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Research paper

Design, synthesis and antitumour evaluation of pyrrolo[1,2-*f*]-phenanthridine and dibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinoline derivatives

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ABSTRACT

A series of 1,2-bis(hydroxymethyl)pyrrolo[1,2-*f*]phenanthridine derivatives and their alkyl (ethyl and isopropyl) carbamates and 12,13-bis(hydroxymethyl)-9,14-dihydro-dibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinoline derivatives were synthesized for antiproliferative evaluation. The preliminary antitumour studies revealed that these two types of bis(hydroxymethyl) derivatives showed significant antitumour activities and were able to inhibit the growth of various human tumour cell lines *in vitro*. Several of the derivatives were demonstrated to cause DNA interstrand cross-links by an alkaline agarose gel shifting assay. These conjugates were cytotoxic to a variety of cancer cell lines by inducing DNA damage, delaying cell cycle progression in the G2/M phase and triggering apoptosis. Compound **21a**, dissolved in a vehicle suitable for intravenous administration, was selected for antitumour studies in animal models. We demonstrated that at a dose that did not cause body weight loss in mice, compound **21a** could significantly suppress the growth of tumour xenografts of human lung cancer H460 and colorectal cancer HCT-116 cells in nude mice. Our present results confirm the antitumour activities of these conjugates.

1. Introduction

Cancer is one of the major leading causes of death worldwide. The design of new antitumour agents is one of the most challenging tasks in the field of medicinal chemistry. Among anticancer agents, DNA alkylating agents have attracted attention and have been widely used as potential therapeutic agents for a long time. Notably, DNA-damaging therapeutic agents are widely used in combination therapy with targeted therapeutics as well as immunotherapeutics in clinical settings [1–4].

Naturally occurring mitomycin C (MMC, **1**, Fig. 1) is a clinically useful chemotherapeutic agent for treating various cancers [5]. Both MMC and synthetic indoloquinone EO9 (**2**) [6], which possess two reactive nucleophilic centres on its pyrrole, are capable of inducing DNA cross-linking via bioreductive activation [7]. Numerous pyrrolizine alkaloids [8–10] and their synthetic analogues bearing a bis(hydroxymethyl)pyrrolidine moiety, such as IPP (**3**) [11], are also capable of inducing DNA interstrand or intrastrand cross-linking (CL), giving them potent antitumour activities [12]. Numerous studies had shown that synthetic bis(hydroxymethyl or alkylcarbamate)pyrroles or pyrrolizines were able to generate an electrophilic centre on the pyrrole ring, and hence reacted with DNA to induce DNA interstrand cross-linking (ICL) via an electrophilic reaction (Fig. 2) [13,14]. Obviously, these agents do not require bioreductive activation to induce DNA CL.

To explore new bifunctional DNA alkylating agents, we previously synthesized 3*a*-azacyclopenta[*a*]indene derivatives (**4**) (wherein R^1 = H or CONH-alkyl; R^2 = alkyl or aryl), which contain a bis(hydroxymethyl)pyrrole alkylating pharmacophore and was viewed as a "benzologue" of IPP (**3**). Among these congeners, compound BO-1012 (**5**) exhibited significant *in vitro* cytotoxicity

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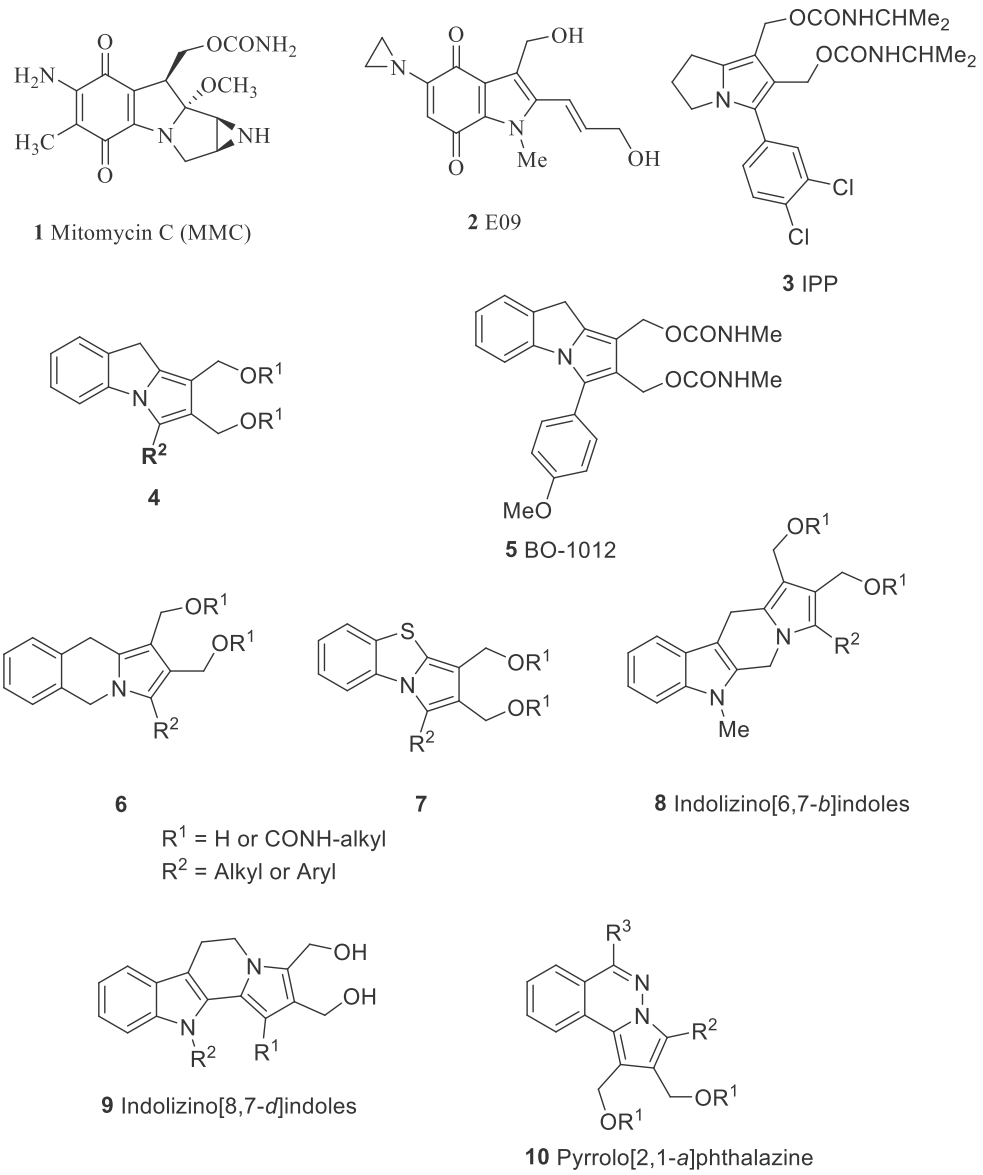


Fig. 1. Structures of antitumour mitomycin C and bis(hydroxymethyl)pyrrole derivatives.

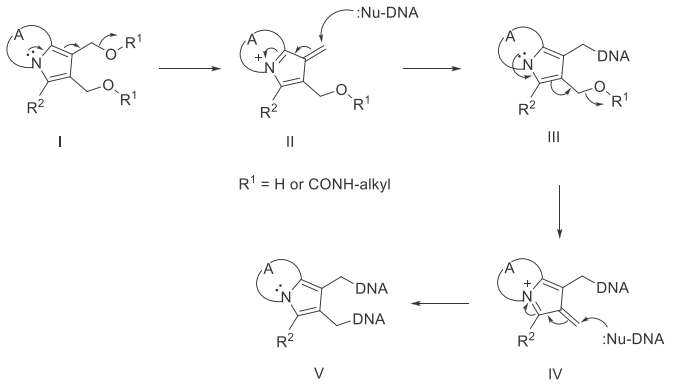


Fig. 2. Plausible DNA cross-linking mechanism by bis(hydroxymethyl or alkylcarbamate)pyrrole derivatives via the formation of electrophilic centre on the pyrrole ring and electrophilic reaction with DNA.

and potent therapeutic efficacy in nude mice bearing cisplatin-resistant lung or bladder cancer [15]. We further synthesized a series of "bioisosteres" of 3*a*-azacyclopenta[*a*]indenes (**4**), namely, 5,10-dihydropyrrolo[1,2-*b*]isoquinolines (**6**) and benzo[*d*]pyrrolo [2,1-*b*]thiazoles (**7**), which also displayed potent antitumour activities by inhibiting the growth of a variety of human leukaemia and solid tumour cell lines *in vitro* and *in vivo* [16,17].

To further broaden the chemical space of bifunctional DNA alkylating agents containing bis(hydroxymethyl) pyrrole alkylating pharmacophores, we took advantage of a hybrid approach to synthesize a series of indolizino[6,7-*b*]indole derivatives (**8**) [18] and indolizino[8,7-*d*]indoles (**9**) [19]. These analogues include β-carboline (DNA topo I and II inhibition moiety) and bis(hydroxymethyl)pyrrole (DNA ICL moiety), as shown in Fig. 1. As expected, these hybrids exhibited multiple modes of action, including induction of DNA ICLs and inhibition of topo I and II [18]. Of these analogues, BO-1978 (**8**, wherein R^1 = H, R^2 = Et) significantly

suppressed the growth of EGFR wild-type and mutant non-small-cell lung cancer (NSCLC) cells in xenograft and orthotopic lung tumour models [18]. Furthermore, the combination of BO-1978 with gefitinib further suppressed EGFR mutant NSCLC cell growth *in vivo* [20]. Additionally, we coupled a bis((hydroxymethyl)pyrrole) pharmacophore with phthalazines (an anti-angiogenic moiety) to generate new pyrrolo[2,1-*a*]phthalazine (**10**) hybrids [21]. We demonstrated that these conjugates were cytotoxic to a variety of cancer cell lines by inducing DNA ICLs and inhibiting the phosphorylation of VEGFR in endothelial cells, leading to cancer cell killing as well as the suppression of vascular formation.

More recently, we synthesized a series of new antitumour bis(hydroxymethyl)pyrrole derivatives (Fig. 3), namely, 1,2-bis(hydroxymethyl)pyrrolo[1,2-*f*]phenanthridine derivatives (Class I) and 12,13-bishydroxymethyl-9,14-dihydrodibenzo[*f,h*]pyrrolo[1,2-*b*]isoquinoline derivatives (Class II), via a hybrid approach for anticancer evaluation. Compounds of Class I contain a phenanthridine moiety, which is commonly found in natural products with anticancer activity [22–25]. For example, an *N*-[2-(dimethylamino)ethyl]phenanthridine-4-carboxamide derivative (**11**, Fig. 4) exhibited DNA-intercalating activity and displayed moderate *in vivo* antitumour activity against P388 leukaemia and Lewis lung carcinoma cells [26]. Likewise, the phenanthridinium scaffold was used to design a number of DNA-intercalating agents with antitumour properties. For instance, ethidium bromide (**12**) is a well-known DNA-intercalating agent commonly used as a fluorescent tag (nucleic acid stain) in molecular biology laboratories for techniques such as agarose gel electrophoresis [27]. Moreover, phenanthriplatin derivatives (**13** and **14**) are hybrid molecules of a phenanthridinium moiety and a platinum(II) diamine. The former was synthesized by directly conjugating cisplatin to the phenanthridinium cation [28], and the latter was formed by tethering platinum via a polymethylene chain (n = 3, 5, 8 and 10) to the phenanthridinium cation (**14**) [29]. The antitumour activity of phenanthriplatins is substantially greater than that of cisplatin and pyriplatin because of the hydrophobicity of the phenanthridine ligand.

Compounds of Class II possess a phenanthroindolizine moiety, as phenanthroindolizidine alkaloids are commonly isolated from plants [30,31]. Various analogues have been synthesized for anticancer studies [32–35]. For instance, (R)-antofine (**15**, Fig. 4) significantly inhibited various cancer cell lines at nanomolar concentrations and induced cell arrest in the G2/M phase in human colon Col2 cells [36,37]. Naturally occurring tylophorine (**16**) and its synthetic analogues exhibited significant inhibitory effects against the growth of human hepatocellular carcinoma HepG2 and human nasopharyngeal carcinoma KB cells and potent tumour growth suppression activity in xenograft models [38]. Tylophorine inhibited cyclic AMP response elements, activator protein-1 sites, or nuclear factor-κB binding site-mediated downstream transcription in HepG2 cells, indicating that phenanthroindolizine derivatives have a mode of action different from those of known antitumour drugs [38].

Based on the above reports, phenanthridine or phenanthroindolizine moieties may play an important role in enhancing the DNA/drug interaction and thus may increase antitumour activity with a novel mechanism of action. We have synthesized several Class I and II derivatives (Fig. 3), including 1,2-bis(hydroxymethyl)pyrrolo[1,2-*f*]phenanthridine derivatives and their corresponding alkylcarbamates (where R^1 = H, Me, Et, aryl; R^2 = CONHEt or CONH*i*-Pr, Class I) and 12,13-bis(hydroxymethyl)-9,14-dihydrodibenzo[*f,h*]pyrrolo[1,2-*b*]isoquinoline derivatives (where R^1 = H, Me, Et, aryl, R^2 = H, Class II). The Class I and II derivatives, bearing various substituents at C3 or C12, respectively, will allow us to study their structure-activity relationships. Additionally, the heterocyclic nitrogen of the pyrrole moiety fused to the phenanthridine ring (Class I) and the 1,2,3,4-tetrahydro-dibenzo[*f,h*]isoquinoline (Class II) allowed us to compare the biological activities of these structurally distinct types of chemical compounds. As mentioned previously, the electronic properties of the substituent(s) on the pyrrole may influence the ability of the compound to induce DNA cross-linking and its antitumour activity. We report herein that the newly synthesized compounds exhibited significant cytotoxicity against various human cancer cell lines and significant tumour suppression *in vivo*. Furthermore, these analogues induced DNA ICLs, cell cycle interference and apoptotic cell death.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of 1,2-Bis(hydroxymethyl)pyrrolo[1,2-f] phenanthridine derivatives (Class I)

The syntheses of 1,2-bis(hydroxymethyl)pyrrolo[1,2-*f*]phenanthridine derivatives (**21a-f**) and their bis(alkylcarbamate) derivatives (**22a-f** and **23a-f**) are shown in Scheme 1. Commercially available phenanthridine (**17**) was treated with bromoacetic acid in acetonitrile to give the *N*-(carboxymethyl)phenanthridinium

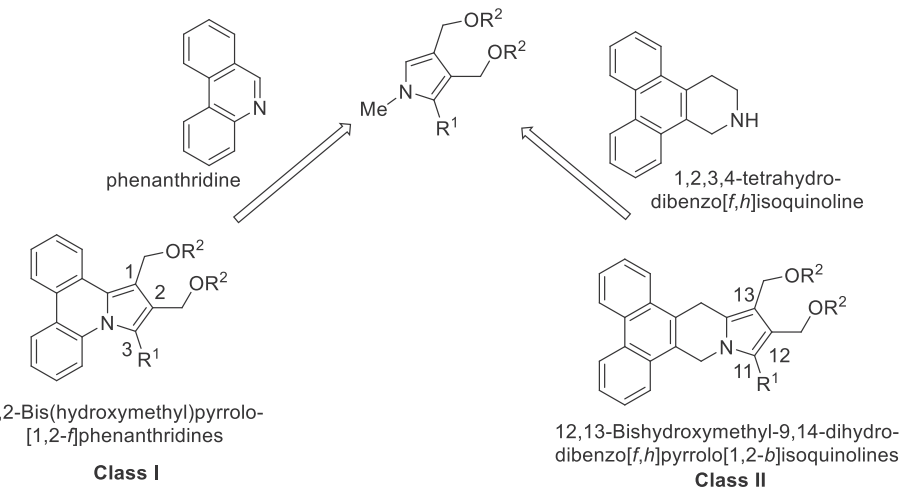


Fig. 3. Design and synthesis of pyrrolo[1,2-*f*]phenanthridine and dibenzo[*f,h*]pyrrolo[1,2-*b*]isoquinoline derivatives via a hybrid approach.

