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Research Article

# Exploring the Antibacterial and Enzyme Inhibitory Potential of Selected β-Carboline Derivatives: *In Vitro* and *In Silico* Insights

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## ABSTRACT

β-Carboline derivatives have a wide range of biological effects such as antiviral, antitumor and antimalarial and are therefore important compounds for drug development. In this study, the effect of some β-carboline derivatives on glucose 6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), and antibacterial activities were investigated. For this purpose, affinity chromatography was used to separate G6PD and 6PGD from human erythrocytes with a specific activity of 1.96 EU/ml protein and 4.569 EU/ml protein, respectively and then in vitro effects of compounds were assayed. It was found that only 1-Isopropyl-2,3,4,9-tetrahydro-1H-beta-carboline, among the selected β-Carboline derivatives, inhibited G6PD with an $IC_{50}$ value of 31.2 µM. It was determined that other derivatives did not have any effect on G6PD and 6PGD activities. Besides antibacterial effects of compounds were examined and compounds were found effective against *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter haemolyticus*. Molecular docking study of isopropyl-2,3,4,9-tetrahydro-1H-beta-carboline molecule to the hG6PD (6E08) receptor was performed and the estimated binding energy was determined as -6.97 kcal/mol. According to the results of antibacterial studies, compounds were docked into some specific proteins of microorganisms as well and possible interactions between receptors and derivatives were discussed.

**Keywords:** Antibacterial, 6-phosphogluconate dehydrogenase, structure-activity relationship, glucose 6-phosphate dehydrogenase, molecular docking.

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## 1. INTRODUCTION

Beta-carbolines are compounds consisting of indole and pyridine rings that are found widely in nature as a component of alkaloids. Beta-carboline and related derivatives are fundamental to a variety of biochemical processes including nitrogen fixation, the action of artificial and natural pharmaceutical compounds, and bacterial chemotaxis. Numerous fungi, marine sources, plants, and animal tissues have all yielded carboline.[1] They are divided into three main groups: fully aromatic carbolines (Cs), 3,4-dihydro-carbolines (DHCs) and 1,2,3,4-tetrahydro-carbolines (THCs). 1,2,3,4-Tetrahydro-β-carbolines (THβCs) are a compound class that has been of interest to pharmacologists and other natural scientists for many years. Beta-carbolines are found in teratogenic plants and in the brain of mammals.[2] Natural alkaloids containing THβC have profound effects on the central nervous system, exhibiting hallucinogenic, vasodilatory and analgesic properties.

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However, due to the breadth of their pharmacological effects and lack of natural selectivity, they have not found much use in modern medicine.[2,3]

THβCs are a favored structure seen in numerous medicinal candidates under research as well as in many medications now on the market. 1,2,3,4- THβCs have diverse biological activities and are continuously being investigated for their use in pharmacological applications. THβCs are of pharmacological interest and have an important role in the development of effective antimalarial medications.[4-6] Manzamine A, a β-carboline alkaloid that was extracted from sea sponge in 1986, is one of the natural compounds that has demonstrated strong antiplasmodial action both *in vitro* and *in vivo*.[7]

The effects of natural β-carboline alkaloids obtained from the plant *Guiera senegalensis*, used in traditional malaria treatment in [Africa], on *Plasmodium falciparum* were investigated and it was found that these compounds were not cytotoxic against human cell lines but showed significant antiplasmodial activity on *P. falciparum*.[8,9] Some THβCs have been reported to exhibit PDE5 inhibitory,[10] antimalarial,[11-13] antiviral[14,15] and antitumor[16-19] activities. Based on the above-mentioned biological activities of THβCs in the current study, it was aimed to investigate the enzyme inhibition potencies of some THβCs (Figure 1) on glucose 6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) that are targeted in malaria treatment in addition to their antibacterial effects. G6PD and 6PGD are enzymes that act in hexose monophosphate pathway or widely known as pentose phosphate pathway (PPP) which provides another pathway for glucose oxidation. About 80–90% of glucose is oxidized by glycolysis in most tissues, and PPP responsible for the remaining 10–20% of the glucose.[20] The oxidative reactions of PPP begin with the dehydrogenation of glucose-6P (G-6P), catalyzed by G6PD, resulting in the production of NADPH. A second NADPH is created in the third stage when 6-phosphogluconate, which is produced by the hydrolysis of 6-phosphogluconolactone, is oxidatively decarboxylated to ribulose 5-phosphate by 6-phosphogluconate dehydrogenase 6PGD.[21-24] Unfortunately, the infected cells benefit from these two outputs of oxidative phases, that are essential for a healthy cell. Disease treatment is disrupted by this circumstance. For instance, the PPP is thought to encourage drug resistance and cancer progression because cancer cells need to proliferate quickly and adapt to hostile settings.[25-27] G6PD plays an important role in the parasite's mechanisms to cope with oxidative stress and has attracted attention as a promising target for the treatment of malaria as well. Studies have found that PPP activity in infected erythrocytes is approximately 78 times higher than uninfected erythrocytes,[28] and in humans, it has been determined that G6PD deficiencies, the initial PPP enzyme, offer some defense against malaria infections.[29] This makes PPP a potential target for antimalarial drugs. G6PD's biological significance extends beyond these illnesses. Additionally, this enzyme is essential to the human immune system's operation. Reactive oxygen species (ROS) are produced by neutrophils and other white blood cells as part of their defense against invasive microorganisms. A significant portion of the NADPH required for ROS production is provided by G6PD. Therefore, G6PD deficiency or inhibition may weaken the immune response, increasing susceptibility to bacterial and viral infections. This role of G6PD has become even more pronounced, especially in cases where the immune system such as sepsis is extremely difficult.[30-32]

In addition to studying the interactions of these inhibitors with target proteins through molecular docking, their drug-likeness properties were thoroughly examined to guide drug design.

