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N^2,N^9-Bis(Substituted benzyl)-β-Carbolineum Bromides as Potential Anticancer Therapeutics: Design, Synthesis, Cytotoxicity, Drug-DNA Intercalation and *In-Silico* Binding Properties

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ABSTRACT

The present study reports a series of novel N^2,N^9-bis(substituted benzyl)-β-carbolineum bromides (**4a-f**) synthesized from L-tryptophan in three steps with excellent yields (>80%). The structures of synthesized compounds **4a-f** were confirmed by ^{1}H- and ^{13}C-NMR, FT-IR, LC-MS (ESI-MS) spectrum and elemental analysis. Meanwhile, the crystal structure for compound **4f** was determined by X-ray single-crystal diffraction. The crystal belongs in monoclinic space group in $P12_1/c_1$ space group with a = 13.253(6) Å, b = 20.809(10) Å, c = 9.116(6) Å, β = 107.215(13)°, V = 2401.4(19) Å^3 and Z = 4, F(000) = 1048, D_c = 1.403 Mg/m^3 and μ = 1.743 mm^{-1}. Compounds **4a-f** were evaluated for their *in-vitro* anticancer activity against selected human cancer cell lines, such as HT-29 (colorectal adenocarcinoma), HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma) and K562 (chronic myelogenous leukaemia, CML). Results showed that compounds **4a-f** exerted excellent cytotoxicity effect with IC_{50} values ranging from 0.36-1.08 μM against K562 human CML cell line. It was found that synthesized β-carbolines are much less toxic towards non-cancer cell lines BALB/c3T3 and Hs-27, in comparison to cisplatin and doxorubicin, which were employed as positive controls. To investigate the binding mode of these compounds against DNA, spectroscopic studies were conducted. Subsequent UV-Visible and *in-silico* (molecular docking) studies revealed that compound **4f** interacts with DNA through intercalation. Based on the present findings, it was suggested that compound **4f** has a great potential to be developed as a novel anticancer agent.

1. Introduction

Cancer is growing as the leading cause of mortality and accounts for significant morbidity worldwide [1]. According to the World Health Organization (WHO), cancer is liable for 8.8 million deaths in 2015 and was predicted to rise over 13.1 million by 2030 [2]. Cancer is characterized by cells' growth of beyond their boundaries and invasion of tissues, making it dreadful disease among all diseases. Despite the enormous efforts to implement novel chemotherapeutic strategies to treat different cancer types, this disease remains one of the significant concerns globally [3]. In spite of the availability of many chemotherapeutic agents, tolerance, resistance [4] and the unwanted toxic side effects of chemotherapy necessitates the development of new and efficient anticancer agents in drug discovery. The critical cellular process that plays an essential role in maintaining tissue growth and homeostasis is apoptosis. The apoptotic pathway's improper regulation has been indicated in various diseases, including cancer [5]. To date, DNA remains as the primary target in cancer treatment due to its fundamental role in cell division (replication) and maintenance (transcription) [6,7]. Therefore, the search towards developing novel compounds which are able to target DNA and induce apoptosis could be a fruitful strategy in cancer therapy development. Naturally, phytochemicals from plants are a promising source in discovering and discovering new therapeutic agents.

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Peganum harmala is a plant traditionally used as an abortifacient agent and an emmenagogue for many years [8]. Previously, β-carboline compounds isolated from the seeds of *Peganum harmala* (Family: Zygophyllaceae) were reported to possess a planar tricyclic pyrido [3,4-β] indole ring system [9]. Previous studies have shown that the alkaloids in these seeds are mostly β-carbolines (harmine, harmane, harmalol, and harmaline) [10]. In addition to their occurrence in plants, β-carbolines are also endogenously synthesized in mammals from tryptophan-derived indoleamines and tryptophan [11]. These compounds' pharmacological effects are varied and possess a broad range of bioactivities, including anticonvulsant, hallucinogenic, antitumor, antiviral, antibacterial, and antiparasitic [12,13]. In particular, these β-carboline molecules are known to induce apoptosis and inhibit cancer cell proliferation [14] through multiple mechanisms of action such as inhibition of DNA-topoisomerase-I & -II [15,16], cyclin-dependent kinases (CDKs) [17,18], Polo-like kinases (PLKs) [19,20] and interaction with DNA, specifically by intercalation with DNA or by binding through the minor groove [21–27]. In addition, recent reports indicated that these compounds also inhibit protein synthesis translation and DNA photocleavage [28,29]. On top of this, due to the presence of aromatic planar tricyclic nucleus, β-carbolines could intercalate DNA by stacking between DNA base pairs [21,24]. This capability has further strengthened the rationale of targeting β-carbolines structure in discovering novel anticancer agents as doxorubicin and dactinomycin, two well-known anticancer drugs for Hodgkin's sarcoma and Ewing's sarcoma treatment, are DNA intercalating agents [30].

In continuing our efforts in developing β-carbolines as significant anticancer agents [31–33], the present study aimed to design and synthesize a series of novel N^2,N^9-bis(substituted benzyl)-β-carbolineum bromides. Meanwhile, evaluation of their *in-vitro* cytotoxicity profile on selected human cancer or non-cancer cell lines and comparison of their cytotoxicity profile with known anticancer drugs were also conducted. In addition, we report for the first time, single X-ray crystal structure of compound **4f** and its orientation that binds to the DNA complex. In order to understand the molecular interaction of compound **4f** with its macromolecular target, the molecular docking technique was performed to provide a clear view of molecular interaction between drug and DNA. The theoretical results were compared with experimental data and are found in good agreement.