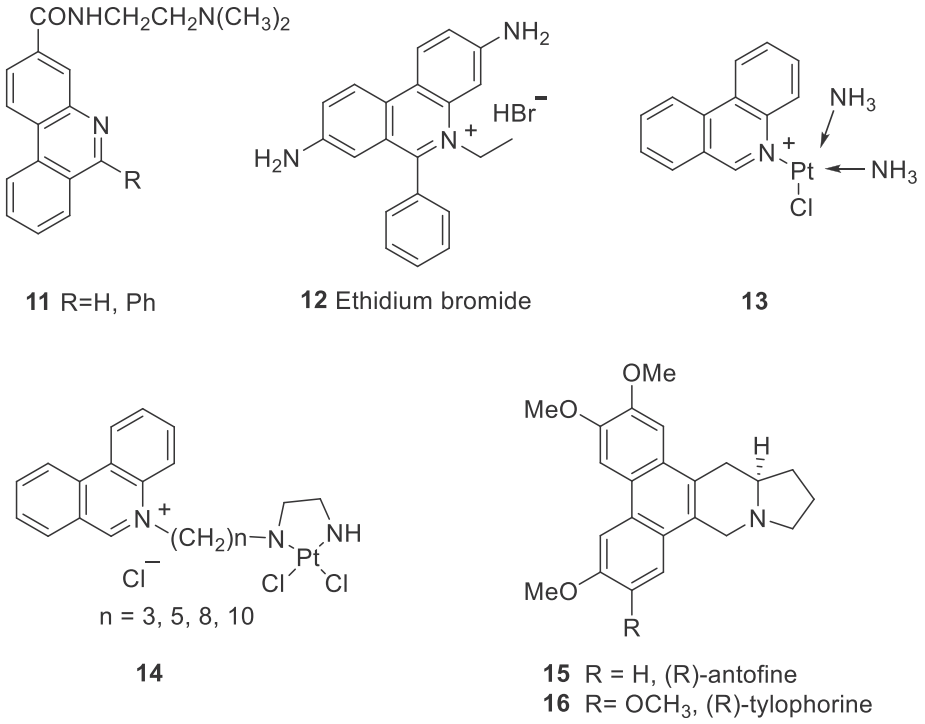
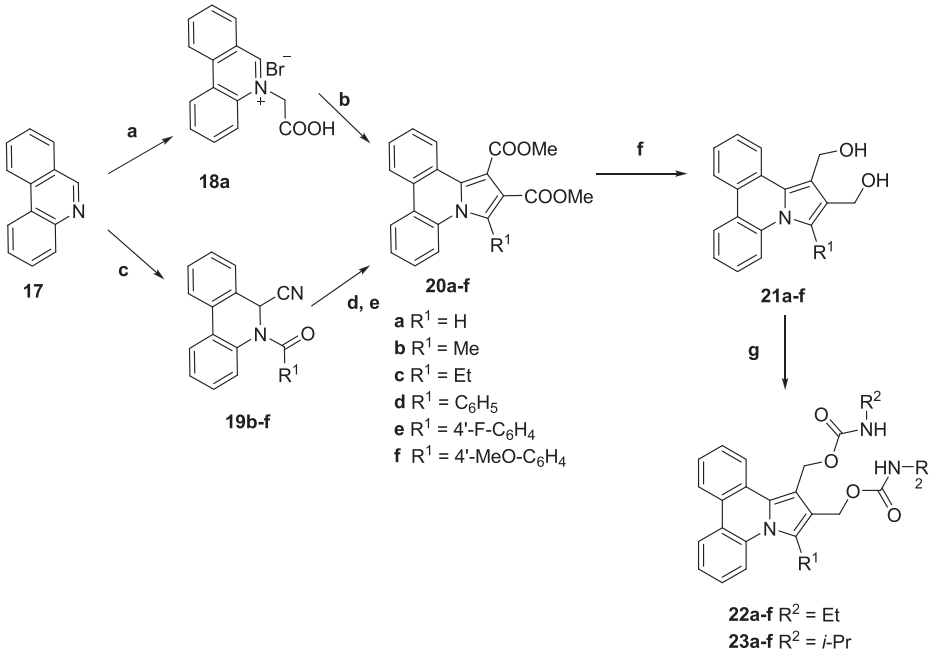


Fig. 4. Structures of several anticancer phenanthridine derivatives (**11**–**14**) and phenanthroindolizine derivatives (**15**–**16**).



[a]Reagents and reaction conditions: (a) Bromoacetic acid, ACN, 80°C; (b) DMAD, TEA, toluene, 100°C; (c) TMSCN, $AlCl_3$, MDC, R^1COCl, rt; (d) HBF_4, AcOH, 60°C; (e) DMAD, DMF, 100°C; (f) $LiAlH_4$, Ether, DCM, 0-10°C, (g) R^2NCO, TEA/THF or DMF

Scheme 1. Chemical synthesis of pyrrolo[1,2-*f*]phenanthridine derivatives[a].

bromide salt (**18a**), which was then reacted with dimethyl acetylenedicarboxylate (DMAD) and trimethylamine (TEA) in toluene at reflux to yield diester **20a** (wherein $R^1 = H$) by using a procedure developed previously [39]. The diester derivatives (**20b-f**) having R^1 substituents other than H were also prepared starting from phenanthridine (**17**). The reaction of **17** with trimethylsilyl cyanide and various acyl chlorides in dichloromethane (DCM) with a catalytic amount of $AlCl_3$ afforded phenanthridine-6-carbonitriles **19b-f** by

following the previously described procedure [21]. Compounds **19b-f** were treated with tetrafluoroboric acid in acetic acid to give the hydrofluoroborate salt intermediates, which were then reacted with DMAD to give desired diesters **20b-f**. The diester functions of **20a-f** were reduced to the corresponding bis(hydroxymethyl) groups (**21a-f**) with lithium aluminium hydride (LAH) in a mixture of ether/DCM in an ice bath. Compounds **21a-f** were then converted to the corresponding ethyl carbamates or isopropyl carbamates (**22a-f** and **23a-f**, respectively) in good yields by treatment with ethyl or isopropyl isocyanates under basic conditions.

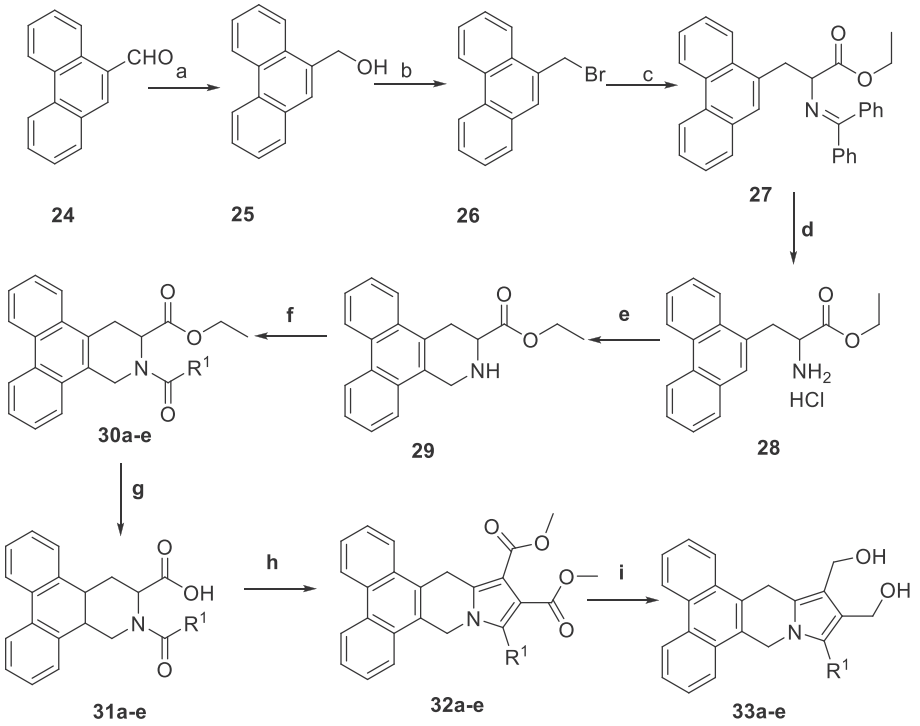
2.1.2. Synthesis of 2,13-bishydroxymethyl-9,14-dihydrodibenzo[f,h] pyrrolo[1,2-b]-isoquinoline derivatives (Class II)

The syntheses of Class II compounds **33a-e** are shown in Scheme 2. Commercially available 9-phenanthrene carboxaldehyde (**24**) was reduced with $NaBH_4$ to the corresponding known alcohol (**25**) [40], which was then treated with PBr_3 to afford 9-(bromomethyl) phenanthrene **(26)** [41]. The reaction of compound **26** with diphenyl methylene-glycine ethyl ester in the presence of K_2CO_3 afforded compound **27**, which was further treated with concentrated HCl to yield compound **28**. The Pictet-Spengler cyclization of **28** by treatment with formaldehyde (37%) in a mixture of DCM/ trifluoroacetic acid (TFA) gave dibenzo[*f,h*]isoquinoline **29**. Compound **29** was reacted with various acid chlorides or acid anhydrides in the presence of TEA to produce *N*-acetyl derivatives **30a-e**. Hydrolysis of **30a-e** under basic conditions (1 *N* aqueous sodium hydroxide in ethanol) yielded corresponding carboxylic acid derivatives **31a-e**, which were further converted to diesters **32a-e** by treatment with DMAD in Ac_2O at 100 °C. The reaction of diesters **32a-e** with LAH in a mixture of ether/DCM yielded bis(hydroxymethyl) derivatives **33a-e**. Attempts to convert **33a-e** into their corresponding bis(alkylcarbamate) derivative congeners failed because of the instability of **33a-e** under the reaction conditions. A similar result was observed in the synthesis of the alkylcarbamate of bis(hydroxymethyl)pyrroloindolizino[8,7-*d*]indoles (**9**) as previously reported [19].

2.2. Biological results

2.2.1. In vitro cytotoxicity

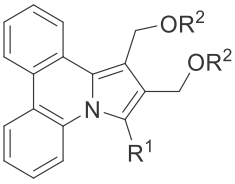
We first evaluated the anti-proliferative activities and studied the structure-activity relationships (SARs) of the newly synthesized compounds against human lymphoblastic leukaemia (CCRF-CEM) and various human solid tumour cells *in vitro*. As shown in Table 1, among the tested bis(hydroxymethyl) derivatives (**21a-f**), **21a** (R^1 = H) was the most cytotoxic against CCRF/CEM cells, with an IC_{50} value of 0.37 μM. The cytotoxicities of the tested compounds clearly decreased as the size of the substituent at C3 increased [e.g.,



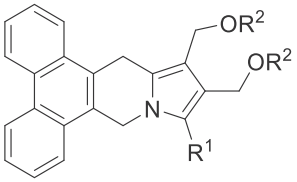
[a]Reagents and reaction conditions: (a) $NaBH_4$/THF, IPA, rt, (b) PBr_3, DCM, 0°C, (c) diphenylmethylene-glycine ethyl ester, K_2CO_3, ACN, 80°C, (d) Ethylacetate: HCl, rt, (e) HCHO, TFA, 50°C, (f) R^1COCl or $(R^1CO)_2O$, TEA, THF, rt, (g) Ethanol/ 1 *N* NaOH solution, rt, (h) DMAD/Ac_2O, 100°C, (i) $LiAlH_4$, ether/DCM, 0°C,

Scheme 2. Substituted phenanthroindolizine derivatives.

Table 1
Cytotoxicities of the newly synthesized pyrrolo[1,2-*f*]phenanthridine derivatives (**21a-f**, **22a-f** and **23a-f**) and dibenzo[*f,h*]pyrrolo[1,2-*b*]isoquinoline (**33a-e**).



21a-f, 22a-f and 23a-f



33a-e

Compound	R^1	R^2	IC_{50} (μM)[a]	
			CCRF-CEM	CEM/VBL
21a	H	H	0.37 ± 0.01	0.38 ± 0.01 [1.03 ×][b]
21b	Me	H	0.91 ± 0.05	0.55 ± 0.01 [0.60 ×]
21c	Et	H	7.39 ± 1.21	2.89 ± 0.18 [0.39 ×]
21d	C_6H_5	H	9.82 ± 1.59	10.93 ± 0.70 [1.11 ×]
21e	4′-F-C_6H_4	H	8.09 ± 0.43	6.44 ± 0.39 [0.80 ×]
21f	4′-MeO-C_6H_4	H	6.34 ± 0.46	4.77 ± 0.74 [0.75 ×]
22a	H	CONHEt	1.28 ± 0.34	1.24 ± 0.55 [0.97 ×]
22b	Me	CONHEt	0.37 ± 0.05	0.37 ± 0.06 [1.00 ×]
22c	Et	CONHEt	1.89 ± 0.20	1.68 ± 0.15 [0.89 ×]
22d	C_6H_5	CONHEt	4.09 ± 0.55	2.48 ± 0.39 [0.60 ×]
22e	4′-F-C_6H_4	CONHEt	3.41 ± 0.34	3.40 ± 0.29 [1.00 ×]
22f	4′-MeO-C_6H_4	CONHEt	3.01 ± 0.58	2.85 ± 0.39 [0.95 ×]
23a	H	CONH-*i*-Pr	1.75 ± 0.19	2.21 ± 1.29 [1.26 ×]
23b	Me	CONH-*i*-Pr	0.35 ± 0.02	0.34 ± 0.02 [0.98 ×]
23c	Et	CONH-*i*-Pr	1.89 ± 0.30	1.55 ± 0.07 [0.82 ×]
23d	C_6H_5	CONH-*i*-Pr	3.15 ± 0.45	0.48 ± 0.31 [1.42 ×]
23e	4′-F-C_6H_4	CONH-*i*-Pr	4.10 ± 0.08	3.17 ± 0.17 [0.77 ×]
23f	4′-MeO-C_6H_4	CONH-*i*-Pr	2.19 ± 0.16	2.76 ± 0.86 [1.26 ×]
33a	Me	-H	0.36 ± 0.03	0.34 ± 0.00 [0.94 ×]
33b	Et	-H	1.38 ± 0.35	1.08 ± 0.01 [0.78 ×]
33c	C_6H_5	-H	3.21 ± 0.58	3.66 ± 0.23 [1.14 ×]
33d	4′-F-C_6H_4	-H	6.50 ± 1.40	6.14 ± 0.66 [0.94 ×]
33e	4′-MeO-C_6H_4	-H	2.99 ± 0.72	2.83 ± 0.55 [0.95 ×]
Vinblastine[c]			1.41 ± 0.10	392.5 ± 44.7 [278 ×]

[a] The data represent the mean ± STDEV from three to six independent experiments.
[b] Resistance factor, IC_{50} CCRF-CEM/IC_{50} CCRF-CEM/VBL.
[c] nM.

H > Me > Et > aryl (Ph, 4′-FPh, and 4′-MeOPh)]. Among the bis(alkylcarbamate)-substituted derivatives (**22a-f** and **23a-f**), their cytotoxicities were influenced by the C3 substituent (R^1) and were in the order C3-Me > H > Et > aryl. A similar SAR was observed among the dibenzo[*f,h*]pyrrolo[1,2-*b*]isoquinoline derivatives (**33a-e**) (Class II); the size and the electronic properties of the substituent at C11 influenced their cytotoxicity. A Me substituent at C11 (R^1) (**33a**) resulted in the most cytotoxic compound among the Class II series (**33a-e**). These results confirmed that the electronegativity of the N atom in the pyrrole affects the DNA ICL and thus the cytotoxicity of the compound, and the electronegativity of the N atom is decreased when the inductive effect of the substituent at C3 or C11 in Class I or Class II compounds, respectively, is reduced.

Intriguingly, except for those without a substituent at C3 (H) (**21a**, **22a** and **23a**), Class I compounds with ethyl or isopropyl substituents on the bis(alkylcarbamate) moiety showed significantly higher cytotoxicities to CCRF/CEM cells relative to those of their Class II counterparts. These results indicate that the substituents at C3 or C11 in Class I or Class II compounds, respectively, influence the biological activity of these bis(hydroxymethyl) derivatives. Furthermore, the C3-4′-MeOPh-substituted compound is more cytotoxic than the corresponding C3-Ph and C3-4′-F-Ph substituted derivatives.

Drug resistance is one of the main concerns in new drug development [42]. To determine whether the newly synthesized compounds effectively inhibit multi-drug resistant (MDR) cancer cells, we compared the *in vitro* cytotoxic activities of the new derivatives against CCRF-CEM cells and its drug-resistant sublines resistant to vinblastine, CCRF-CEM/VBL (approximately 278-fold more resistant than the sensitive parent cell lines). As shown in Table 1, 15 out of the 23 compounds tested showed a resistance factor (RF) ≤ 1. The RFs of these new compounds were between 0.39 and 1.42, indicating that they are not substrates of p-glycoprotein, which may allow them to overcome MDR.

We further evaluated the effects of selected new derivatives on the inhibition of cell growth against a panel of human solid tumour cell lines *in vitro*, including colon carcinoma HCT-116, lung cancer H1650 and H460 and pancreatic cancer PacaS1 cells. The antiproliferative activities of the tested compounds are summarized in Table 2. Class I compounds with H or Me substituents at C3 displayed the most potent cytotoxicities against the tested tumour cell lines. In Class II, it was also shown that the C11-alkyl (Me and Et) derivatives were generally more cytotoxic than the corresponding C11-aryl congeners. In general, the values of IC50 were less than 3 folds among the tumour cell lines tested, except compound **33a**. H460 cells were the most sensitive to compound **33a**, while HCT-116 to compound **21a**.

2.2.2. DNA cross-linking study

Our previous studies revealed that bis(hydroxymethyl)pyrrole analogues [8] are able to induce DNA ICLs. To determine whether the newly synthesized compounds are also capable of causing DNA cross-linking, linearized pBR322 DNA was treated with potent Class I and II bis(hydroxymethyl) derivatives (**21a**, **21b**, **33a**, and **33b**) at various concentrations (1, 5, 10 and 20 μM) and melphalan (1 and 5 μM) as a positive control. The DNA ICLs were determined by an alkaline agarose gel shifting assay. The results, shown in Fig. 5, indicated that compounds **33a** and **33b** (Class II) induced more DNA ICLs than **21a** and **21b** (Class I), suggesting that the electron density on the N atom of the pyrrole heterocycle influence potency of the drug-induced DNA ICLs. As stated previously, the heterocyclic N of the pyrrole moiety in Class I and Class II compounds is fused to the phenanthridine ring and the dihydroisoquinoline, respectively. These results clearly show that the lone pair electrons on the N atom in Class II compounds may improve the leaving group ability of the hydroxymethyl function in **33a** and **33b** relative to the

Table 2
In vitro cytotoxicities of new pyrrolo[1,2-*f*]phenanthridine and dibenzo[*f,h*]pyrrolo[1,2-*b*]isoquinoline derivatives against human solid tumour cell growth *in vitro*.

