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Synthesis, Adme, and in Silico Molecular Docking Study of Novel N-substituted β-Carboline Analogs as a Potential Inhibitor 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 Insilco 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. silico docking, the study was performed to determine the maximum interaction between designed ligands and 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 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 the literature search the majority of natural and synthetic β-carboline derivatives have been reported as potential anticancer agents such as antileishmanial, antitrypanosomal, [8,9] antiplatelet aggregation, anti-alzheimer, [10] anti-thrombotic, [11] anti-Parkinson [12] and as DYRK1A inhibitors. [13] Mechanistically, the anticancer activity of *β*-carboline derivatives was connected via diverse mechanisms such as intercalating into DNA, inhibiting, topoisomerases I and II,16 blocking the process of cell mitosis, or targeting a specific cancer signaling pathway,17 IkappaB kinase (IKK),18 CDK,1 PLK20etc. Some of the reported potent anticancer *β*-carboline derivatives as shown in (Figure 1.).

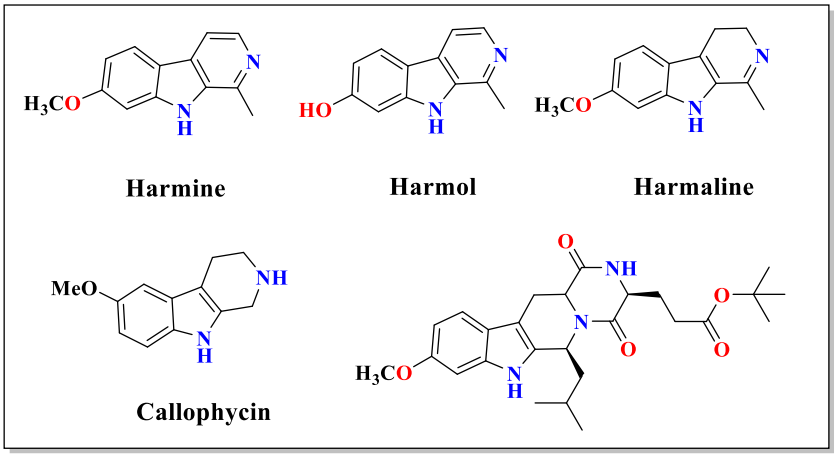
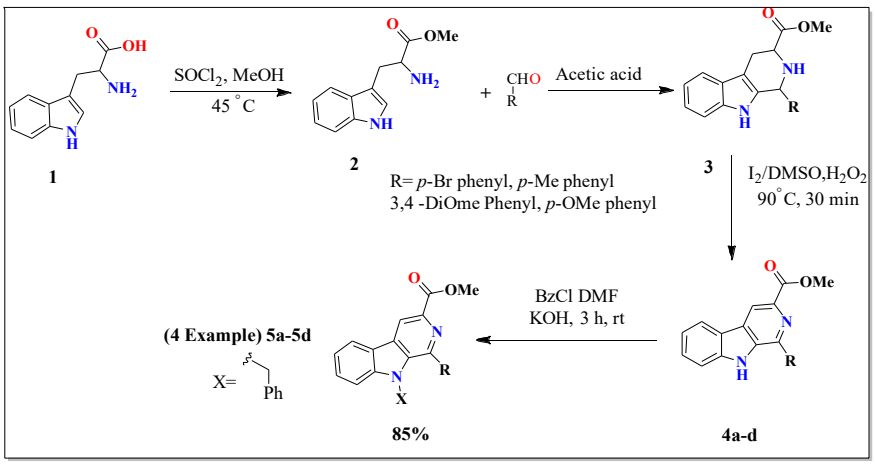


Figure 1. Alkaloid harmine has been identified as an inhibitor of ABCG2.

This research aimed to synthesize new *β*-carboline derivatives with *Gaikwad et al.* 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.

