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Short Communication

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 were designed, synthesized (4a-b) by using conventional synthetic methods. ^{1}H NMR, IR, and mass spectral data were used to evaluate the structures of synthesized compounds. Besides the in silico molecular docking, have been done on these newer synthesized compounds in the active pocket of Protein kinase inhibition by staurosporine PDB:1aq1 complex, It shows a good binding interaction in the active pocket PDB:1aq1 enzyme. The ADME and cytotoxicity properties suggest that this compound is best for further studies.

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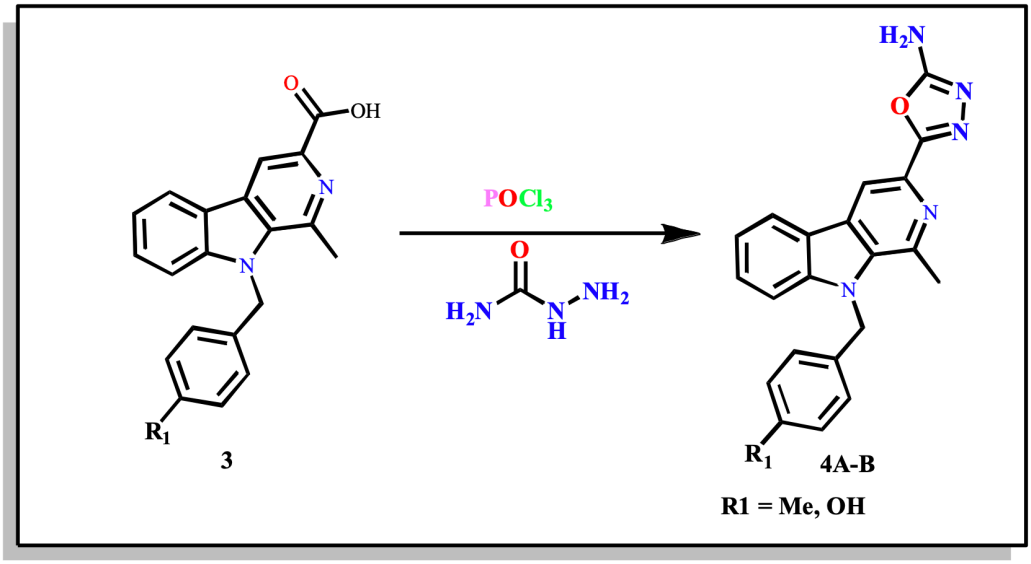

Introduction

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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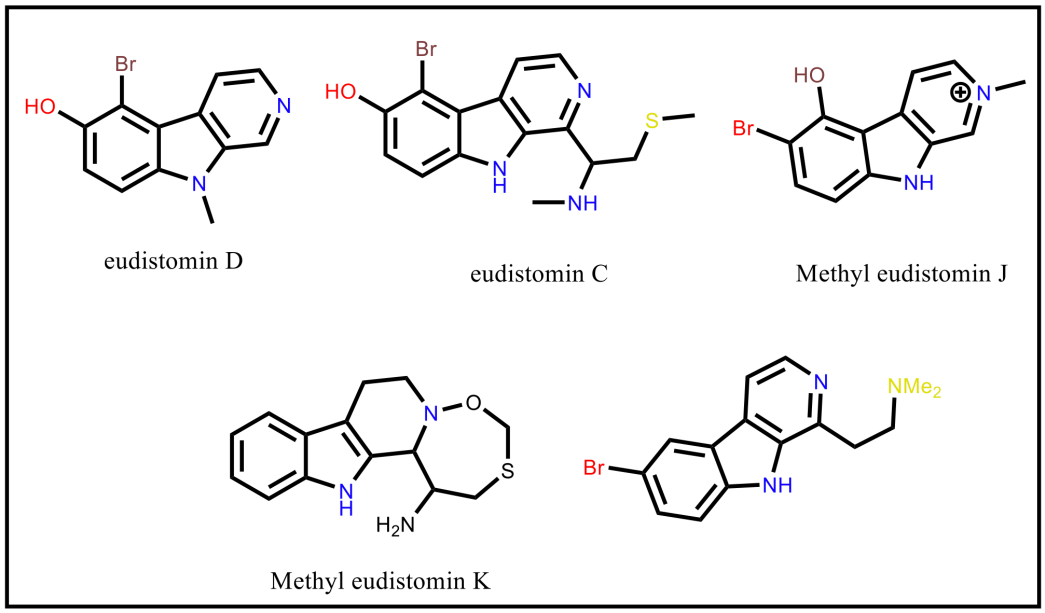