Compd.	IC_{50} (μM)			
	HCT-116	H1650	H460	PacaS1
21a	1.12 ± 0.37	3.73 ± 1.1	3.14 ± 0.32	1.41 ± 0.36
21b	1.88 ± 0.44	4.46 ± 0.88	2.87 ± 0.25	3.50 ± 0.25
21c	11.54 ± 1.24	13.56 ± 3.48	9.48 ± 1.28	6.20 ± 0.27
21d	73.32 ± 5.63	38.98 ± 2.20	37.46 ± 2.34	36.67 ± 1.75
22a	1.80 ± 0.36	5.73 ± 0.84	4.25 ± 0.75	4.41 ± 0.95
22b	2.10 ± 0.49	4.47 ± 0.89	1.20 ± 0.17	2.26 ± 0.48
22c	8.53 ± 0.14	10.12 ± 3.24	9.09 ± 0.34	5.05 ± 0.49
22d	21.02 ± 0.44	18.15 ± 0.57	9.03 ± 1.40	11.13 ± 0.50
23a	2.27 ± 0.35	9.62 ± 2.05	7.20 ± 0.97	15.31 ± 0.80
23b	2.12 ± 0.30	5.17 ± 0.87	1.19 ± 0.20	1.80 ± 0.10
23c	16.20 ± 1.03	10.21 ± 0.90	4.98 ± 1.07	4.58 ± 0.10
23d	16.24 ± 2.27	16.30 ± 1.95	14.91 ± 2.10	13.76 ± 2.50
33a	1.57 ± 0.29	2.69 ± 0.22	0.18 ± 0.07	1.80 ± 1.13
33b	2.70 ± 0.74	2.39 ± 0.11	1.33 ± 0.03	2.66 ± 1.75
33c	5.99 ± 0.76	7.95 ± 0.34	2.34 ± 0.11	4.45 ± 0.66
Cisplatin	11.82 ± 0.27	10.35 ± 0.19	3.60 ± 0.45	27.86 ± 3.13

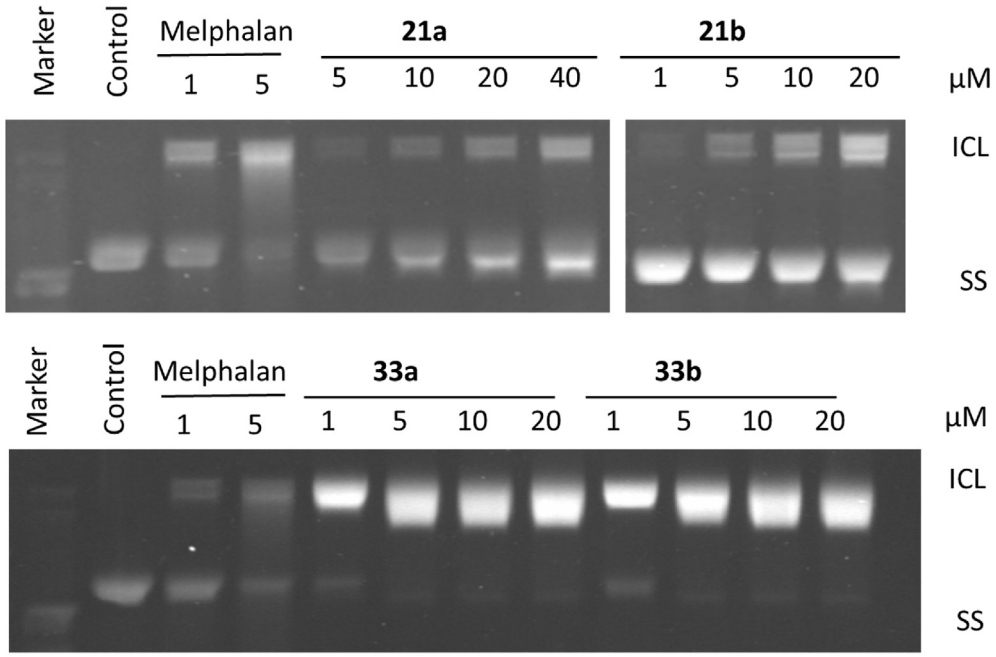


Fig. 5. Representative DNA cross-linking gel shift assay for **21a**, **21b**, **33a** and **33 b** at the indicated concentrations. The control lane shows single stranded DNA (SS), while the interstrand cross-linking (ICL) shown in all the test lanes is DNA double-stranded cross-linking. Melphalan (1 and 5 μM) was used as a positive control.

analogous groups in **21a** and **21b**. Therefore, one can expect compounds **33a** and **33b** to be stronger DNA cross-linkers than **21a** and **21b**. Although **21a** showed lower activity in forming DNA interstrand cross-links than **33a** *in vitro*, their IC_{50} values for all tested cell lines were similar. We can therefore infer that **21a** may have functions other than DNA cross-linking. However, we cannot rule out other possibilities, such as that **21a** and **33a** may have different pharmacokinetics, including differential cellular uptake rates and different stabilities in the medium.

2.2.3. Cell cycle inhibition

DNA interacting/damaging agents are known to induce cell cycle perturbations and arrest cell cycle progression predominantly at the G2/M boundary. Therefore, the effect of compound **21a** on cell cycle progression was further investigated in human colorectal cancer HCT-116 and non-small-cell lung cancer H460 cells. HCT-116 cells were treated with **21a** at concentrations of 0.375, 0.75, and 1.5 μM for 12, 24, and 36 h. Similarly, H460 cells were treated with compound **21a** at 0.75, 1.5, and 3 μM. At the end of treatment, cells were harvested by trypsination, fixed with ethanol, stained with propidium iodide (PI), and subjected to flow cytometric analysis. As shown in Fig. 6, the cell cycle progression was influenced by compound **21a**. Accordingly the cell cycle progression profiles, we noticed the temporary and dose-dependent increase in G2/M phase at 24 h after treatment in either HCT-116 and H460 cells. At 36 h after treatment, the jammed G2/M phase was likely progressing to the next cycle. However, at a higher concentration (i.e., 1.5 μM in HCT-116 cells and 3.0 μM in H460 cells), we observed the appearance of a large proportion of the sub-G1 phase at 36 h, indicating that the cells underwent apoptosis. These observations implicate that compound **21a** targets DNA, induces DNA damage, and subsequently triggers apoptotic cell death. However, we could not exclude other toxic mechanisms.

2.2.4. Induction of apoptosis

To confirm that compound **21a** triggered apoptotic cell death, we performed an annexin V binding assay. H460 cells were treated with compound **21a** at various concentrations (1.5, 3.0 and 6.0 μM) for 48 h, stained with annexin V-FITC and PI, and subjected to flow cytometry analysis. As shown in Fig. 7A, there was a significant time-dependent increase in the percentage of annexin V^+ cells after 48 h of treatment with compound **21a** at various concentrations (1.5, 3, and 6 μM). The frequencies of annexin V^+ cells are summarized in Fig. 7B. These results confirm that the cytotoxic effects of **21a** in H460 cells were due to its ability to induce apoptosis.

2.2.5. In vivo antitumour activity

Although **33a** was as cytotoxic as **21a**, inhibiting all the tested tumour cell lines *in vitro* and serving as a stronger DNA cross-linker than **21a**, this congener was not selected for *in vivo* antitumour activity analysis because of its poor solubility. Alternatively, compound **21a** has good solubility in a mixture of ethanol/PEG400/Cremophor-EL/0.9% saline (10:10:10:70; v/v/v/v) that can be administered via intravenous injection (*i.v.*). We therefore selected compound **21a** for antitumour activity evaluation using human tumour xenografts in animal models. The therapeutic efficacy of **21a** was analysed in nude mice bearing human lung cancer H460 and human colorectal cancer HCT-116 xenografts by *i.v.* administration at 30 mg/kg once every day for five days (QD × 5), and this five-day cycle was repeated for twice with a 2-day interval. Oxaliplatin administered at 7.5 mg/kg once a week for 2 weeks (Q7D × 2) through *i.v.* was used as a positive control. As shown in Fig. 8A and B (left), compound **21a** suppressed approximately 66 and 72% of tumour growth compared to the vehicle control in H460 and HCT-116 xenografts, respectively, at day 24. However, the tumour size in H460 and HCT-116 xenografts was reduced by approximately 34 and 40%, respectively, in mice treated with oxaliplatin at day 24. Based on the average body weight changes (Fig. 8A and B, right), neither **21a** nor oxaliplatin showed significant systematic toxicity in mice at the doses used. These results demonstrated that compound **21a** was more effective than oxaliplatin in inhibiting these *in vivo* models.

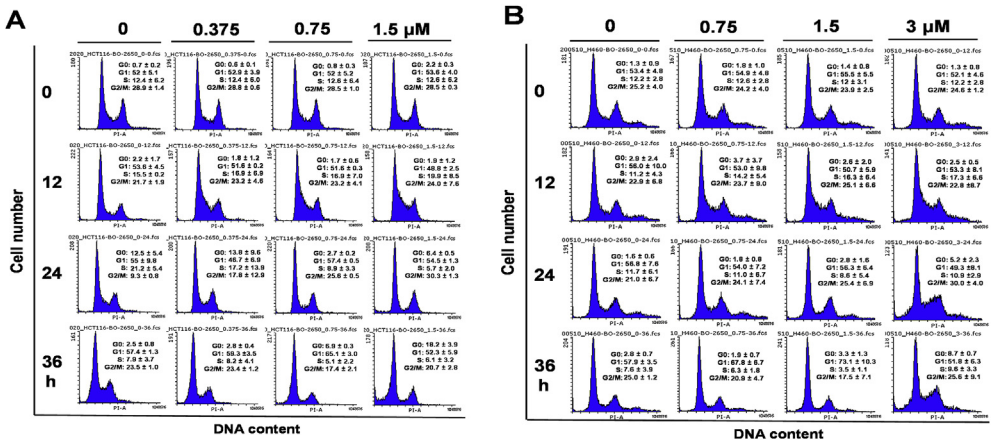


Fig. 6. Cell cycle progression interference by compound **21a.** **(A)** Human colorectal cancer HCT-116 cells; and **(B)** Human non-small cell lung cancer H460 cells. The cells were treated with various concentrations of compound **21a** for 12, 24, and 36 h and subjected for flow cytometric analysis.

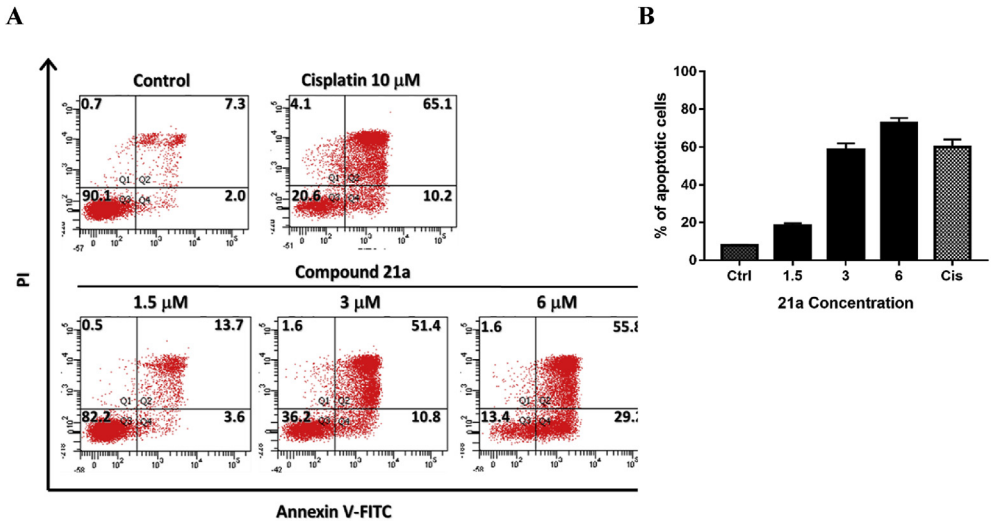


Fig. 7. **(A)** Effects of compound **21a** on the induction of apoptosis in human non-small-cell lung adenocarcinoma H460 cells. The cells were harvested and analysed for apoptosis by flow cytometric analysis of annexin V binding and cell membrane integrity (PI staining) after treatment with **21a** (1.5, 3.0 and 6 μM) or cisplatin (10 μM) for 48 h. **(B)** Apoptotic cell analysis. Percentages of annexin V^+ were calculated.

3. Conclusion

In the current study, we designed and synthesized a series of pyrrolo[1,2-*f*]phenanthridine (Class I) and dibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinoline (Class II) derivatives by coupling a DNA cross-linking bis(hydroxymethyl)pyrrole pharmacophore with a phenanthridine or phenanthroindolizine moiety and evaluated their antitumour activities. We demonstrated that compounds in Classes I and II showed potent cytotoxicities against the growth of lymphoblastic leukaemia CCRF/CEM and human colon carcinoma HCT-116, lung cancer H1650 and H460, and pancreatic cancer PacaS1 cells *in vitro*. The SAR studies showed that compounds having a Me or Et substituent at R^1 in both classes of compounds are generally more cytotoxic than the corresponding aryl-substituted compounds. Interestingly, bis(hydroxymethyl)dibenzo[*f*,*h*]pyrrolo[1,2-*b*]- isoquinolines (e.g., **33a** and **33b**) are more cytotoxic and induce more DNA cross-linking than the corresponding bis(hydroxymethyl) pyrrolo[1,2-*f*]phenanthridines (e.g., **21a** and **21b**). Whether bis(hydroxymethyl)pyrrolo[1,2-*f*]phenanthridines have other biological activities warrants further investigation. The results of the present study further confirmed that the substituents on the pyrrole affect the degree of electronic perturbation in the participating pyrrole and thus modulate the properties of the leaving OH or alkylcarbamate group, the ability to induce DNA cross-linking and the antitumour activity. Among the newly synthesized compounds, we selected compound **21a** for further antitumour activity evaluation in human tumour xenograft models because of its potent *in vitro* cytotoxicity and better solubility in the intravenous injection vehicle. We compared the therapeutic efficacy of **21a** with that of oxaliplatin in nude mice bearing H460 and HCT-116 xenografts. **21a** showed significant tumour growth inhibition in both the human lung and colorectal cancer models. Moreover, these compounds are capable of inducing DNA cross-linking, interfering with cell cycle progression and triggering cell apoptosis.

We previously constructed various hybrid molecules by coupling β-carboline (Topo I/II inhibitory moiety) or phthalazine (an anti-angiogenic moiety) with bis(hydroxymethyl)pyrrole pharmacophorer. It was revealed that these hybrids displayed multiple modes of action with significant antitumour activity in tumour xenograft models. In comparison with that, we applied

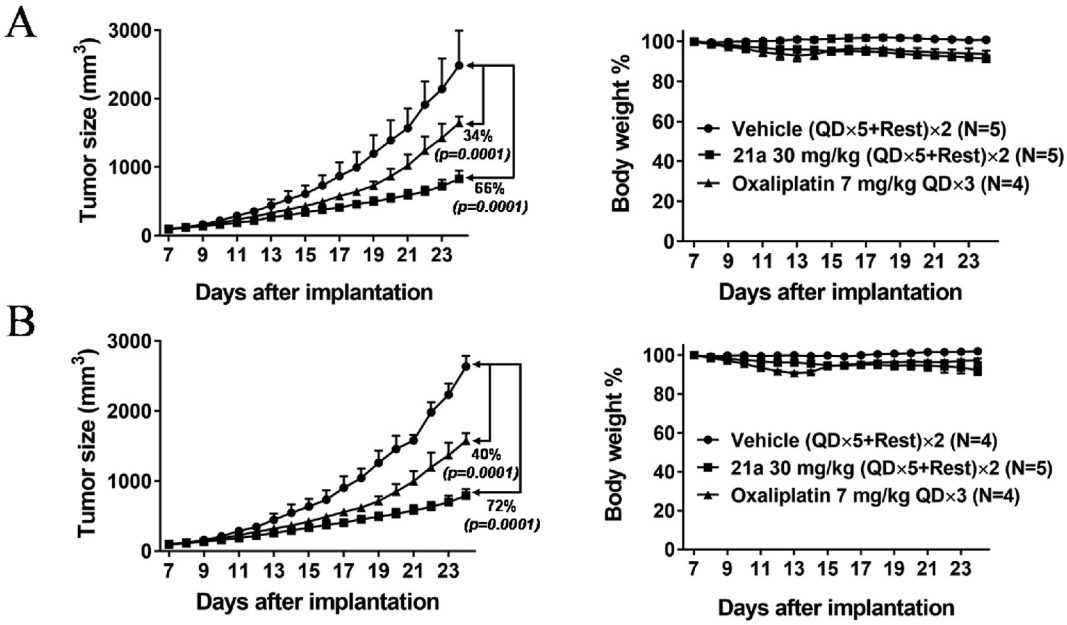


Fig. 8. Therapeutic effect of **21a**: 30 mg/kg (QD × 5+Rest) × 2 cycle, oxaliplatin: 7.5 mg/kg once per week for two cycles by *i.v.* in nude mice bearing H460 and HCT-116 xenografts. **(A)** Average tumour size changes (left) and average body weight changes (right) in the H460 model; **(B)** average tumour size changes (left) and average body weight changes (right) in the HCT-116 model. The tumour volume and body weight changes are presented as the mean ± SD of each group. ****, $P < 0.0001$.

phenanthridine or phenanthroindolizine moieties for preparing hybrids in the present studies. It showed that the main mechanism of action of the new hybrids is DNA ICL. Due to the planar structure of phenanthridine and phenathriondolizine, we may infer that these moieties may intercalate into DNA and hence enhance the interaction as well as cleavage activity of these newly synthesized compounds. As a result, the newly synthesized compounds are less potent than those previously synthesized. Nevertheless, the current studies suggest that bis(hydroxymethyl) pyrrole is a valuable scaffold for designing powerful DNA cross-linking agents with potential antitumour activity for clinical applications. The anticancer activity of conjugation of other functional moiety to bis(hydroxymethyl)pyrrole warrants our further investigation.

