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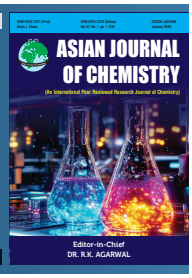

Biological Efficacy Studies of Theoretically Examined L-Valine based Schiff Base

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This study reported the effectiveness of the newly synthesized Schiff base, 2-{(*E*)-[(2,4-dihydroxyphenyl)methylidene]amino}-3-methylbutanoic acid (LVDHBSB) from chiral L-valine and 2,4-dihydroxy benzaldehyde and characterized with UV, FTIR and ^{1}H NMR techniques. The QSAR, DFT and docking properties *in silico* studies of the novel Schiff base were also performed. From the results, this work conducted the brine shrimp toxicity, antimicrobial, antidiabetic and antioxidant activities to confirm the biological efficacy of novel L-valine based Schiff base compound. The docking analysis against seven proteins showed the binding scores of -3 to -9 kcal/mol. The Schiff base is supported regardless of the docking values (from -30.49 to -49.86 kcal/mol) in the offline workbench-3. Experimental biological tests exposed good results in brine shrimp lethal assay (LC_{50} = 208.45 µg/mL), antibacterial activity (ZI = 9-13 mm/150 µg/mL, MIC = 100-150 µg/mL), antidiabetic activity (IC_{50} = 504.54 µg/mL) and antioxidant (IC_{50} = 1773 µg/mL) respectively. The compound exhibited good antimicrobial activity within the toxicity limit and the other results are higher than LC_{50}.

Keywords: L-Valine, Schiff base, QSAR, Docking studies, Biological studies.

INTRODUCTION

Following the recent viral pandemic, a transformative phase in medical research has emerged, aimed at developing more robust safeguards against potentially lethal diseases [1]. An abundance of natural resources has the ability to provide several advantages to human health, one of which is an improvement in those individuals health [2]. COVID-19 is a supplement to the living system that includes Ayurveda, Siddha, Unani and the recently permitted homeopathic system [3]. Likely, synthetic molecules are also playing a vital role in the medicinal field. Apart from various kinds of molecules, Schiff bases are receiving more interest in research [4]. In the quest to develop effective antimalarial drugs, Schiff bases have emerged as promising molecules. Amine condensed with carbonyl group Schiff base analogs make them versatile chelating agents. A large body of studies indicates that nitrogen atoms in the imine (-CH=N-) group, which have a single pair of electrons in their sp^2 hybridized orbitals, have important biological implications [5,6].

Organometallic complexes derived from amino acid-Schiff bases may contain new types of antibacterial and anticancer activities. Due to their unique biological characteristics, including DNA binding, anticancer and various therapeutic effects, L-enantiomers of amino acids have recently garnered increased interest [7-9]. A crucial part of drug development is identifying active compounds, which is achieved by a series of biological trials. A molecule is considered marketable if it meets certain criteria. It is common practice to prioritize identifying exposure, solubility, *in vivo* effectiveness and oral bioavailability, but to neglect tracking the generic development of drug-likeness as a coherent descriptor. Various precise descriptions of medicinal characteristics are thought to be more closely linked to key

factors that take their place [10]. Standard deviations for drug-likeness-related metrics [11].

Density functional theory (DFT) is a popular method for learning and predicting molecular features using computational means. Computational techniques such as QSAR and DFT are effective methods for accurately predicting the geometry of various organic compounds, including all kinds of products [12-14]. The energy gaps of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) on the best possible structures of compounds using the B3LYP functional model at density functional theory (DFT) in the aqueous phase used to predict the drug likeness related Koopmans parameters. Several biological theories suggest that the difference between the energies of the HOMO and LUMO orbitals can determine these parameters [15-17]. The Koopmans parameters are determined by the energy gap and the Hartree-Fock (HF) method with a 3-21G basis. The molecular docking studies are also receiving more interest from the researchers due to the basic theoretical confirmation for the prepared structures and their binding ability with disease-causing pathogens before clinical trials [18,19]. In addition, the research of the derived compounds was completed with their usefulness in experimental biological efficacy investigations.

