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Research Article

Synthesis, ADME, Toxicity, and in Silico Molecular Docking Study of Novel β-Carboline Derivatives as Potential Inhibitor Anticancer Agents

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In the present study, a series of new 5-(9-benzyl-1-methyl-9H-pyrido[3,4-b] indol-3-yl)-1,3,4-oxadiazol-2-amine compounds were designed and synthesized (4a-b) by using conventional synthetic methods. ^{1}H NMR, IR, and mass spectral data were used to evaluate the structures of the synthesized compounds. Besides, in silico molecular docking has been done on these newly synthesized compounds in the active pocket of Protein kinase inhibition by the staurosporine PDB:1aq1 complex. It shows a good binding interaction in the active pocket of the PDB:1aq1 enzyme. The ADME and cytotoxicity properties suggest that this compound is best for further studies.

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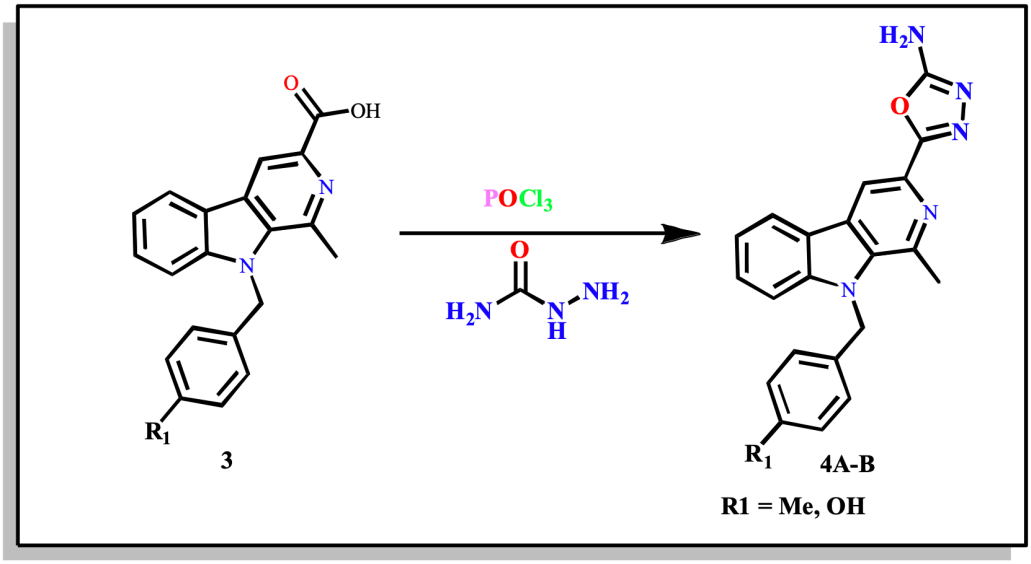

Introduction

Cancer is a significant challenge for society, as it remains one of the leading causes of mortality and morbidity worldwide. Each year, more than 27% of global deaths are attributed to cancer, affecting approximately 20 million individuals and resulting in an average of 9.5 million cancer-related deaths[1]. The current approaches to cancer management are diverse, encompassing chemotherapy, radiotherapy, and surgical interventions tailored to the type and severity of the disease. While substantial progress has been made through research, there are still hurdles to overcome. One of the key issues faced by researchers is 'drug resistance,' which has unfortunately limited the effectiveness of many treatments. Nonetheless, there is great potential in exploring natural products, which have been a valuable source of therapeutic agents in the treatment of various diseases[2]. Many widely used antitumor drugs, such as eudistomin D, eudistomin C, Methyl eudistomin K, and Methyl eudistomin J, have emerged from natural sources (**see Figure 1**). Continuing to investigate these resources and addressing the challenges of drug resistance could lead to more effective cancer therapies and improved patient outcomes in the future.