Figure 1. Bio-active natural product

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 is 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 °C[16], obtain methyl 1-methyl-9H-pyrido[3,4-b] indole-3-carboxylate (2). The obtained intermediates subsequently N-alkylated with benzyl bromide in K_2CO_3 and DMF solvent at 65 °C, for 5 h gives 3A-B (**Scheme 1**).

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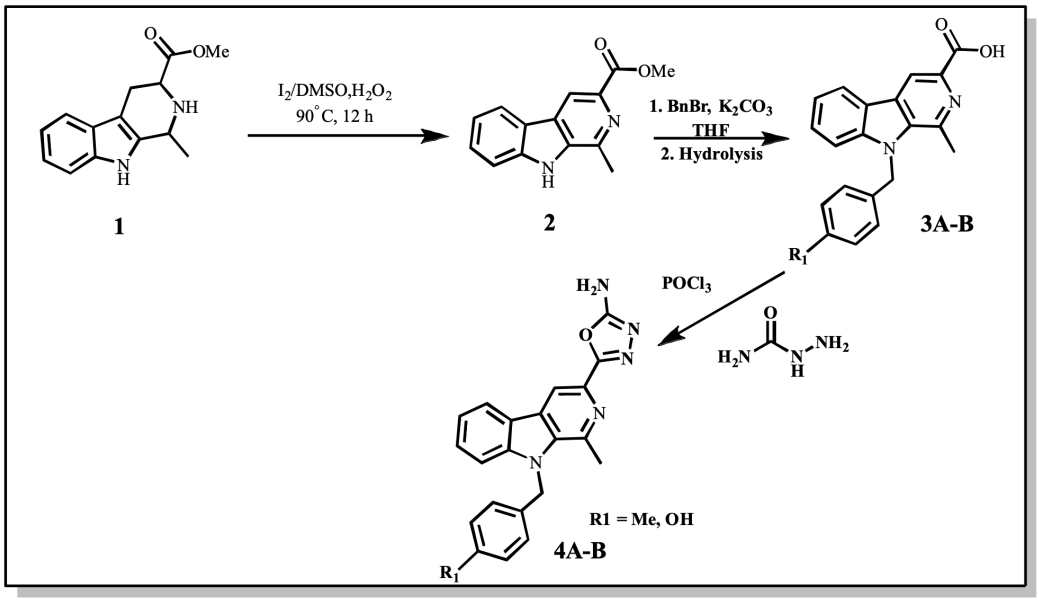

Scheme 1. Synthesis of novel 5-(9-benzyl-1-methyl-9*H*-pyrido[3,4-b] indol-3-yl)-1,3,4-oxadiazol-2-amine