Chemical compounds have many important features, including biological activity. However, there is always the chance that chemical substances used in drug can have negative side effects. An effective drug is defined as a chemical that has target selectivity and sufficient ADME properties. Several methods exist for determining whether a medicinal substance or harmful chemical has the innate capacity to alter any one or more physiological processes in a living being. In this work, an novel Schiff base prepared from 2,4-dihydroxy benzaldehyde and aliphatic L-valine is considered for its biological efficacies [19]. In order to determine the efficacy through theoretical and subsequent biological research, an experimental biological evaluation, preliminary QSAR and spectral characterization were used to promote the molecule. Furthermore, the molecular docking studies is also carried out between the titled inhibitor compound and the target selected proteins. The study also evaluated the antimicrobial acitivity against three different types of bacteria (two Gram-negative and one Gram-positive) as well as the brine shrimp fatal assay to check toxicity.

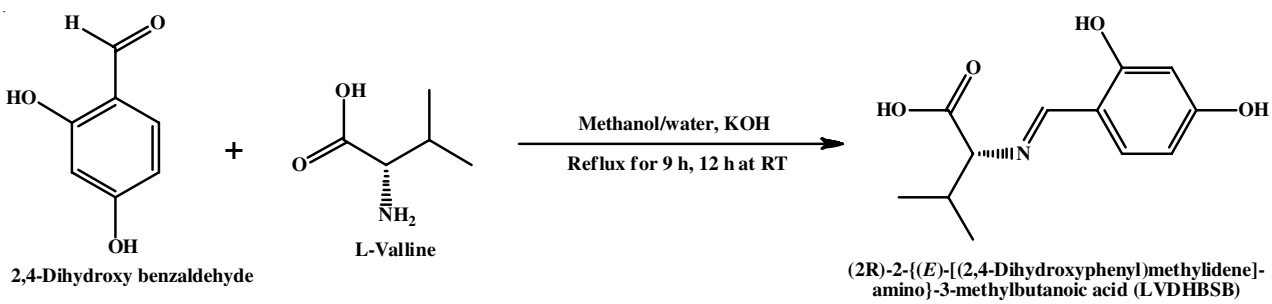
EXPERIMENTAL

All the chemicals L-valine, ethanol, KOH and 2,4-dihydroxy benzaldehyde were procured from Sigma-Aldrich Chemicals, India and used as such. The progress of reaction was monitored on the TLC plates using a 60:40 hexane and ethyl acetate solvent system. The melting point was measured using a Sunsim electric melting point instrument and is uncorrected. The UV and fluorescence spectra were measured using the Perkin-Elmer LS25 and LS45 equipment, respectively. The vibrational and ^{1}H NMR spectrum in DMSO solvent were recorded using the FTIR Jasco-6300 and Bruker NMR400 spectrometers, respectively.

The antimicrobial susceptibility was evaluated against *E. coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 35657 and *Staphylococcus aureus* MTCC 1430 using the agar well diffusion method. Brine shrimp eggs were procured from Aquamarine in Guindy, Chennai, India. The toxicity tests at Besant Nagar, Marina Beach, Chennai, Tamil Nadu, India and used it for brine shrimp toxicity in March and April of 2024.

Synthesis of 2-{(*E*)-[(2,4-dihydroxyphenyl)methylidene]amino}-3-methylbutanoic acid (LVDHBSB): A 250 mg of KOH with 2 mmol of L-valine were dissolved in 50 mL of 2:1 methanol and water solution. The reaction was heated to 60 ºC for 30 min followed by the addition of 2,4-dihydroxybenzaldehyde (2.2 mmol) in 4 equal amounts over the course of 15 min. Before being progressively heated to 70-80 ºC, the reaction mass was agitated at room temperature for 25 min. The temperature was maintained at 9 h under a sealed silver foil. Once the mixture had cooled to room temperature, it was agitated continuously for 12 h. The *t*-butanol:water:acetic acid (5:2:0.5) solvent system was used to monitor the reaction using TLC. Upon completion of the reaction, the mixture was filtered and rinsed with water at 60 ºC, after which the light brown solid was allowed to dry. A 30 mL solution of methanol:water (1:1) was added, refluxed for 1 h and then cool to room temperature (**Scheme-I**). The resulting solid was filtrated, rinsed with hot methanol and then recrystallized with 1:1 mixture of methanol and water to obtain light yellow product (yield: 69%; m.p.: 248 ºC).