4. Experimental protocols

4.1. Materials and methods

All commercial chemicals and solvents were reagent grade. Melting points were determined in open capillaries on a Fargo melting point apparatus and are uncorrected. Thin-layer chromatography was performed on silica gel G60 F254 plates (Merck, Merck KGaA, Darmstadt, Germany) with short-wave UV light for visualization. The purity of all the tested compounds was ≥95% based on analytical HPLC. High-resolution mass spectrometry (HRMS) was conducted on a Waters HDMS G1 instrument with ESI^+, centroid mode, and the samples were dissolved in MeOH. ^1H NMR spectra and ^{13}C NMR spectra were recorded on a Bruker AVANCE 500 DRX and/or a 400 MHz Bruker Top-Spin spectrometer in the solvents indicated. The proton chemical shifts are reported in parts per million (δ ppm) relative to $(CH_3)_4Si$, coupling constants (J) are reported in Hertz (Hz), and multiplicities are given by the following abbreviations: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet; and br s, broad singlet. The HPLC chromatograms and ^1H NMR, ^{13}C NMR and HRMS spectra of the new compounds are presented in Appendix A.

4.2. Chemistry

4.2.1. 5-(Carboxymethyl)phenanthridin-5-ium bromide (**18a**)

Bromoacetic acid (4.65 g, 33.0 mmol) was added to a stirred solution of phenanthridine (**17**, 5.0 g, 28.0 mmol) in acetonitrile (50 mL) at rt. The mixture was heated at reflux for 48 h until a solid product was obtained. After cooling, the product was collected by filtration, washed with acetonitrile and dried to give compound **18a**. Yield 5.8 g (65%); mp 260–262 °C; ^1H NMR (DMSO-d_6) δ 6.15 (s, 2H, CH_2), 8.11–8.18 (m, 3H, ArH), 8.45–8.49 (m, 2H, ArH), 8.61–8.62 (m, 1H, ArH), 9.18–9.23 (m, 2H, ArH), 10.54 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 58.11, 119.81, 123.13, 123.40, 124.98, 125.41, 130.43, 130.66, 132.23, 133.12, 133.69, 134.67, 138.81, 157.04, 167.39; HRMS [ESI^+] calcd for $C_{15}H_{12}NBrO_2$, 239.0946 $[M + H\text{-}Br]^+$, found 239.0884.

4.2.2. 5-Acetyl-5,6-dihydrophenanthridine-6-carbonitrile (**19b**)

Trimethylsilyl cyanide (5.0 mL, 40.0 mmol) and a catalytic amount of $AlCl_3$ were added to a stirred solution of **17** (3.6 g, 20.0 mmol) in DCM (60 mL) under an argon atmosphere. To this reaction mixture was dropwise added acetyl chloride (2.2 mL, 30.0 mmol). After stirring for 4 h at rt, the reaction was poured into cold water, and the organic layer was separated and washed with water, 5% sodium hydroxide aqueous solution and water, dried over sodium sulfate and concentrated to dryness in vacuo to yield **19b**. Yield 4.6 g (92%); mp 170–172 °C; ^1H NMR (DMSO-d_6) δ 2.22 (s, 3H, $COCH_3$), 7.26 (s, 1H, CH), 7.45–7.52 (m, 3H, ArH), 7.58–7.60 (m, 1H, ArH), 7.69–7.71 (m, 1H, ArH), 7.77–7.79 (m, 1H, ArH), 8.06–8.08 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 21.94, 117.26, 124.30, 124.89, 125.53, 126.87, 127.11, 128.83, 128.90, 129.96, 130.30, 130.41, 134.21, 168.70; HRMS [ESI^+] calcd for $C_{16}H_{12}N_2O$, 249.1028 $[M+H]^+$, found 249.1034.

By following the same synthetic procedure as that of **19b**, the following compounds were synthesized:

4.2.3. 5-Propionyl-5,6-dihydrophenanthridine-6-carbonitrile (**19c**)

Compound **19c** was prepared from **17** (3.6 g, 20.0 mmol), trimethylsilyl cyanide (5.0 mL, 40.0 mmol) and propionyl chloride

(2.7 mL, 30.0 mmol). Yield 5.0 g (95%); mp 152–154 °C; ^{1}H NMR (DMSO-d_6) δ 0.98 (t, J = 7.3 Hz, 3H, CH_3), 2.31–2.32 (m, 1H, CH_2), 2.77–2.85 (m, 1H, CH_2), 7.27 (s, 1H, CH), 7.45–7.51 (m, 3H, ArH), 7.56–7.59 (m, 1H, ArH), 7.69–7.70 (m, 1H, ArH), 7.77–7.78 (m, 1H, ArH), 8.05–8.08 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 9.28, 26.60, 117.30, 124.30, 124.95, 125.67, 126.85, 127.14, 127.28, 128.81, 128.84, 130.13, 130.28, 130.45, 133.96, 172.21; HRMS [ESI$^+$]: calcd for $C_{17}H_{14}N_2O$, 263.1184 [M+H]$^+$, found 263.1183.

4.2.4. 5-Benzoyl-5,6-dihydrophenanthridine-6-carbonitrile (**19d**)

Compound **19d** was prepared from **17** (3.6 g, 20.0 mmol), trimethylsilyl cyanide (5.0 mL, 40.0 mmol) and benzoyl chloride (3.5 mL, 30.0 mmol). Yield 5.2 g (82%); mp 144–146 °C; ^{1}H NMR (DMSO-d_6) δ 6.76–6.77 (m, 1H, ArH), 7.10–7.12 (m, 2H, 1 × CH and 1 × ArH), 7.29–7.36 (m, 5H, ArH), 7.46–7.53 (m, 2H, ArH), 7.62–7.65 (m, 1H, ArH), 7.83–7.85 (m, 1H, ArH), 8.06–8.07 (m, 1H, ArH), 8.13–8.15 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 45.10, 117.22, 124.27, 124.94, 125.52, 126.10, 126.40, 127.13, 128.33, 128.45, 129.03, 129.32, 130.25, 130.42, 131.57, 133.35, 168.19; HRMS [ESI$^+$] calcd for $C_{21}H_{14}N_2O$, 311.1184 [M+H]$^+$, found 311.1182.

4.2.5. 5-(4-Fluorobenzoyl)-5,6-dihydrophenanthridine-6-carbonitrile (**19e**)

Compound **19e** was prepared from **17** (3.6 g, 20.0 mmol), trimethylsilyl cyanide (5.0 mL, 40.0 mmol) and 4-fluorobenzoyl chloride (3.5 mL, 30.0 mmol). Yield 5.6 g (85%); mp 156–158 °C; ^{1}H NMR (DMSO-d_6) δ 6.77–6.79 (m, 1H, ArH), 7.09 (s, 1H, CH), 7.12–7.19 (m, 3H, ArH), 7.29–7.32 (m, 1H, ArH), 7.38–7.41 (m, 2H, ArH), 7.49–7.51 (m, 1H, ArH), 7.59–7.63 (m, 1H, ArH), 7.81–7.83 (m, 1H, ArH), 8.04–8.06 (m, 1H, ArH), 8.11–8.12 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 45.17, 115.47, 115.64, 117.18, 124.26, 124.98, 125.53, 126.11, 126.47, 127.13, 128.41, 129.03, 129.30, 129.79, 129.81, 130.21, 130.40, 131.90, 131.97, 134.73, 162.64, 164.63, 167.16; HRMS [ESI$^+$] calcd for $C_{21}H_{13}FN_2O$, 329.1090 [M+H]$^+$, found 329.1089.

4.2.6. 5-(4-Methoxybenzoyl)-5,6-dihydrophenanthridine-6-carbonitrile (**19f**)

Compound **19f** was prepared from **17** (3.6 g, 20.0 mmol), trimethylsilyl cyanide (5.0 mL, 40.0 mmol) and 4-methoxybenzoyl chloride (4.1 mL, 30.0 mmol). Yield 5.4 g (79%); mp 148–150 °C; ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 3H, OCH_3), 6.78–6.79 (m, 1H, ArH), 6.88–6.89 (m, 1H, ArH), 7.04 (1H, s, CH), 7.14–7.17 (m, 1H, ArH), 7.29–7.33 (m, 3H, ArH), 7.49–7.52 (m, 1H, ArH), 7.61–7.64 (m, 1H, ArH), 7.82–7.83 (m, 1H, ArH), 8.05–8.07 (m, 1H, ArH), 8.12–8.13 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 45.32, 55.36, 113.76, 117.34, 124.19, 124.94, 125.12, 125.25, 125.88, 126.08, 127.10, 128.41, 128.95, 129.40, 130.34, 130.36, 131.39, 135.38, 161.82, 167.80; HRMS [ESI$^+$] calcd for $C_{22}H_{16}N_2O_2$, 341.1290 [M+H]$^+$, found 341.1369.

4.2.7. Dimethyl pyrrolo[1,2-f]phenanthridine-1,2-dicarboxylate (**20a**)

DMAD (9.7 mL, 80.0 mmol) was slowly added to a stirred suspension of **18a** (5.0 g, 16.0 mmol) and TEA (2.6 mL, 19.0 mmol) in toluene (80 mL) at rt. The reaction mixture was stirred at 90 °C for 2 h (monitored by TLC). The mixture was cooled to rt, and the solvent was removed in vacuo. The crude product was purified by silica gel column chromatography (SiO_2, hexane:ethyl acetate = 80:20 v/v) to give **20a**. Yield 2.6 g (50%); mp 175–177 °C; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H, $COOCH_3$), 3.96 (s, 3H, $COOCH_3$), 7.55–7.58 (m, 1H, ArH), 7.60–7.62 (m, 2H, ArH), 7.67–7.68 (m, 1H, ArH), 7.95–7.97 (m, 1H, ArH), 8.49–8.50 (m, 1H, ArH), 8.56–8.57 (m, 1H, ArH), 8.59–8.61 (m, 1H, ArH), 8.90 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 51.73, 52.73, 111.41, 116.63, 116.74, 118.74, 121.29, 122.99, 123.48, 123.54, 124.39, 125.69, 125.83, 126.17, 128.16, 128.95, 129.68, 131.37, 163.27, 167.32; HRMS [ESI$^+$] calcd for $C_{20}H_{15}NO_4$, 356.0899 [M+Na]$^+$, found 356.0893.

4.2.8. Dimethyl 3-methylpyrrolo[1,2-f]phenanthridine-1,2-dicarboxylate (**20b**)

To a solution of **20b** (4.0 gm, 16.0 mmol) in hot acetic acid (100 mL) was dropwise added tetrafluoroboric acid (HBF_4) (3.2 mL, 17.6 mmol). The solution was stirred for 30 min at 60–70 °C. The mixture was cooled, the white precipitate was collected by filtration, and the filter cake was washed with ether to give the desired hydrofluoroborate salt. The solid salt was added to a solution of DMAD (5.0 mL, 40.0 mmol) in dimethylformamide (DMF) (25 mL) and heated at 95–100 °C for 14 h. The reaction mixture was concentrated in vacuo, and the residue was crystallized from methanol to give **20b**. Yield 3.6 g (64%); mp 145–147 °C; ^{1}H NMR (DMSO-d_6) δ 3.11 (s, 3H, CH_3), 3.81 (s, 3H, $COOCH_3$), 3.93 (s, 3H, $COOCH_3$), 7.54–7.58 (m, 3H, ArH), 7.62–7.65 (m, 1H, ArH), 7.92–7.94 (m, 1H, ArH), 8.39–8.40 (m, 1H, ArH), 8.51–8.53 (m, 1H, ArH), 8.60–8.61 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 16.13, 51.75, 52.65, 111.29, 115.25, 118.86, 122.73, 123.05, 123.21, 123.83, 124.43, 125.41, 125.74, 127.78, 128.59, 128.87, 132.42, 132.90, 164.22, 167.79; HRMS [ESI$^+$] calcd for $C_{21}H_{17}NO_4$, 348.1236 [M+H]$^+$, found 348.1247.

By following the same synthetic procedure as that of **20b**, the following compounds were synthesized:

4.2.9. Dimethyl 3-ethylpyrrolo[1,2-f]phenanthridine-1,2-dicarboxylate (**20c**)

Compound **20c** was prepared from **19c** (4.0 g, 15 mmol), HBF_4 (3.0 mL, 16.5 mmol) and DMAD (4.7 mL, 37.5 mmol). Yield 3.2 g (58%); mp 121–123 °C; ^{1}H NMR (DMSO-d_6) δ 1.38 (t, J = 7.3 Hz, 3H, CH_3), 3.58 (m, 2H, CH_2), 3.82 (s, 3H, $COOCH_3$), 3.94 (s, 3H, $COOCH_3$), 7.55–7.59 (m, 3H, ArH), 7.67–7.70 (m, 1H, ArH), 7.91–7.93 (m, 1H, ArH), 8.24–8.26 (m, 1H, ArH), 8.51–8.53 (m, 1H, ArH), 8.61–8.62 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 13.26, 20.59, 51.80, 52.66, 111.50, 115.05, 118.30, 122.73, 123.02, 123.19, 123.87, 124.63, 125.56, 125.62, 125.85, 127.82, 128.89, 129.02, 132.60, 137.71, 164.07, 167.81; HRMS [ESI$^+$] calcd for $C_{22}H_{19}NO_4$, 362.1392 [M+H]$^+$, found 362.1385.

4.2.10. Dimethyl 3-phenylpyrrolo[1,2-f]phenanthridine-1,2-dicarboxylate (**20d**)

Compound **20d** was prepared from **19d** (5.0 g, 16.0 mmol), HBF_4 (3.2 mL, 17.6 mmol) and DMAD (5.0 mL, 40.0 mmol). Yield 3.2 g (48%); mp 132–134 °C; ^{1}H NMR (DMSO-d_6) δ 3.59 (s, 3H, $COOCH_3$), 3.95 (s, 3H, $COOCH_3$), 7.04–7.06 (m, 1H, ArH), 7.12–7.16 (m, 1H, ArH), 7.39–7.42 (m, 1H, ArH), 7.47–7.49 (m, 2H, ArH), 7.54–7.59 (m, 3H, ArH), 7.61–7.63 (m, 2H, ArH), 8.15–8.17 (m, 1H, ArH), 8.55–8.58 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 51.63, 52.68, 111.24, 117.93, 118.66, 123.06, 123.28, 123.43, 123.78, 124.69, 125.62, 126.11, 126.88, 127.83, 128.25, 128.89, 129.22, 130.18, 132.29, 132.54, 132.60, 163.60, 167.12; HRMS [ESI$^+$] calcd for $C_{26}H_{19}NO_4$, 410.1392 [M+H]$^+$, found 410.1393.

4.2.11. Dimethyl 3-(4-fluorophenyl)pyrrolo[1,2-f]phenanthridine-1,2-dicarboxylate (**20e**)

Compound **20e** was prepared from **19e** (5.3 g, 16.0 mmol), HBF_4 (3.2 mL, 17.6 mmol) and DMAD (5.0 mL, 40.0 mmol). Yield 3.1 g, (45%); mp 148–150 °C; ^{1}H NMR (DMSO-d_6) δ 3.60 (s, 3H, $COOCH_3$), 3.95 (s, 3H, $COOCH_3$), 7.07–7.09 (m, 1H, ArH), 7.22–7.24 (m, 1H, ArH), 7.37–7.44 (m, 3H, ArH), 7.54–7.57 (m, 2H, ArH), 7.61–7.64 (m, 2H, ArH), 8.12–8.14 (m, 1H, ArH), 8.55–8.60 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 51.66, 52.71, 111.34, 115.87, 116.04, 118.61, 123.08, 123.28, 123.35, 123.74, 124.74, 125.66, 126.11, 126.81, 127.99, 128.26, 128.94, 131.64, 132.27, 132.55, 132.62, 161.53, 163.49, 167.18; HRMS [ESI$^+$] calcd for $C_{26}H_{18}FNO_4$, 450.1118 [M+Na]$^+$, found 450.1135.

*4.2.12. Dimethyl 3-(4-methoxyphenyl)pyrrolo[1,2-f] phenanthridine-1,2-dicarboxylate (**20f**)*

Compound **20f** was prepared from **19f** (4.4 g, 13.0 mmol), HBF_4 (2.6 mL, 14.2 mmol) and DMAD (4.0 mL, 32.5 mmol). Yield 3.0 g (53%); mp 138–140 °C; ^{1}H NMR (DMSO-d_6) δ 3.62 (s, 3H, $COOCH_3$), 3.88 (s, 3H, OCH_3), 3.95 (s, 3H, $COOCH_3$), 7.09–7.11 (m, 2H, ArH), 7.18–7.20 (m, 2H, ArH), 7.38–7.41 (m, 3H, ArH), 7.61–7.63 (m, 2H, ArH), 8.15–8.17 (m, 1H, ArH), 8.54–8.58 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 51.62, 52.65, 55.22, 111.15, 114.34, 117.95, 118.61, 123.04, 123.24, 123.42, 123.85, 124.39, 124.65, 125.54, 126.11, 126.68, 127.91, 128.16, 128.86, 159.75, 163.70, 167.19; HRMS [ESI$^+$] calcd for $C_{27}H_{21}NO_5$, 462.1317 $[M+Na]^+$, found 462.1314.