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 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 Viena docking software[18]. The compound -(9-benzyl-1-methyl-9H-pyrido[3,4-b] indol-3-yl)-1,3,4-oxadiazol-2-amine 4A exhibit 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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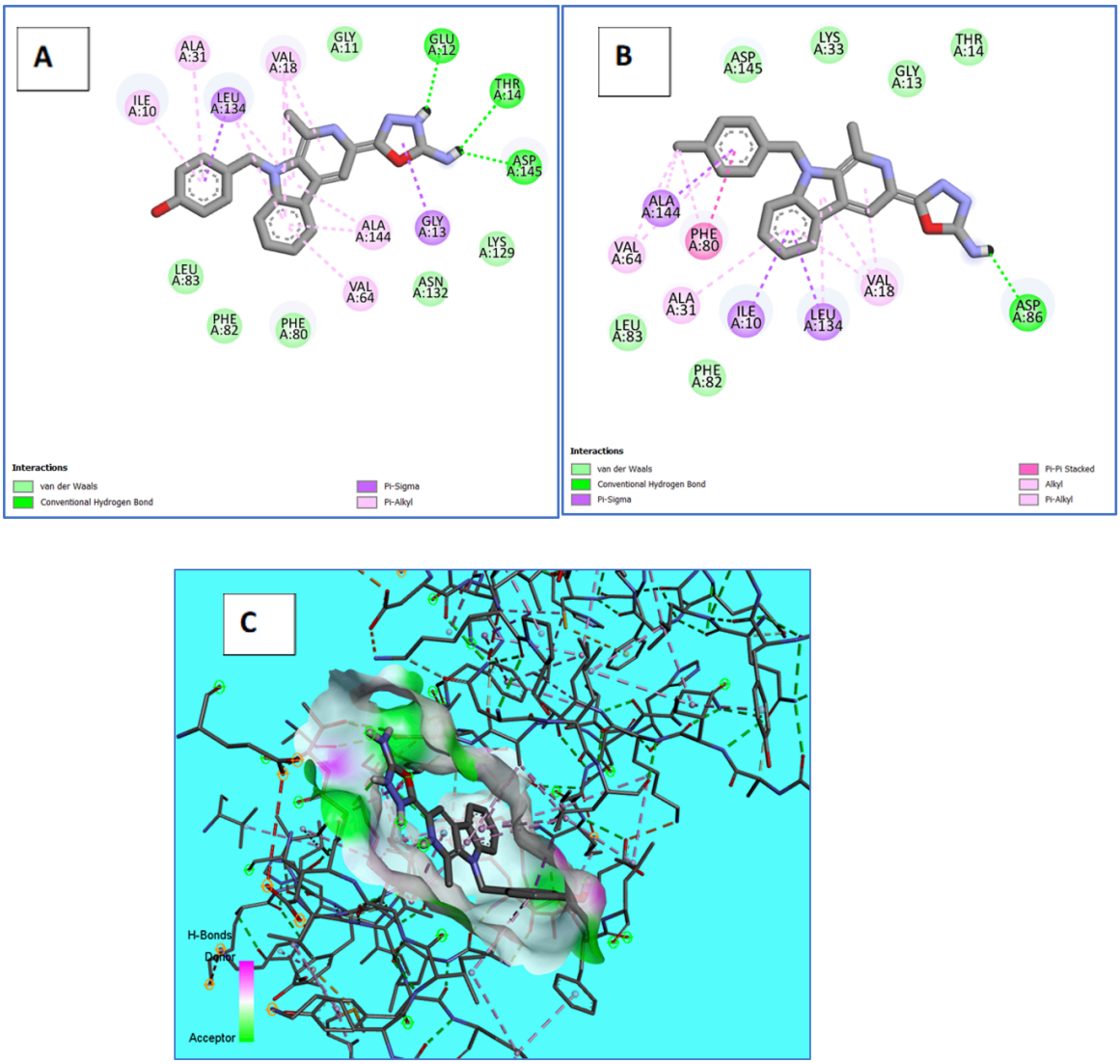

Figure 2. 2D image shows the bonding interaction of ligand **4A (A)** and 4B (**B**) in the active pocket of enzyme **1**PDB:1aq1.

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 the 11.8675 kcal/mol binding energy and **4B** showing the hydrogen bond with an essential amino acid amino acid GLU:12, THR:14, and ASP:145 amino acids and (**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, LEU:134 amino acids. Similarly, compound 4B exhibits similar bonding interaction and to native ligand staurosporine hydrogen bonding interaction with amino acids ASP:86 and 11.6600 kcal/mol binding energy. 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 the 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

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the high GI absorption and CYP1A2 inhibitor, while unable to cross the BBA barrier. Likewise, the molecules 4A shows the Log Kp (skin permeation) -5.85 cm/s, while 4B show 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.

