^{o}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)-9H-pyrido[3,4-b]indole-3-carboxylate (**3c**).* Pale yellow solid; 72% yield; mp: 252-254 ^{o}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)-9H-pyrido[3,4-b]indole-3-carboxylate (**3d**).*White solid : 76% yield; mp: 197-198 ^{o}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)-9H-pyrido[3,4-b]indole-3-carboxylate (**3e**).* Yellow solid: 76% yield; mp: 229-230 ^{o}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 ^{o}C to 0 ^{o}C in an ice-salt (NaCl) bath. To the reaction mixture LAH (0.076 mol) was added slowly portion wise and continued stirring at room temperature for about 4 h. After completion of the reaction further the reaction mixture was cooled to 0 ^{o}C and the excess of LAH was quenched with Na_2SO_4 paste, filtered on Buckner funnel washed with MeOH (2 x 20 mL).The filtrate was dried over anhydrous Na_2SO_4 concentrated under vacuum. The crude obtained (0.014 mol) was taken in dry CH_2Cl_2 (100 mL). To that DMP (0.021 mol) was added, stirred at room temperature for about 2 h. After the completion of reaction the reaction mixture was washed with Water (2x100 mL) the organic layer was dried over anhydrous Na_2SO_4, 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)-9H-pyrido[3,4-b]indole-3-carbaldehyde (**4a**).*Pale yellow solid; 80% yield; mp: 218-220 ^{o}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)-9H-pyrido[3,4-b]indole-3-carbaldehyde (**4b**).* Pale Yellow solid: 84% yield; mp: 225-228 ^{o}C; ^{1}H NMR

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(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)-9H-pyrido[3,4-b]indole-3-carbaldehyde (**4c**).*Pale yellow solid: 84% yield; mp 280-283 ^{o}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* = 7.9Hz, 1H), 8.28 (t, *J* = 8.1Hz, 2H), 8.01 (d, *J* = 8.1Hz, 2H), 7.61-7.71 (m, 2H), 7.36 (t, *J*= 7.9Hz, 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)-9H-pyrido[3,4-b]indole-3-carbaldehyde(**4d**).*white solid of 90% yield; mp: 189-190 ^{o}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)-9H-pyrido[3,4-b]indole-3-carbaldehyde (**4e**).* Pale Yellow solid: 80% yield; mp: 213-214 ^{o}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** (1equiv) and respective *o*-phenylenediamine (1equiv) in ethanol (20 mL), 10 mol% of catalyst $(La(NO_3)_3.6H_2O)$ was added. The reaction mixture was heated at 60 ^{o}C for a stipulated time mentioned in Table 2. After the completion of reaction, ethanol was removed under vacuum. The reaction crude was dissolved in ethyl acetate (50 mL) and washed with water (2 x 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-1H-benzo[d]imidazol-2-yl)-1-(4-methoxyphenyl)-9H-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 ^{o}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-1H-benzo[d]imidazol-2-yl)-9H-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 ^{o}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-1H-benzo[d]imidazol-2-yl)-1-(4-methoxyphenyl)-9H-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 ^{o}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-1H-benzo[d]imidazol-2-yl)-1-(4-methoxyphenyl)-9H-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 ^{o}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-(1H-benzo[d]imidazol-2-yl)-1-(4-methoxyphenyl)-9H-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 ^{o}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)-9H-pyrido[3,4-b]indol-3-yl)-1H-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 ^{o}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)-1H-benzo[d]imidazol-2-yl)-9H-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 ^{o}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}CNMR (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-1H-benzo[d]imidazol-2-yl)-9H-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 ^{o}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)-9H-pyrido[3,4-b]indol-3-yl)-1H-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 ^{o}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-1H-benzo[d]imidazol-2-yl)-9H-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 ^{o}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-1H-benzo[d]imidazol-2-yl)-1-(3,4-difluorophenyl)-9H-pyrido[3,4-b]indole (**5k**).* This compound was prepared according to the general procedure, employing **4b** (200 mg, 0.64 mmol) and4-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 ^{o}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,