## 2. EXPERIMENTAL

### 2.1. Preparation of Hemolysate

Human erythrocytes were obtained from the Turkish Red Crescent Erzurum Branch (Türk Kızılayı Erzurum Şubesi in Turkish). For the hemolysis process, 10 ml of human erythrocytes were taken and treated with 50 ml of ice water and centrifuged at 13000 x g for 1 hour. The upper hemolysate was carefully removed with a dropper and the precipitate containing cell membrane waste was discarded.[33]

### 2.2. Enzyme Inhibition Studies

#### 2.2.a. Isolation of G6PD

Previously prepared 2', 5' ADP-Sepharose 4B column was equilibrated by using 100 mM $CH_3COOK$-$KH_2PO_4$ solution (pH 6.0). As mentioned in Adem and Çiftci (2012),[34] hemolysate was put into the column and the enzyme was eluted through the use of 0.08 M $KH_2PO_4$ solution (pH 7.85), which has 10 mM EDTA, 0.5 mM $NADP^+$, and 80 mM KCl. The eluates having G6PD activity were collected in 1.5 mL fractions and stored at -20 ºC to be used in inhibition studies.[35,36]

#### 2.2.b. Activity Measurement of G6PD

At 340 nm, the G6PD activity was evaluated using a spectrophotometer at 25 ºC by modifying the Beutler (1975)'s method.[35,37] Assay medium consisted of 200 mM Tris-HCl buffer at pH 7.8 (containing 1 mM EDTA and 20 mM $MgCl_2$), 0.6 mM G6P, and 0.2 mM $NADP^+$.

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### 2.2.c. Isolation of 6PGD

2', 5' ADP-Sepharose 4B column equilibrated with 0.1 M $CH_3COOK$-$KH_2PO_4$ buffer at pH 6.0 was used again for isolation of 6PGD. The hemolysate was put into the column, and the identical G6PD elution solution with 5 mM $NADP^+$ rather than 0.5 mM $NADP^+$ was used to elute the enzyme. The eluates having 6PGD activity were collected in 1.5 mL fractions and stored at -20 ºC to be used in inhibition studies.[36,38] Although the purification procedure appeared to be the same as for G6PD, the concentration of $NADP^+$ used in the elution process of 6PGD was 10 times more concentrated than the process of G6PD.

### 2.2.d. Activity Measurement of 6PGD

A spectrophotometer was used to measure 6PGD activity at 340 nm at 25 °C by adapting the Beutler (1975) technique.[37,38] Assay medium consisted of 200 mM Tris-HCl buffer at pH 7.8 (containing 1 mM EDTA and 20 mM $MgCl_2$), 0.6 mM 6-PGA, and 0.2 mM $NADP^+$.

### 2.3. Investigation of *In Vitro* Effects of Tetrahydro β-Carboline Derivatives on G6PD and 6PGD Activities

For compare inhibitory potency of compounds, $IC_{50}$ values were calculated by assaying G6PD and 6PGD activities at various concentration of (**1**) 1-Isopropyl-2,3,4,9-tetrahydro-1H-beta-carboline, (**2**) 1-Methyl-2,3,4,9-tetrahydro-1H-β-carboline-1-carboxylic acid, and (**3**) 2,3,4,9-tetrahydro-1H-beta-carboline. The activity measured without the inhibitor was taken as 100% activity. Activities in the presence of inhibitors were calculated as activity% and Activity % vs. [inhibitor] plots were drawn. $IC_{50}$ values, or inhibitor doses that halves activity, were computed by setting the y-axis of the exponential curve to 50%.

### 2.4. Antibacterial Activity

### 2.4.a. Used Pathogen Species and Culture Conditions

Gram-negative *Escherichia coli* NCTC 9001, *Klebsiella pneumoniae* ATCC 13883, *Acinetobacter haemolyticus* ATCC 19002, and gram-positive *Staphylococcus aureus* NCTC 10788, *Enterococcus faecalis* ATCC 33186, and *Bacillus cereus* ATCC 14579 bacteria, which are known to be pathogens and frequently used for antibacterial tests,[39] were used for the antibacterial activity. Stock cultures of pathogenic species are stored at -80 ºC. For the antibacterial test, it was first revived by adding it to Luria-Bertani (LB) Agar medium for stock cultures. After one day of incubation at 37 ºC, single colonies were selected, inoculated in a liquid medium (LB Broth), and incubated in a shaking incubator at 37 ºC. The growth in liquid culture was adjusted to the range of 0.08-0.13 by measuring the absorbance at 625 nm in the spectrophotometer.[40,41] Culture liquids of pathogens with adjusted microbial density were used for antibacterial testing.

