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IN VITRO ANTIOXIDANT ACTIVITY, IN SILICO MOLECULAR DOCKING AND ADME PREDICTION STUDIES OF A SERIES OF AMINO SUBSTITUTED β - CARBOLINES

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ABSTRACT
In the present study, we report the in vitro antioxidant activity, in silico molecular docking and ADME prediction studies of a series of aminosubstituted β-Carbolines. Antioxidant activity was tested by using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. Among the tested bioactive products, βCD7 and βCD3, were shown promising antioxidant activity (IC_{50} values 42.79±0.38 µg/ml and 43.13±0.42 µg/ml respectively) compared with the standard drug ascorbic acid (IC_{50} value 34.34±0.21 µg/ml). As a result, we used molecular docking experiments against human peroxiredoxin 5 for βCDs with possible binding energies (-7.01 Kcal/mol to -5.30 Kcal/mol) to investigate repressive mechanisms and reflect a good predicted interactions between the ligand and protein. Further, ADME properties were evaluated in silico by using Lipinski's parameters, topological polar surface area and percentage absorption. Based on the in vitro and in silico studies a series of βCDs may be helpful for further studying SAR and designing more potent antioxidants.

KEYWORDS: β-Carboline, Antioxidant activity, Molecular docking, ADME properties, Lipinski's parameters.

1 INTRODUCTION

β-Carbolines (Pyrido[3,4-b]indole), both natural and synthetic derivatives, have a significant influence on chemical biology and drug discovery. Harmalin was the first known β-Carboline alkaloid, isolated from Peganum harmala in 1841. The presence of β-Carbolines in nature is usual due to its simple biogenesis from tryptophan/tryptamine. It is an important pharmacophore with a wide range of therapeutic activities. β -Carbolines (βCs, A), 3, 4-Dihydro-β-Carbolines (DHβCs, B), and 1,2,3,4-Tetrahydro β-Carbolines (THβCs, C) are the three common types of βCs (1-9) **(Figure 1).**

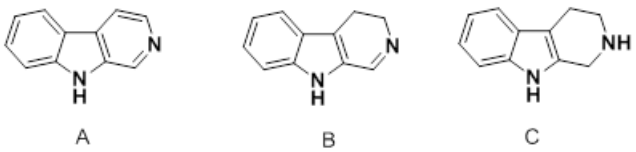

Figure 1: The basic structural units of βCs (A), DHβCs (B) and THβCs (C).

Living organisms produces Reactive oxygen species (ROS) as a result of normal cellular metabolism. They function in physiological cell processes at low to moderate concentrations, but at high concentrations, they cause adverse modifications to cell components (10-15). "Oxidative stress" is defined as a change in the oxidant/antioxidant equilibrium in favour of oxidants. Chronic disorders such as neurodegenerative diseases, diabetes, cardiovascular disease, and cancer all have oxidative stress as a primary factor to their pathogenesis (16-19). Antioxidants are substances that counteract the effects of oxidants. Koteppa Pari *et al* (20), Moura DJ *et al* (21) and Nicole *et al* (22) were some of the previously reported β-Carboline derivatives as antioxidants.

The synthesis and antibacterial activity of a series of title compounds (βCD1- βCD10) were previously reported **(Figure 2)** (23)**.** The potential of βCs and the significance of the search for redesigned antioxidant agents led our group to an ongoing study of this group of compounds. As a result, we report in-vitro antioxidant assessment and molecular docking studies of β-Carbolines in this work. In addition, the Lipinski's parameters, TPSA, and % ABS were explored in silico to study the ADME properties of βCDs.