QSAR prediction: The computational properties were utilized to assess the drug similarity of the synthesized compounds. This research used Molsoft's prediction tool to determine the QSAR properties since there were a lot of molecules referred for the prediction [20]. The synthetic molecules were observed to comply with the five principles established by Lipinski rule of five. As per the rule, molecules that have strong membrane permeability should have the following parameters: molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10, number of hydrogen bond donors ≤ 5, total polar surface



Scheme-I: Preparation of LVDHBSB

area < 140 A^2 and Log P ≤ 5 [21]. The Chemsketch was used to convert smiles notation.

DFT Koopmans's parameters: The theoretical density functional theory was carried out using the softwares Spartan14 and Gausian09. In this work, the Schiff base structure was constructed using Spartan14 and its characteristics in the aqueous phase were estimated using HF at 3-21G. Then, this research executed the program through the same basis set till it finished. The software's local monitor was used to observe the progress of the calculation. The structural QSAR properties were investigated with the Spartan14 wave function tool [22]. Electrostatic potential map structure, theoretical HOMO and LUMO energies and related diagrams were also created. The FM orbital energy gap explains the charge transfer interaction within the molecule as well as the Koopmans parameters [23]. The parameters were calculated using the following formulae.

Electronegativity:

$$\chi = -\frac{1}{2}(E_{HOMO} + E_{LUMO})$$

Chemical potential:

$$(\mu) = -\chi$$

Global hardness:

$$\eta = \frac{1}{2}(E_{LUMO} - E_{HOMO})$$

Global softness:

$$S = \frac{1}{2}\eta$$

Softness:

$$\sigma = \frac{1}{\eta}$$

Global electrophilicity index:

$$\omega = \frac{\mu^2}{2\eta}$$

Docking: The docking analysis evaluated the efficacy of the synthesized LVDHBSB by using the capacity of molecules to attach to the active sites of proteins in biological systems. The molecules with the lowest dock scores are the ones with the strongest affinity. Docking analyses the protein-ligand recognition forces, including electrostatic forces, hydrogen van der Waals bonding and ligand placement within the active site. After conducting an adequate literature review, this work downloaded the protein PDB files from the web server https://www.rcsb.org/pdb/home/home.do. Protein binding coordinates were subsequently predicted using a fuzzy oil drop model on an online server (http://bioinformatics.cm-uj.krakow.pl/activesite/). The docking simulations were conducted using the smiling notation of the compounds and the simple and quick mcule 1-click docking software. The software revealed four docking scores for the Schiff base through an analysis of the compounds' affinities. Utilizing the optimal score pose, docking was executed in the offline CLC drug discovery workbench-3. The hydrophobic interaction of protein at 13 Å was examined to identify the requisite amino acids, and the most prevalent amino acids were chosen for the docking study. This work used the dynamic amino acid residues such as aspartame and glutamine to determine docking affinity with Schiff base (LVDHBSB) [24].

Brine shrimp lethal assay: The brine shrimp fatal experiment was carried out to ascertain the LC_{50} or deadly concentration of the LVDHBSB. This assay is an initial toxicity evaluation for pharmaceuticals to determine their compatibility with mammals. The brine shrimp lethal assay for the Schiff base was performed by following the reported method [25]. A preliminary toxicity study was conducted for LVDHBSB, to assess medication compatibility after brine shrimp larvae were hatched. One liter of seawater containing 1 g of Artemia cyst eggs was inoculated and maintained under oxygenation. The beaker was illuminated for 30 h by a 60 W tungsten bulb. The toxicity assessment was performed on nauplii post-completion of their development during the hatching period and simultaneously the assay was also performed on Schiff base solutions that were serially diluted to concentrations of 31.25, 62.5, 125, 250 and 500 µg/mL. Using 0.5 mL of DMSO, a transparent solution was prepared to test the samples and mixed thoroughly. The solutions were prepared using seawater up to 100 mL in a standard flask. Each experiment used 10 newly hatched nauplii and 10 mL of test fluid. The test solutions were kept at room temperature for 24 h before being concentrated with a 25W tungsten lamp. After 24 h, the proportion of mortality was calculated and the number of live nauplii was tallied. Each concentration was tested in triplicate using the same protocol. From the regression equations, the LC_{50} value of the synthesized Schiff base was calculated for cytotoxic activity against Brine Shrimp nauplii. To validate the test procedure, DMSO was employed as a negative control. The statistical analyses were conducted in Excel 2010. Observations of the brine shrimp were carried out using the LYNX-Lawrence Mayo microscope in conjunction with capture pro 2.8.8 software.