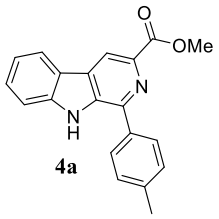
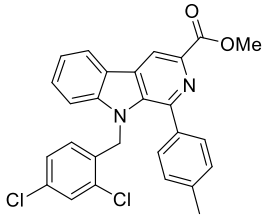
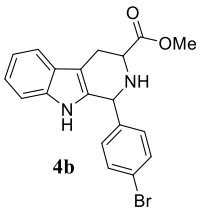
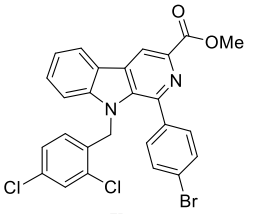
A series of novel *β*-carboline derivatives were designed and computationally optimized to investigate the interaction between designed ligands and selected proteins. The silico docking studies were performed to find out the maximum interaction between designed ligands and protein with 1pye CDK_2 inhibitor. Best binding post with a minimum energy of 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 esterification of L-tryptophan 1 using $SOCl_2$ and methanol, which was subjected to the Pictet Spengler condensation with appropriate aldehydes in the presence of ammonium chloride (NH_4Cl) [17] provided the tetrahydro-*β*-carboline methyl carboxylate 3a-3d (Scheme1.). Further Oxidation of tetrahydro-β-carboline methyl ester with previously developed protocol i.e. iodine in DMSO/H_2O_2, gives to the methyl 1-phenyl-9*H*-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 in KOH as compared with other bases. The *β*-carboline methyl ester was treated with various benzyl chloride in KOH for 3h product N-alkylated novel *β*-carboline derivatives with up to 75%. All the novel compounds were characterized by spectroscopic techniques (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)-9*H*-pyrido[3,4-b]indole-3-carboxylate.

For the synthesis of compound 5a Initially, the TH*β*C was 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 (Scheme13) 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 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 of the product display all the peaks at the aromatic region. The peak at δ 11.58 (s, 1H) corresponds to N-H group. The peak at δ 3.98 3H, s belongs to the OCH_3 group and the peak at δ2.40 for CH_3. In ^{13}CNMR spectra of 4a in DMSO, the peak appears at 166.68 indicating the presence of 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 1H 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 Chemical Formula: $C_{20}H_{17}N_2O_2$ Exact Mass: 317.1290 observed at 317.1291. Then pure compound 4a was in our hands we treated with treated with 2,4-dichloro-benzyl chloride in KOH/DMSO for 3 h afforded yellow oil 5a with 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 4a molecule. While the peak is at 2941 for C-H stretching. Of alkyl group. The peak at 1704 cm-1 corresponds to C=O stretching for carbonyl ester.

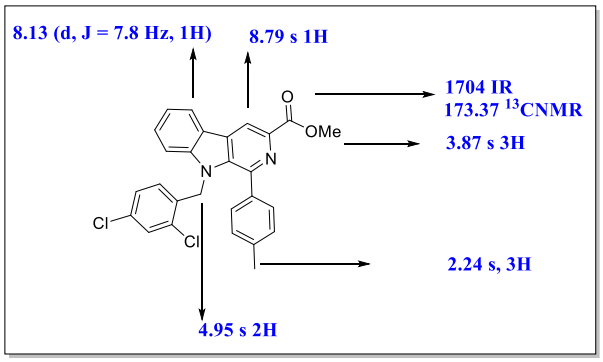


Figure 2. ^{1}H NMR and ^{13}C NMR of compound 5a.

^{1}H NMR spectrum (figure 2) showed aromatic C-5 of C-H protons appeared as doublets at 8.13 (d, J = 7.8 Hz, 1H) and a singlet at δ 8.79 (1H) for C-4 of C-H proton respectively. Benzylic CH_2 proton appeared as a singlet at δ 4.95 for 2H and another singlet at δ 3.87 corresponds to OCH_3 group and a singlet at 2.24 corresponding to CH_3 group. In ^{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 observed at δ 70.80, 58.46, and 20.87. Corresponding to CH_2, OCH_3 and CH_3 groups was observed (figure 2). Finally, the Structure of compound was confirmed by mass m/z [M+H] calculated for Chemical Formula: $C_{27}H_{21}Cl_2N_2O_2$ Exact Mass: 475.0980 observed at 475.

3. Experimental Section:

Preparation of N-9-alkyl-*β*-carboline methyl ester

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

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 have utilized the state-of-the-art Insilco docking software Auto dock Viena [18] to design and analyze a series of novel N-substituted *β*-

Carboline. The docking study has been performed with the Crystal Structure of Crystal structure of CDK_2 with inhibitor with PDB: 1PYE [19-20] in complex with the ligand aminoimidazo[1,2-a]pyridines were retrieved from the Protein Data Bank (http://www.rscb.org/pdb).