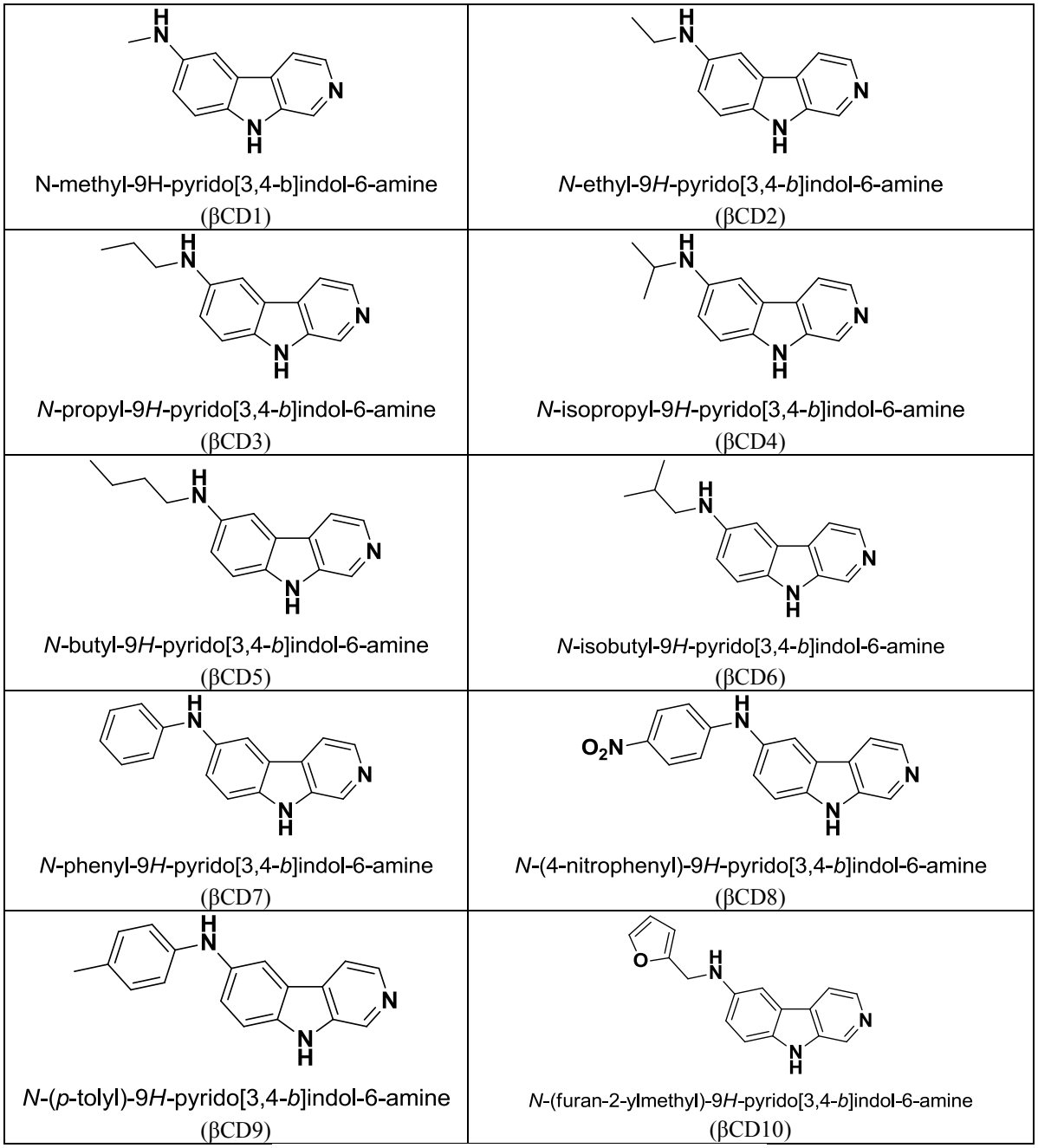

Figure 2: β-Carboline derivatives (βCD1-βCD10).

2 MATERIALS AND METHODS

All of the reagents were purchased from commercial suppliers. The well-known Pictet Spengler reaction was used to create a new series of β-Carboline derivatives. Antioxidant properties of βCDs were determined spectrophotometrically.

2.1 Biological evaluation

2.1.1 Antioxidant activity

The DPPH assay method (24), radical scavenging DPPH has a violet coloring, the intensity of which decreases in the presence of antioxidants proportionally to the ability to "sweep off" free radicals by the tested compound. About 2 ml of different doses of βCDs or standard were added to the 2 ml of DPPH solution (0.1 mM). After 30 minutes of incubation at 37° c in the dark, the absorbance

was measured at 517 nm and all tests were performed in triplicate. Further, to establish the antiradical potential, Graphpad prism 8 statistical linear regression analysis program was utilized in the 95% confidence interval ($p < 0.001$). **Table 1 and Figure 3** summarizes the calculated IC_{50} values.