Antimicrobial studies: On the MHA plates, the well were prepared and filled with 200 µg mL^{-1} of LVDHBSB, 50 µL of DMSO as negative control and 50 µg of gentamicin as positive control. The plates were kept at 37 ºC for 24 h for incubation. Then, the zone of inhibition was measured in millimeters to identify the microbial growth. The inhibition zone in mm was measured from the triplicated trials. The micro dilution approach was used to measure the minimal inhibitory concentration (MIC) [26]. The unoculums of bacteria were prepared in 5 mL of nutrient broth and cultured at 37 ºC for 48 h. The Schiff base was prepared at various concentrations (500, 400, 250, 200, 125, 100, 62.5, 31.25, 15.62, 81, 3.90 µg/mL) and the MIC was determined using the well-known technique.

Antidiabetic activity: The potential of newly synthesized Schiff base to demonstrate the antidiabetic activity can be assessed by the α-amylase inhibition assay and the α-glucosidase inhibition assay [27]. The α-amylase inhibition test was also conducted according to the standard protocol. Standard and test samples (20-100 µg/mL) were each measured using 100 µL. In brief, 250 µL of α-amylase (1 mg/mL) in 0.2 M sodium phosphate buffer (pH 6.9) into each tube and incubated at 37 ºC for 20 min. In the subsequent step, 250 µL of 0.5% starch

solution in 0.2 M sodium phosphate buffer having pH of 6.9 was added to every tube. The solutions were then maintained at 37 ºC for a further 15 min. Then, 1 mL of 3,5-dinitro salicylic acid was used to stop the process. After 10 min in an incubator set at 100 ºC, the tubes were removed and allowed to cool to room temperature. This work measured the absorbance at 540 nm after diluting the reaction mixture with 10 mL of distilled water. The intensity of the colour is proportional to the concentration. Comparing the test samples to the standard revealed their inhibitory propensity. To determine the inhibition percentage, the following formula related to absorbance was applied.

$$\alpha\text{-Amylase inhibition } (\%) = \frac{OD_{sample} - OD_{control}}{OD_{sample}} \times 100$$

Antioxidant assay: The anticancer potential of the synthesized compound was assessed using antioxidant assay. The DPPH assay is widely used to assess antioxidant activity and based on the mechanism having hydrogen-donating antioxidant to convert alcoholic DPPH to DPPH-H. A decrease in DPPH absorbance at 517 nm signifies the antioxidant's radical scavenging activity [28,29]. DPPH became purple to yellow as absorbance fell and antioxidants donated hydrogen to stabilize it. At first, 3.7 mL of methanol was added in all test tubes. In order to prepare the blank, 100 µL of 100% methanol and 200 µL of DPPH were added after the solvent was added. Moreover, 100 µg, 200 µg, 300 µg, 400 µg and 500 µg of test samples were added to each of the remaining test tubes after 100 µL of ascorbic acid was mixed followed by the addition of 200 µL of DPPH reagent. Then incubated each test tube including the blank, in a dark environment at room temperature for at least 30 min. The absorbance of each sample at 517 nm was measured by applying the following formula:

$$\text{Antioxidant activity } (\%) = \frac{Abs_{blank} - Abs_{test}}{Abs_{blank}} \times 100$$

RESULTS AND DISCUSSION

A newly synthesized Schiff base LVDHBSB from L-valine condensed with 2,4-dihydroxy benzaldehyde was characterized with ^{1}H NMR, FT-IR and UV spectroscopy. The initial absorption spectrum of the chiral LVDHBSB was obtained in DMSO solvent, as illustrated in Fig. 1. The absorption bands at 211, 288 and 331 nm appeared for LVDHBSB. The strong peak at 288 nm is attributed to the π→π* transition, which involves the -C=N- group and the benzene ring. The absorption spectrum of LVDHBSB has more intense and red-shifted due to the presence of methyl group. Furthermore, the compound exhibited a significant absorption at 211 nm for n→π*.