### 2.4.b. Agar Well Diffusion Method for Antibacterial Test

The Agar well diffusion method[42] was used to test the antibacterial properties of substances. Concentrations of 6 mg/ml were prepared using DMSO for the samples. Culture liquids of pathogens, whose microbial density was adjusted spectrophotometrically were spread homogeneously on the Mueller Hinton Agar (MHA) medium with a swab, 6 mm diameter wells were opened and 50 µl of each sample was added to the wells. As a positive control, Ampicillin and Kanamycin antibiotics, stored at -20 ºC, were used at 200 µg/ml concentrations. Additionally, DMSO was used as a negative control to examine the solvent effect. The prepared petri dishes were incubated at 37 ºC for 18-24 hours. The zone diameters were measured by millimeters using calipers and recorded after incubation. All analyses were performed in three replicates and average inhibition diameters were obtained.

### 2.4.c. Determination of MIC and MBC

The microdilution method was applied to a 96-well plate to calculate the MIC (Minimum Inhibitory Concentration).[43]100 µl of sterile medium (LB Broth) was added to the microplate wells and 100 µl of sample was added to the medium in the first column. Samples were adjusted to the final concentration ranging from 3 mg/ml to 0.023 mg/ml. 10 µl of the test bacteria brought to the appropriate microbial density as described above was added to the labelled wells, one strain per microplate line. The final volume of each well is 110 µl. While only medium and bacteria were added to the growth control column, only medium was added to the sterility control column. The plate was incubated at 37 ºC for 18 hours. After incubation, the plate wells are examined and the formation of a white pellet at the bottom of the microwells is associated with bacterial growth. The lowest concentration at which bacterial growth does not occur is determined as the MIC (mg/ml).

After the microdilution test, 50 µl samples were taken from the MIC well and three previous wells and spread into MHA medium using Drigalski for MBC (Minimum Bactericidal Concentration) determination. Petri dishes were incubated at 37 ºC for 24 h. The lowest concentration at which no bacterial colonies were observed after incubation was determined as MBC (mg/ml).[44] The bactericidal effectiveness of the substances was interpreted by calculating the MBC/MIC ratio from the data obtained.

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### 2.5. Molecular Docking

Using AutoDock, potential binding energies, types of interactions, and docking mechanisms between compounds and receptor proteins and $K_i$ constants were estimated.[45] Ligands were prepared with the Autodock tool and *pdbqt files were created. G6PD (PDB code: 6E08),[46] *Escherichia coli* NCTC 9001 (PDB code: 2VF5),[47] *Klebsiella pneumoniae* ATCC 13883 (PDB code: 4LZH),[48] and *Acinetobacter haemolyticus* ATCC 19002 (PDB code: 4EVY)[49] crystal structures (*pdb file type) was downloaded from http://www.rcsb.org and receptors were prepared by AutoDock. The results were captured by using Discovery Studio Visualizer.

### 2.6. Drug Similarity Analyzes

Drug similarity analysis of the derivatives was carried out using the SwissADME web server, considering Lipinski filters.[50]

### 2.7. Estimation of Toxicity

Toxicities of compounds were predicted Toxicity Estimation Software Tool (T.E.S.T). This program uses a compound's structure to forecast toxicity and the consensus model was selected above the nearest neighbor and hierarchical clustering models because it averages the output of multiple models while accounting for their limitations.[51]

## 3. RESULTS and DISCUSSION

### 3.1. Enzyme Inhibition Studies

Oxidative reactions of PPP, which plays an important role in many metabolic reactions in cells, is an important helper in protecting erythrocyte cells from oxidative damage. PPP serves as the primary source of both ribose 5-phosphate and NADPH in Plasmodium. Studies have found that PPP activity in infected erythrocytes is approximately 78 times higher than in uninfected erythrocytes[28] and in humans, it has been established that G6PD deficits offer some protection against malaria infections.[29] This makes PPP a possible target for antimalarial medications. Ramírez-Nava et al. (2021) found new inhibitors that affect HsG6PD activity and alter both the structural and thermal stability of the protein. The mechanisms of these inhibitors showed both competitive and non-competitive effects. In addition, they demonstrated that the inhibitors bind to the protein outside the active site, which is crucial for the design of next-generation inhibitors.[52] In another study, it has been reported that the β-carboline derivatives used in the study showed strong antiplasmodial activity both *in vitro* and *in vivo*.[5,7] Based on their antimalarial activities the *in vitro* effects of carboline derivatives on G6PD and 6PGD that are target in the treatment of malaria were investigated. For this propose, firstly, G6PD was isolated with a protein specific activity of 1.96 EU/ml, and 6PGD was isolated with a protein specific activity of 4.569 EU/ml human erythrocytes. Then, the effects of the derivatives (Figure 1) on the isolated enzymes were studied and the inhibitor concentrations ($IC_{50}$) that reduced the enzyme activity by half were calculated.