% Inhibition= $(C_{ab} – S_{ab}/ C_{ab}) \times 100$.

(C_{ab} – Absorbance of control; S_{ab} – Absorbance of sample).

2.2 Computational studies

2.2.1 Computational tools

At first, the ligand structures of title compounds (βCD1-βCD10) were generated using Chem Draw 12.0 software to execute all in- silico studies.

2.2.2 Molecular docking

The goal of molecular docking studies is to predict a ligand's predominant binding mode (s) to a protein (macromolecule). In-silico antioxidant molecular docking was done using the Autodock vina to better understand the inhibitory mechanism as well as the mechanism of interrelatedness of the produced molecules.

The protein model will represent the crystal structure of human peroxiredoxin 5 (PDB code 1HD2) with a resolution of 1.5Å. Water molecules were removed from the protein, polar hydrogen's were added, kolman united atom charges were assigned, and the file was saved in .pdbqt format. Chemdraw 12.0 software was used to draw 2D structure and converted into 3D structure by using Chemdraw 3D and energy minimization carried out by MM2 for each ligand and saved as sdf format later by using Open Bebal software converted into suitable formats. Molecular docking analysis performed by PyMol and Biovia discovery studio.

2.2.3 Study of ADME properties

Using the Molinspiration tools (http://www.molinspirations.com), an in-silico study of the ADME characteristics of βCDs were carried out by investigating their lipinski's parameters, TPSA and % ABS calculations (25-27).

3 RESULTS AND DISCUSSION

3.1 Antioxidant activity

The antiradical activity of the 10 βCDs was determined by using the DPPH assay, and their IC_{50} values (The concentration of the compounds that caused 50 % inhibition) were calculated **(Table 1 & Figure 3)**. βCD7 and βCD3 were showed good DPPH scavenging activity with IC_{50} values 42.79±0.38 µg/ml and 43.13±0.42 µg/ml respectively which are comparable to standard drug ascorbic acid (**IC_{50}** value 34.34±0.21 µg/ml).

Table 1: Antioxidant activity of DPPH scavenger radical for βCDs and Ascorbic acid.

Compound	IC_{50} (Mean ± SEM) µg/ mL
βCD1	55.00±1.39
βCD2	49.97±0.62
βCD3	43.13±0.42
βCD4	50.69±0.10
βCD5	56.35±4.03
βCD6	54.69±1.15
βCD7	42.79±0.38
βCD8	50.80±0.85
βCD9	55.74±4.27
βCD10	56.08±2.98
AA	34.34±0.21

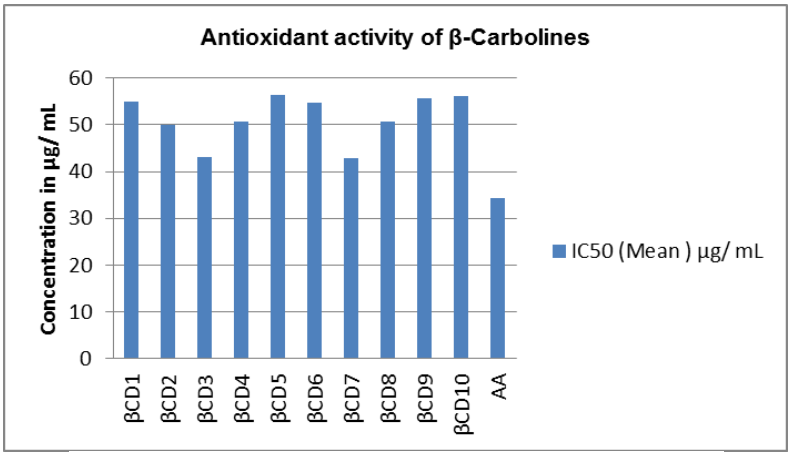

Figure 3: Antioxidant activity of βCDs and Ascorbic acid.