2. Materials and Methods

2.1. General

All reagents used in this study were purchased from Aldrich Co. Ltd. and used directly without further purifications. Melting point of synthesized compounds was determined using Stuart SMP 20 Melting Point B-545 apparatus and was uncorrected. Fourier-transform infrared (FT-IR) spectra were recorded on a Nicolet 6700 FT-IR spectrometer (Thermo Scientific, MA, USA) in the mid-IR region (400-4,000 cm^{-1}) using Attenuated Total Reflection (ATR) technique. 1D (^{1}H- and ^{13}C-) and 2D (DEPT90, DEPT135, COSY, HSQC, HMBC) NMR spectra were recorded on Bruker AV 500 MHz and 125 MHz instruments in $CDCl_3$. The chemical shifts (δ) were reported in ppm relative to the TMS as internal standard and *J* values were reported in Hertz. The electrospray ionization mass spectrometry (ESI-MS) was recorded on an LC-MS THERMO QUEST Finnigan LCQ DUO system. Elemental analysis (CHN) was conducted using Thermo Finnigan Flash EA 1112 elemental analyzer. Thin-layer chromatography (TLC) was performed on glass plates coated with silica gel G254 (e-Merck) and exposed to iodine vapours to monitor the reactions' progress to certify the purity of the reaction products.

2.2. Synthesis

2.2.1. Procedure for the synthesis of intermediates ***2*** and ***3***

According to the previously published method, intermediate **2** was synthesized in good yield using Pictet-Spengler cyclization [34,35]. The reaction involved L-tryptophan (**1**) in the presence of the formaldehyde afforded the corresponding diastereoisomeric mixture 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**2**). The synthesis of aromatic β-carbolines (**3**) involves a simple method using sequential decarboxylation and aromatization of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**2**) with 10 mol% of $CuCl_2$ without any catalyst [36]. Thus, a convenient protocol for the synthesis of aromatic β-carbolines (**3**) *via* copper(II)-mediated decarboxylation and subsequent aromatization of tetrahydro-β-carboline acid intermediate **2** in the absence of a ligand/catalyst was developed. This sequence of reactions can be easily scaled up to produce enough compounds for further transformations.

2.2.2. General procedure for the synthesis of compounds ***4a-h***

Compounds **4a-h** were synthesized according to the modified reported procedure [37]. A mixture of β-carbolines (**3**, 0.2 g, 5.0 mmol) and anhydrous DMF (12.5 mL) was stirred at room temperature for 10 minutes. 60% NaH (7.5 mmol) and substituted benzyl bromide (10.0 mmol) were added. The mixture was heated for 3 hrs at 60°C than continued with refluxed for 5 hrs. Upon completion, the solution was poured into ice-cold water (H_2O, 100 mL) and extracted with ethyl acetate (2 × 100 mL). The combined organic layers were washed with water, brine, dried over anhydrous sodium sulphate, filtered and evaporated to afford compounds **4a-h** as solid powder/crystals. Pure compounds **4a-h** were obtained from recrystallization using ethanol. Physical properties, FT-IR, NMR, Mass and Elemental analysis data for compounds **4a-h** have been placed in the supplementary file.

2.3. Single-crystal X-ray diffraction (XRD) study of compound ***4f***

A supersaturated solution was prepared by dissolving compound **4f** in ethanol at ambient temperature. The prepared solution was slightly warmed and allowed to evaporate slowly at room temperature. After seven days, good quality transparent crystal appeared were allowed to grow to a maximum possible dimension and then harvested. The single crystal obtained was used for X-ray diffraction studies.

A crystal of the compound **4f** suitable for an X-ray diffraction study, with needle habit and having appropriate dimensions of 0.25 mm × 0.17 mm × 0.10 mm, was glued to glass fiber mounted on a Bruker APEX II Duo CCD diffractometer. The diffraction data were collected at temperature 100 K using graphite monochromated Mo-K_α radiation (λ = 0.71073 Å) at a sample-to-detector distance of 5 cm with APEX2 software [38]. The data integration and reduction were carried out with SAINT software, and the empirical absorption corrections were applied to the collected reflections with SADABS program [38]. The complex's structure was solved by direct methods and refined using a full-matrix least-squares method on F^2 using the SHELXTL program [39]. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were placed in calculated positions with C–H = 0.93 and 0.97 Å and refined using a riding model with $U_{iso}(H) = 1.2U_{eq}(C)$. Data for publication were prepared by using SHELXTL [39] and PLATON [40]. Details of the data collections condition and the parameters of the refinement process are summarized in Table 1. Atomic coordinates, thermal parameters and