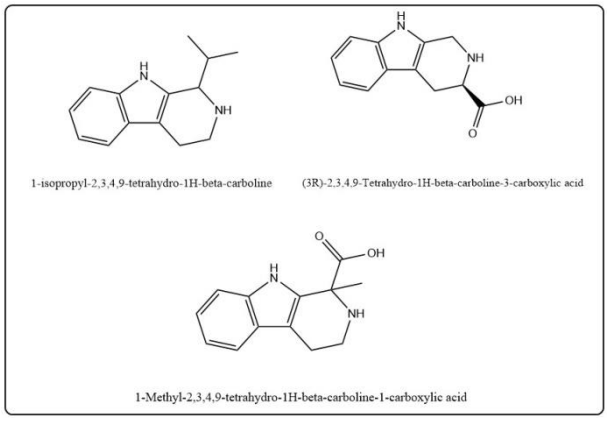


**Figure 1.** The chemical structures of tetrahydro-β-carbolines used in this study.

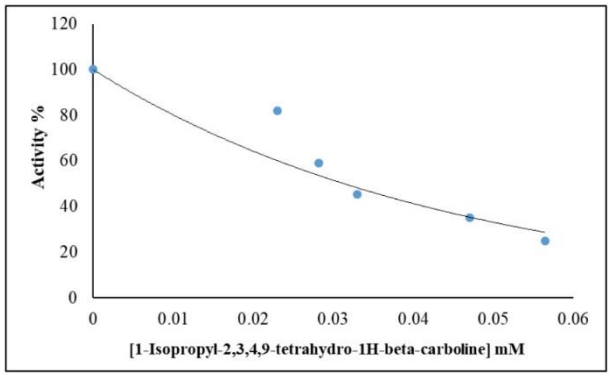


**Figure 2.** Activity %-[1-Isopropyl-2,3,4,9-tetrahydro-1H-beta-carboline] plot, indicating the inhibition on enzymes G6PD.

### 3.2. Antibacterial Activity Studies

Compounds **1**, **2**, and **3** were tested against a total of six test bacteria, three gram-positive and three gram-negative, by the agar well diffusion method, and their antibacterial effects were evaluated according to whether there was an inhibition zone or not. Inhibition zones of samples were observed in three gram-negative bacterial strains (*E. coli NCTC* 9001, *K. pneumoniae* ATCC 13883, *A. haemolyticus* ATCC 19002), and the zone diameters were measured and averaged by repeating 3 times. In Table 1 results of antibacterial studies were summarized, inhibition diameters, MIC values and MBC values of samples (mm) were given. It was seen from Table 1 that the most sensitive strain of all three samples is *A. haemolyticus* (19, 13, and 12 mm, respectively). While the first sample exhibited an inhibitory effect on *E. coli* (11 mm), it was observed that the other two samples had no effect. When the

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inhibition effect of the samples against *K. pneumoniae*, another gram-negative bacterium, was examined, it was observed that they exhibited inhibition zones of 14, 11, and 10 mm, respectively. In addition, none of the samples inhibited the gram-positive bacteria.

**Table 1.** Inhibition zone diameters (mm) obtained with the microdilution method and MIC-MBC (mg/ml) of samples in a concentration of 6 mg/ml.

| | | | Inhibition diameter of Samples (mm) | | | MIC of Samples (mg/ml) | | | MBC of Samples (mg/ml) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Microorganisms** | **Positive control** | **Negative control** | **1** | **2** | **3** | **1** | **2** | **3** | **1** | **2** | **3** |
| ***Escherichia coli* NCTC 9001** | 19 mm (Ampicillin) | - | 11.0±0.23 | - | - | 0.375 | - | - | 0.75 | - | - |
| ***Staphylococcus aureus* NCTC 10788** | 17 mm (Kanamycin) | - | - | - | - | - | - | - | - | - | - |
| ***Klebsiella pneumoniae* ATCC 13883** | 25 mm (Kanamycin) | - | 14.0±0.0 | 11.33±1.32 | 10.0±0.8 | 0.375 | 0.375 | 0.375 | 0.75 | 3 | 3 |
| ***Enterococcus faecalis* ATCC 33186** | 19 mm (Kanamycin) | - | - | - | - | - | - | - | - | - | - |
| ***Acinetobacter haemolyticus* ATCC 19002** | 23 mm (Ampicillin) | - | 19.0±0.35 | 13.0±0.44 | 12.4±0.33 | 0.1875 | 0.375 | 0.375 | 0.375 | 1.5 | 1.5 |
| ***Bacillus cereus* ATCC 14579** | 23 mm (Kanamycin) | - | - | - | - | - | - | - | - | - | - |

*Samples in which no zone was detected around the 6 mm diameter well are expressed as (-)

As can be seen in Figure 3, DMSO, used as a solvent, was added to the negative control wells of the petri dishes in which the test bacteria were spread, and it was determined that it had no inhibition effect against any bacteria. MIC and MBC analysis were performed using only strains where the substances showed inhibitory effects (*E. coli*, *K. pneumoniae*, and *A. haemolyticus* for **1**; *K. pneumoniae* and *A. haemolyticus* for **2** and **3**) and are given in Table 1. According to the values in Table 1, the MIC of derivative **1**, which inhibits the growth of *E. coli*, is 0.375 mg/ml, while the MBC is 0.75 mg/ml. All three substances inhibited the growth of *Klebsiella pneumoniae*, and the MIC of all was determined as 0.375 mg/ml. MBC values were determined as 0.75 mg/ml for compound **1** and 3 mg/ml for derivatives **2** and **3**. All the test compounds inhibited the growth of *Acinetobacter haemolyticus*, and the MIC for derivative **1** was 0.1875 mg/ml, while it was 0.375 mg/ml for derivatives **2** and **3**. MBC is 0.375 mg/ml for compound **1** and 1.5 mg/ml for compounds **2** and **3**.