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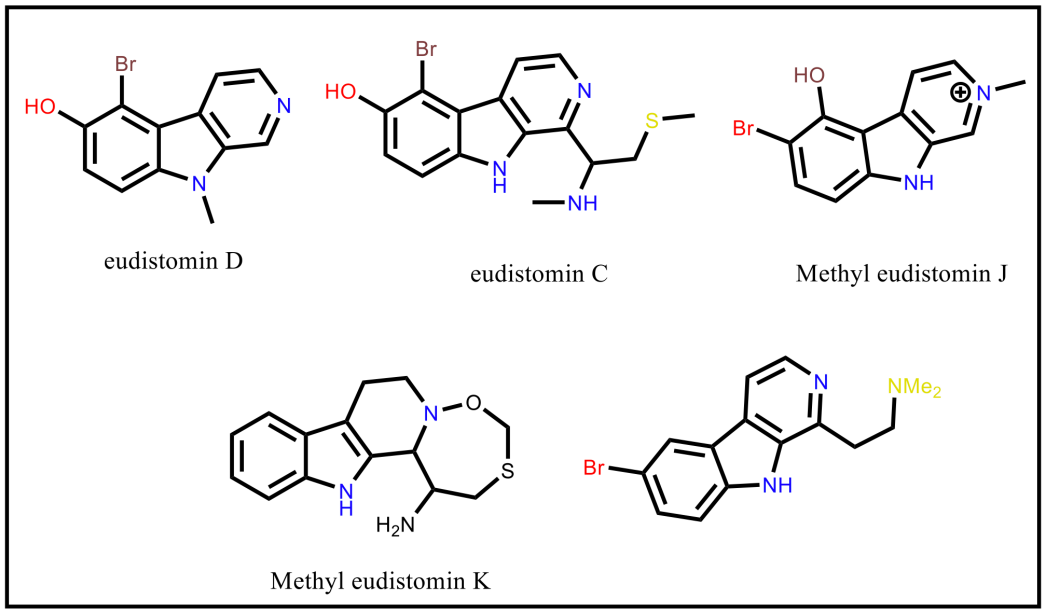

Figure 1. Bio-active natural product

The development of new drugs for the treatment of cancer is facing several issues such as the high cost, length of time required for drug development, and regulatory challenges of performing the necessary clinical evaluations across multiple geographical areas. Hence, due to these limitations, very few classes of anticancer drugs have been developed[3]. The β-carboline scaffold has been widely used in medicinal chemistry and drug development processes. In recent years, more emphasis has been given to seeking new uses and applications of this heterocycle[4]. The modified β-carboline-containing compounds have numerous medicinal and biological activities, i.e., antitumor[5], antibacterial[6], antifungal[7], antiviral[8] and antidiabetic properties[9]. β-carboline derivatives are present in commercially available and widely used[10]

As part of our ongoing study on bioactive benzimidazoles and their analogs, certain fresh and physiologically active benzimidazoles were previously synthesized[11][12][13][14][15]. We have synthesized novel 5-(9-benzyl-1-methyl-9*H*-pyrido[3,4-b] indol-3-yl)-1,3,4-oxadiazol-2-amine scaffolds as part of our ongoing study on these compounds.

Results and discussion

The overall strategy for preparing the target compounds (4A-B) is depicted in Scheme 1. Initially, the starting material L-tryptophan methyl ester (1) undergoes a Pictet-Spengler condensation reaction with the corresponding aldehyde to yield methyl 1-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b] indole-3-carboxylate (**2**). The obtained intermediate methyl 1-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b] indole-3-carboxylate (1) was subsequently aromatized with I_2 in DMSO at 90 ^{0}C[16], to obtain methyl 1-methyl-9H-pyrido[3,4-b] indole-3-carboxylate (2). The obtained intermediates were subsequently N-alkylated with benzyl bromide in K_2CO_3 and DMF solvent at 65 ^{0}C for 5 h, giving 3A-B (**Scheme 1**).