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 having 11.9975 kcal/mol binding energy and 5a showing the hydrogen bond with an essential amino acid amino acid ASP145, ASP:86, PHE:82, ILE:10, and LEU:83. The LEU:83 showed hydrogen bonding with Cl atom of benzyl ring with LEU:83 amino acid having distance C=O-----Cl (2.6 Å), LEU:83. While amino acid LYS:33 form hydrogen bonding with NH of amino acid with carbonyl ester group and show NH-----C=O carbonyl Oxygen having distance (2.9 Å). The ASP:86 shows the distance between COOH---CH_3, (2.5 Å) (Figure 3, (A)).

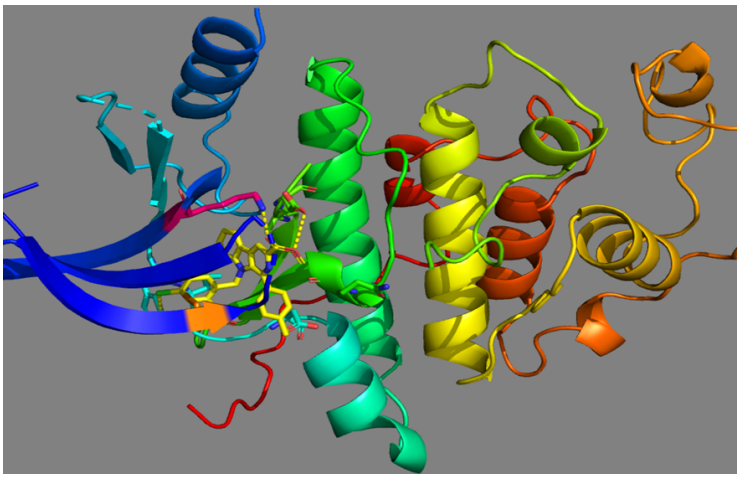


Figure 3. Cartoon model representing the mode of interaction of protein molecule**.** (yellow) to the ligand 5a).

5. ADME Study:

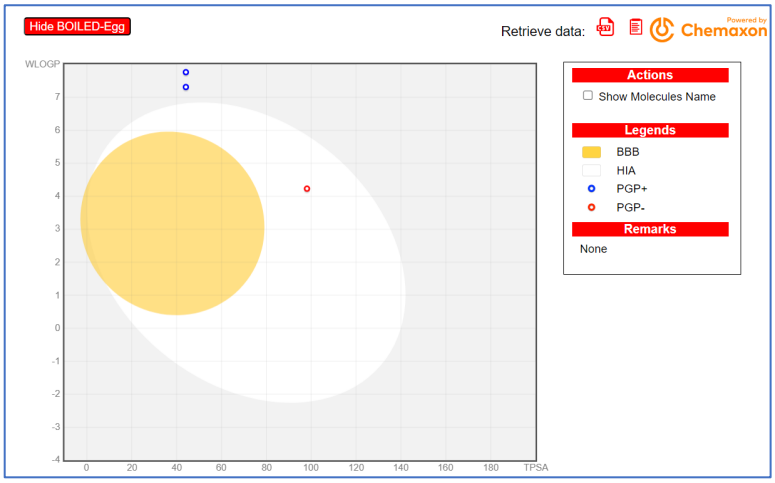


Figure 4. ADME Boiled egg diagram**.**

All the compounds fall in the 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 activity in neurotoxicity, respiratory toxicity, aromatase, immunological, BBB barrier, and ecotoxicity, 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 in these areas. The details regarding the toxicological endpoints for each of the compounds and the differences observed could be visualized through the toxicity radar charts [21] (Figure 5).