*4.2.13. Pyrrolo[1,2-f]phenanthridine-1,2-diyldimethanol (**21a**)*

To a stirred suspension of LAH (0.48 g, 12.5 mmol) in diethyl ether (25 mL) was portionwise added **20a** (1.7 g, 5.0 mmol) in DCM (50 mL) at 0 to −5 °C. The reaction mixture was stirred at this temperature for 50 min. The excess LAH was decomposed by adding water (1 mL), NH_4OH (1 mL) and more water (1 mL) at 0 °C. The mixture was filtered through a pad of Celite, and the filter cake was washed several times with DCM. The combined filtrate and washings were sequentially washed with water and brine, dried over sodium sulfate and concentrated to dryness in vacuo. The residue was crystalized from ether to give **21a.** Yield 1.25 g (89%); mp 194–196 °C; ^{1}H NMR (DMSO-d_6) δ 4.65 (d, J = 5.1 Hz, 2H, OCH_2), 4.82 (d, J = 4.9 Hz, 2H, OCH_2), 4.89 (t, J = 5.1 Hz, 1H, OH, exchangeable), 4.93 (t, J = 4.9 Hz, 1H, OH, exchangeable), 7.40–7.43 (m, 1H, ArH), 7.46–7.49 (m, 1H, ArH), 7.56–7.60 (m, 2H, ArH), 8.08 (s, 1H, ArH), 8.16–8.18 (m, 1H, ArH), 8.41–8.43 (m, 1H, ArH), 8.46–8.51 (m, 1H, ArH), 8.51–8.53 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 54.29, 55.40, 111.90, 115.29, 117.76, 120.59, 122.72, 124.04, 124.15, 124.45, 124.85, 125.58, 125.85, 126.12, 128.21, 128.35, 129.15, 132.53; HRMS [ESI$^+$] calcd for $C_{18}H_{15}NO_2$, 260.1075 $[M + H\text{-}H_2O]^+$, found 260.1101.

By following the same synthetic procedure as that of **21a**, the following compounds were synthesized:

*4.2.14. (3-Methylpyrrolo[1,2-f]phenanthridine-1,2-diyl)dimethanol (**21b**)*

Compound **21b** was prepared from **20b** (2.1 g, 6.0 mmol) and LAH (0.6 g, 15 mmol). Yield 1.6 g (91%); mp 158–160 °C; ^{1}H NMR (DMSO-d_6) δ 2.86 (s, 3H, CH_3), 4.60 (d, J = 5.1 Hz, 2H, OCH_2), 4.63 (t, J = 5.0 Hz, 1H, OH, exchangeable), 4.82 (d, J = 5.0 Hz, 2H, OCH_2), 4.90 (t, J = 5.1 Hz, 1H, OH, exchangeable), 7.41–7.44 (m, 2H, ArH), 7.52–7.57 (m, 2H, ArH), 8.31–8.33 (m, 1H, ArH), 8.42–8.43 (m, 2H, ArH), 8.52–8.54 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 15.53, 53.31, 54.27, 117.43, 117.63, 122.40, 122.43, 123.80, 124.07, 124.34, 124.69, 125.37, 125.43, 125.83, 126.39, 128.11, 128.33, 134.30; HRMS [ESI$^+$] calcd for $C_{19}H_{17}NO_2$, 274.1226 $[M + H\text{-}H_2O]^+$, found 274.1243.

*4.2.15. (3-Ethylpyrrolo[1,2-f]phenanthridine-1,2-diyl)dimethanol (**21c**)*

Compound **21c** was prepared from **20c** (2.5 g, 7.0 mmol) and LAH (0.65 g, 17.5 mmol). Yield 1.85 g (88%); mp 167–169 °C; ^{1}H NMR (DMSO-d_6) δ 1.32 (t, J = 7.3 Hz, 3H, CH_3), 3.32 (m, 2H, CH_2), 4.60 (d, J = 5.0 Hz, 2H, OCH_2), 4.65 (t, J = 4.9 Hz, 1H, OH exchangeable), 4.82 (d, J = 4.9 Hz, 2H, OCH_2), 4.92 (t, J = 5.0 Hz, 1H, OH exchangeable), 7.42–7.44 (m, 2H, ArH), 7.53–7.56 (m, 1H, ArH), 7.58–7.61 (m, 1H, ArH), 8.17–8.19 (m, 1H, ArH), 8.42–8.44 (m, 2H, ArH), 8.53–8.55 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 14.46, 20.30, 53.20, 54.28, 117.09, 117.73, 123.37, 123.86, 124.16, 124.21, 124.66, 125.50, 125.67, 125.77, 126.43, 128.33, 128.46, 130.20, 133.39; HRMS [ESI$^+$] calcd for $C_{20}H_{19}NO_2$, 288.1339 $[M + H\text{-}H_2O]^+$, found 288.1387.

*4.2.16. (3-Phenylpyrrolo[1,2-f]phenanthridine-1,2-diyl)dimethanol (**21d**)*

Compound **21d** was prepared from **20d** (2.85 g, 7.0 mmol) and LAH (0.65 g, 17.5 mmol). Yield 2.1 g (85%); mp 210–212 °C; ^{1}H NMR (DMSO-d_6) δ 4.36 (d, J = 4.1 Hz, 2H, OCH_2), 4.77 (t, J = 4.1 Hz, 1H, OH, exchangeable), 4.93 (d, J = 3.7 Hz, 2H, OCH_2), 5.02 (t, J = 4.4 Hz, 1H, OH, exchangeable), 7.11–7.12 (m, 2H, ArH), 7.29–7.30 (m, 1H, ArH), 7.46–7.54 (m, 6H, ArH), 7.59–7.62 (m, 1H, ArH), 8.46–8.51 (m, 3H, ArH); ^{13}C NMR (DMSO-d_6) δ 53.37, 54.34, 118.19, 118.47, 122.47, 122.58, 123.83, 124.46, 124.83, 125.39, 126.09, 126.32, 127.15, 127.37, 127.65, 127.94, 128.05, 128.87, 129.90, 133.27, 133.64; HRMS [ESI$^+$] calcd for $C_{24}H_{19}NO_2$, 336.1383 $[M + H\text{-}H_2O]^+$, found 336.1416.

*4.2.17. (3-(4-Fluorophenyl)pyrrolo[1,2-f]phenanthridine-1,2-diyl) dimethanol (**21e**)*

Compound **21e** was prepared from **20e** (2.15 g, 5.0 mmol) and LAH (0.48 g, 12.5 mmol). Yield 1.7 g (91%); mp 190–192 °C; ^{1}H NMR (DMSO-d_6) δ 4.34 (d, J = 4.9 Hz, 2H, OCH_2), 4.80 (t, J = 4.9 Hz, 1H, OH, exchangeable), 4.92 (d, J = 4.7 Hz, 2H, OCH_2), 5.03 (t, J = 4.7 Hz, 1H, OH, exchangeable), 7.10–7.12 (m, 1H, ArH), 7.16–7.19 (m, 1H, ArH), 7.29–7.32 (m, 1H, ArH), 7.36–7.40 (m, 2H, ArH), 7.48–7.52 (m, 3H, ArH), 7.59–7.62 (m, 1H, ArH), 8.46–8.52 (m, 3H, ArH); ^{13}C NMR (DMSO-d_6) δ 53.30, 54.30, 115.89, 115.96, 118.07, 118.40, 122.50, 122.58, 123.89, 124.53, 124.83, 125.39, 126.14, 126.26, 126.84, 127.21, 127.52, 127.86, 128.50, 130.04, 130.07, 131.97, 132.03, 133.20, 160.86, 162.82; HRMS [ESI$^+$] calcd for $C_{24}H_{18}FNO_2$, 354.1294 $[M + H\text{-}H_2O]^+$, found 354.1316.

*4.2.18. (3-(4-Methoxyphenyl)pyrrolo[1,2-f]phenanthridine-1,2-diyl)dimethanol (**21f**)*

Compound **21f** was prepared from **20f** (2.0 g, 4.5 mmol) and LAH (0.43 g, 11.3 mmol). Yield 1.54 g (88%); mp 177–179 °C; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H, OCH_3), 4.35 (d, J = 5.0 Hz, 2H, OCH_2), 4.72 (t, J = 5.0 Hz, 1H, OH, exchangeable), 4.92 (d, J = 4.9 Hz, 2H, OCH_2), 5.00 (t, J = 4.9 Hz, 1H, OH, exchangeable), 7.09–7.11 (m, 2H, ArH), 7.13–7.16 (m, 1H, ArH), 7.21–7.23 (m, 1H, ArH), 7.27–7.30 (m, 1H, ArH), 7.37–7.39 (m, 2H, ArH), 7.46–7.49 (m, 1H, ArH), 7.58–7.61 (m, 1H, ArH), 8.44–8.50 (m, 3H, ArH); ^{13}C NMR (DMSO-d_6) δ 53.46, 54.41, 55.18, 114.32, 117.99, 118.33, 122.45, 122.54, 123.75, 124.41, 124.76, 125.31, 125.81, 125.95, 126.41, 126.75, 127.43, 127.46, 127.90, 128.45, 131.23, 133.50, 159.00; HRMS [ESI$^+$] calcd for $C_{25}H_{21}NO_3$, 366.1494 $[M + H\text{-}H_2O]^+$, found 366.1516.

*4.2.19. General procedures for the preparation of bis(alkylcarbamate) derivatives (**22a-f** and **23a-f**)*

Alkyl isocyanate (4.0 equivalents) and TEA (4.0 equivalents) were added to a solution of bis(hydroxymethyl) derivative (**21a-e**, 1.0 equivalent) in anhydrous THF or DMF. The reaction mixture was stirred at ambient temperature for 24–48 h under an argon atmosphere. After completion of the reaction, the reaction mixture was concentrated to dryness in vacuo. The desired product was obtained by crystallization.

*4.2.19.1. Pyrrolo[1,2-f]phenanthridine-1,2-diylbis(methylene)bis(ethylcarbamate) (**22a**).* Compound **22a** was prepared from **21a** (0.42 g, 1.5 mmol), TEA (0.84 mL, 6.0 mmol) and ethyl isocyanate (0.48 mL, 6.0 mmol). Yield 0.38 g (60%); mp 145–147 °C; ^{1}H NMR (DMSO-d_6) δ 1.01 (t, J = 7.3 Hz, 6H, 2 × CH_3), 3.01–3.04 (m, 4H, 2 × CH_2), 5.20 (s, 2H, OCH_2), 5.40 (s, 2H, OCH_2), 7.09–7.13 (br s, 2H, 2 × NH, exchangeable), 7.47–7.62 (m, 4H, ArH), 8.17–8.26 (m, 3H, ArH), 8.54–8.56 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 15.02, 35.05, 57.19, 57.50, 112.99, 114.13, 115.49, 120.67, 123.09, 123.46, 123.59, 124.24, 124.68, 125.20, 125.38, 126.32, 126.53, 128.60, 129.33, 132.06, 155.99, 156.18; HRMS [ESI$^+$] calcd for $C_{24}H_{25}N_3O_4$, 244.1125 $[M + H\text{-}2(OCONHC_2H_5)]^+$, found 244.1144.

*4.2.19.2. (3-Methylpyrrolo[1,2-f]phenanthridine-1,2-diyl)bis(-methylene)bis(ethylcarbamate) (**22b**).* Compound **22b** was prepared from **21b** (0.3 g, 1.0 mmol), TEA (0.55 mL, 4.0 mmol) and ethyl isocyanate (0.33 mL, 4.0 mmol). Yield 0.24 g (52%); mp 172–174 °C; ^{1}H NMR (DMSO-d_6) δ 0.98 (t, J = 7.3 Hz, 3H, CH_3), 1.01 (t, J = 7.3 Hz, 3H, CH_3), 2.87 (s, 3H, CH_3), 2.97–3.04 (m, 4H, 2 × CH_2), 5.22 (s, 2H, OCH_2), 5.40 (s, 2H, OCH_2), 7.05 (br s, 1H, NH, exchangeable), 7.12 (br s, 1H, NH, exchangeable), 7.45–7.50 (m, 2H, ArH), 7.55–7.60 (m, 2H, ArH), 8.13–8.15 (m, 1H, ArH), 8.33–8.34 (m, 1H, ArH), 8.47–8.49 (m, 1H, ArH), 8.55–8.57 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 15.00, 15.50, 35.06, 56.14, 57.37, 112.73, 117.78, 121.58, 122.46, 122.77, 123.18, 124.15, 124.37, 125.11, 125.66, 126.12, 126.31, 128.29, 128.57, 133.80, 156.04, 156.16; HRMS [ESI$^+$] calcd for $C_{25}H_{27}N_3O_4$, 258.1282 [M + H-2($OCONHC_2H_5$)]$^+$, found 258.1289.

*4.2.19.3. ((3-Ethylpyrrolo[1,2-f]phenanthridine-1,2-diyl)bis(-methylene)bis(ethylcarbamate)) (**22c**).* Compound **22c** was prepared from **21c** (0.32 g, 1.0 mmol), TEA (0.55 mL, 4.0 mmol) and ethyl isocyanate (0.33 mL, 4.0 mmol). Yield 0.3 g, (64%); mp 180–182 °C; ^{1}H NMR (DMSO-d_6) δ 0.99 (t, J = 7.1 Hz, 3H, CH_3), 1.02 (t, J = 7.1 Hz, 3H, CH_3), 1.30 (t, J = 6.0 Hz, 3H, CH_3), 2.99–3.05 (m, 4H, 2 × CH_2), 3.34 (m, 2H, CH_2), 5.22 (s, 2H, OCH_2), 5.40 (s, 2H, OCH_2), 7.05 (br s, 1H, NH, exchangeable), 7.13 (br s, 1H, NH, exchangeable), 7.48–7.50 (m, 2H, ArH), 7.55–7.58 (m, 1H, ArH), 7.61–7.64 (m, 1H, ArH), 8.13–8.14 (m, 1H, ArH), 8.19–8.23 (m, 1H, ArH), 8.48–8.50 (m, 1H, ArH), 8.57–8.58 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 14.20, 15.00, 20.31, 35.02, 35.05, 57.98, 57.39, 112.80, 117.39, 121.50, 22.38, 122.76, 123.22, 124.30, 124.46, 125.08, 125.69, 126.20, 126.62, 128.58, 128.67, 132.04, 133.47, 155.97, 156.15; HRMS [ESI$^+$] calcd for $C_{26}H_{29}N_3O_4$, 272.1438 [M + H-2($OCONHC_2H_5$)]$^+$, found 272.1464.

*4.2.19.4. (3-Phenylpyrrolo[1,2-f]phenanthridine-1,2-diyl)bis(-methylene)bis(ethylcarbamate) (**22d**).* Compound **22d** was prepared from **21d** (0.7 g, 2.0 mmol), TEA (1.1 mL, 8.0 mmol) and ethyl isocyanate (0.64 mL, 8.0 mmol). Yield 0.53 g (54%); mp 200–202 °C; ^{1}H NMR (DMSO-d_6) δ 1.02 (t, J = 4.9 Hz, 6H, 2 × CH_3), 2.99–3.05 (m, 4H, 2 × CH_2), 4.92 (s, 2H, OCH_2), 5.47 (s, 2H, OCH_2), 7.05–7.12 (m, 4H, 2 × NH, exchangeable, and 2 × ArH), 7.33–7.34 (m, 1H, ArH), 7.43–7.44 (m, 2H, ArH), 7.55–7.63 (m, 5H, ArH), 8.20–8.21 (m, 1H, ArH), 8.52–8.53 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 14.99, 35.00, 35.08, 56.32, 56.38, 113.21, 118.26, 122.48, 122.95, 123.08, 123.76, 124.41, 124.52, 125.58, 125.69, 126.72, 127.57, 127.67, 128.63, 128.74, 129.10, 129.66, 129.99, 132.81, 133.01, 155.77, 156.14; HRMS [ESI$^+$]: calcd for $C_{30}H_{29}N_3O_4$, 320.1438 [M + H-2($OCONHC_2H_5$)]$^+$, found 320.1451.

*4.2.19.5. (3-(4-Fluorophenyl)pyrrolo[1,2-f]phenanthridine-1,2-diyl) bis(methylene)bis(ethylcarbamate) (**22e**).* Compound **22e** was prepared from **21e** (0.37 g, 1.0 mmol), TEA (0.55 mL, 4.0 mmol) and ethyl isocyanate (0.33 mL, 4.0 mmol). Yield 0.28 g (55%); mp 175–177 °C; ^{1}H NMR (DMSO-d_6) δ 0.99 (t, J = 6.9 Hz, 3H, CH_3), 1.02 (t, J = 7.1 Hz, 3H, CH_3), 2.94–3.06 (m, 4H, 2 × CH_2), 4.91 (s, 2H, OCH_2), 5.46 (s, 2H, OCH_2), 7.04 (br s, 1H, NH, exchangeable), 7.10–7.11 (m, 1H, ArH), 7.15 (br s, 1H, NH, exchangeable), 7.20–7.22 (m, 1H, ArH), 7.34–7.42 (m, 3H, ArH), 7.47–7.50 (m, 2H, ArH), 7.54–7.57 (m, 1H, ArH), 7.61–7.64 (m, 1H, ArH), 8.20–8.21 (m, 1H, ArH), 8.53–8.55 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 15.01, 35.01, 35.09, 56.23, 57.36, 113.20, 116.08, 116.25, 118.18, 122.52, 122.98, 123.37, 123.78, 124.49, 124.61, 125.53, 125.71, 126.79, 127.76, 128.51, 128.78, 129.44, 132.21, 132.28, 132.77, 155.75, 156.14, 161.16, 163.12; HRMS [ESI$^+$]: calcd for $C_{30}H_{28}FN_3O_4$, 338.1344 [M + H-2($OCONHC_2H_5$)]$^+$, found 338.1376.

*4.2.19.6. (3-(4-Methoxyphenyl)pyrrolo[1,2-f]phenanthridine-1,2-diyl)bis(methylene)bis(ethyl-carbamate) (**22f**).* Compound **22f** was prepared from **21f** (0.4 g, 1.0 mmol), TEA (0.55 mL, 4.0 mmol) and ethyl isocyanate (0.33 mL, 4.0 mmol). Yield 0.35 g (64%); mp 155–157 °C; ^{1}H NMR (DMSO-d_6) δ 0.99 (t, J = 7.1 Hz, 3H, CH_3), 1.02 (t, J = 7.2 Hz, 3H, CH_3), 2.97–3.06 (m, 4H, 2 × CH_2), 3.86 (s, 3H, OCH_3), 4.90 (s, 2H, OCH_2), 5.45 (s, 2H, OCH_2), 7.04 (br s, 1H, NH, exchangeable), 7.10–7.22 (m, 5H, 1 × NH, exchangeable, and ArH), 7.32–7.35 (m, 3H, ArH), 7.52–7.55 (m, 1H, ArH), 7.60–7.63 (m, 1H, ArH), 8.18–8.19 (m, 1H, ArH), 8.51–8.53 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 15.01, 35.00, 35.08, 55.19, 56.45, 57.43, 113.06, 114.54, 118.08, 122.46, 122.94, 123.69, 124.35, 124.49, 125.02, 125.63, 125.67, 126.62, 127.35, 127.67, 128.71, 129.64, 131.34, 133.04, 155.82, 156.16, 159.34; HRMS [ESI$^+$] calcd for $C_{31}H_{31}N_3O_5$, 350.1544 [M + H-2($OCONHC_2H_5$)]$^+$, found 350.1565.