The FT-IR spectrum of LVDHBSB was recorded using KBr method and presented in Fig. 2. The IR spectrum of novel Schiff base displayed significant peaks at 1610-1590 cm^{-1} (s), 1500-1490 cm^{-1} and 1250 cm^{-1}. In addition, the Schiff base showed the broad peak at 3200 cm^{-1} (broad) for hydroxy groups. The symmetric and asymmetric stretching frequencies for carboxyl group are observed at 1300 cm^{-1} and 1750 cm^{-1}, respectively. In ^{1}H NMR, the spectrum revealed the singlet peak for –COOH group at 11.71 ppm (Fig. 3). Moreover, -OH protons

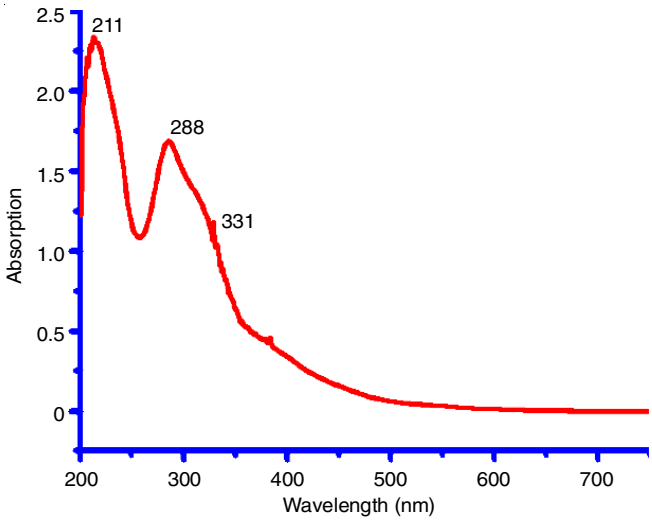


Fig. 1. Absorption spectrum of LVDHBSB

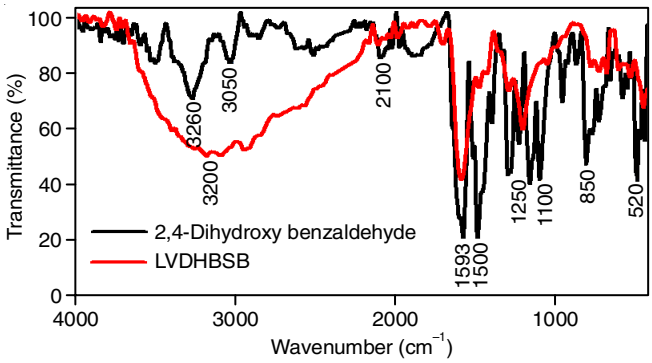


Fig. 2. FT-IR spectra of LVDHBSB

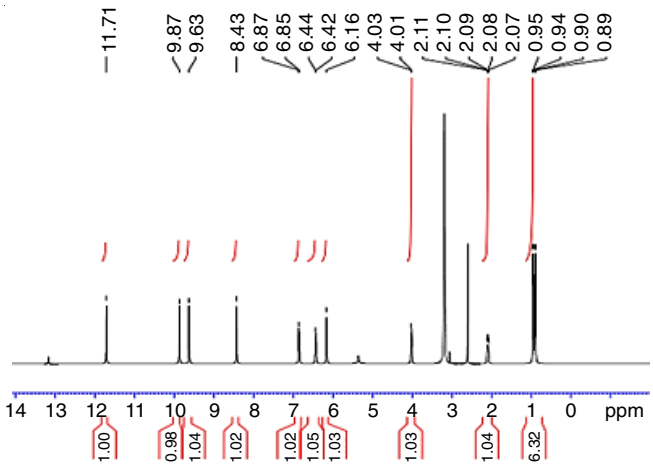


Fig. 3. ^{1}H NMR spectrum of LVDHBSB

showed chemical shift at 9.87 ppm and 9.63 ppm, respectively. The azomethine (-CH=N-) proton of LVDHBSB was observed at 8.43 ppm and displayed the specific aromatic protons between 6.16 and 6.86 ppm.