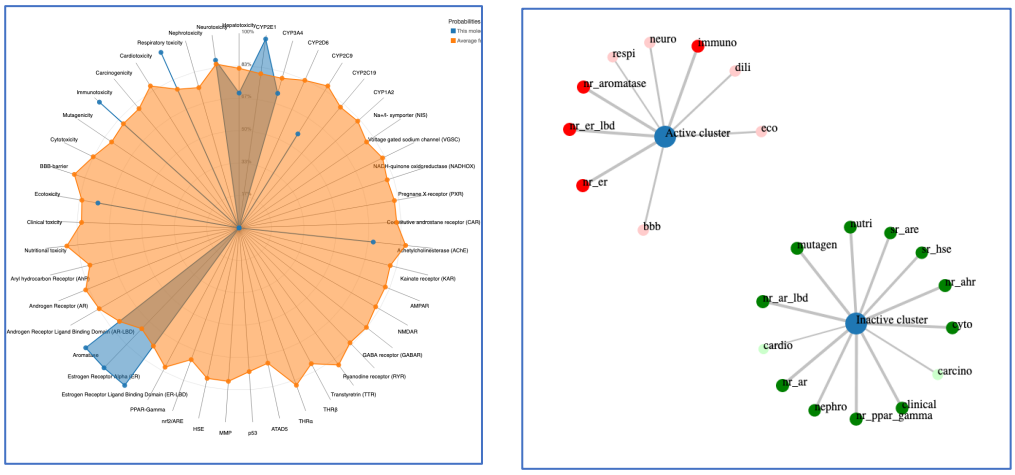


Figure 5. Toxicity radar chart for reference compound Rifampicin (Blue: probabilities for activity - user-defined molecule Orange: probabilities for activity - average for active molecules).

6. Conclusions:

Investigated the molecular docking model and their interaction between designed ligands and selected proteins. The synthesized ligand was identified with 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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Data Availability Statement:

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Reference.

1. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, **2018**, *68*, 394–424.
2. Sathishkumar, K.; Chaturvedi, M.; Das, P.; Stephen, S.; Mathur, P. Cancer incidence estimates for 2022 & projection for 2025: Result from National Cancer Registry Programme, India. *Indian J Med Res.* **2022**, *156*, 598–607.