*4.2.19.7. Pyrrolo[1,2-f]phenanthridine-1,2-diylbis(methylene)bis(isopropylcarbamate) (**23a**).* Compound **23a** was prepared from **21a** (0.42 g, 1.5 mmol), TEA (0.84 mL, 6.0 mmol) and isopropyl isocyanate (0.6 mL, 6.0 mmol). Yield 0.36 g (53%); mp 166–168 °C; ^{1}H NMR (DMSO-d_6) δ 1.04–1.06 (m, 12H, 4 × CH_3), 3.60–3.64 (m, 2H, 2 × CH), 5.19 (s, 2H, OCH_2), 5.39 (s, 2H, OCH_2), 7.02–7.06 (br s, 2H, 2 × NH, exchangeable), 7.45–7.48 (m, 1H, ArH), 7.52–7.55 (m, 1H, ArH), 7.58–7.64 (m, 2H, ArH), 8.16–8.21 (m, 2H, ArH), 8.28 (s, 1H, ArH), 8.52–8.57 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 22.54, 12.31, 57.03, 57.37, 113.04, 114.13, 115.49, 120.67, 123.10, 123.45, 123.60, 124.26, 124.69, 125.20, 125.39, 126.29, 126.53, 128.60, 129.35, 132.07, 155.30, 155.52; HRMS [ESI$^+$] calcd for $C_{26}H_{29}N_3O_4$, 244.1126 [M + H-2($OCONHC_3H_7$)]$^+$, found 244.1156.

*4.2.19.8. (3-Methylpyrrolo[1,2-f]phenanthridine-1,2-diyl)bis(-methylene)bis(isopropylcarbamate) (**23b**).* Compound **23b** was prepared from **21b** (0.3 g, 1.0 mmol), TEA (0.55 mL, 4.0 mmol) and isopropyl isocyanate (0.4 mL, 4.0 mmol). Yield 0.3 g (63%); mp 186–188 °C; ^{1}H NMR (DMSO-d_6) δ 1.02–1.05 (m, 12H, 4 × CH_3), 2.88 (s, 3H, CH_3), 3.59–3.65 (m, 2H, 2 × CH), 5.22 (s, 2H, OCH_2), 5.40 (s, 2H, OCH_2), 6.96–6.97 (br s, 1H, NH, exchangeable), 7.02–7.04 (br s, 1H, NH, exchangeable), 7.46–7.50 (m, 2H, ArH), 7.54–7.60 (m, 2H, ArH), 8.14–8.15 (m, 1H, ArH), 8.33–8.35 (m, 1H, ArH), 8.48–8.50 (m, 1H, ArH), 8.56–8.58 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 15.51, 22.52, 42.24, 42.31, 55.96, 57.20, 112.80, 117.80, 121.61, 122.46, 122.78, 123.21, 124.17, 124.39, 125.11, 125.68, 126.13, 126.30, 128.32, 128.55, 133.81, 155.35, 155.50; HRMS [ESI$^+$] calcd for $C_{27}H_{31}N_3O_4$, 258.1283 [M + H-2($OCONHC_3H_7$)]$^+$, found 258.1302.

*4.2.19.9. (3-Ethylpyrrolo[1,2-f]phenanthridine-1,2-diyl)bis(-methylene)bis(isopropylcarbamate) (**23c**).* Compound **23c** was prepared from **21c** (0.31 g, 1.0 mmol), TEA (0.55 mL, 4.0 mmol) and isopropyl isocyanate (0.4 mL, 4.0 mmol). Yield 0.28 g (58%); mp 196–198 °C; ^{1}H NMR (DMSO-d_6) δ 0.99–1.06 (m, 12H, 4 × CH_3), 1.29 (t, J = 6.4 Hz, 3H, CH_3), 3.31–3.35 (m, 2H, CH_2), 3.59–3.65 (m, 2H, 2 × CH), 5.21 (2H, s, OCH_2), 5.40 (s, 2H, OCH_2), 6.96–6.97 (br s, 1H, NH, exchangeable), 7.05–7.06 (br s, 1H, NH, exchangeable), 7.49–7.51 (m, 2H, ArH), 7.54–7.57 (m, 1H, ArH), 7.62–7.65 (m, 1H, ArH), 8.13–8.14 (m, 1H, ArH), 8.22–8.24 (m, 1H, ArH), 8.49–8.50 (m, 1H, ArH), 8.57–8.59 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 14.18, 20.33, 22.53, 23.26, 42.30, 55.80, 57.22, 112.88, 117.40, 121.55, 122.40, 122.78, 123.27, 124.33, 124.49, 125.10, 125.72, 126.22, 126.63, 128.57, 128.70, 132.04, 132.49, 155.32, 155.50; HRMS [ESI$^+$] calcd for $C_{28}H_{33}N_3O_4$, 272.1439 [M + H-2($OCONHC_3H_7$)]$^+$, found 272.1461.

*4.2.19.10. (3-Phenylpyrrolo[1,2-f]phenanthridine-1,2-diyl)bis(-methylene)bis(isopropylcarbamate) (**23d**).* Compound **23d** was prepared from **21d** (0.7 g, 2.0 mmol), TEA (1.1 mL, 8.0 mmol) and isopropyl isocyanate (0.8 mL, 8.0 mmol). Yield 0.57 g (55%); mp 212–214 °C; ^{1}H NMR (DMSO-d_6) δ 1.05 (d, J = 5.5 Hz, 12H, 4 × CH_3), 3.57–3.65 (m, 2H, 2 × CH), 4.91 (s, 2H, OCH_2), 5.47 (s, 2H, OCH_2),

6.98 (br s, 1H, NH, exchangeable), 7.10–7.14 (m, 3H, 1 × NH, exchangeable, and ArH), 7.33–7.34 (m, 1H, ArH), 7.42–7.45 (m, 2H, ArH), 7.55–7.64 (m, 5H, ArH), 8.19–8.20 (m, 1H, ArH), 8.52–8.54 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 22.54, 42.34, 56.12, 57.20, 113.29, 118.28, 122.51, 122.98, 123.18, 123.78, 124.44, 124.55, 125.61, 125.70, 126.73, 127.60, 127.65, 128.65, 128.74, 129.12, 129.66, 130.00, 132.81, 133.02, 155.10, 155.50; HRMS [ESI$^+$] calcd for $C_{32}H_{33}N_3O_4$, 320.1409 [M + H-2(OCONHC$_3$H$_7$)]$^+$, found 320.1449.

*4.2.19.11. (3-(4-Fluorophenyl)pyrrolo[1,2-f]phenanthridine-1,2-diyl) bis(methylene)bis(isopropylcarbamate) (**23e**).* Compound **23e** was prepared from **21e** (0.37 g, 1.0 mmol), TEA (0.55 mL, 4.0 mmol) and ethyl isocyanate (0.4 mL, 4.0 mmol). Yield 0.16 g (55%); mp 184–186 °C; ^{1}H NMR (DMSO-d_6) δ 1.02–1.06 (m, 12H, 4 × CH$_3$), 3.56–3.66 (m, 2H, 2 × CH), 4.91 (s, 2H, OCH$_2$), 5.47 (s, 2H, OCH$_2$), 6.97 (br s, 1H, NH, exchangeable), 7.08–7.11 (m, 2H, 1 × NH, exchangeable, and ArH), 7.19–7.22 (m, 1H, ArH), 7.34–7.41 (m, 3H, ArH), 7.48–7.49 (m, 2H, ArH), 7.54–7.57 (m, 1H, ArH), 7.60–7.63 (m, 1H, ArH), 8.19–8.20 (m, 1H, ArH), 8.54–8.55 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 22.53, 22.35, 56.03, 57.17, 116.07, 116.25, 118.19, 122.53, 122.98, 123.79, 124.49, 124.61, 125.55, 125.71, 126.78, 127.69, 127.76, 128.49, 128.74, 129.46, 132.20, 132.26, 132.77, 155.05, 155.49, 161.16, 163.12; HRMS [ESI$^+$] calcd for $C_{32}H_{32}FN_3O_4$, 338.1345 [M + H-2(OCONHC$_3$H$_7$)]$^+$, found 338.1394.

*4.2.19.12. (3-(4-Methoxyphenyl)pyrrolo[1,2-f]phenanthridine-1,2-diyl)bis(methylene)bis(iso-propylcarbamate) (**23f**).* Compound **23f** was prepared from **21f** (0.4 g, 1.0 mmol), TEA (0.55 mL, 4.0 mmol) and isopropyl isocyanate (0.4 mL, 4.0 mmol). Yield 0.38 g (66%); mp 170–172 °C; ^{1}H NMR (DMSO-d_6) δ 1.03–1.07 (m, 12H, 4 × CH$_3$), 3.57–3.66 (m, 2H, 2 × CH), 3.87 (s, 3H, OCH$_3$), 4.90 (s, 2H, OCH$_2$), 5.45 (s, 2H, OCH$_2$), 6.97 (br s, 1H, NH, exchangeable), 7.06–7.12 (m, 3H, 1 × NH, exchangeable, and ArH), 7.16–7.22 (m, 2H, ArH), 7.34–7.35 (m, 3H, ArH), 7.50–7.55 (m, 1H, ArH), 7.59–7.62 (m, 1H, ArH), 8.17–8.19 (m, 1H, ArH), 8.51–8.53 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 22.53, 42.24, 42.33, 55.20, 56.25, 57.29, 113.14, 114.55, 118.10, 122.47, 122.95, 123.71, 124.35, 124.51, 125.05, 125.63, 125.68, 126.61, 127.32, 127.67, 128.69, 129.63, 131.35, 133.05, 155.14, 155.52, 159.35; HRMS [ESI$^+$] calcd for $C_{33}H_{35}N_3O_5$, 350.1545 [M + H-2(OCONHC$_3$H$_7$)]$^+$, found 350.1563.

*4.2.20. Phenanthren-9-ylmethanol (**25**)*

Sodium borohydride (5.7 g, 150.0 mmol) was suspended in a solution of 9-phenanthrene carboxaldehyde (**24**, 10.3 g, 50.0 mmol) in dry THF (200 mL). Isopropanol (100 mL) was slowly added at rt. The mixture was allowed to stir for 1 h until the reaction was complete. The solvents were evaporated under reduced pressure to afford a dry residue, and the crude product was triturated with water (1000 mL), filtered and washed with water to give **25**. Yield 10.0 g (96%); mp 146–148 °C; ^{1}H NMR (DMSO-d_6) δ 5.02 (d, J = 4.1 Hz, 2H, OCH$_2$), 5.42 (t, J = 4.7 Hz, 1H, OH exchangeable), 7.61–7.72 (m, 4H, ArH), 7.88 (s, 1H, ArH), 7.96–7.98 (m, 1H, ArH), 8.12–8.14 (m, 1H, ArH), 8.79–8.80 (m, 1H, ArH), 8.85–8.86 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 61.40, 122.71, 123.24, 126.49, 126.73, 126.85, 128.35, 129.43, 129.84, 129.89, 131.20, 136.02; HRMS [ESI$^+$] calcd for $C_{15}H_{12}O$, 191.0860 [M + H-H$_2$O]$^+$, found 191.0861.

*4.2.21. 9-(Bromomethyl)phenanthrene (**26**)*

Phosphorus tribromide (4.6 mL, 24.0 mmol) was slowly added to a solution of **25** (10.0 g, 48.0 mmol) in DCM (100 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h. The reaction mixture was sequentially washed with 5% NaHCO$_3$ aqueous solution (100 mL), 10% sodium thiosulfate solution (100 mL) and water (100 mL). The separated organic layer was dried over sodium sulfate and concentrated under reduced pressure to afford **26**. Yield 10.28 g (79%), mp 118–120 °C; ^{1}H NMR (DMSO-d_6) δ 5.27 (s, 2H, CH$_2$), 7.66–7.79 (m, 4H, ArH), 7.98–7.99 (m, 1H, ArH), 8.08 (s, 1H, ArH), 8.25–8.27 (m, 1H, ArH), 8.82–8.84 (m, 1H, ArH), 8.89–8.91 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 33.57, 122.90, 123.47, 124.81, 126.95, 127.08, 127.23, 127.71, 128.68, 128.86, 129.08, 130.18, 130.35, 130.72, 131.99; HRMS [ESI$^+$] calcd for $C_{15}H_{11}Br$, 271.0122 [M+H]$^+$, found 271.0182.

*4.2.22. Ethyl 2-((diphenylmethylene)amino)-3-(phenanthren-9-yl) propanoate (**27**)*

Diphenylmethylene-glycine ethyl ester (5.91 g, 22 mmol) and K$_2$CO$_3$ (16.66 g, 120.0 mmol) were stirred in acetonitrile (100 mL) for 30 min at rt. **26** (5.45 g, 20.0 mmol) was added, and the reaction mixture was heated to reflux and stirred at this temperature for 20 h. The K$_2$CO$_3$ was removed by filtration, and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography and eluted with 0–4% ethyl acetate in hexane to give **27.** Yield 7.4 g (80%); mp 69–71 °C; ^{1}H NMR (DMSO-d_6) δ 1.09 (t, J = 8.8 Hz, 3H, CH$_3$), 3.45–3.50 (m, 1H, CH$_2$), 3.71–3.75 (m, 1H, CH$_2$), 4.00–4.14 (m, 1H, OCH$_2$), 4.37–4.41 (m, 1H, CH), 6.46 (s, 1H, ArH), 7.10–7.14 (m, 2H, ArH), 7.26–7.35 (m, 3H, ArH), 7.38–7.45 (m, 5H, ArH), 7.52–7.68 (m, 5H, ArH), 7.83–7.85 (m, 1H, ArH), 8.76–8.78 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 14.20, 37.08, 37.13, 61.12, 65.58, 122.32, 122.93, 124.18, 126.01, 126.18, 126.41, 126.56, 127.20, 127.31, 127.66, 127.72, 128.69, 128.76, 129.81, 131.17, 131.90, 135.59, 135.64, 139.29, 170.90, 170.98, 171.99, 172.04; HRMS [ESI$^+$] calcd for $C_{32}H_{27}NO_2$, 458.2120 [M+H]$^+$, found 458.2140.

*4.2.23. Ethyl 2-amino-3-(phenanthren-9-yl)propanoate hydrochloride (**28**)*

Con. HCl (3.0 mL) was added to a stirred solution of **27** (4.6 g, 10.0 mmol) in ethyl acetate (50 mL). The reaction mixture was stirred for 3 h at rt. The solid product was collected by filtration, washed with ethyl acetate, and dried to afford **28.** Yield 2.92 g (88%); mp 223–225 °C; ^{1}H NMR (DMSO-d_6) δ 0.76 (m, 3H, CH$_3$), 3.47–3.51 (m, 1H, CH$_2$), 3.85–3.95 (m, 3H, 1 × CH$_2$ and 1 × OCH$_2$), 4.21–4.24 (m, 1H, CH), 7.64–7.77 (m, 5H, ArH), 7.92–7.94 (m, 1H, ArH), 8.26–8.28 (m, 1H, ArH), 8.82–8.84 (m, 1H, ArH), 8.90–8.92 (m, 4H, NH$_2$HCl and ArH); ^{13}C NMR (DMSO-d_6) δ 13.42, 34.11, 52.41, 61.43, 122.77, 123.69, 124.10, 126.82, 126.99, 127.03, 127.19, 128.24, 128.82, 129.39, 129.68, 130.15, 130.29, 130.94, 169.06; HRMS [ESI$^+$] calcd for $C_{19}H_{19}NO_2$, 294.1494 [M+H]$^+$, found 294.1512.

*4.2.24. Ethyl 1,2,3,4-tetrahydrodibenzo[f,h]isoquinoline-3-carboxylate (**29**)*

Formalin solution (37%, 3 mL) was added to a stirred solution of **28** (3.3 g, 10.0 mmol) in DCM (95 mL). TFA (5 mL) was slowly added, and the reaction mixture was then stirred for 12 h at rt. The reaction mixture was extracted with DCM (3 × 100 mL), and the organic layer was washed with saturated NaHCO$_3$ aqueous solution, dried over sodium sulfate and concentrated to dryness in vacuo. The crude product was purified by column chromatography and eluted with 0–3% MeOH to yield **29**. Yield 2.6 g (85%); mp 122–123 °C; ^{1}H NMR (CDCl$_3$) δ 1.36 (t, J = 7.2 Hz, 3H, CH$_3$), 3.23–3.28 (m, 1H, CH$_2$), 3.48–3.52 (m, 1H, CH$_2$), 3.87–3.90 (m, 1H, CH), 4.27–4.34 (m, 2H, OCH$_2$), 4.45 (d, J = 16.3 Hz, 1H, NCH$_2$), 4.65 (d, J = 16.3 Hz, 1H, NCH$_2$), 7.59–7.64 (m, 4H, ArH), 7.84–7.86 (m, 1H, ArH), 8.01–8.03 (m, 1H, ArH), 8.69–8.71 (m, 2H, ArH); ^{13}C NMR (CDCl$_3$) δ 14.28, 29.05, 45.65, 55.69, 61.23, 122.45, 122.86, 122.96, 123.04, 126.11, 126.86, 127.26, 128.37, 129.22, 129.47, 129.54, 131.14, 173.22; HRMS [ESI$^+$] calcd for $C_{20}H_{19}NO_2$, 306.1494 [M+H]$^+$, found 306.1485.