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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-1H-benzo[d]imidazol-2-yl)-9H-pyrido[3,4-b]indole (**5l**).* This compound was prepared according to the general procedure, employing **4b** (200 mg, 0.64 mmol) and4-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 ^oC; 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; ^1H 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-1H-benzo[d]imidazol-2-yl)-1-(3,4-difluorophenyl)-9H-pyrido[3,4-b]indole (**5m**).* This compound was prepared according to the general procedure, employing **4b** (200 mg, 0.64 mmol) and4-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 ^oC; 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; ^1H 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-(1H-benzo[d]imidazol-2-yl)-1-(3,4-difluorophenyl)-9H-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 ^oC; 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; ^1H 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-1H-benzo[d]imidazol-2-yl)-1-(4-(trifluoromethyl)phenyl)-9H-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 ^oC; 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; ^1H 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-(1H-benzo[d]imidazol-2-yl)-1-(4-(trifluoromethyl)phenyl)-9H-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 ^oC; 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; ^1H 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-1H-benzo[d]imidazol-2-yl)-1-(4-(trifluoromethyl)phenyl)-9H-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 ^oC; 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; ^1H 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-1H-benzo[d]imidazol-2-yl)-1-(4-fluorophenyl)-9H-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 ^{o}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-(1H-benzo[d]imidazol-2-yl)-1-(4-fluorophenyl)-9H-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 ^{o}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-1H-benzo[d]imidazol-2-yl)-9H-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 ^{o}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-1H-benzo[d]imidazol-2-yl)-1-(4-fluorophenyl)-9H-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 ^{o}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)-9H-pyrido[3,4-b]indol-3-yl)-1H-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 ^{o}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-(1H-benzo[d]imidazol-2-yl)-1-(3,4,5-trimethoxyphenyl)-9H-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 ^{o}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-1H-benzo[d]imidazol-2-yl)-1-(3,4,5-trimethoxyphenyl)-9H-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 ^{o}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-1H-benzo[d]imidazol-2-yl)-1-(3,4,5-trimethoxyphenyl)-9H-pyrido[3,4-b]indole (**5y**).*This compound

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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 ^{o}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-1H-benzo[d]imidazol-2-yl)-1-(3,4,5-trimethoxyphenyl)-9H-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 ^{o}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-(3H-imidazo[4,5-b]pyridin-2-yl)-1-(4-methoxyphenyl)-9H-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 ^{o}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-(3H-imidazo[4,5-b]pyridin-2-yl)-9H-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 ^{o}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-(3H-imidazo[4,5-b]pyridin-2-yl)-1-(4-(trifluoromethyl)phenyl)-9H-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 ^{o}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 have cultured in Eagle's minimal essential medium (MEM) containing nonessential amino acids, 1mM sodium pyruvate, and 10% FBS. Hela (human epithelial cervical cancer), A549 (human lung carcinoma epithelial) and L929 (Mice 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 maintained under humidified atmosphere of 5% CO_2 at 37 ^{o}C. Cells were trypsinized when sub confluent from T75 flasks/90mm dishes and seeded on to 96 well test plates at a concentration of 1×10^4 cells/mL in complete medium, treated with compounds at desired concentrations and harvested as required.[63]

Cytotoxicity

Cell proliferation and viability was determined by 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 that 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 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 ^oC. 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 TECAN multimode reader. The growth percentage of each treated well of 96 well plate have 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 defines as a concentration of compound that produced 50% absorbance reduction relative to control.[64]

CD studies

Circular dichroism experiments were carried out using JASCO 815 CD spectro polarimeter (Jasco, Tokyo, Japan). All the CD titrations were performed in 100 mM KBPES buffer (pH 7.0) at 25 ^oC. CD spectrum was recorded from 200 to 350 nm and for each experiment, $15x10^{-6}$ 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 ABI Lambda 40 UV-Vis spectrophotometer (Foster City, USA) at 25 ^oC using 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 ligand was prepared in 1:1 water: methanol mixture and diluted to required concentration in suitable buffer solutions. The quartz cells were thoroughly cleaned with distilled water and 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 were 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 ^oC for 1 h. After incubation, the ligand-DNA mixture was resolved by using 0.8% agarose gel. Sample treated with 2 µM ethydiumbromide was considered as 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 ligands were added and the total volume was maintained at 20 µL. The DNA samples with complexes were taken in 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 dark reaction, 100 µM ligand and 0.45 µg pBR322 plasmid DNA was taken in a PCR tube and the samples were wrapped with aluminium foil and placed them in dark. Then, samples were analysed by gel electrophoresis on a 0.8% agarose horizontal slab gel containing 0.5 µg/ml ethidium bromide in Tris-EDTA buffer (40 mM Tris, 20 mM acetic acid and 1mM EDTA, pH 8.0) at 10 V Cm^{-1}. Gels were photographed under UV light with the 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 procedure.[66] 0.5 µg of pBR322 DNA was incubated with 1 unit of Topo I (Invitrogen) in 1X NEB buffer (50 mM potassium acetate, 20 mM tris acetate buffer, 10 mM magnesium acetate, 1 mM DTT). The ligands to be studied was added to the Topo I-DNA complex and incubated at 37 ^oC for 30 min, allowing the formation of ternary enzyme-DNA-ligand complexes. Then, the enzyme was inactivated by increasing the temperature to 65 ^oC. 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 positive control.

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Notes and references

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A series β-carboline-benzimidazole conjugate were synthesized using lanthanum nitrate as a novel catalyst and evaluated for their anticancer activity.