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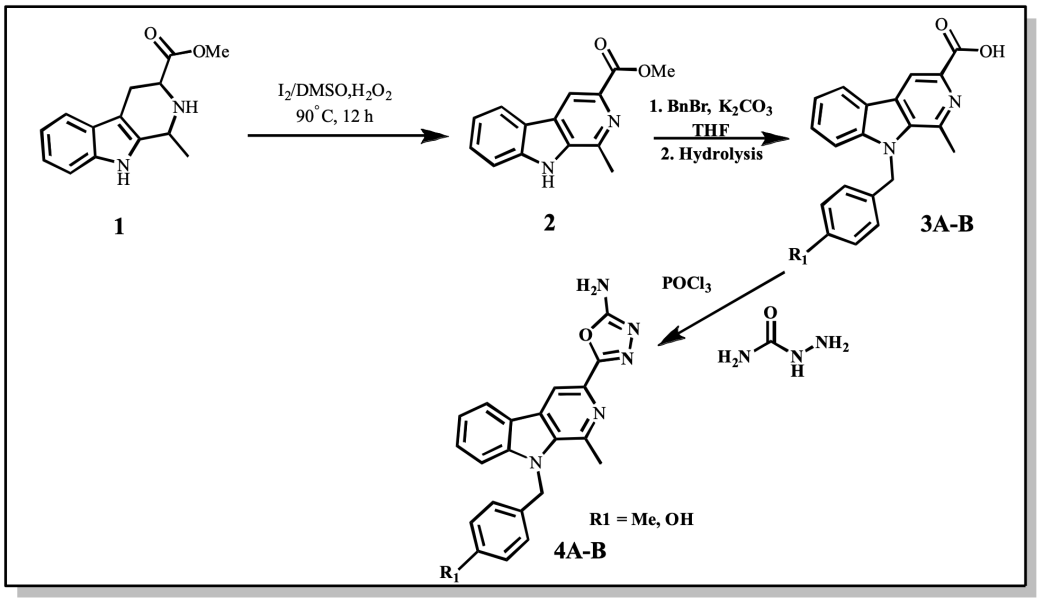

Scheme 1. Synthesis of novel 5-(9-benzyl-1-methyl-9*H*-pyrido[3,4-b] indol-3-yl)-1,3,4-oxadiazol-2-amine

The obtained **3A-B** intermediates were refluxed with hydrazine carboxamide in POCl3 for 4 h, giving new 5-(9-benzyl-1-methyl-9H-pyrido[3,4-b] indol-3-yl)-1,3,4-oxadiazol-2-amine derivatives with a 40% yield. The synthesized final compounds were characterized with ^{1}HNMR and ^{13}CNMR spectroscopic techniques. The ^{1}H NMR peak for the CH_2 proton appears at 5.23 as a singlet, while the peak for the Me proton appears at 2.81 for 3H and 2.30 s (3H), and the aromatic proton appears around δ 7.09 to 8.10 ppm for 8 protons.

Molecular docking

For the development and discovery of potential drug candidates, we have performed molecular docking with the CDK_2 inhibitor protein with an enzyme Protein kinase inhibition by staurosporine PDB:1aq1 complex[17]. The protein PDB:1aq1 was isolated from the protein data bank in the PDB format. The in-silico docking has been done with the help of Auto Dock Vienna docking software[18]. The compound -(9-benzyl-1-methyl-9H-pyrido[3,4-b] indol-3-yl)-1,3,4-oxadiazol-2-amine 4A exhibits good docking results with the enzyme **1**PDB:1aq1, having 10.9975 kcal/mol binding energy. The compound also exhibits the best hydrogen bonds with the essential amino acids.