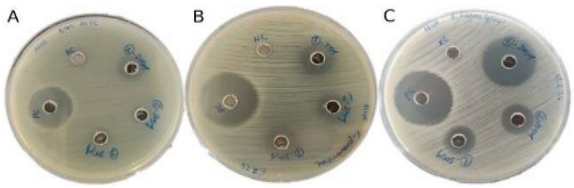


**Figure 3.** Agar well diffusion test for 6 mg/ml concentrations of substances a) *E. coli* NCTC 9001, b) *K. pneumoniae* ATCC 13883, c) *A. haemolyticus* ATCC 19002.

*NC: Negative control-DMSO, PC: Antibiotic control. The tests were repeated three times and the average of the resulting zone diameters was given.

The ratio of MBC, defined as the lowest concentration that can kill 99.9% of bacteria, and MIC, defined as the lowest concentration that prevents the growth of bacteria, defines that the test substances will exhibit a bactericidal or bacteriostatic effect.[53] When the MBC/MIC ratio is less than 2, the effect is referred to be bactericidal; when it is greater than 4, the effect is referred to as bacteriostatic.[53-55] Based on this information, the MBC/MIC ratio was calculated from the data obtained because of the antibacterial test, and the antibacterial effectiveness of the test substances was interpreted. The first substance had an MBC/MIC value of ≤2 for all three bacteria and showed a bactericidal effect. Other substances showed a bacteriostatic effect because their MBC/MIC ratio was ≥4.

### 3.3. *In silico* Studies

Molecular docking study of 1-Isopropyl-2,3,4,9-tetrahydro-1H-beta-carboline molecule, which only has an inhibitory effect on G6PD, was performed on the hG6PD (6E08) receptor. The estimated binding energy was determined as -6.97 kcal/mol. Interaction types and attachment model are given in Figure 4.

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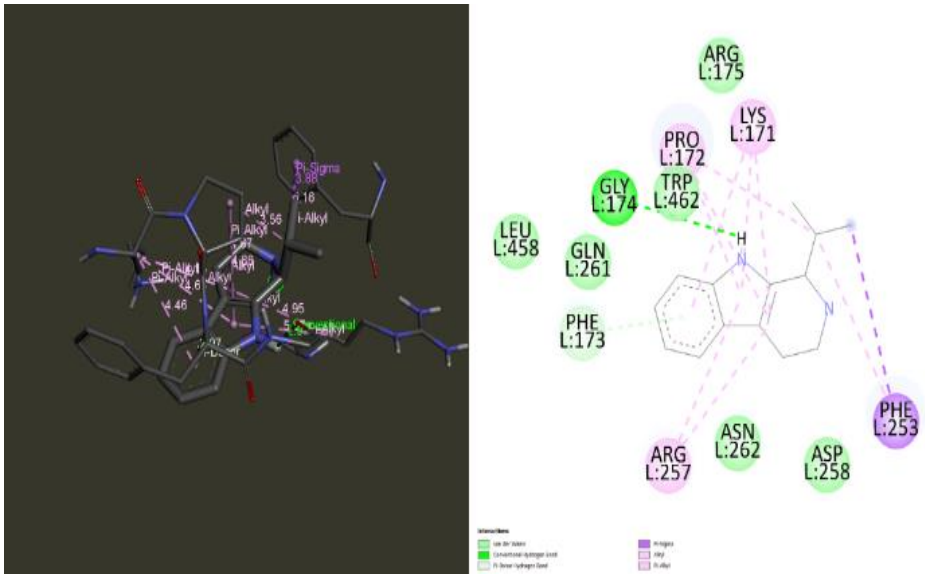


**Figure 4.** The docking poses and 2D ligand-receptor interaction diagrams of 1-Isopropyl-2,3,4,9-tetrahydro-1H-beta-carboline at the active site of hG6PD receptor.

A H bond appears to form between the pyrrole residue of the molecule and Gly174. It also made hydrophobic alkyl and pi-alkyl interactions with Lys171, Pro172 and Arg257. The -$CH_3$ residue of the molecule showed pi-sigma interaction with Phe253.

According to results of antibacterial studies, compounds were docked into some specific proteins of microorganisms. For *E. coli* 2VF5 receptor protein, Glucosamine-6-phosphate synthase (GlmS), was targeted. The synthesis of glucosamine-6-phosphate (GlcN-6-P), which serves as a precursor in peptidoglycan biosynthesis, is catalyzed by GlmS. Peptidoglycan is one of the main components required for the structural integrity and shape of bacterial cell walls. Therefore, since GlmS plays a critical role in the synthesis of the cell wall in bacteria, it is the target protein for weakening the cell wall in terms of antibacterial effect.[56] Figure 5, represents possible interaction modes and types for *E.coli* GlmS and compound **1**. Compound **1** had -7.08 kcal/mol estimated free binding energy. Benzene ring of compound exhibited a pi-anion interaction with negative site of Glu488. Pyrrole moiety of compound formed an unfavorable donor-donor clash with Thr302 residue of receptor. Also compound **1** acted as a hydrogen donor against Ser401 in a hydrogen bond.

