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Synthesis, ADME, and In Silico Molecular Docking Study of Novel N-Substituted β-Carboline Analogs as a Potential Anticancer Agent †

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Abstract: The present study designed and computationally optimized a series of novel β-carboline derivatives to investigate the interaction between designed ligands and selected proteins. Therefore, to find better intercalating agents, β-carboline was used as a basic skeleton, and a series of novel β-carboline derivatives with various aryl groups at C-1 sites and a benzyl group at N-9 position were designed and synthesized and in silico-evaluated for their anticancer activity. The structures of the compounds were identified by employing a range of spectroscopic techniques, including IR, ^{1}H NMR, ^{13}C NMR, and elemental analyses. The silico docking study was performed to determine the maximum interaction between designed ligands and those with protein 1pye **CDK$_2$** inhibitor. The results of the molecular docking study with enzyme 1PYE indicate that the scores and binding modes are similar to those of the co-crystallized ligand. This similarity confirms the anticancer activity of the studied compound, suggesting its potential as a promising candidate for further development as an anticancer agent. In silico **ADME** prediction involves using computational methods to assess the absorption, distribution, metabolism, and excretion of compounds, as well as forecasting their drug-like properties.

Keywords: β-carboline; molecular modeling; ADME; cytotoxicity

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1. Introduction

Cancer is the second most dangerous disease in the world. According to the 2018 WHO report, about 9.6 million people died from cancer globally [1]. Cancer has many types and can affect any part of the body. It is mostly aggressive and lethal. Predictions indicate that the incidence rates will increase in the coming years due to lifestyle changes, such as increased sunbathing and tanning. Over the next 20 years, the number of cancer cases is projected to increase from 14 million in 2012 to 22 million [2]. The academic and pharmaceutical sectors are focused on discovering new cancer chemotherapeutic drugs [3]. Despite significant progress in cancer treatment, challenges like patient compliance, drug resistance, and side effects have motivated the search for novel cancer drugs of clinical significance [4,5]. The breast cancer-resistant protein, now known as ABCG2 as per the gene naming convention, was originally identified in 1998 [6]. It is composed of 655 amino acids and has a molecular weight of 72 kDa. ABCG2 contains a single transmembrane domain (TMD) and a solitary nucleotide-binding domain (NBD), making it a half transporter. Its functional transporter is likely formed through tetramerization [7]. The alkaloid harmine has been identified as an inhibitor of ABCG2 [8]. It is worth noting that all three compounds—Ko143, FTC, and harmine—share a common substructure known as the

tetrahydroβcarboline or β-carboline moiety, which is illustrated in Figure 1. As per a literature search, the majority of natural and synthetic β-carboline derivatives have been reported as potential anticancer agents with antileishmanial, antitrypanosomal [8,9], anti-platelet aggregation, anti-Alzheimer [10], anti-thrombotic [11], and anti-Parkinson [12] effects and as DYRK1A inhibitors [13]. Mechanistically, the anticancer activity of β-carboline derivatives is connected via diverse mechanisms such as intercalating into DNA, inhibiting topoisomerases I and II, blocking the process of cell mitosis, or targeting a specific cancer signaling pathway, such as IkappaB kinase (IKK), 18 CDK, 1 PLK20, etc. Some of the reported potent anticancer *β*-carboline derivatives are shown in Figure 1.

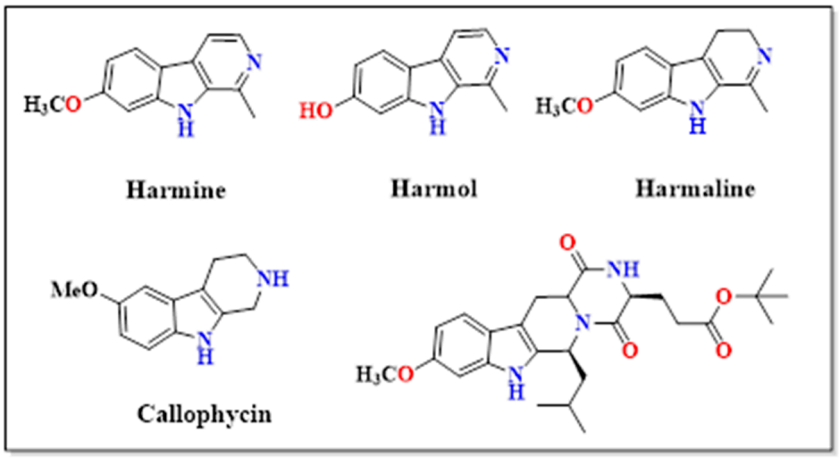
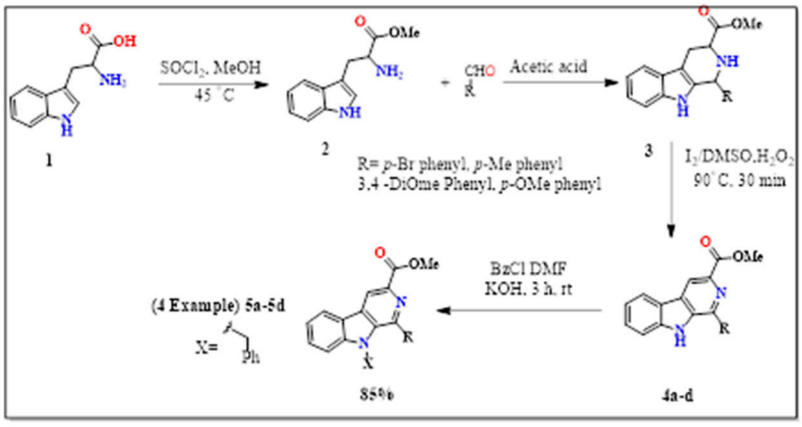