3. Anand, U.; Dey, A.; Chandel, A.K.S.; Sanyal, R.; Mishra, A.; Pandey, D.K.; De Falco, V.; Upadhyay, A.; Kandimalla, R.; Chaudhary, A.; Dhanjal, J.K. Cancer chemotherapy and beyond Current status, drug candidates, associated risks and progress in targeted therapeutics. *Genes & Diseases* **2023**, *10*, 1367–1401.
4. Kinnel. B.; Singh, S.K.; Oprea-Ilies, G.; Singh, R. Targeted Therapy and Mechanisms of Drug Resistance in Breast Cancer. *Cancers* **2023**, *19*, 1320. doi: 10.3390/cancers15041320.
5. Housman, G.; Byler, S.; Heerboth, S.; Lapinska, K.; Longacre, M.; Snyder, N.; Sarkar, S. Drug resistance in cancer: an overview. *Cancers* **2014**, *6*, 1769–1792.
6. Ajith, A.K.; Subramani, S.; Manickam, A.H.; Ramasamy, S. Chemotherapeutic resistance genes of breast cancer patients-an overview. *Advanced Pharmaceutical Bulletin* **2022**, *12*, 649.
7. Kluth, M. Molecular in vitro analysis of the human ABC transporter MDR3 (Doctoral dissertation). 2014.
8. Gaikwad, S.; Kováčiková, L.; Pawar, P.; Gaikwad, M.; Boháč, A.; Dawane, B. An updates: Oxidative aromatization of THβC to β-carbolines and their application for the β-carboline alkaloids synthesis. *Tetrahedron* **2024**, *155*, 133903.
9. Manda, S.; Khan, S.I.; Jain, S.K.; Mohammed, S.; Tekwani, B.L.; Khan, I.A.; Vishwakarma, R.A.; Bharate, S.B. Synthesis, antileishmanial and antitrypanosomal activities of N-substituted tetrahydro-β-carbolines. *Bioorganic Med. Chem. Lett.* **2014**, *24*, 3247–3250, https://doi.org/10.1016/j.bmcl.2014.06.030.
10. Yao, K.; Zhao, M.; Zhang, X.; Wang, Y.; Li, L.; Zheng, M.; Peng, S. A class of oral N-[(1S, 3S)-1-methyl-1, 2, 3, 4-tetrahydro-*β*-carboline-3-carbonyl]-N′-(amino-acid-acyl) hydrazine: Discovery, synthesis, in vitro anti-platelet aggregation/in vivo anti-thrombotic evaluation and 3D QSAR analysis. *European journal of medicinal chemistry* **2011**, *46*, 3237–3249.
11. Yao, K.; Zhao, M.; Zhang, X.; Wang, Y.; Li, L.; Zheng, M.; Peng, S. A class of oral N-[(1S, 3S)-1-methyl-1, 2, 3, 4-tetrahydro-β-carboline-3-carbonyl]-N′-(amino-acid-acyl) hydrazine: Discovery, synthesis, in vitro anti-platelet aggregation/in vivo anti-thrombotic evaluation and 3D QSAR analysis. *European journal of medicinal chemistry* **2011**, *46*, 3237–3249.
12. Banerjee, R.; Kumar, M., Gaurav, I., Thakur, S., Thakur, A., Singh, K., Karak, S., Das, R. and Chhabra, M., In-silico Prediction of the Beta-carboline Alkaloids Harmine and Harmaline as Potent Drug Candidates for the Treatment of Parkinson's disease. *Antiinflamm Antiallergy Agents Med Chem.* **2021**, *20*, 250–263.
13. Rüben, K.; Wurzlbauer, A.; Walte, A.; Sippl, W.; Bracher, F.; Becker, W. Selectivity Profiling and Biological Activity of Novel β-Carbolines as Potent and Selective DYRK1 Kinase Inhibitors. *PLOS ONE* **2015**, *10*, e0132453, https://doi.org/10.1371/journal.pone.0132453.
14. Gaikwad, S.V.; Nadimetla, D.N.; Kobaisi, M.A.; Devkate, M.; Joshi, R.; Shinde, R.G.; Gaikwad, M.V.; Nikalje, M.D.; Bhosale, S.V.; Lokhande, P.D., 2019. Iodine-DMSO-Catalyzed Chemoselective Biomimetic Aromatization of Tetrahydro-β-carbolines-3-carboxylic Acid: Mechanism Study with DFT-Calculation. *Chemistry Select* **2019**, *4*, 10054–10059.
15. Gaikwad, S.V.; Gaikwad, M.V.; Lokhande, P.D. Iodine-DMSO catalyzed chemoselective oxidative aromatization and deallylation, nondeallylation of aryl allyl ether of tetrahydro-*β*-carboline. *Journal of Heterocyclic Chemistry* **2021**, *58*, 1408–1414.
16. Gaikwad, S., Kamble, D. and Lokhande, P., 2018. Iodine-catalyzed chemoselective dehydrogenation and aromatization of tetrahydro-β-carbolines: A short synthesis of Kumujian-C, Eudistomin-U, Norharmane, Harmane Harmalan and Isoeudistomine-M. *Tetrahedron Letters* **2018**, *59*, 2387–2392.
17. Gaikwad, M.V.; Gaikwad, S.V.; Kamble, R.D. Mild and efficient ammonium chloride catalyzed Greener synthesis of tetrahydro-β-carboline. *Curr. Res. Green Sustain. Chem.* **2022**, *5*, 100268, https://doi.org/10.1016/j.crgsc.2022.100268.
18. Gaikwad, S., González, C.M., Vilariño, D., Lasanta, G., Villaverde, C., Mouriño, A., Verlinden, L., Verstuyf, A., Peluso-Iltis, C., Rochel, N. and Berkowska, K., 2021. Lithocholic acid-based design of noncalcemic vitamin D receptor agonists. Bioorganic Chemistry, 111, p.104878.
19. Madej, T.; Lanczycki, C.J.; Zhang, D.; Thiessen, P.A.; Geer, R.C.; Marchler-Bauer, A.; Bryant, S.H. MMDB and VAST+: tracking structural similarities between macromolecular complexes. *Nucleic Acids Res.* **2013**, *42*, D297–D303, https://doi.org/10.1093/nar/gkt1208.
20. Gaikwad, M.; Gaikwad, S.; Kamble, R. Synthesis of novel series of 1-(6-hydroxy-4-(1H-indol-3-yl)-3, 6-dimethyl-4, 5, 6, 7-tetrahydro-1H-indazol-5-yl) ethan-1-oneas evaluations of their antimicrobial activity with insilco docking study. *J. Med. Chem. Sci.* **2022,** 5, 239–248.
21. Banerjee, P.; Kemmler, E.; Dunkel, M.; Preissner, R. ProTox 3.0: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res.* **2024**, *52*, W513–W520, https://doi.org/10.1093/nar/gkae303.