Table 1
Crystallographic data and structure refinement of compound **4f**

Chemical Formula	$C_{27}H_{27}BrN_2O_3$	
Formula Weight	507.41 g•mole^{-1}	
Temperature	301.0 K	
Wavelength	0.71073 Å	
Crystal System	Monoclinic	
Space group	$P12_1/c_1$	
Unit cell dimensions	a = 13.253(6) Å	α = 90°
	b = 20.809(10) Å	β = 107.215(13)°
	c = 9.116(4) Å	γ = 90°
Volume	2401.4(19) Å^3	
Z	4	
Density (calculated)	1.403 Mg/m^3	
Absorption coefficient	1.743 mm^{-1}	
F(000)	1048	
Crystal size	0.32×0.23×0.13 mm^3	
Theta range for data collection	2.534 to 28.266 °	
Index ranges	-17 ≤ *h* ≤ 17; -27 ≤ *k* ≤ 27; -12 ≤ *l* ≤ 12	
Reflections collected	36610	
Independent reflections	5909 [R(int) = 0.0848]	
Completeness to theta = 25.242 °	99.7 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	5909 / 3 / 310	
Goodness-of-fit on F2	1.005	
Final R indices [I>2sigma(I)]	R1 = 0.0479, wR2 = 0.1052	
R indices (all data)	R1 = 0.1146, wR2 = 0.1318	

bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre (CCDC) with reference number CCDC 1995165.

2.4. In-vitro cytotoxicity studies

The cytotoxic activity of N^2,N^9-bis(substituted benzyl)-β-carbolineum bromides (**4a-f**) were investigated through MTT assay [41]. The cytotoxicity effect was tested against several selected human cancer cell lines, namely hepatocellular carcinoma (HepG2), colorectal adenocarcinoma (HT-29), cervical carcinoma (HeLa) and chronic myelogenous leukaemia (CML) (K562), while non-cancer cell lines were mouse embryonic fibroblast (BALB/3T3 clone A31) and human foreskin fibroblast (Hs-27). All cell lines were obtained from American Type Culture Collection (ATCC) (Rockville, USA). HepG2 and HeLa cells were cultured in Eagle's Minimum Essential Medium (EMEM), HT-29 cells were cultured in McCoy's 5a Medium Modified, K562 cells were cultured with Iscove's Modified Dulbecco's Medium (IMDM), while BALB/3T3 clone A31 (BALB/c3T3) and Hs-27 cells were cultured with Dulbecco's modified Eagle's medium (DMEM). Complete media for HepG2, HT-29, HeLa, K562 and Hs-27 cell lines were supplemented with 10% fetal bovine serum (FBS). In contrast, a complete medium for BALB/3T3 clone A31 cell line was supplemented with 10% calf bovine serum (CBS). All cell lines were adherent cells except for K562 cell line, which was suspension cells. Cell suspension (5-10 × 10^4 cells/mL in 100 μL per well) was seeded into 96-well plates and incubated at 37°C in a humidified atmosphere of 5% CO_2 for 24 hrs to allow cell adherent and growth. After incubation, cells were treated with compounds **4a-h** at 100, 50, 25, 12.5, 6.25, and 3.125 μM for 48 hrs. Then, 20 µL of 5 mg/mL MTT reagents dissolved in PBS was added per well, and plates were incubated in the dark at 37°C for 3 hrs. The medium was carefully removed, and 100 μL DMSO was added to dissolve the formazan crystals. The absorbance was measured at 570 nm against the reference wavelength of 630 nm using a Microplate reader (Multiskan GO) by Thermo Fisher Scientific (Waltham, USA). Cytotoxicity of compounds **4a-h** against the cell lines was expressed as the compound concentration required for 50% inhibition of the cell population (IC_{50}). Cisplatin and doxorubicin were used as positive control drugs.

2.5. UV-Visible spectral studies

UV-Vis spectroscopy was carried out to determine the binding mode of compound **4f** to calf thymus DNA (CT-DNA, Type I) using UV-1800 UV-Vis recording spectrophotometer (Shimadzu Cooperation, Kyoto, Japan) at 25°C. The experiments were carried out using quartz cuvettes to minimize the binding of derivatives to the cuvettes' surface. Stock solution with fixed concentration (10 µM) of the compound **4f** was dissolved in a solvent mixture of 1% DMSO and 99% PBS (0.01 M, pH 7.4), and CT-DNA was prepared by dissolving in an appropriate amount of PBS at pH 7.4. UV-Visible absorption titrations were performed by adding 2 µL CT-DNA solution to the quartz cuvettes containing approximately 10 µM compound **4f.** Absorption spectra were from 200-800 nm. All the solutions used were freshly prepared before commencing the experiment, and titration was carried out until absorbance saturation occurs.

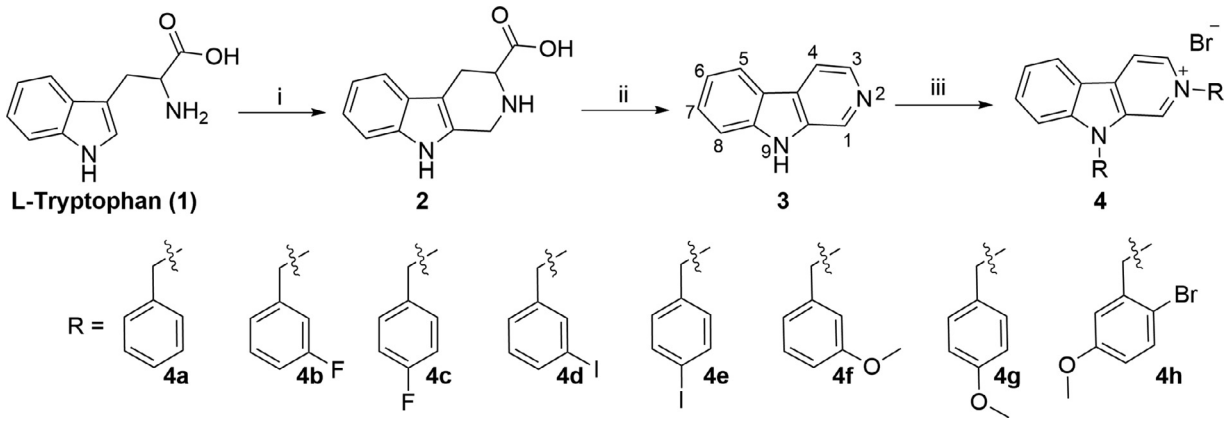
For molecules interacting with DNA, the intrinsic binding constant/association constant (K_b) can be evaluated spectrophotometrically according to the following Benesi-Hildebrand equation [42].