Figure 1. Alkaloid harmine has been identified as an inhibitor of ABCG2.

This research aimed to synthesize new *β*-carboline derivatives with Gaikwad et al.'s methods [14,15]. Our goal was to investigate the inhibitory properties of these derivatives in the context of breast cancer, particularly in comparison to the inhibitory effects of known compounds.

2. Results and Discussion

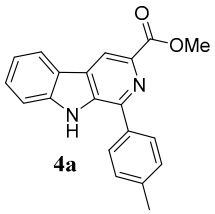
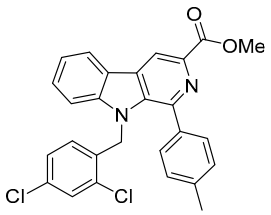
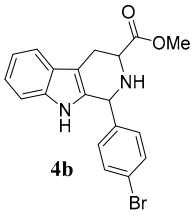
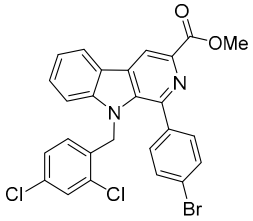
A series of novel *β*-carboline derivatives were designed and computationally optimized to investigate the interaction between designed ligands and selected proteins. The silico-docking studies were performed to find out the maximum interaction between designed ligands and protein with 1pye CDK_2 inhibitor. The best binding post with the minimum energy for the designed ligand was selected for synthesis. The synthetic routes for the preparation of novel derivatives are outlined in Scheme 1 [16].



Scheme 1. Synthesis of novel N-9 alkyl-substituted *β*-carboline.

Initially, the L-tryptophan methyl ester 2 was prepared by the esterification of L-tryptophan 1 using $SOCl_2$ and methanol, which was subjected to Pictet Spengler condensation with appropriate aldehydes in the presence of ammonium chloride (NH_4Cl) [17] and which provided the tetrahydro-*β*-carboline methyl carboxylate **3a**-**3d** (Scheme 1). Further oxidation of the tetrahydro-β-carboline methyl ester with the previously developed protocol, i.e., iodine in DMSO/H_2O_2, gave the methyl 1-phenyl-9H-pyrido [3,4-b]indole-3-carboxylate [16]. Finally, the N-alkylation reaction of β-carboline was carried out with different bases like K_2CO_3, NaOH, triethyl amine, and KOH in DMF solvent. We found better yield as well as less time needed for KOH as compared with other bases. The *β*-carboline methyl ester was treated with various benzyl chlorides with KOH base for 3 h to produce N-alkylated novel *β*-carboline derivatives with up to 71% yield (Table 1). All the novel compounds were characterized by spectroscopic technique (IR,^{1}H NMR ^{13}C NMR, HRMS, MS) data.

Table 1. Physiochemical data of N-9 alkyl-substituted β-carboline.