3.2 Molecular docking studies

Molecular docking studies were performed on βCD1 - βCD10 to substantiating the in vitro results with in -silico studies. This research will focus on the enzyme 1HD2. The binding energies of the tested βCDs to the target macromolecule 1HD2 ranged from -7.01 Kcal/mol to -5.30 Kcal/mol. βCD8 (-7.01 Kcal/mol), βCD7 (-6.83 Kcal/mol), βCD9 (-6.61 Kcal/mol), and βCD10 (-6.51 Kcal/mol) were shown to have a high affinity for 1HD2.

Even yet, there was not always a link between the results of the in silico and in vitro studies. Despite the fact that βCD1 (-6.01 Kcal/mol), βCD6 (-6.0 Kcal/mol), βCD9 (-6.61 Kcal/mol), βCD10 (-6.51 Kcal/mol) and βCD8 (-

7.01 Kcal/mol) had high binding affinity for 1HD2 (-6.80 Kcal/mol) showed low antioxidant activity.

Furthermore, the interactions in βCD7 and βCD3 binding energies matched the in vitro DPPH assay experimental findings from antioxidant studies. The antioxidant properties of βCDs may be attributed to contact between the βCDs and the 1HD2, according to in vitro evidence and molecular docking studies. **Table 2** shows the docking results, and **Figure 4, 5 & 6** shows the receptor interactions of the βCD7 & βCD3.

Table 2: Molecular docking studies of βCDs.

Compound	Dock score (Kcal/mol)	No. of Hydrogen bonds	Interacting amino acids	H-Bond distance (Å)
βCD1	-6.01	0	-	-
βCD2	-5.50	1	GLY 85	3.06
βCD3	-5.91	2	GLY 85, GLU 91	2.25, 2.81
βCD4	-5.72	1	VAL 94	2.17
βCD5	-5.30	0	-	-
βCD6	-6.0	0	-	-
βCD7	-6.83	2	ALA 90, VAL 94	2.44, 2.34
βCD8	-7.01	1	GLU 91	2.73
βCD9	-6.61	0	-	-
βCD10	-6.51	0	-	-
AA (Ascorbic acid)	-6.80	4	GLU 16, ARG 86, ALA 90, GLU 91	2.2, 3.0, 2.63, 2.63

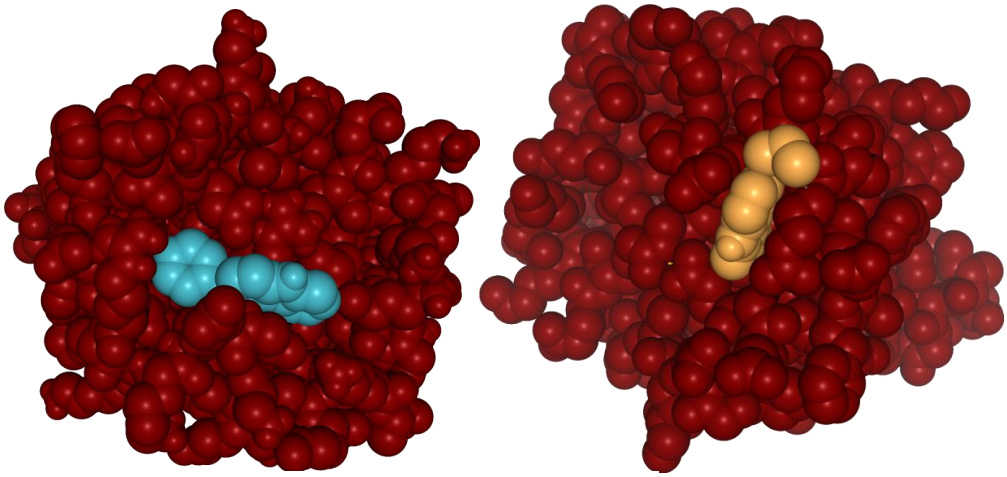

Figure 4: 3D Receptor positioning of the most active candidates βCD7 (left side) & βCD3 (right side).