$$\frac{A_0}{A-A_0} = \frac{\varepsilon G}{\varepsilon H-G-\varepsilon G} + \frac{\varepsilon G}{\varepsilon H-G-\varepsilon G} \times \frac{1}{K_b[DNA]}$$

Where K_b is the association/binding constant, *A* and A_0 are the absorbances of the drug and its complex with DNA, respectively, and ε_G and $\varepsilon_{H\text{-}G}$ are the absorption coefficients of the drug and the drug-DNA complex, respectively. The association constant can be obtained from the intercept-to-slope ratios of $A_0/(A\text{-}A_0)$ *vs* 1/[DNA] plots. The K_b can also be determined from the intercept-to-slope ratios of the plot of [DNA] *vs* [DNA]/ε_a-ε_f, where ε_a (or ε_G) and ε_f (or $\varepsilon_{H\text{-}G}$), are the absorption coefficients of the drug and the drug-DNA complex, respectively. A slight shift in the λ_{max} may be observed on the addition of CT-DNA. This blue shift (hypochromic) or redshift (bathochromic) predicts the interaction of compounds with DNA.

2.6. Molecular docking study

Docking simulations of compound **4f** were carried out according to the method described previously [43]. The energy mini-



Scheme 1. Synthesis of the compounds **4a-h**. *Reagents and reaction conditions*: (i) NaOH, HCHO, stirrer at rt for 3 hrs, reflux 3 hrs; (ii) $CuCl_2$, DMF, 130°C, 1h; (iii) Substituted-benzylbromides, NaH, DMF, reflux, 5 hrs.

mized structure of compound **4f** was sketched with ChemDraw ultra (2D and 3D). The crystal structure coordinates (doxorubicin) were obtained from RCSB-Protein Data Bank, and suitable corrections were made using Protein Preparation Wizard from Schrödinger package [44]. The three-dimensional structures of target protein function as a receptor [d(CGATCG)$_2$ oligonucleotide (PDB ID: 1D12)] and was retrieved from the protein data bank (PDB). All the heteroatoms coupled with proteins, including water molecules, bound ligands and any co-crystallized solvent, were discarded from the PDB file. The missing assignments like proper bonds, bond orders, hybridization and charges were assigned using the Molegro Virtual Viewer [45]. Regarding the ligand, compound **4f** was sketched using Hyperchem 8.0 and prepared in PDB format, and their geometries were optimized using molecular mechanics. Finally, docking studies were performed on the compound **4f** by using AutoDock 4.2 docking software, and the results were visualized using Discovery Studio Visualization software.

3. Results and Discussion

3.1. Synthesis

Synthesis of 2,9-bis(substituted benzyl)-β-carbolineum bromides (**4a-h**) was straightforward as depicted in Scheme 1. Cyclization of L-tryptophan (**1**) with formaldehyde was performed using Pictet-Spengler reaction in the presence of NaOH to afford 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**2**). Subsequently, synthesis of *9H*-pyrido [3,4-b]indole (**3**) was accomplished by heating compound **2** at 130°C with $CuCl_2$ (10 mol%) in refluxing DMF. In the final step, N^2,N^9-benzylation of compound **3** was performed using substituted benzyl bromides in the presence of NaH in DMF at refluxing conditions produced the desired 2,9-bis(substituted benzyl)-β-carbolineum bromides (**4a-h**) in good yield.

The chemical structures were confirmed by proton NMR (^{1}H, ^{13}C, ^{1}H-^{1}H COSY correlation spectrum and ^{1}H-^{13}C coupling patterns), mass spectra (ESI-MS) (Fig. S1-S27) and elemental analysis data, as well as single-crystal X-ray analysis for compound **4f** only. ^{1}H-NMR spectra of the compounds **4a-h** showed the presence of characteristic protons in C-1 position in the region of δ 10.1~11.3 ppm. Two sharp singlets for methylene protons in between at δ 5.8~6.3 ppm, respectively, thus confirming the formation of the N^2,N^9-benzylated products **4a-h** (Fig. S1-S27 & Table S1-S4). Furthermore, to confirm the structure using crystallography, we were trying to get a good crystal for 2,9-bis(substituted benzyl)-β-carbolineum bromides (**4a-h**) and succeeded to get a single crystal for compound **4f**. Therefore, we have selected compound **4f** for X-ray diffraction study and confirmed the DNA-drug intercalation.