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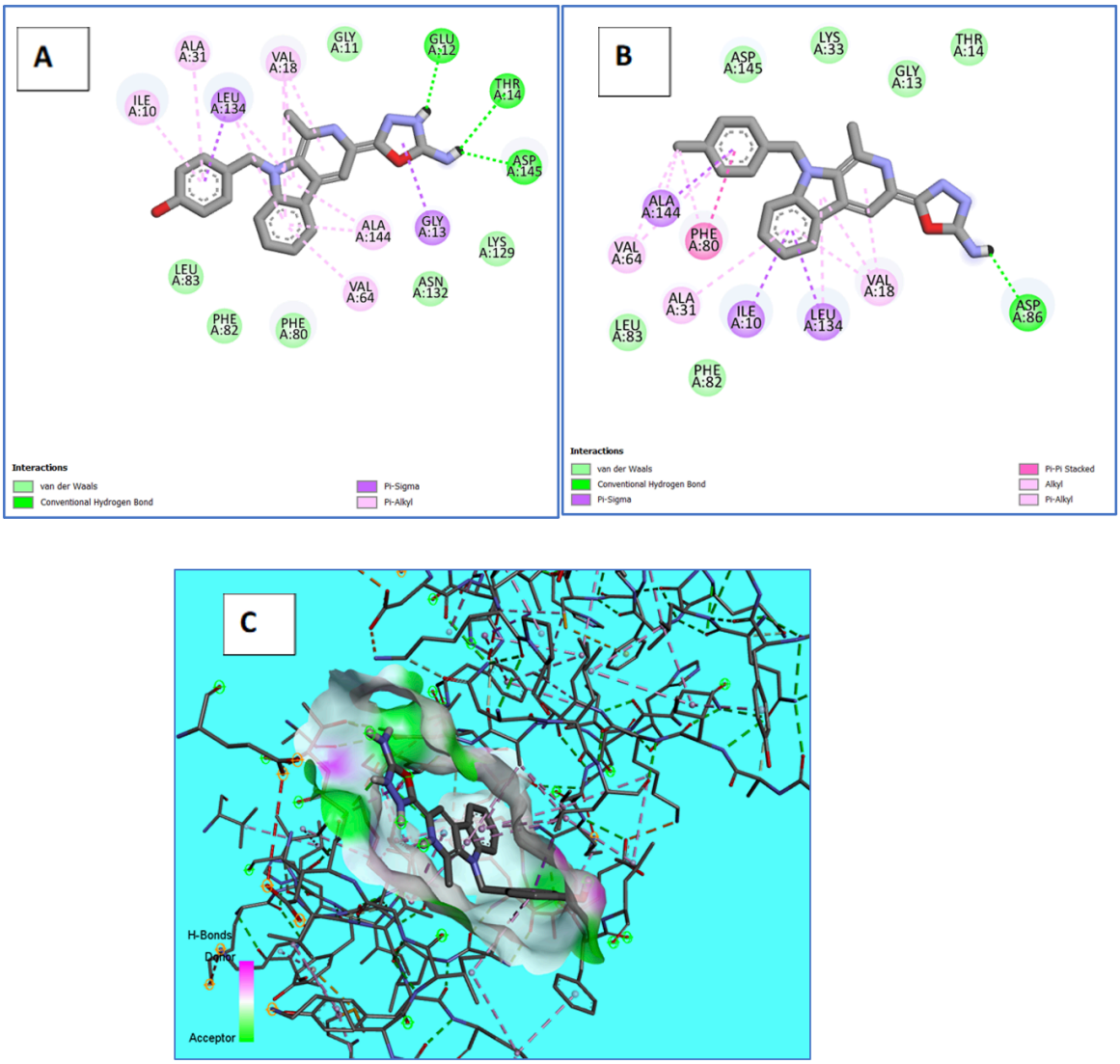

Figure 2. 2D image shows the bonding interaction of ligand **4A (A)** and 4B (**B**) in the active pocket of enzyme 1PDB:1aq1.

The compound (**4A**) 4-((3-(5-amino-1,3,4-oxadiazol-2-yl)-1-methyl-9H-pyrido[3,4-b] indol-9-yl) methyl) phenol shows an 11.8675 kcal/mol binding energy, and **4B** shows the hydrogen bond with essential amino acids GLU:12, THR:14, and ASP:145. The compound (**4A**) 4-((3-(5-amino-1,3,4-oxadiazol-2-yl)-1-methyl-9H-pyrido[3,4-b] indol-9-yl) methyl) phenol shows the Pi-alkyl interaction with GLY:13, Val:18, and LEU:134 amino acids. Similarly, compound 4B exhibits similar bonding interaction to the native ligand staurosporine, with hydrogen bonding interaction with amino acids ASP:86 and a binding energy of 11.6600 kcal/mol. These results indicate that this compound could be useful for further research.

ADME

Molecular Prediction ADME Parameters and Swiss Target Prediction

The ADME studies are predicted by using the Swiss Target Prediction online tools. These two compounds, 4A and 4B, are a bit good for ADME as the results are outlined in Table 1. This result tells us that both molecules are active for further pharmacokinetic studies. The molecules 4A and 4B are very active for high GI absorption and as CYP1A2 inhibitors, while unable to cross the BBB barrier. Likewise, molecule 4A shows the Log Kp (skin permeation) -5.85 cm/s, while

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4B shows the Log Kp (skin permeation) -6.37 cm/s. Nearly all the molecules demonstrate drug-likeness characteristics, adhering to Lipinski's rule with zero violations, and their bioavailability scores are almost identical.