*4.2.25. Ethyl 2-acetyl-1,2,3,4-tetrahydrodibenzo[f,h]isoquinoline-3-carboxylate (**30a**)*

To a stirred suspension of **29** (2.75 g, 9.0 mmol) and TEA (1.9 mL, 13.5 mmol) in THF (50 mL) was dropwise added acetic anhydride (1.0 mL, 10.8 mmol) at rt, and the mixture was stirred for 10 h at rt. The solvent was evaporated in vacuo to afford a dry residue, which was then diluted with chloroform (100 mL) and washed with a saturated solution of $NaHCO_3$ (100 mL). The organic layer was dried over dried sodium sulfate and concentrated to dryness, and the resulting residue was crystallized from ether to give **30a**. Yield 1.8 g (58%); mp 110–112 °C; ^{1}H NMR (DMSO-d_6) δ 0.99 (t, J = 7.2 Hz, 3H, CH_3), 2.25–2.50 (br s, 3H, $COCH_3$), 3.27–3.31 and 3.42–3.47 (each m, 1H, CH_2), 3.83–3.96 (m, 1H, CH_2), 3.96–4.04 (m, 2H, OCH_2), 4.45 (d, J = 18.0 Hz, 1H, NCH_2), 5.10 (d, J = 16.8 Hz, 1H, NCH_2), 5.75 (m, 1H, CH), 7.68–7.71 (m, 4H, ArH), 7.96–7.98 (m, 1H, ArH), 8.11–8.12 (m, 1H, ArH), 8.84–8.86 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 13.85, 21.88, 27.24, 43.81, 48.94, 54.17, 61.16, 122.82, 123.27, 123.40, 125.24, 125.83, 126.65, 126.70, 127.27, 127.32, 127.44, 128.45, 128.87, 128.99, 129.97, 170.40, 170.57; HRMS [ESI$^+$] calcd for $C_{22}H_{21}NO_3$, 348.1600 [M+H]$^+$, found 348.1605.

By following the same synthetic procedure as that of **30a**, the following compounds were synthesized:

*4.2.26. Ethyl 2-propionyl-1,2,3,4-tetrahydrodibenzo[f,h]isoquinoline-3-carboxylate (**30b**)*

Compound **30b** was prepared from **29** (2.75 g, 9.0 mmol), TEA (1.9 mL, 13.5 mmol) and propionic anhydride (1.4 mL, 10.8 mmol). Yield 1.9 g (58%); mp 127–129 °C; ^{1}H NMR ($CDCl_3$) δ 1.06–1.11 (m, 3H, CH_3), 1.26–1.30 (m, 3H, CH_3), 2.50–2.57 (m, 1H, $COCH_2$), 2.65–2.72 (m, 1H, $COCH_2$), 3.33–3.40 (m, 1H, CH_2), 3.93–4.01 (m, 1H, CH_2), 4.02–4.09 (m, 2H, OCH_2), 4.79 (d, J = 17.8 Hz, 1H, NCH_2), 5.11 (d, J = 16.1 Hz, 1H, NCH_2), 5.99–5.99 (m, 1H, CH), 7.64–7.68 (m, 4H, ArH), 7.87–7.88 (m, 1H, ArH), 8.05–8.07 (m, 1H, ArH), 8.69–8.73 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$) δ 9.11, 14.02, 26.76, 27.18, 43.49, 49.57, 61.40, 121.85, 122.92, 123.23, 123.39, 124.53, 126.46, 126.62, 126.95, 127.13, 127.15, 128.91, 129.58, 129.63, 130.50, 171.81, 174.10; HRMS [ESI$^+$] calcd for $C_{23}H_{23}NO_3$, 362.1756 [M+H]$^+$, found 362.1768.

*4.2.27. Ethyl 2-benzoyl-1,2,3,4-tetrahydrodibenzo[f,h]isoquinoline-3-carboxylate (**30c**)*

Compound **30c** was prepared from **29** (3.38 g, 11.0 mmol), TEA (3.1 mL, 22.0 mmol) and benzoyl chloride (1.93 mL, 16.6 mmol). Yield 2.3 g (53%); mp 190–192 °C; ^{1}H NMR (DMSO-d_6) δ 0.98–1.12 (m, 3H, CH_3), 3.51–3.53 (m, 1H, CH_2), 3.77–3.90 (m, 1H, CH_2), 3.97–4.12 (m, 2H, OCH_2), 4.77 (d, J = 17.8 Hz, 1H, NCH_2), 4.96 (d, J = 16.6 Hz, 1H, NCH_2), 5.89 (m, 1H, CH), 7.56–7.73 (m, 9H, ArH), 8.05–8.16 (m, 2H, ArH), 8.84–8.87 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 13.93, 27.12, 45.57, 55.68, 61.42, 122.51, 123.22, 123.37, 123.51, 124.70, 126.08, 126.70, 126.79, 127.01, 127.37, 127.54, 128.11, 128.85, 129.03, 130.00, 130.23, 135.49, 170.05, 171.17; HRMS [ESI$^+$] calcd for $C_{27}H_{23}NO_3$, 410.1756 [M+H]$^+$, found 410.1728.

*4.2.28. Ethyl 2-(4-fluorobenzoyl)-1,2,3,4-tetrahydrodibenzo[f,h]isoquinoline-3-carboxylate (**30d**)*

Compound **30d** was prepared from **29** (3.38 g, 11.0 mmol), TEA (3.1 mL, 22.0 mmol) and 4-fluorobenzoyl chloride (1.96 mL, 16.6 mmol). Yield 2.6 g (55%); mp 167–169 °C; ^{1}H NMR (DMSO-d_6) δ 0.96–1.12 (m, 3H, CH_3), 3.51–3.55 (m, 1H, CH_2), 3.78–3.90 (m, 1H, CH_2), 3.97–4.11 (m, 2H, OCH_2), 4.74 (d, J = 18.4 Hz, 1H, NCH_2), 5.11 (d, J = 17.0 Hz, 1H, NCH_2), 5.87 (m, 1H, CH), 7.36–7.39 (m, 2H, ArH), 7.63–7.73 (m, 6H, ArH), 8.05–8.15 (m, 2H, ArH), 8.85–8.89 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 13.84, 27.05, 45.68, 55.75, 61.43, 115.95, 122.51, 123.23, 123.37, 123.50, 124.64, 126.16, 126.71, 126.83, 127.40, 127.55, 128.59, 128.87, 129.03, 129.45, 129.99, 131.88, 163.87, 169.90, 170.39; HRMS [ESI$^+$] calcd for $C_{27}H_{22}FNO_3$, 428.1662 [M+H]$^+$, found 428.1660.

*4.2.29. Ethyl 2-(4-methoxybenzoyl)-1,2,3,4-tetrahydrodibenzo[f,h]isoquinoline-3-carboxylate (**30e**)*

Compound **30e** was prepared from **29** (3.38 g, 11.0 mmol), TEA (3.1 mL, 22.0 mmol) and 4-methoxybenzoyl chloride (2.24 mL, 16.6 mmol). Yield 2.7 g (56%); mp 182–184 °C; ^{1}H NMR (DMSO-d_6) δ 1.00–1.10 (m, 3H, CH_3), 3.51–3.52 (m, 1H, CH_2), 3.78–3.82 (m, 1H, CH_2), 3.84 (s, 3H, OCH_3), 3.99–4.09 (m, 2H, OCH_2), 4.74 (d, J = 17.1 Hz, 1H, NCH_2), 5.11 (d, J = 16.3 Hz, 1H, NCH_2), 5.84 (m, 1H, CH), 7.09 (m, 2H, ArH), 7.56–7.73 (m, 6H, ArH), 8.02–8.11 (m, 2H, ArH), 8.85–8.86 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 13.86, 27.14, 45.81, 55.27, 61.30, 64.89, 114.03, 122.47, 123.19, 123.37, 123.47, 124.91, 126.65, 126.77, 127.35, 127.47, 128.84, 129.00, 129.34, 130.02, 160.60, 170.08, 170.15; HRMS [ESI$^+$] calcd for $C_{28}H_{25}NO_4$, 440.1862 [M+H]$^+$, found 440.1827.

*4.2.30. 2-Acetyl-1,2,3,4-tetrahydrodibenzo[f,h]isoquinoline-3-carboxylic acid (**31a**)*

To a stirred suspension of **30a** (1.75 g, 5.0 mmol) in ethanol (25 mL) was added 1 *N* aqueous sodium hydroxide solution (5 mL). The mixture was stirred for 3 h at rt and then concentrated to dryness under reduced pressure. The residue was dissolved in water (50 mL) and acidified with 1 *N* aqueous hydrochloric acid with stirring. The resulting white precipitate was collected by filtration, rinsed with water and dried to afford **31a**. Yield 1.4 g (85%); mp 192–194 °C; ^{1}H NMR (DMSO-d_6) δ 2.17 and 2.33 (each s, 3H, $COCH_3$), 3.18–3.22 and 3.28–3.33 (each m, 1H, CH_2), 3.85–3.90 (m, 1H, CH_2), 4.48 and 5.35 (each d, J = 18.0 Hz, 1H, NCH_2), 5.07 and 5.62 (each m, 1H, CH), 5.11 and 5.16 (each d, J = 17.2 Hz, 1H, NCH_2), 7.68–7.71 (m, 4H, ArH), 8.06–8.08 (m, 1H, ArH), 8.10–8.12 (m, 1H, ArH), 8.83–8.88 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 21.97, 27.02, 40.00, 48.99, 54.62, 122.79, 123.15, 123.23, 123.49, 125.38, 126.31, 126.48, 126.57, 127.27, 127.38, 128.76, 128.84, 128.96, 130.15, 170.37, 172.51; HRMS [ESI$^+$] calcd for $C_{20}H_{17}NO_3$, 320.1287 [M+H]$^+$, found 320.1312.

By following the same synthetic procedure as that of **31a**, the following compounds were synthesized:

*4.2.31. 2-Propionyl-1,2,3,4-tetrahydrodibenzo[f,h]isoquinoline-3-carboxylic acid (**31b**)*

Compound **31b** was prepared from **30b** (1.8 g, 5.0 mmol) and 1 *N* aqueous sodium hydroxide solution (5 mL) in ethanol (25 mL). Yield 1.4 g (84%); mp 203–205 °C; ^{1}H NMR (DMSO-d_6) δ 1.03–1.11 (m, 3H, CH_3), 2.36–2.41 (m, 1H, $COCH_2$), 2.67–2.69 (m, 1H, $COCH_2$), 3.14–3.22 (m, 1H, CH_2), 3.86–3.90 (m, 1H, CH_2), 4.62 and 5.35 (each d, J = 17.8 Hz, 1H, NCH_2), 4.96 and 5.59 (each m, 1H, CH), 5.10 and 5.14 (each m, 1H, NCH_2), 7.66–7.70 (m, 4H, ArH), 8.02–8.03 (m, 1H, ArH), 8.08–8.10 (m, 1H, ArH), 8.83–8.84 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 9.11, 25.79, 27.97, 43.07, 49.46, 53.99, 122.73, 123.09, 123.22, 123.47, 125.61, 126.34, 126.52, 126.59, 127.20, 127.30, 128.71, 128.82, 128.92, 130.26, 172.60, 173.18; HRMS [ESI$^+$] calcd for $C_{21}H_{19}NO_3$, 334.1443 [M+H]$^+$, found 334.1439.

*4.2.32. 2-Benzoyl-1,2,3,4-tetrahydrodibenzo[f,h]isoquinoline-3-carboxylic acid (**31c**)*

Compound **31c** was prepared from **30c** (2.08 g, 5.0 mmol) and 1 *N* aqueous sodium hydroxide solution (5 mL) in ethanol (25 mL). Yield 1.75 g (90%); mp 243–245 °C; ^{1}H NMR (DMSO-d_6) δ 3.46–3.48 (m, 1H, CH_2), 3.78–3.92 (m, 1H, CH_2), 4.82 and 5.56 (each d, J = 17.9 Hz, 1H, NCH_2), 4.93 and 5.13 (each d, J = 16.8 Hz, 1H, NCH_2), 5.85 (m, 1H, CH), 7.55–7.72 (m, 9H, ArH), 8.07–8.15 (m, 2H, ArH), 8.83–8.87 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 27.40, 45.59, 55.65, 122.48, 123.22, 123.37, 123.56, 124.83, 126.30, 126.75, 127.04, 127.34,

127.56, 128.71, 128.83, 128.89, 129.03, 129.96, 130.08, 135.73, 171.05, 171.94; HRMS [ESI$^+$] calcd for $C_{25}H_{19}NO_3$, 382.1443 [M+H]$^+$, found 382.1418.

*4.2.33. 2-(4-Fluorobenzoyl)-1,2,3,4-tetrahydrodibenzo[f,h] isoquinoline-3-carboxylic acid (**31d**)*

Compound **31d** was prepared from **30d** (1.93 g, 4.5 mmol) and 1 *N* aqueous sodium hydroxide solution (5 mL) in ethanol (25 mL). Yield 1.44 g (80%); mp 206–208 °C; ^{1}H NMR (DMSO-d_6) δ 3.44–3.46 (m, 1H, CH_2), 3.78–3.91 (m, 1H, CH_2), 4.80 and 5.53 (each d, J = 18.0 Hz, 1H, NCH_2), 4.88 and 5.81 (each m, 1H, CH), 4.92 and 5.15 (each d, J = 16.8 Hz, 1H, NCH_2), 7.34–7.38 (m, 2H, ArH), 7.63–7.71 (m, 6H, ArH), 8.04–8.14 (m, 2H, ArH), 8.86–8.87 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 27.40, 45.68, 55.84, 115.87, 122.45, 123.21, 123.36, 123.54, 124.82, 126.43, 126.65, 126.72, 127.33, 127.53, 128.68, 128.86, 129.01, 129.38, 129.84, 130.10, 132.30, 163.77, 170.20, 171.68; HRMS [ESI$^+$] calcd for $C_{25}H_{18}FNO_3$, 400.1349 [M+H]$^+$, found 400.1349.

*4.2.34. 2-(4-Methoxybenzoyl)-1,2,3,4-tetrahydrodibenzo[f,h] isoquinoline-3-carboxylic acid (**31e**)*

Compound **31e** was prepared from **30e** (2.3 g, 5.2 mmol) and 1 *N* aqueous sodium hydroxide solution (5 mL) in ethanol (25 mL). Yield 1.80 (84%); mp 222–224 °C; ^{1}H NMR (DMSO-d_6) δ 3.17–3.22 (m, 1H, CH_2), 3.84 (s, 3H, OCH_3), 3.93–3.96 (m, 1H, CH_2), 4.52 and 5.39 (each m, 1H, CH), 4.83 and 5.37 (each d, J = 17.6 Hz, 1H, NCH_2), 4.89 and 5.52 (each d, J = 17.6 Hz, 1H, NCH_2), 6.99–7.04 (m, 2H, ArH), 7.52–7.73 (m, 6H, ArH), 8.00–8.14 (m, 2H, ArH), 8.80–8.86 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 18.57, 28.77, 40.88, 57.22, 57.67, 113.54, 122.30, 123.00, 123.22, 123.67, 125.90, 126.16, 126.21, 126.99, 127.24, 127.81, 128.68, 128.80, 129.21, 129.28, 130.77, 159.90, 170.43, 172.09; HRMS [ESI$^+$] calcd for $C_{26}H_{21}NO_4$, 412.1549 [M+H]$^+$, found 412.1546.

*4.2.35. Dimethyl 1-methyl-9,14-dihydrodibenzo[f,h]pyrrolo[1,2-b] isoquinoline-12,13-dicarboxylate (**32a**)*

To a stirred suspension of **31a** (1.3 g, 4.0 mmol) in Ac_2O (20 mL) was dropwise added DMAD (0.74 mL, 6.0 mmol) at rt. The mixture was then heated at 100 °C for 5 h. After completion of the reaction as indicated by TLC, the solvent was evaporated in vacuo to afford a dry residue, and the residue was triturated with cold MeOH (50 mL). The separated solid product was collected by filtration, washed with cold MeOH and dried to give **32a**. Yield: 1.4 g (86%); mp 200–202 °C; ^{1}H NMR ($CDCl_3$) δ 2.56 (s, 3H, CH_3), 3.91 (s, 6H, 2 × $COOCH_3$), 4.49 (s, 2H, CH_2), 5.11 (s, 2H, NCH_2), 7.67–7.69 (m, 4H, ArH), 7.77–7.78 (m, 1H, ArH), 8.07–8.08 (m, 1H, ArH), 8.65–8.70 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$) δ 10.82, 25.54, 29.71, 43.39, 51.31, 51.69, 109.05, 113.86, 120.74, 121.85, 122.91, 123.31, 123.81, 124.25, 126.67, 127.02, 127.23, 127.33, 128.35, 129.66, 129.74, 129.81, 131.77, 132.56, 165.34, 166.51; HRMS [ESI$^+$] calcd for $C_{25}H_{21}NO_4$, 422.1368 [M+Na]$^+$, found 400.1362.