Table 2
The X-H...Cg(π-ring) interactions of compound 4f

X–H···Cg	d(H···Cg)	<X-H···Cg	d(X···Cg)
C1–H1C···Cg*5*	2.88	136	3.634(17)
C13–H13···Cg*3*	2.72	161	3.613(17)

List of centroids: **Cg3** = C2-C7; **Cg5**= C21-C26

Table 3
Hydrogen bond geometry (Å, °)

D-H···A	d(D-H)	d(H···A)	d(D···A)	<(DHA)
O3-H3B···Br1	1.09	2.85	3.360(16)	109
C17-H17···O3	0.92	2.36	3.275(16)	173
C18-H18···Br1	0.93	2.90	3.805(18)	164
C24-H24···O2	0.93	2.48	3.212(15)	135

3.2. X-ray diffraction (XRD) study

Crystal structure of compound **4f** is discussed in this section. The ORTEP diagram of the molecular structure of compound **4f** ($C_{27}H_{27}BrN_2O_3$) with assigned atom-numbering scheme was presented in Fig. 1. The packing diagram with intermolecular interactions in the unit cells is shown in Fig. 2. In compound **4f**, all bond lengths, angles and torsion angles are within the normal range and comparable with the recently published related structures [46–48].

From the crystallography data in Table 1, the block-like yellow crystal of the compound **4f** belong to monoclinic was crystallized in *P12$_1$/c* space group with a = 13.253(6) Å, b = 20.809(10) Å, c = 9.116(6) Å, β = 107.215(13) °, V = 2401.4(19) Å^3 and Z = 4. The angles between the mean-square planes of the benzene/pyrrole and pyrrole/pyridyl rings of the **4f** structure were 1.40 ° and 1.16 ° respectively, defining that the structure is almost planar. Two 3-methylanisole substituents are bonded to each nitrogen of the norharman's molecule, with the torsion angles of -95.45 (1) ° and -100.40(1) ° along the N1, C8, C6, C7 and N2, C20-C22 respectively. Both 3-methylanisole moieties are perpendicular to each other, with the mean-square planes angle of 70.78 °. In the crystal packing structure (Fig. 2), the compound **4f** was stabilized by C-H···π-Ring interactions, as well as by intermolecular hydrogen bonding listed in Table 2 and Table 3, respectively. The crystallization molecule water, which is present in the crystal structure, agreed with the results obtained from elemental analysis data. Unfortunately, due to the large thermal ellipsoid of O3 of the water molecule, the hydrogen atoms were unable to be refined from the

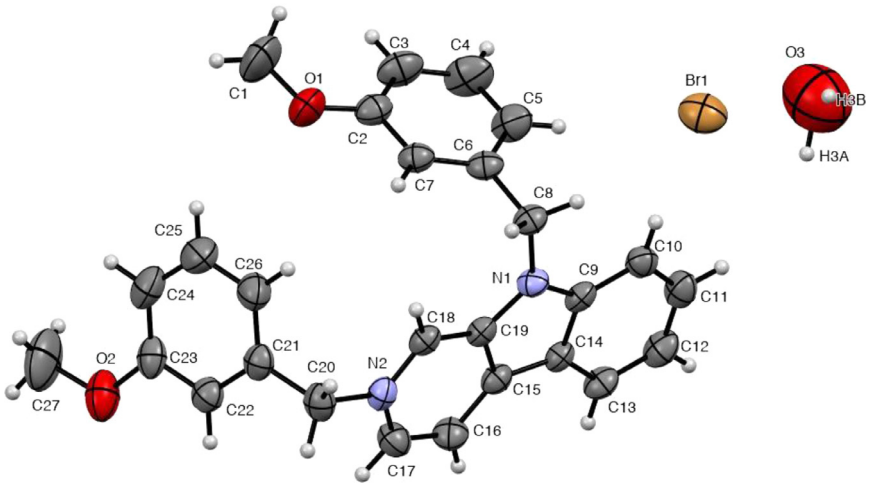


Fig. 1. The molecule structure of compound **4f** with the atom labelling scheme showing 50% probability displacement ellipsoid.