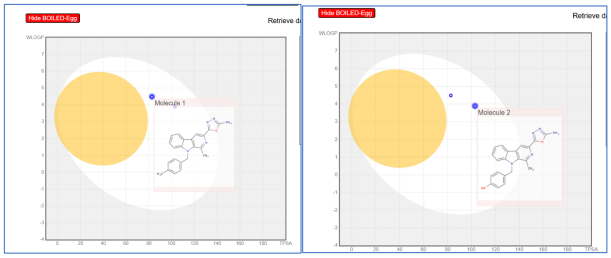

Figure 3. ADME Boiled egg diagram

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Pharmacokinetics	
GI absorption High	GI absorption High
BBB permeant No	BBB permeant No
P-gp substrate Yes	P-gp substrate Yes
CYP1A2 inhibitor Yes	CYP1A2 inhibitor Yes
CYP2C19 inhibitor Yes	CYP2C19 inhibitor No
CYP2C9 inhibitor Yes	CYP2C9 inhibitor No
CYP2D6 inhibitor Yes	CYP2D6 inhibitor Yes
CYP3A4 inhibitor Yes	CYP3A4 inhibitor No
Log Kp (skin permeation) -5.85 cm/s	Log Kp (skin permeation) -6.37 cm/s
Lipophilicity	
Log Po/w (iLOGP) 2.47	Log Po/w (iLOGP) 3.19
Log Po/w (XLOGP3) 3.09	Log Po/w (XLOGP3) 3.81
Log Po/w (WLOGP) 3.89	Log Po/w (WLOGP) 4.49
Log Po/w (MLOGP) 2.37	Log Po/w (MLOGP) 3.12
Log Po/w (SILICOS-IT) 2.94	Log Po/w (SILICOS-IT) 3.94
Consensus Log Po/w 2.95	Consensus Log Po/w 3.71

Table 1. Pharmacokinetics Properties.

Toxicity

Using our ProTox 3.0 prediction pipeline[18], the synthesized compounds have been predicted with toxicity class 4 for acute oral toxicity with an LD50 value of 1000 mg/kg, with a prediction accuracy of 100.00%.

The synthesized compounds 4A and 4B were predicted to be active for neurotoxicity, respiratory toxicity, BBB permeability, carcinoma, and clinical toxicity under the 4^{th} category.