In silico studies: The characterized compound structure was drawn in Chemsketch and converted to smiles notations. After that, it was sent to an online molsoft server in order to predict the theoretical pharmacophore sites' drug-like properties. Table-1 displays the pertinent data collected for this

TABLE-1
MOLSOFT, DFT QSAR PROPERTIES AND KOOPMANS PARAMETERS OF L-VALINE BASED SCHIFF BASE (LVDHBSB)

Parameters	Molsoft QSAR	DFT QSAR	DFT Koopmans parameters	
			Parameters	Values
Molecular formula	$C_{12}H_{15}NO_4$	$C_{12}H_{15}NO_4$	HOMO	-0.3488
Molecular weight	231	231	LUMO	-0.0139
HBA*	5	4	$E_{HOMO}+E_{LUMO}$	-0.3627
HBD*	3	3	Electronegativity (χ)	0.1814
Log P	1.82	-0.19	Chem. potential (μ)	-0.1814
Log S (mol/L)	-2.48	60.11	$E_{LUMO}-E_{HOMO}$	0.3349
PSA ($A^{2)}$	71.88	73.12	Global hardness (η)	0.1674
Vol ($A^{3)}$	231.5	–	Softness (S)	2.9861
Drug-likeness	-0.43	–	Electrophilicity index (ω)	0.0982
Polarizability	–	59.57	Softness (σ)	5.9723
Max. epot	–	329.3		
Min. epot	–	-262.26		

study, in addition to the numerous data. The results showed that the compounds met all of the necessary Lipinski criteria. However, as shown in Fig. 4a, the molecule had a drug likeness score of -0.43. When contrasted with paracetamol (0.00), it is a significant amount and somewhat closer to the market based drugs like phenformin- +0.04, acetylcysteine- +0.3, dalfampridine- −1.44). Additionally, there was a strong agreement between the compound and the Lipinski five rules. The Schiff base compound showed significant polar surface area above 70 A^2. DFT verified the online parameters for the compound's offline QSAR computation. The predicted offline values show minimal variation from the online parameters. The presence of two hydroxyl groups, along with imine, carboxylic and chiral features, leads to these significant results. For biological experiments, the structure reactivity parameters of LVDHBSB were computed using Spartan-14-DFT. The computed relevant DFT parameters are given in Table-1.

It is well-known that molecular interactions can be revealed by electrostatic potential maps and three-dimensional charge distributions. The wave functions for molecular electrostatic potentials (MEP) were obtained from the molecular orbital density functional theory (DFT) calculations. The maximum electron density is represented by red. Hydrogen has the least electronegative value and oxygen the most. Fig. 4b shows the computed molecular potential surfaces. A drug predominantly binds to its dynamic sites (receptors) due to the molecule's deviation in electrostatic potential. Both sides of the binding site will have different electrostatic potentials, which is well known [30]. The bioactivity of LVDHBSB may be due to its relatively high green area and modest red region content.

With the help of mol2 file, the HOMO and LUMO gap (Fig. 4c) was calculated and Koopmans parameters were derived. According to the results, the obtained molecule is biologically active due to its high softness and energy gap of -0.3349. The proteins that were downloaded and the projected active site coordinates for the docking study are shown in Table-2 using online server.