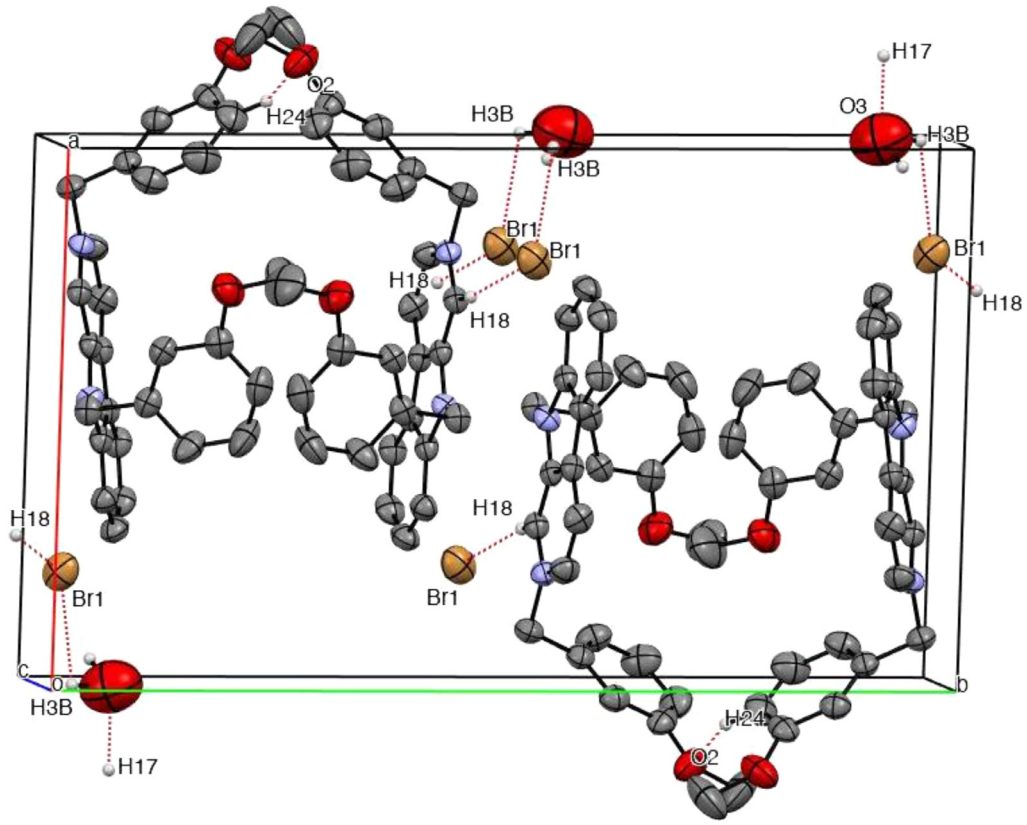


Fig. 2. Crystal packing of compound **4f** viewed down the c-axis. Non-contact hydrogen atoms were omitted for clear view. Red dashed lines indicate hydrogen bonds.

density map. Therefore, a riding model of hydrogen atom was used in order to elucidate the water molecule.

3.3. Cytotoxicity of compounds *4a-h*

Cytotoxicity of compounds **4a-h** against four types of human cancer cell lines and two non-cancer cell lines was evaluated using MTT assay [41]. Cisplatin and doxorubicin were used as positive controls. As shown in Table 4, among eight compounds, **4a, 4b, 4c, 4f** and **4h** exhibited strong cytotoxicity against all the human cancer cell lines tested. Compounds **4f** and **4h** exerted better cytotoxicity generally for all cancer cell lines, but the IC_{50} values were particularly the lowest for K562 cell lines. In addition, cytotoxicity evaluation for compounds **4a, 4b, 4c, 4d, 4f** and **4h** against non-cancer cell lines BALB/c3T3 and Hs-27 shows excellent results, as it was found that the compounds were less toxic to the cells than the commercial anticancer drug doxorubicin.

Even though compounds **4f** and **4h** showed different levels of excellent potency against all cancer cell lines, compound **4f** generated higher IC_{50} values for non-cancer cell lines, thereby making it safer to normal cells. As observed in Table 4, the IC_{50} values of **4f** were 5.35, 6.10, 11.65 and 0.72 μM for HT-29, HeLa, HepG2 and

Table 4
Cytotoxicity of compounds **4a-h** on various cancer and non-cancer cell lines

Entry	IC_{50} (μM)					
	Human cancer cell lines				Non-cancer cell lines	
	HT-29	HeLa	HepG2	K562	BALB/c3T3	Hs-27
4a	8.3±1.75	12.4±1.45	ND	1.08±0.10	45.3±2.55	ND
4b	14.3±1.46	17.4±1.32	14.1±3.22	3.8±0.45	35.8±5.25	25.5±2.82
4c	11.8±1.33	24±1.02	10.3±6.10	4.2±1.45	26.5±2.12	23.1±2.55
4d	ND	ND	ND	2.44±0.22	ND	0.56±0.05
4e	ND	ND	ND	2.43±0.13	ND	ND
4f	5.35±1.32	6.10±1.98	11.65±6.25	0.72±0.05	39.31±3.53	10.68±1.35
4g	ND	ND	ND	0.36±0.14	ND	ND
4h	4.0±1.75	5.9±1.28	3.4±1.05	0.78±0.0	9.8±0.30	5.6±1.28
Cis[a]	97.21±0.57	57.36±5.24	7.75±1.45	5.75±1.53	>100	28.81±3.80
Dox[b]	ND	ND	ND	0.22 ±0.05	0.73±0.01	2.76±0.61

*Data are expressed as mean IC_{50} values ±SD from at least three independent experiments, each performed in triplicate; Positive control drugs are **Cis**[a] = Cisplatin; **Dox**[b] = Doxorubicin; ND = Not determined

Table 5
Selectivity index (SI) values for compounds **4a-h** for K562 cell line, in comparison to non-cancer cell lines

Entry	SI for K562	
	BALB/c3T3	Hs-27
4a	41.94	ND
4b	9.42	6.71
4c	6.31	5.5
4d	ND	0.23
4e	ND	ND
4f	54.6	14.8
4g	ND	ND
4h	12.56	7.20
Cisplatin[a]	23.2	5.0
Doxorubicin[a]	3.3	12.5

*SI data are expressed as mean from at least three independent experiments;
[a] positive control drug

K562 cell lines, respectively. This proved that **4f** was most potent against K562 cell line, evidently by the lowest IC_{50} generated compared to the rest of the cell lines. Anticancer drug cisplatin showed less cytotoxicity against all of the cell lines tested, as observed from higher IC_{50} values generated. Cisplatin showed 18, 9.4 and 8-folds less cytotoxicity than compound **4f** against HT-29, HeLa and K562 cell lines, respectively. But in case of HepG2 cell lines, cisplatin showed 1.5-folds cytotoxicity than compound **4f**. It should be noted that compound **4f** showed 54-folds and 3.9-folds less toxicity than doxorubicin against non-cancer cell lines BALB/c3T3 and Hs-27, respectively.