Figure. 4 The toxicity radar chart for **4A and 4A**

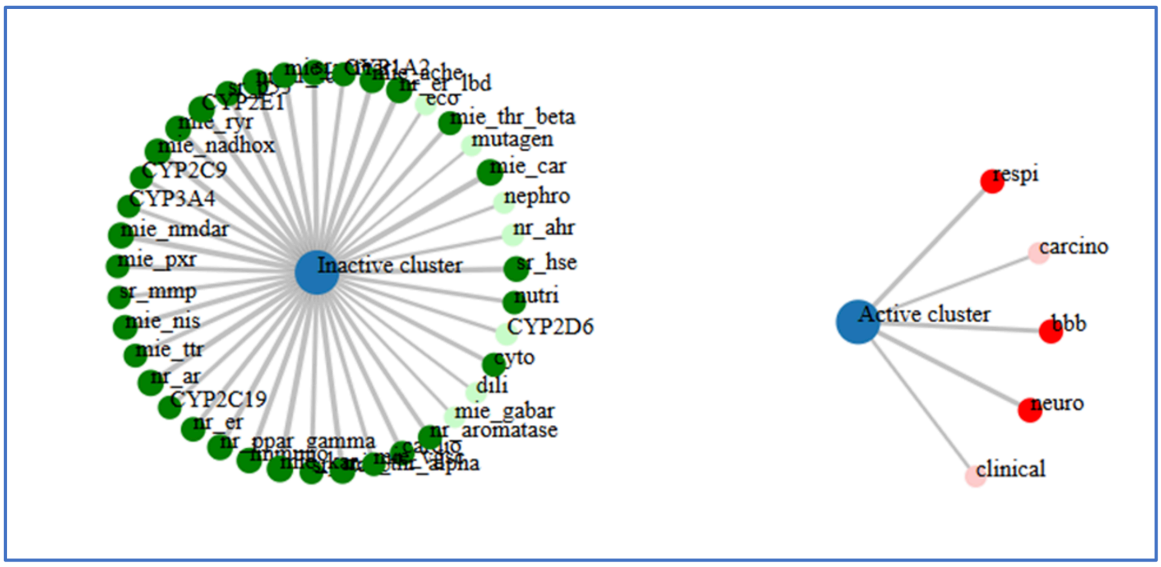

Figure 5. The network chart for 4A and 4B

Experimental section

Aromatization of THβC:

A mixture of THβC methyl ester 0.300 g (0.892 mmol, 1 equiv), Iodine 0.056 g (0.223 mmol, 0.25 equiv), was taken in DMSO, and H_2O_2 (3 to 5 drops) was added. The resulting reaction mixture was heated at 100°C with stirring for 3-4 h. After the consumption of the starting material (monitored by TLC) using ethyl acetate, allow the mixture to cool down to room temperature. Add an aqueous solution of sodium thiosulphate to the reaction mixture and ice. The resulting mixture was filtered, and the obtained solid was purified by column chromatography.

Synthesis of N-9-alkyl-β-carboline-3-methyl ester (3A-B)

In a dry 10 mL round-bottom flask, tetrahydro-β-carboline ester (1 equiv), benzyl bromide (1 equiv), and K_2CO_3 (2.5 equiv) in dry DMF (10 mL) solvent were taken. The resulting reaction mixture was heated at 60°C with stirring for 12 h. After the consumption of the starting material (monitored by TLC) using ethyl acetate and hexane, allow the mixture to cool down to room temperature. After completion of the reaction, the reaction mixture was quenched in 2 M HCl solution; the white precipitates formed. The crude product was extracted with ethyl acetate 3 times and concentrated under high vacuum. The crude product was purified by column chromatography.

Preparation of -((3-(5-amino-1,3,4-oxadiazol-2-yl)-1-methyl-9H-pyrido[3,4-b] indol-9-yl) methyl) phenol (4A-B)

In a dry 50 mL tube, take methyl 1-methyl-9-(4-methylbenzyl)-9H-pyrido[3,4-b] indole-3-carboxylic acid 0.100 g in an excess of $POCl_3$, and reflux the reaction mixture for over 3 h. Check the TLC, and the crude residue was purified by flash chromatography on silica to obtain 5-(1-methyl-9-(4-methylbenzyl)-9H-pyrido[3,4-b] indol-3-yl)-1,3,4-oxadiazol-2-amine 0.050 g.

^{1}H NMR (400 DMSOd_6) δ 8.11 (dd, J = 7.2, 1.1 Hz, 1H), 7.72 (dd, J = 7.4, 1.2 Hz, 1H), 7.81 (s, 1H), 7.66 (td, J = 7.2, 1.1 Hz, 1H), 7.34 (td, J = 7.2, 1.1 Hz, 1H), 7.00 (d, J = 7.5 Hz, 2H), 7.09 (d, J = 7.1 Hz, 2H), 5.20 (s, 1H), 2.81 (s, 3H), 2.30 (s, 3H).

Conclusions

We have prepared the novel 4-((3-(5-amino-1,3,4-oxadiazol-2-yl)-1-methyl-9H-pyrido[3,4-b] indol-9-yl) methyl) phenol (4A-B) derivatives having an amino group and a 3,4-oxadiazole ring in the beta carboline scaffolds. The final compounds are characterized by ^{1}H NMR and ^{13}C NMR spectral analysis. The molecular docking shows very good binding energy; compound

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4A exhibits -11.8675 kcal/mol binding energy, while compound 4B displays -11.6600 kcal/mol binding energy. Based on the in silico results, these molecules could be best for further research in cancer discovery.

Statements and Declarations

Conflicts of interest

All the authors declare that there are no conflicts of interest.

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Declarations

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