Sr/No	Product	%Yield	Product	%Yield
1	 **4a**	90%	 **5a**	71%
2	 **4b**	91%	 **5b**	68%

Synthesis of Methyl 9-(2,4-Dichlorobenzyl)-1-(p-Tolyl)-9H-Pyrido [3,4-b]Indole-3-Carboxylate

For the synthesis of compound **5a**, the TH*β*C was initially synthesized from the PS condensation of tryptophan methyl ester with 4-methylbenzaldehyde, which gave THBC methyl ester **4a** as a white solid in 90% yield (Scheme 1) followed by oxidation of TH*β*C by using I_2 in DMSO; H_2O_2 afforded *β*-carboline methyl ester **4a**. The compound Methyl 1-(p-tolyl)-9H-pyrido [3,4-*β*]indole-3-carboxylate was confirmed by spectral characterization. The compound purified by column chromatography yielded a light yellow solid and melted at 190–194°. The IR spectrum of **4a** showed the presence of a N-H broad peak at 3228 cm^{-1}. The IR stretching band at 1712 cm-1 indicates the presence of C=O ester. In the ^{1}H NMR spectrum, the product displayed all the peaks in the aromatic region. The peak at δ 11.58 (s, 1H) corresponds to the N-H group. The peak at δ 3.98 3H, s belongs to the OCH_3 group, as well as the peak at δ2.40 for CH_3. In the ^{13}CNMR spectra of **4a** in DMSO, the peak appears at 166.68, indicating the presence of a C=O carbonyl ester group, and the two singlets appear at the shielded region at 52.31 ppm and 21.42 ppm for 3H hydrogen belonging to OMe and CH_3 at carbon. In ^{1}H and ^{13}C, NMR showed the absence of all aliphatic signals and the presence of additional signals in the aromatic region, which confirmed compound **4a**. In HRMS (ESI+) m/z [M+H] calculated for the chemical formula $C_{20}H_{17}N_2O_2$, an exact mass of 317.1290 was observed at 317.1291. Then, pure compound **4a** was in our hands, which we treated with 2,4-dichloro-benzyl chloride in KOH/DMSO for 3 h, and which afforded yellow oil **5a** with a 71% yield.

The product **5a** was purified by column chromatography and yielded 71% yellow oil. The formation of product **5a** was confirmed by analytical methods. In the primary investigation, the IR peak for N-H was absent in the spectrum, indicating that N-benzylation occurs on the **4a** molecule, while the peak is at 2941 for C-H stretching of the alkyl group. The peak at 1704 cm^{-1} corresponds to C=O stretching for the carbonyl ester.

The ^{1}H NMR spectrum (Figure 2) showed aromatic C-5 of C-H protons appearing as doublets at 8.13 (d, J = 7.8 Hz, 1H) and a singlet at δ 8.79 (1H) for C-4 of the C-H proton, respectively. The benzylic CH_2 proton appeared as a singlet at δ 4.95 for 2H, and another singlet at δ 3.87 corresponds to the OCH_3 group, and a singlet at 2.24 corresponds to the CH3 group. In the ^{13}C NMR spectrum, two signals were observed at δ 173.73 and 166.40, corresponding to carbonyl carbons, and another signal for C-3 carbon. The three signals were observed at δ 70.80, 58.46, and 20.87, corresponding to the CH_2, OCH_3, and CH_3 groups. Finally, the structure of the compound was confirmed by mass m/z [M+H] calculated for the chemical formula $C_{27}H_{21}Cl_2N_2O_2$, with an exact mass of 475.0980 observed at 475.

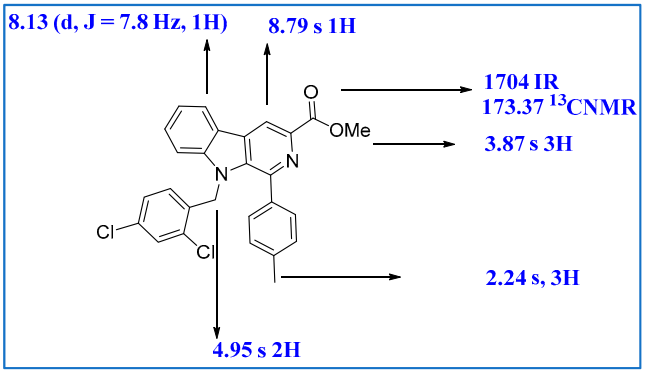


Figure 2. ^{1}H NMR and ^{13}C NMR of compound **5a**.

3. Experimental Section

3.1. Preparation of N-9-Alkyl-β-Carboline Methyl Ester

Tetrahydro-β-carboline ester (1 equiv.), ethyl chloro acetate (1 equiv.), KOH (1.1 equiv.), and DMSO were placed in a dry 10 mL round-bottom flask. The resulting reaction mixture was heated at 60 °C with stirring for 3 h. After consumption of the starting material (monitored by TLC) using ethyl acetate and hexane, the mixture was allowed to cool down at room temperature. Then, 10% hydrochloric acid was added to the reaction mixture for neutralization, which was checked with pH paper. The resulting mixture was extracted in ethyl acetate (3 × 20), with an organic layer dried over sodium sulfate, filtered, and concentrated, and the obtained solid was purified using column chromatography.