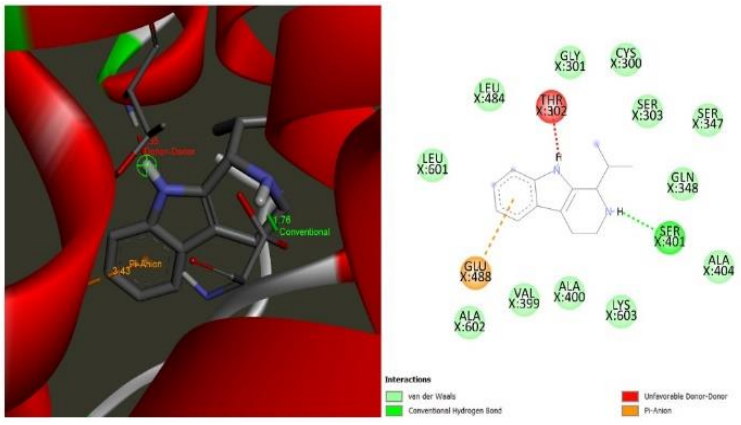


**Figure 5.** The docking poses and 2D ligand-receptor interaction diagrams of 1-Isopropyl-2,3,4,9-tetrahydro-1H-beta-carboline and 2VF5 receptor for L, Glucosamine-6-phosphate synthase of *E. coli.*

For *K. pneumoniae* compounds were docked into 4LZH receptor protein, L, D-transpeptidase, estimated free binding energies and estimated $K_i$ constants of compounds of compounds given in Table 2. Compound **2** had the lowest binding energy (as -7.90 kcal/mol) so the highest affinity against receptor. As seen from Figure 6, compound had three hydrogen bonds by its carboxyl group with Asn64, Arg107, and Ile123. Via its pyrrole moiety it made amide-pi stacked interaction with Gly201 and pi-alkyl interaction with Arg203 residues.

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**Table 2.** *In silico* free binding energies of compounds predicted by AutoDock 1.5.

| Compound Number | | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|---|
| **Microorganisms** | **Receptor Protein** | **Estimated Free Binding Energy (kcal/mol)** | **Estimated $K_i$ (µM)** | **Estimated Free Binding Energy (kcal/mol)** | **Estimated $K_i$ (µM)** | **Estimated Free Binding Energy (kcal/mol)** | **Estimated $K_i$ (µM)** |
| ***Escherichia coli* NCTC 9001** | 2VF5 | -7.08 | 6.45 | - | - | - | - |
| ***Klebsiella pneumoniae* ATCC 13883** | 4LZH | -7.25 | 4.81 | -7.90 | 1.62 | -7.20 | 5.29 |
| ***Acinetobacter haemolyticus* ATCC 19002** | 4EVY | -7.11 | 6.11 | -6.17 | 30.05 | -6.10 | 33.79 |

For *A. haemolyticus*, compounds were docked into 4EVY receptor protein, aminoglycoside antibiotic 6'-N-acetyltransferase AAC(6')-Ig an enzyme that provides resistance to aminoglycoside antibiotics and inactivates the antibiotic by acetylating it in bacterial cells. On this basis, the high activity of this enzyme may complicate the clinical use of aminoglycosides; therefore, research on inhibitors of such enzymes attracts attention. Compound **1** had the highest affinity against receptor protein with -7.11 kcal/mol estimated free binding energy (Table 2).

A closer look at the interaction types has shown that compound 1 acted as a hydrogen donor for one hydrogen bond with Asp114 (Figure 7). Via its pi bonds in pyrrole moiety, it made pi-anion interaction with the -$COO^-$ group of Asp114 amino acid. Besides, its pi bonds in benzene ring made pi-alkyl interaction with Leu77 residue.

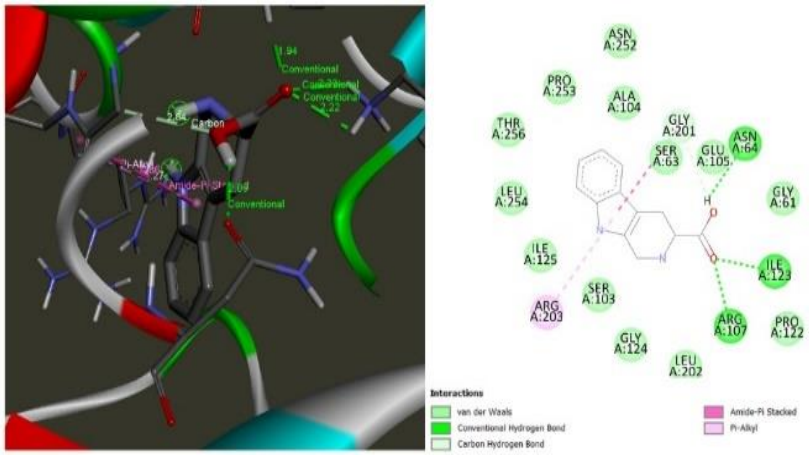


**Figure 6.** The docking poses and 2D ligand-receptor interaction diagrams of (3R)-2,3,4,9-Tetrahydro-1H-beta-carboline-3-carboxylic acid and 4LZH receptor for L, D-transpeptidase of *K. pneumoniae*.