The cytotoxicity profile of compound **4f** might be due to the β-carboline skeleton's planarity, as shown by the crystal structure. The unsaturated congener of fully aromatized β-carboline has the planar conformation and potent cytotoxicity owing to their ability to intercalate into DNA double helix, which can further cause cell apoptosis [49,50].

As shown in Table 4, the IC_{50} values for **4f** are higher for non-cancer cell lines BALB/c3T3 (IC_{50} = 39.3 μM) and Hs-27 (IC_{50} = 10.68 μM) compared to the IC_{50} value for CML cell lines K562 (IC_{50} = 0.72 μM). Therefore, selectivity index (SI) values were calculated based on the equation (SI = IC_{50} non-cancer cells/ IC_{50} cancer cells), stated in the materials and methods sections and are the results are summarized in Table 5. SI values for compound **4f** for K562 were 54.6 and 14.8 for BALB/c3T3 and Hs-27, respectively. It could also be observed that these SI values are higher than the SI values derived from the positive control drugs cisplatin and doxorubicin. While cisplatin has SI values of 23.2 and 5.0 for BALB/c3T3 and Hs-27, respectively, doxorubicin has SI values of 3.3 and 12.5 for BALB/c3T3 and Hs-27, respectively. These results suggested that compound **4f** is highly selective in killing CML cells, with less toxicity against non-cancer cells. In fact, selectivity to K562, as shown by compound **4f**, is higher than commercial drugs cisplatin and doxorubicin, thus highlighting the potential of compound **4f** to be developed as an effective and selective anticancer drug.

3.4. DNA binding

DNA spectra have been widely used to explore its intercalation with small molecules. In order to confirm the intercalation of **4a-f**, calf thymus DNA (CT-DNA) was selected as the model DNA, **4f** was selected as a model compound of **4a-f**, and a combination of CT-DNA and **4f** was selected as a model system for simulating the interactions of **4a-f** towards DNA. UV spectra and molecular docking were measured with this simulation system. The results provided important spectral evidence for the intercalation of **4f** with CT-DNA and proved that this simulation system should be generally useful to define intercalation of β-carboline with DNA.

3.4.1. UV-Visible spectral analysis

Initially, the UV-Vis spectra of pure CT-DNA at 260 nm was recorded (Fig. S28). The UV spectra of a CT-DNA solution alone in PBS buffer (pH 7.4) and a CT-DNA solution (pH 7.4) plus the representative compound **4f** in PBS buffer were determined determined on a UV-1800 UV-Vis spectrophotometer from 200 to 800 nm. Based on the origin, the hypochromic must lie in the mechanism of interaction of β-carboline **4f** with CT-DNA were investigated (Fig. 3). Before adding DNA, the UV spectra of compound **4f** showed three maxima bands, one peak in the lower wavelength region at 262 nm and two peaks in the higher wavelength region at 315 nm and 390 nm, respectively. A considerable decrease in absorption due to hypochromic effect was observed upon adding 2 μM of DNA to 10 μM solution of compound **4f**. Compound **4f** induced hypochromic effect (93%) and bathochromic shift (2 nm) from 260 to 262 nm compared with the UV spectrum of CT-DNA alone. The maxima at 262 nm were not suitable for the compound's interaction due to the overlapping of compound **4f** and DNA spectra as the DNA molecule shows absorption at 260 nm.

On the other hand, the strong absorption of compound **4f** in the near UV region (292-330 nm) was observed due to the aromatic system's long-living triplet excited state. As shown in Fig. 3, increasing DNA concentrations decreased the absorbance, and the hypochromic effect was observed with no significant shift in peak position. The absorption peak for **4f**-DNA at 315 nm showed a de-

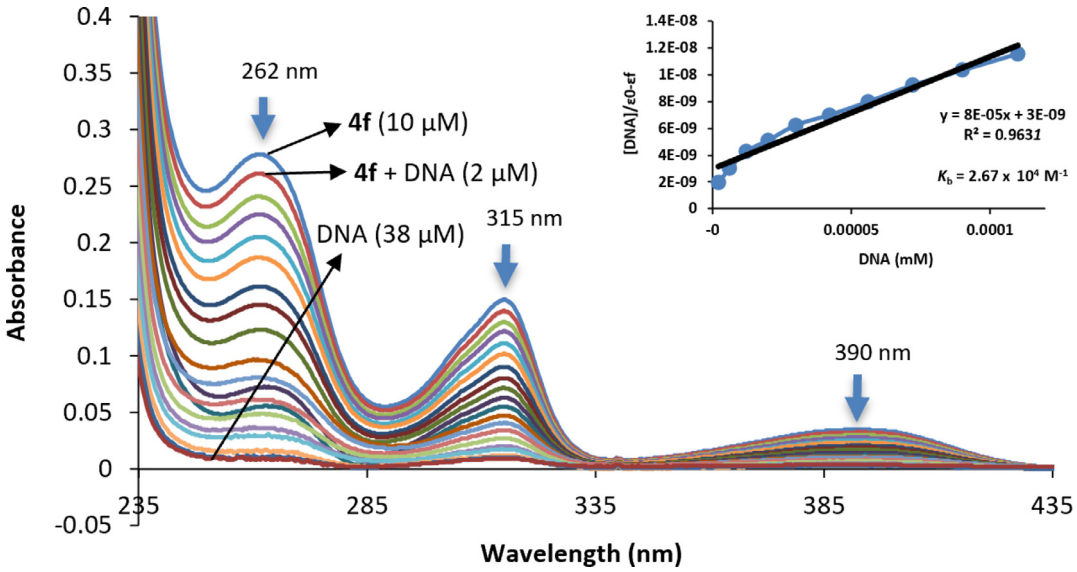


Fig. 3. UV-Visible spectroscopy study of β-carboline derivative (**4f**) with CT-DNA. UV-Visible absorption titrations were performed by adding 2 μL CT-DNA solution each time to the quartz cuvettes containing approximately 10 μM compound **4f.** Absorption spectra were from 200-800 nm.