3.2. Methyl 1-(4-Methylphenyl)-9-(2,4-Dichlorobenzyl)-9H-Pyrido [3,4-b]Indole-3-Carboxylate

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.13 (d, J = 7.8 Hz, 1H), 7.42 (t, J = 7.7 Hz, 1H), 7.24 (dd, J = 8.6, 3.1 Hz, 2H), 7.19–7.16 (m, 1H), 7.07 (d, J = 1.6 Hz, 1H), 6.96 (d, J = 7.7 Hz, 2H), 6.87 (d, J = 7.7 Hz, 2H), 6.79 (d, J = 8.4 Hz, 1H), 4.95 (s, 2H), 3.87 (s, 3H), 2.24 (s, 3H). δ 13C (101 MHz, $CDCl_3$) 173.37, 166. 40, 144.48, 142.33, 138.69, 137.25, 134.62, 133.62, 133.40, 133.38, 133.16, 132.89, 131.64, 129.60, 129.41, 128.78, 127.44, 126.93, 121.83, 121.44, 116.68,110. 31, 70.80, 42.67, 20.87.

4. Molecular Docking

CADD is a constructive approach to drug discovery, as it allows us to screen and analyze a vast number of compounds efficiently and effectively. In our quest to develop inhibitors for the treatment of cancer, we conducted a molecular docking study with a series of β-carboline derivatives. We utilized the state-of-the-art in silico-docking software AutoDock Vina [18] to design and analyze a series of novel N-substituted β-Carbolines.

The docking study was performed with the crystal structure of CDK_2 with an inhibitor with PDB: 1PYE [19,20] in complex with ligand aminoimidazo [1,2-a]pyridines, which were retrieved from the Protein Data Bank (https://www.rcsb.org/). The compound methyl 9-(2,4-dichlorobenzyl)-1-(*p*-tolyl)-9*H*-pyrido [3,4-b]indole-3-carboxylate **5a** was docked in the pocket of 1PYE, and had 11.9975 kcal/mol binding energy, and **5a** showed the hydrogen bond with an essential amino acid ASP145, ASP:86, PHE:82, ILE:10, and LEU:83. The LEU:83 showed hydrogen bonding with the Cl atom of the benzyl ring, with the LEU:83 amino acid having a distance C=O-----Cl (2.6 Å), LEU:83. Meanwhile, amino acid LYS:33 formed hydrogen bonds with the NH of amino acid with the carbonyl ester group and showed NH-----C=O carbonyl oxygen bond distance is 2.9 Å. The ASP:86 showed the distance within COOH---CH3: 2.5 Å (Figure 3).

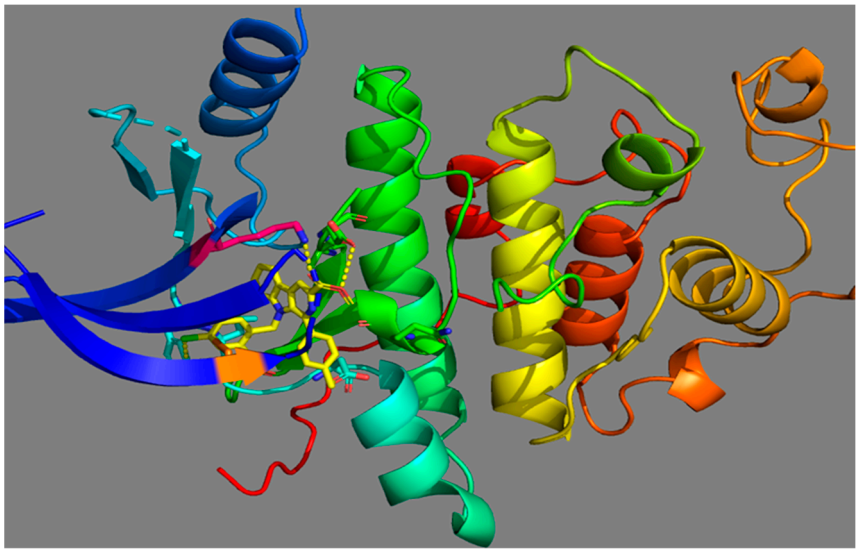


Figure 3. Cartoon model representing the mode of interaction of the protein molecule (yellow) and ligand **5a**.