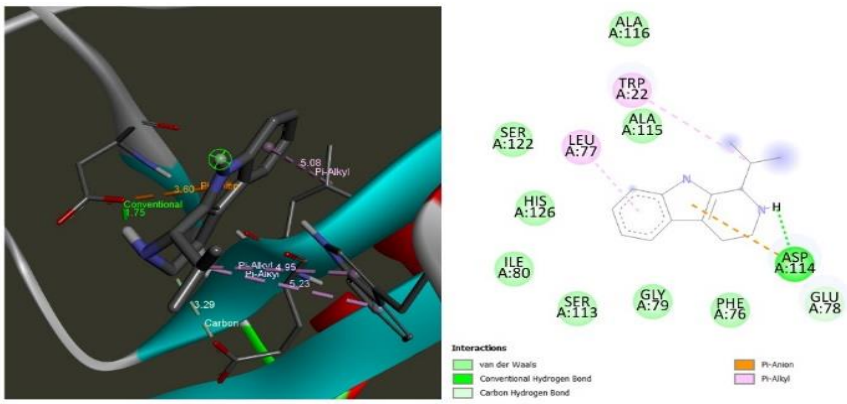


**Figure 7.** The docking poses and 2D ligand-receptor interaction diagrams of 1-Isopropyl-2,3,4,9-tetrahydro-1H-beta-carboline and 4EVY receptor for aminoglycoside antibiotic 6'-N-acetyltransferase AAC(6')-Ig of *A. haemolyticus.*

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Table 3 provides the derivatives' physicochemical properties. When evaluating parameters such as miLogP, TPSA, H-bond acceptors/donors, Mw, and n violations of derivatives meet Lipinski's requirements. The SwissADME server predicted BBB transitions for derivatives other than 1-Methyl-2,3,4,9-tetrahydro-1H-β-carboline-1-carboxylic acid (Table 3). This can be explained by the effect of the miLogP value (-0.3), which indicates the low lipophilicity of the compound. For BBB permeability, it is necessary to have both suitable polar surface area and sufficient lipophilicity. It is often easier for compounds having a logPS value larger than "-2" to pass through the brain than it is for those with a logPS value less than "-3". Additionally, the BOILED-EGG (Brain Or IntestinaL EstimateD permeation method) is shown in Figure 8. It has also been anticipated that these substances will have high gastrointestinal (GI) absorption. It is easier for medications that are quickly absorbed by the GI tract to reach the target concentration and optimal level.[57-59]

Although compounds **1** and **3** were predicted to be P-gp substrates, these compounds appeared to have both high GI absorption and BBB crossing potential. This was thought to be due to the compounds having sufficient lipophilicity, low TPSA, and favorable molecular properties

**Table 3.** Results of physicochemical parameter analysis.

| **Properties** | **Rules** | **Compounds** | | |
|---|---|---|---|---|
| | | **1** | **2** | **3** |
| Molecular Weight (g/mol) | <500 | 214.31 | 216.24 | 230.26 |
| Num. rotatable bonds | <10 | 1 | 1 | 1 |
| Number of H-bond acceptors | <10 | 1 | 3 | 3 |
| Number of H-bond donors | <5 | 2 | 3 | 3 |
| TPSA (Å$^2$) | 20<TPSA<130 | 27.82 | 65.12 | 65.12 |
| ABS % | | 99.40 | 86.53 | 86.53 |
| miLogP | ≤4,15 | 3.23 | -0.30 | 1.80 |
| nviolations | ≤1 | 0 | 0 | 0 |
| GI absorption | | high | high | high |
| BBB permeant | | yes | no | yes |
| P-gp substrate | | yes | no | yes |
| Log Kp (skin permeation) cm/s | (-9,7<log Kp<-3,5) | -5.58 | -8.44 | -8.28 |
| Synthetic accessibility (sa) | | 2.64 | 2.46 | 2.66 |

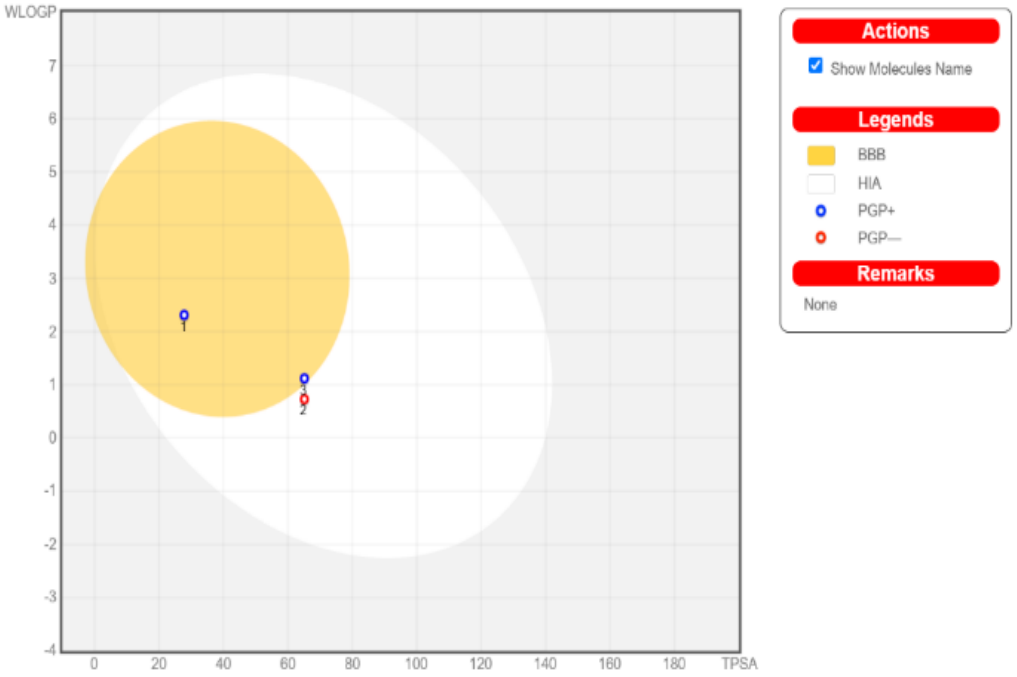


**Figure 8.** BOILED-EGG model of derivatives.

*Dots in the yellow area are molecules that are predicted to passively pass through the blood-brain barrier. The dots in the white area are molecules passively estimated by the gastrointestinal tract. Red dots are for molecules predicted not to be excreted from the central nervous system by P-glycoprotein