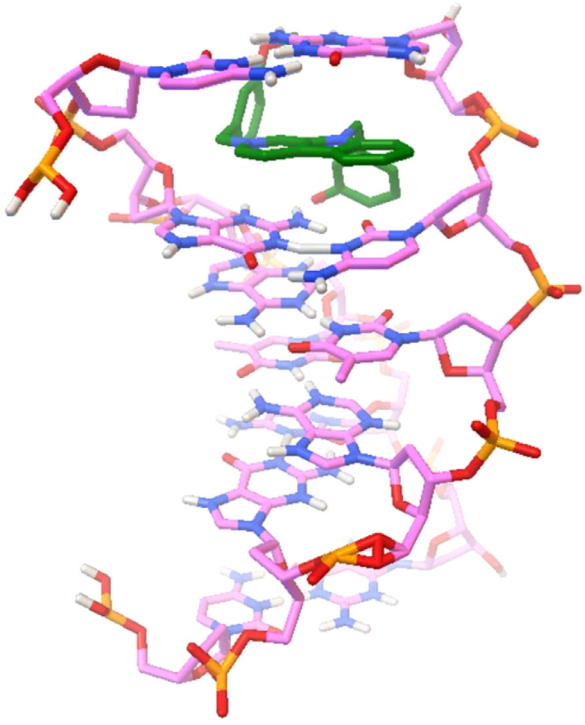


Fig. 4. The docking interaction of compound **4f** with d(CGATCG)$_2$ oligonucleotide

crease in the intensity with redshift and shown hypochromities with 94% and 93%, respectively. These spectra suggest that a binding event has occurred, which may be attributed to the intercalation of compound **4f**. Based on the variations in the absorption spectra of **4f** upon its binding to CT-DNA, the binding constant (K_b) was calculated according to the Benesi-Hildebrand equation [42]. The linear plots for calculating intrinsic binding constant (K_b) was found to be 2.67 $\times$ 10^4 M^{-1}. The hypochromic and bathochromic shifts are considered evidence of intercalation of DNA with small molecules [30]. Thus, the UV changes seen in the UV experiment are direct evidence for intercalation of **4f** with CT-DNA.

3.4.2. Molecular Docking Studies

Compound **4f** was subjected to the docking study with Autodock 4.2 in order to predict its binding mode against the targeted d(CGATCG)$_2$ oligonucleotide, which was retrieved from the Protein Data Bank (PDB ID: 1D12). The docked conformations of compound **4f** were scored with LigandFit/LigandScore function in Discovery Studio Visualizer. The conformation with the highest LigandFit score will be selected as the final docking conformation. Fig. 4 represents the best conformation of compound **4f** against d(CGATCG)$_2$ oligonucleotide, of which its total LigandFit score was calculated as -10.65. Based on the results obtained, **4f** was found to fit nicely into the respective DNA structure and intercalated between nitrogenous base pairs of DNA.

Meanwhile, it is also found to form π–π stacking interactions with purine and pyrimidine bases of the targeted DNA. All these observations further support the results obtained from our UV-Visible spectra-based analysis in which **4f** was found to intercalate CT-DNA. In contrast, no hydrogen bonding was observed between **4f** and the CT-DNA, suggesting that **4f** interacts hydrophobically with the respective DNA.

4. Conclusions

In summary, a novel 2,9-bis(substituted benzyl)-β-carbolineum bromides (**4a-h**) were successfully synthesized from L-tryptophan (**1**) in excellent yields. Among eight, five compounds exhibited potent *in-vitro* cytotoxicity against selected cancer cell lines (K562, HT-29, HeLa and HepG2), with the most potent inhibitory activity exhibited against K562 cell line. Cytotoxicity against non-cancer cell lines BALB/c3T3 and Hs-27 revealed that the synthesized compounds were less toxic than the known anticancer drug doxorubicin. Among the compounds tested, 2,9-bis(3-methoxybenzyl)-β-carbolineum bromide (**4f**) exhibited excellent cytotoxicity against all the cell lines, with the highest selectivity on K562 cell line. The subsequent DNA-binding affinity analysis and molecular docking suggested that compound **4f** could interact with DNA through intercalation.

Supplementary data

NMR (^{1}H, ^{13}C, COSY, HSQC and HMBC) and Mass Spectra of compounds **4a-h** can be obtained in the supporting

information files. Crystallographic data for compound **4f** has been deposited at the Cambridge Crystallographic Data Centre with CCDC number 1995165. The data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by e-mailing to data_request@ccdc.cam.ac.uk, or by contacting The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033.

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Declaration of competing interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.130771.

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