5. ADME Study and Toxicity

All the compounds fell in toxicity class "**4**", including the reference compound Rifampicin, where class **1** is the most toxic whereas class **3** is the least toxic. The compounds **4a**–**4d** demonstrated neurotoxicity, respiratory toxicity, aromatase, immunological, BBB barrier, and ecotoxicity activities, which indicate potential adverse effects on the nervous system, respiratory system, blood-brain integrity, and environmental health. By contrast, the compounds demonstrated inactivity in cardiotoxicity, cytotoxicity, nutritional toxicity, clinical toxicity, mutagenicity, nuclear receptor signaling pathways, stress response pathways, molecular signaling events, and metabolism, suggesting a plausible lack of adverse effects. The details regarding the toxicological endpoints for each of the compounds and the differences observed can be visualized through the toxicity radar charts [21] (Figure 4). The Edan–Egg model's visual analysis highlights that molecule 5a can only be passively absorbed by the gastrointestinal tract (Figure 5).

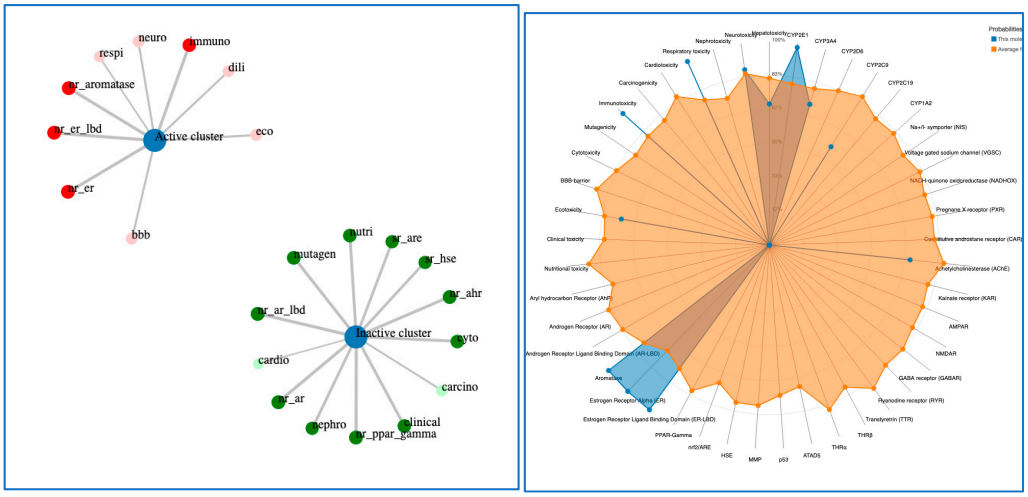


Figure 4. Toxicity radar chart for reference compound Rifampicin. Blue: probabilities for activity—user-defined molecule. Orange: probabilities for activity—average for active molecules.

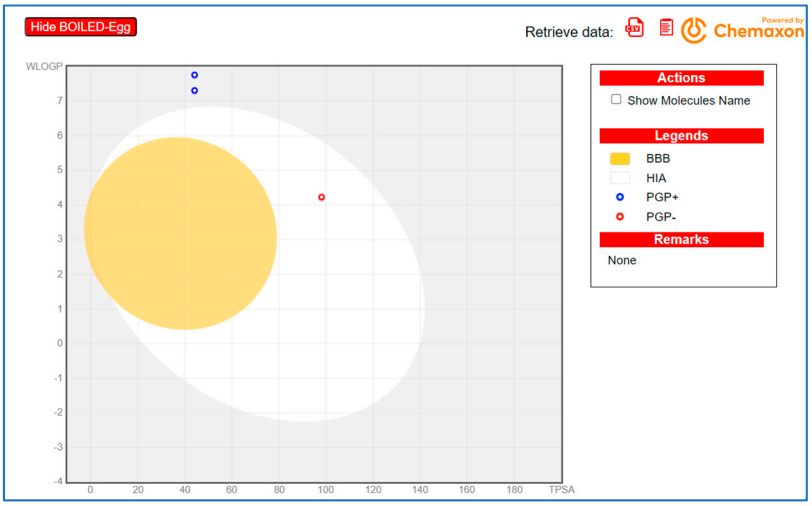


Figure 5. ADME boiled egg diagram.

6. Conclusions

The molecular docking model and the interactions between designed ligands and selected proteins were investigated. The synthesized ligand was identified using different spectroscopic techniques. The compounds **5b** and **5c** revealed promising anticancer activity, with the best binding score, and exhibited good ADME properties with a fall in the toxicity class "**4**", including the reference compound Rifampicin. Hence, it is evident that compound **5c** exhibits the highest potency and should be prioritized for further investigation. These compounds therefore display promising drug-like properties, and their PK/toxicity and ADME prediction profiles support their potential as candidates for further investigation for cancer research.

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Conflicts of Interest: The author declares no conflicts of interest.

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