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These compounds have a high synthetic accessibility (sa) score, indicating ease of synthesis. Additionally, all derivatives were found to have good intestinal solubility when compared to the literature (>30% abs).[60] All of the compounds had skin permeability values that were less than the reference value (-2.5 logKp), indicating that they might be used to create transdermal medications.

Early assessment of toxicological risks is a critical step in the drug discovery process. In this study, toxicity estimations were performed using the T.E.S.T. (Toxicity Estimation Software Tool) program; similar toxicological analyses were reported using the OSIRIS Property Explorer software in different studies with similar purposes.[61-66] A compound is classified as bioaccumulative if its bioaccumulation factor (BF) value is greater than 2000, and as multi-bioaccumulative if the value is greater than 5000.[67] In Table 4, toxicity values of compounds were summarized.

**Table 4.** Predicted values of bioaccumulation factor, developmental toxicity, mutagenicity and toxicity values of derivatives against rat.

| Compound ID | Bioaccumulation Factor | Developmental toxicity | | Mutagenicity | | Rat | |
|---|---|---|---|---|---|---|---|
| | | Value | Result | Value | Result | $LD_{50}$ (mg/kg) | Category |
| **1** | 1.37 | 0.67 | T | 0.77 | + | 318.60 | NT |
| **2** | 1.45 | 0.84 | T | -0.02 | - | 3589.08 | NT |
| **3** | 2.36 | 0.92 | T | 0.03 | - | 1897.91 | NT |

T: toxicant; NT: nontoxicant; -: negative; +: positive.

All of the derivatives in this investigation are nonbioaccumulative in nature because they have BF values as 1.37, 1.45, and 2.36 (all of them are less than 2000). Developmental toxicity values, which show compounds affect healthy development both before and after birth, [68] were calculated as 0.67, 0.84, and 0.92 for compounds **1**, **2**, and **3**. Based on this, all three derivatives were found to be toxic, suggesting a potential risk to developing fetuses when exposed during pregnancy. The results of the mutagenicity test showed that compound **1** was developmental poison and mutagenic. The compounds' estimated $LD_{50}$ values as 318.60, 3589.08, and 1897.91 mg/kg. Table 4 details the toxicity test results.

## 4.CONCLUSION

In conclusion, this study aimed to reveal the inhibition potencies of tetrahydro-β-carboline derivatives that play a crucial role in the development of drugs based on PPP inhibition in some diseases. Only 1-Isopropyl-2,3,4,9-tetrahydro-1H-beta-carboline derivative inhibited G6PD with an $IC_{50}$ value of 31.2 µM. Although the PPP enzymes were not inhibited significantly, the antibacterial activities of these tetrahydro-β-carboline derivatives demonstrated encouraging antibacterial activity. It was observed that while 1-isopropyl-2,3,4,9-tetrahydro-1H-beta-carboline was effective against *E.Coli*, all three of them acted as antibacterial agents against *K. pneumoniae* and *A. haemolyticus* with having more than 10 mm inhibition zone diameters. To estimate interaction types and free binding energies molecular docking was performed for G6PD and some specific proteins for microorganisms. Future studies may examine ways to improve the tetrahydro-β-carboline scaffolds' inhibitory effects on PPP enzymes So, it this study, only compound 1 was found to affect the activity of G6PD. While compound **1** was effective against *E. coli* derivative **2** and **3** had antibacterial impact against *E. coli*, *K. pneumoniae*, and *A. haemolyticus*. Molecular interaction of compounds with receptor proteins specified for bacteria and hG6PD receptor were predicted by molecular docking estimated free binding energies of compounds were calculated less than -6.00 kcal/mol. Drug similarity characters and toxicities of derivatives were also elucidated by computational methods. The compounds were found to not deviate from Lipinski's rule of five. Compound **1** was estimated to be able to pass BBB, and all three compounds were predicted to have high GI absorption. According to toxicity predictions all derivatives were nonbioaccumulative and developmental toxic or enhance their antibacterial processes. Overall, it is thought that the evaluation of the inhibition potencies on G6PD and 6PGD and antibacterial properties of the compounds reported to have many biological activities will provide a comprehensive perspective for the development of new therapeutic agents. In addition, the study is important in terms of determining the potential drug features and toxicological risks of compounds by the *in silico* method. This study provides a multifaceted evaluation by integrating *in vitro* biological tests as well as *in silico* analyses and contributes to the literature.

## Data Availability

The crystal structures of receptor proteins were downloaded from protein data bank (http://www.rcsb.org/pdb). This manuscript contains all further supporting information related to the investigations mentioned here.

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## Competing Interests

*The authors declare no conflict of interest.*

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