By following the same synthetic procedure as that of **32a**, the following compounds were synthesized:

4.2.36. Dimethyl 11-ethyl-9,14-dihydrodibenzo[f,h]pyrrolo[1,2-b] isoquinoline-12,13-dicarboxylate (32b)

Compound **32b** was prepared from **31b** (1.35 g, 4.0 mmol) and DMAD (0.74 mL, 6.0 mmol) in Ac_2O (20 mL). Yield 1.4 g (84%); mp 245–247 °C; ^{1}H NMR ($CDCl_3$) δ 1.32 (t, J = 7.5 Hz, 3H, CH_3), 2.95 (q, J = 7.5 Hz, 2H, CH_3), 3.91 (s, 6H, 2 × $COOCH_3$), 4.42 (s, 2H, CH_2), 5.14 (s, 2H, NCH_2), 7.63–7.66 (m, 4H, ArH), 7.72–7.73 (m, 1H, ArH), 8.01–8.03 (m, 1H, ArH), 8.61–8.66 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$) δ 14.50, 18.43, 25.35, 42.88, 51.24, 51.65, 109.06, 113.42, 120.72, 121.73, 122.81, 123.22, 123.70, 124.18, 126.58, 126.93, 127.14, 127.25, 128.25, 129.55, 129.66, 132.28, 137.07, 165.24, 166.45; HRMS [ESI$^+$] calcd for $C_{26}H_{23}NO_4$, 436.1525 [M+Na]$^+$, found 436.1520.

*4.2.37. Dimethyl 11-phenyl-9,14-dihydrodibenzo[f,h]pyrrolo[1,2-b] isoquinoline-12,13-dicarboxylate (**32c**)*

Compound **32c** was prepared from **31c** (1.55 g, 4.0 mmol) and DMAD (0.74 mL, 6.0 mmol) in Ac_2O (20 mL). Yield 1.6 g (85%); mp 262–264 °C; ^{1}H NMR (DMSO-d_6) δ 3.65 (s, 3H, $COOCH_3$), 3.85 (s, 3H, $COOCH_3$), 4.58 (s, 2H, CH_2), 5.32 (s, 2H, CH_2), 7.46–7.48 (m, 1H, ArH), 7.53–7.66 (m, 7H, ArH), 7.69–7.72 (m, 1H, ArH), 7.73–7.76 (m, 1H, ArH), 8.04–8.06 (m, 1H, ArH), 8.81–8.82 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 25.12, 44.10, 51.21, 51.68, 108.55, 116.35, 122.18, 123.13, 123.18, 123.25, 123.36, 126.76, 126.99, 127.40, 127.51, 127.76, 128.58, 128.61, 128.92, 129.13, 129.18, 129.65, 130.18, 132.29, 132.66, 163.96, 165.91; HRMS [ESI$^+$] calcd for $C_{30}H_{23}NO_4$, 462.1705 [M+H]$^+$, found 462.1722.

*4.2.38. Dimethyl 11-(4-fluorophenyl)-9,14-dihydrodibenzo[f,h] pyrrolo[1,2-b]isoquinoline-12,13-dicarboxylate (**32d**)*

Compound **32d** was prepared from **31d** (1.2 g, 3.0 mmol) and DMAD (0.55 mL, 4.5 mmol) in Ac_2O (15 mL). Yield 1.15 g (80%); mp 236–238 °C; ^{1}H NMR ($CDCl_3$) δ 3.75 (s, 3H, $COOCH_3$), 3.93 (s, 3H, $COOCH_3$), 4.53 (s, 2H, CH_2), 5.04 (s, 2H, NCH_2), 7.23–7.26 (m, 2H, ArH), 7.38–7.39 (m, 1H, ArH), 7.51–7.63 (m, 6H, ArH), 8.03–8.04 (m, 1H, ArH), 8.60–8.61 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$) δ 25.58, 44.37, 51.39, 51.96, 109.16, 115.68, 115.85, 116.80, 121.15, 121.72, 122.76, 123.08, 123.65, 123.86, 126.07, 126.58, 126.93, 127.09, 127.25, 128.07, 129.47, 129.55, 129.64, 132.01, 132.38, 132.45, 133.55, 162.04, 164.02, 164.79, 166.32; HRMS [ESI$^+$] calcd for $C_{30}H_{22}FNO_4$, 480.1611 [M+H]$^+$, found 480.1624.

*4.2.39. Dimethyl 11-(4-methoxyphenyl)-9,14-dihydrodibenzo[f,h] pyrrolo[1,2-b]- isoquinoline-12,13-dicarboxylate (**32e**)*

Compound **32e** was prepared from **31e** (1.65 g, 4.0 mmol) and DMAD (0.74 mL, 6.0 mmol) in Ac_2O (20 mL). Yield 1.67 g (85%); mp 248–250 °C; ^{1}H NMR ($CDCl_3$) δ 3.76 (s, 3H, OCH_3), 3.93 (s, 6H, 2 × $COOCH_3$), 4.64 (s, 2H, CH_2), 5.15 (s, 2H, NCH_2), 7.06–7.08 (m, 2H, ArH), 7.46–7.49 (m, 3H, ArH), 7.51–7.54 (m, 1H, ArH), 7.59–7.62 (m, 1H, ArH), 7.65–7.67 (m, 2H, ArH), 8.10–8.11 (m, 1H, ArH), 8.64–8.66 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$) δ 25.72, 44.46, 51.34, 51.94, 55.33, 108.95, 114.04, 116.36, 121.52, 121.89, 122.19, 122.82, 123.11, 123.75, 124.05, 126.55, 126.91, 127.08, 127.27, 128.23, 129.54, 129.71, 131.81, 133.05, 133.37, 159.95, 164.96, 166.62; HRMS [ESI$^+$] calcd for $C_{31}H_{25}NO_5$, 514.1630 [M+Na]$^+$, found 514.1672.

*4.2.40. (11-Methyl-9,14-dihydrodibenzo[f,h]pyrrolo[1,2-b] isoquinoline-12,13-diyl) dimethanol (**33a**)*

To a stirred suspension of LAH (0.29 g, 7.5 mmol) in diethyl ether (100 mL) was dropwise added **32a** (1.2 g, 3.0 mmol) in DCM (200 mL) at 0 to −5 °C. The reaction mixture was stirred for 8 h at rt. The excess LAH was decomposed by the addition of water (1 mL), NH_4OH (1 mL) and more water (1 mL). The mixture was filtered through a pad of Celite, and the filter cake was washed several times with DCM. The filtrate was sequentially washed with water and brine, dried over sodium sulfate and concentrated to dryness in vacuo. The residue was crystalized from ether to give **33a**. Yield 0.8 g (78%); mp 128–130 °C; ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3H, CH_3), 4.42–4.47 (m, 5H, 1 × OCH_2, 1 × CH_2 and 1 × OH, exchangeable), 4.48 (t, J = 5.2 Hz, 1H, OH, exchangeable), 4.56 (d, J = 5.2 Hz, 2H, OCH_2), 5.44 (s, 2H, NCH_2), 7.73–7.77 (m, 4H, ArH), 8.12–8.14 (m, 1H, ArH), 8.23–8.25 (m, 1H, ArH), 8.89–8.90 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 9.37, 23.20, 25.48, 42.67, 54.15, 54.20, 116.29, 119.34, 120.66, 123.13, 123.17, 123.23, 123.42, 123.85, 124.80, 126.58, 126.74, 127.27, 127.33, 128.66, 128.97, 129.22, 129.94; HRMS [ESI$^+$] calcd for $C_{23}H_{21}NO_2$, 326.1545 [M + H-H_2O]$^+$, found 326.1564.

By following the same synthetic procedure as that of **33a**, the following compounds were synthesized:

4.2.41. (11-Ethyl-9,14-dihydrodibenzo[f,h]pyrrolo[1,2-b] isoquinoline-12,13-diyl) dimethanol (**33b**)

Compound **33b** was prepared from **32b** (1.25 g, 3.0 mmol) and LAH (0.29 g, 7.5 mmol). Yield 0.78 g (72%); mp 137–139 °C; ^{1}H NMR (DMSO-d_6) δ 1.23 (t, J = 7.4 Hz, 3H, CH_3), 2.88 (q, J = 7.4 Hz, 2H, CH_3), 4.40–4.48 (m, 5H, 1 × OCH_2, 1 × CH_2 and 1 × OH, exchangeable), 4.50 (t, J = 5.3 Hz, 1H, OH, exchangeable), 4.57 (d, J = 5.3 Hz, 2H, OCH_2), 5.51 (s, 2H, NCH_2), 7.74–7.77 (m, 4H, ArH), 8.17–8.19 (m, 1H, ArH), 8.26–8.27 (m, 1H, ArH), 8.90–8.91 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 15.94, 16.78, 23.19, 54.08, 54.19, 116.42, 119.12, 120.69, 123.18, 123.23, 123.53, 123.85, 124.94, 126.58, 126.76, 127.31, 127.34, 128.64, 128.97, 129.22, 129.33, 129.93; HRMS [ESI$^+$] calcd for $C_{24}H_{23}NO_2$, 340.1701 [M + H-H_2O]$^+$, found 340.1704.

4.2.42. (11-Phenyl-9,14-dihydrodibenzo[f,h]pyrrolo[1,2-b] isoquinoline-12,13-diyl) dimethanol (**33c**)

Compound **33c** was prepared from **32c** (1.5 g, 3.25 mmol) and LAH (0.30 g, 8.12 mmol). Yield 1.05 g (79%); mp 170–172 °C; ^{1}H NMR (DMSO-d_6) δ 4.37 (d, J = 5.0 Hz, 2H, OCH_2), 4.56 (s, 2H, CH_2), 4.63 (t, J = 5.0 Hz, 1H, OH, exchangeable), 4.68–4.69 (m, 3H, 1 × OCH_2 and 1 × OH, exchangeable), 5.49 (s, 2H, NCH_2), 7.43–7.46 (m, 1H, ArH), 7.54–7.57 (m, 1H, ArH), 7.64–7.70 (m, 5H, ArH), 7.74–7.78 (m, 2H, ArH), 8.30–8.32 (m, 1H, ArH), 8.88–8.90 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 23.35, 43.95, 54.17, 54.40, 117.87, 121.74, 122.32, 123.24, 123.39, 123.53, 123.68, 123.92, 125.02, 126.60, 126.86, 127.03, 127.43, 128.35, 128.51, 128.97, 129.04, 129.23, 129.78, 130.09, 131.73; HRMS [ESI$^+$] calcd for $C_{28}H_{23}NO_2$, 388.1701 [M + H-H_2O]$^+$, found 388.1702.

4.2.43. (11-(4-Fluorophenyl)-9,14-dihydrodibenzo[f,h]pyrrolo[1,2-b]isoquinoline-12,13-diyl) dimethanol (**33d**)

Compound **33d** was prepared from **32d** (1.0 g, 2.1 mmol) and LAH (0.2 g, 5.2 mmol). Yield 0.75 g (85%); mp 152–156 °C; ^{1}H NMR (DMSO-d_6) δ 4.35 (d, J = 5.1 Hz, 2H, OCH_2), 4.54 (s, 2H, CH_2), 4.65 (t, J = 5.1 Hz, 1H, OH, exchangeable), 4.66–4.69 (m, 3H, 1 × OCH_2 and 1 × OH, exchangeable), 5.45 (s, 2H, NCH_2), 7.37–7.41 (m, 1H, ArH), 7.65–7.71 (m, 5H, ArH), 7.73–7.79 (m, 2H, ArH), 8.28–8.30 (m, 1H, ArH), 8.87–8.89 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ 23.33, 43.86, 54.12, 54.30, 115.32, 115.49, 117.78, 124.81, 122.44, 123.23, 123.35, 123.49, 123.60, 123.90, 124.91, 126.61, 126.86, 127.41, 127.99, 128.17, 128.34, 128.96, 129.23, 129.78, 132.10, 132.16, 160.48, 162.42; HRMS [ESI$^+$] calcd for $C_{28}H_{22}FNO_2$, 406.1601 [M + H-H_2O]$^+$, found 406.1579.

4.2.44. (11-(4-Methoxyphenyl)-9,14-dihydrodibenzo[f,h]pyrrolo [1,2-b]isoquinoline-12,13-diyl) dimethanol (**33e**)

Compound **33e** was prepared from **32e** (1.5 g, 3.0 mmol) and LAH (0.28 g, 7.5 mmol). Yield 1.1 g (83%); mp 181–183 °C; ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 3H, OCH_3), 4.36 (d, J = 4.9 Hz, 2H, OCH_2), 4.51 (s, 2H, CH_2), 4.66 (t, J = 4.9 Hz, 1H, OH, exchangeable), 4.67–4.68 (m, 3H, 1 × OCH_2 and 1 × OH, exchangeable), 5.39 (s, 2H, CH_2), 7.11–7.13 (m, 2H, ArH), 7.55–7.56 (m, 2H, ArH), 7.65–7.67 (m, 3H, ArH), 7.71–7.77 (m, 2H, ArH), 8.27 (m, 1H, ArH), 8.86 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 23.33, 43.84, 54.24, 54.50, 55.14, 113.97, 117.58, 121.25, 122.29, 122.87, 123.19, 123.34, 123.64, 123.87, 124.00, 125.03, 126.55, 126.81, 127.38, 128.35, 128.82, 128.94, 129.20, 129.79, 131.42, 158.44; HRMS [ESI$^+$] calcd for $C_{29}H_{25}NO_3$, 418.1807 [M + H-H_2O]$^+$, found 418.1817.

4.3. Biological experiments

4.3.1. Cytotoxicity assays

The cytotoxic effects of the newly synthesized compounds were determined in T-cell acute lymphocytic leukaemia (CCRF-CEM) and their vinblastine-resistant sub-cell lines (CCRF-CEM/VBL) and a panel of human solid tumour cell lines, including colon carcinoma HCT-116, lung cancer H1650 and H460, and pancreatic cancer PacaS1 cells, by a Presto blue assay with a 72-h incubation period using a microplate spectrophotometer as previously described [18]. The tested compounds were freshly prepared by a two-fold serial dilution in DMSO from 100 μM. After the addition of phenazine methosulfate-XTT solution, the cells were incubated at 37 °C for 3 h, and the absorbances at 450 and 630 nm were detected with a microplate reader (EL 340). The IC_{50} values were determined from the dose-effect relationship at six or seven concentrations of each drug using CompuSyn software by Chou and Martin based on the median-effect principle and plot [24,25]. The ranges given for the IC_{50} values are the mean ± SE (n = 4).

4.3.2. Alkaline agarose gel shift assay

The formation of DNA cross-linking was analysed by an alkaline agarose gel electrophoresis assay as previously described [16]. Briefly, purified pEGFP-N1 plasmid DNA (1500 ng) was mixed with various concentrations (1–20 μM) of **21a**, **21b**, **33a**, and **33b** in 40 μL of binding buffer (3 mM sodium chloride/1 mM sodium phosphate, pH 7.4, and 1 mM EDTA). The reaction mixture was incubated at 37 °C for 2 h. At the end of the reaction, the plasmid DNA was linearized by digestion with BamHI-HF and precipitated with ethanol. The DNA pellets were dissolved and denatured in alkaline buffer (0.5 *N* NaOH-10 mM EDTA). A 20-μL aliquot of DNA solution (1000 ng) was mixed with 4 μL of 6 X alkaline loading dye and then electrophoretically resolved on a 0.8% alkaline agarose gel with NaOH-EDTA buffer at 4 °C. Electrophoresis was carried out at 18 V for 22 h. After staining the gels with an ethidium bromide solution, the DNA was then visualized under UV light.

4.3.3. Cell cycle analysis

The effects of compound **21a** on cell cycle progression were analysed by flow cytometry as previously described [18]. Briefly, 1 × 10^5 HCT-116 and H460 cells were seeded in 6-well plate and incubated overnight at 37 °C in 5% CO_2 incubator. The cells were then treated with various concentrations of compound **21a** for different time periods. At the end of treatment, the attached cells were trypsinized, fixed in ice-cold 70% EtOH, and stored at −20 °C overnight. The cells were then stained with 4 μg/mL propidium iodide (PI) in phosphate-buffered saline (PBS) containing 0.1 mg/mL RNase A and 1% Triton X-100 and subjected to flow cytometry analysis (FACScan flow cytometer, Becton Dickinson, San Jose, CA). The cell cycle phase distribution was analysed with ModFit LT 3.0 software (Verity Software House, Topsham, ME) based on the DNA histograms.

4.3.4. Apoptosis assay

As previously described [21], H460 cells were treated with compound **21a** or cisplatin for 24, 48, and 72 h. Apoptotic cell death was determined using an Annexin V-FITC Apoptosis Detection Kit (eBioscienceTM, San Diego, CA, USA) and a flow cytometer according to the manufacturer's instructions. Annexin V-positive cells, including the bottom right and top right quadrants, represented the early and late apoptotic populations, respectively.

4.3.5. Antitumour activity

The antitumour activity of compound **21a** was assessed in xenograft tumour models. All animals (5 weeks of age) were

obtained from the National Laboratory Animal Centre, Taipei, Taiwan, and kept in-house under 12 h light and 12 h darkness for a week prior the experiment. To implant xenograft tumours, H460 (5×10^6) or HCT-116 (5×10^6) cells were suspended in 100 μL of 50:50 media and Matrigel and injected into the right top flank of the mice. When the tumour size reached 80–100 mm^3 in size, vehicle or drug was *i.v.* administered. The solution of **21a** was freshly prepared in a mixture of ethanol/PEG400/Cremophor-EL/0.9% saline (10:10:10:70; v/v/v/v), and oxaliplatin was dissolved in 5% dextrose saline. Animals remained to survive when compound **21a** was administrated at dose of 150 mg/kg that is the maximum soluble dose. The maximum tolerant dose of compound 21a is presumed over 150 mg/kg. For treatment, compound **21a** at a dose of 30 mg/kg was *i.v.* injected once every day for five days (QD × 5), and this five-day cycle was repeated twice with a 2-day interval. Oxaliplatin (7.5 mg/kg) was given via *i.v.* injection once a week for two weeks [43]. The growth of the tumour was measured every day using callipers. The tumour size was calculated by the following formula: Tumour volume = (length × $width^2$)/2. Mouse body weights were also measured and recorded as an indicator of systemic toxicity.

Declaration of competing interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2020.112516.

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