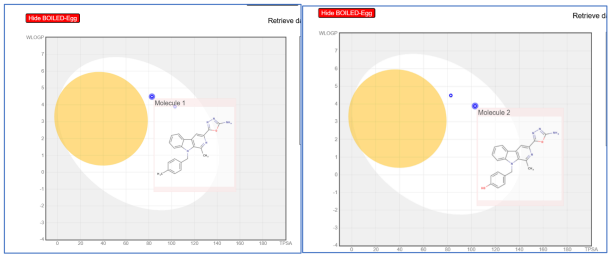

Figure 3. ADME Boiled egg diagram

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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 has been predicted with toxicity class 4 for acute oral toxicity with LD50 value of 1000 mg/kg, with a prediction accuracy of 100.00%.

The synthesized compounds 4A and 4B was 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**

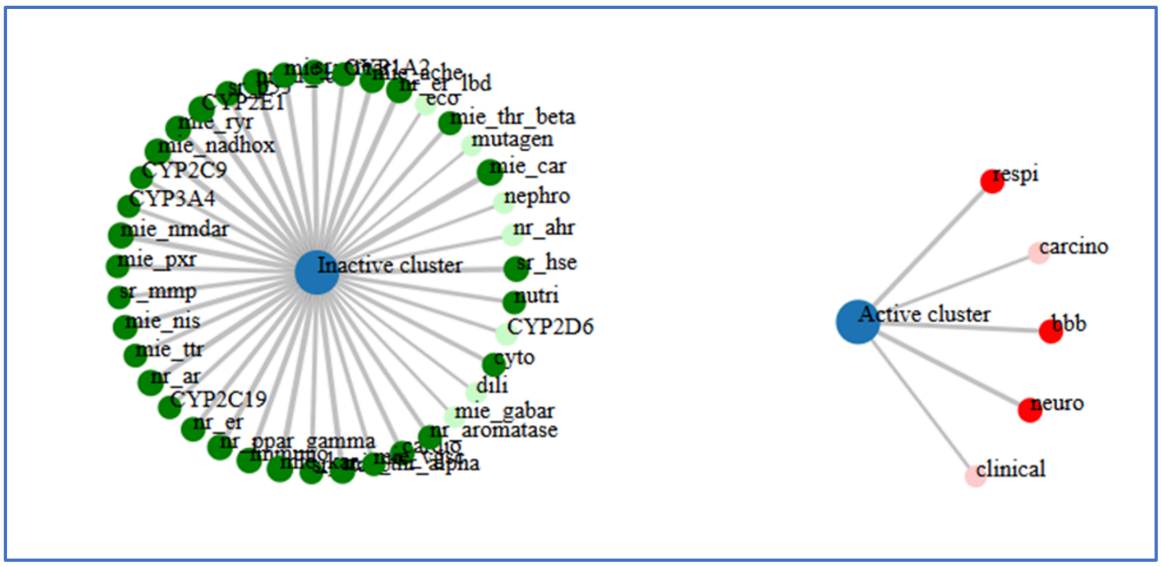

Figure 5. The network chart for 4A and 4B

Experimental section

Aromatization of THβC:

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

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

In a dry 10 ml of dry round bottom flask tetrahydro-β-carboline ester (1 equiv) Benzyl bromide (1 equiv.), K_2CO_3 (2.5 equiv.) in a dry DMF (10 mL) solvent was taken. The resulting reaction mixture was heated at 60°C with stirring for 12h. After consumption of starting material (monitored by TLC) using ethyl acetate and hexane, allow the mixture to cool down at room temperature. After completion of the reaction, the Rm was quenched in the 2m HCl solution, the white ppts formed, the crude product was extracted with ethyl acetate 3 times, and concentrated over the high vacuum, the crude product was purified on 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 a excess of $POCl_3$, reflux the reaction mixture over 3h, check the TLC and crude residue was purified by flash chromatography on silica, 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 prepare 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 heaving amno group and,3,4-oxadiazole ring in the beta carboline scaffolds. The final compounds are characterized with ^{1}HNMr, ^{13}CNMR spectral analysis. The molecular docking shows a very good binding

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energy, compound 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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