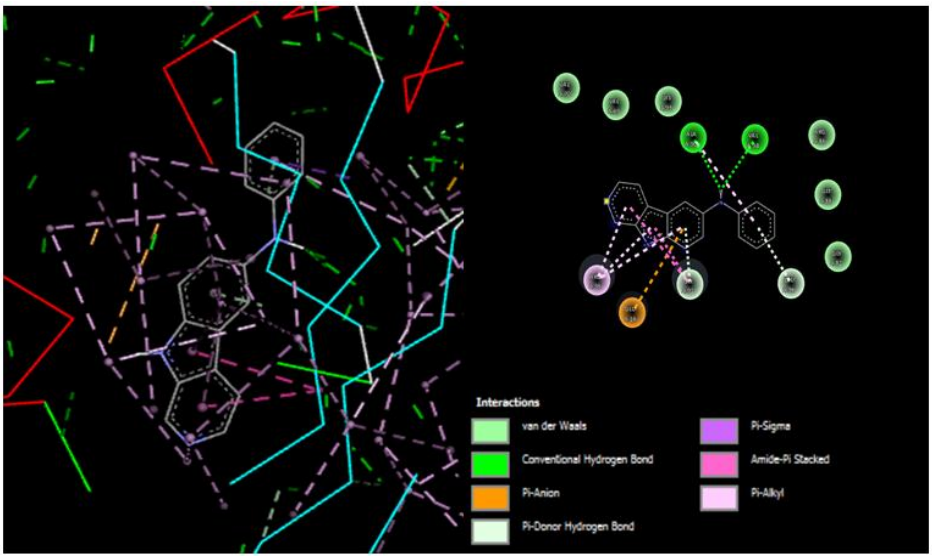

Figure 5: Binding mode of βCD7 into the active site of 1HD2 (left side: 3D interactions & right side: 2D interactions).

Figure 6: Binding mode of βCD3 into the active site of 1HD2 (left side: 3D interactions & right side: 2D interactions).

3.3 Study of ADME properties

Our results **(Table 3)** revealed that the βCDs: i) MW < 500 (197.24-304.3); ii) nHBA< 10 (3-6); iii) nHBD< 5 (2); iv) Lipophilicity < 5 (2.21-4.79) and v) nr < 5 (1-4). All parameters are in agreement with lipinski's rule and the calculated %ABS of all βCDs ranged between 79.15 % and 94.96 %, indicating that these compounds have good permeability in the cellular plasma membrane.

Table 3: % ABS, TPSA, Lipinski's parameters, Log S for compounds βCD1- βCD10.

				Lipinski's parameters					
Com	**%Abs**	**TPSA**	**Log P**	**MW**	**nHBA (ON)**	**nHBD (OHNH)**	**Nr**	**Nv**	**Log S**
βCD1	94.96	40.71	2.21	197.24	3	2	1	0	3.62
βCD2	94.96	40.71	2.58	211.27	3	2	2	0	3.84
βCD3	94.96	40.71	3.08	225.29	3	2	3	0	4.16
βCD4	94.96	40.71	3.54	225.29	3	2	2	0	4.16
βCD5	94.96	40.71	3.64	239.32	3	2	4	0	4.37
βCD6	94.96	40.71	3.33	239.32	3	2	3	0	4.48
βCD7	94.96	40.71	4.34	259.31	3	2	2	0	4.99
βCD8	79.15	86.53	4.3	304.31	6	2	3	0	-5
βCD9	94.96	40.71	4.79	273.34	3	2	2	0	5.27
βCD10	90.42	53.85	2.86	263.3	4	2	3	0	3.77

Note- %ABS= 109-(0.345XTPSA); TPSA= Topological Polar Surface Area; LogP= Octanol-Water partition coefficient; MW= Molecular weight; nHBA (ON) = Number of hydrogen bond acceptors; nHBD (OHNH) = Number of hydrogen bond donors; nr = Number of rotatable bonds; nv = Number of violations; LogS= Solubility

4. CONCLUSION

All the βCDs were tested for the antioxidant properties by DPPH assay. Among the tested compounds βCD7 and βCD3 were found to be most effective antioxidants. Furthermore, molecular docking studies with protein 1HD2 for receptor-ligand interactions were conducted, revealing that all of the βCDs had a high binding affinity to 1HD2. Finally, an in-silico study identified βCDs as potential candidates for new drugs.

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