TABLE-2
PROTEIN BINDING SITE
COORDINATES FOR DOCKING STUDY

Target PDB	Pathogen	X	Y	Z
1SHV	*E. coli*	-9.071	24.805	-9.754
1A2N	*E. coli*	48.8023	21.5255	41.3349
1HSK	*S. aureus*	179.9707	148.9228	163.3638
1S1J	Zipa cell	26.0168	14.1613	3.9038
1XCW	α-Amylase	50.56	16.4744	42.5543
3K0K	Breast cancer	-23.4849	56.5041	2.4167
3FDN	Aurora A kinase	-3.6403	-32.0212	3.734

The present biological work-related proteins were docked against LVDHBSB using the mcule software and the four docking scores were recorded in Table-3. A strong binding score of -3 kcal/mol to -8 kcal/mol was observed for LVDHBSB in this study. The presence of active functional groups and chirality might be to blame for this. In addition, the study found

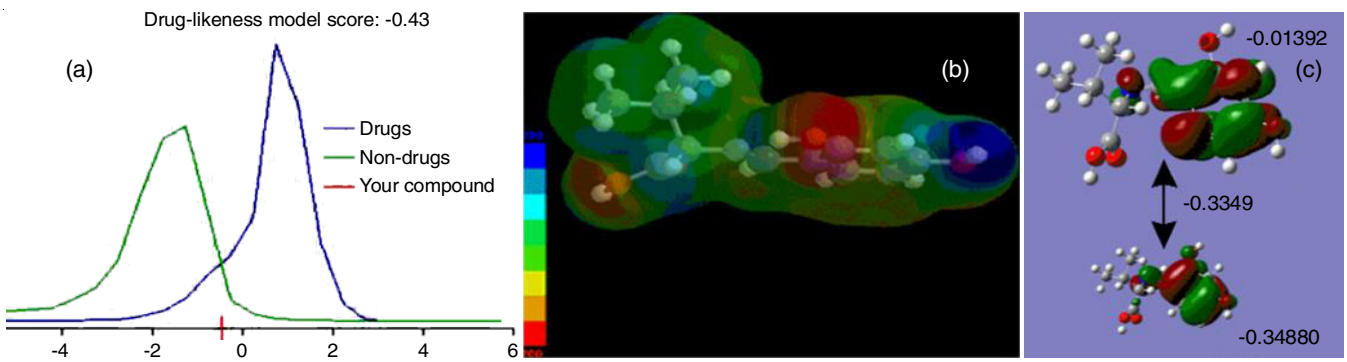


Fig. 4. (a) Drug likeness score between standard drugs and toxic chemicals, (b) MEP and (c) HOMO-LUMO energy gap of the LVDHBSB

TABLE-3
MCULE DOCKING SCORES (kcal/mol) OF LVDHBSB

PDB ID	Pose 1	Pose 2	Pose 3	Best score
1SHV	-3.9	-3.8	-3.7	-4.0
1a2N	-6.3	-6.1	-6.1	-6.6
1HSK	-9	-8	-2	-8.0
1S1J	-5.1	-5.0	-4.9	-5.4
1XCW	-6.7	-6.6	-6.3	-6.8
3K0K	-4.9	-4.9	-4.4	-5.1
3FDN	-6.8	-6.7	-5.7	-3

that the docking result was affected by the substitution effect. A high score for 1HSK and a low score for 3FDN were observed when comparing all targets.

The online docking values were verified and consistency was checked using CLC-3 drug discovery work bench. The best score poses were downloaded and some of the images are displayed in Fig. 5a-c. All the PDB format of Schiff base structures and proteins were imported separately to the CLC for docking studies. The binding site was selected at 15-20 Å radius and the scores were recorded and the docking scores are shown in Table-4. From the recorded docking scores, L-valine based Schiff base exhibited good docking score against all the targets. When compared the scores, bacterial proteins have shown the scores between 43.03 kcal/mol and 44.8 kcal/mol. The remaining proteins related to cancer and diabetics also exposed the similar range of scores. Some of the docking images are shown in Fig. 5d-h. By analyzing the binding affinity of chemicals with both static and dynamic proteins, the software confirms the hydrogen bonding connection.

Toxicity studies: Dead shrimp counts were determined after 24 h and three replicates of each trial were conducted. A regression method was employed to determine the LC_{50} of the synthesized LVDHBSB Schiff base and found to be 208.45 µg/mL. The phenolic -OH methyl group of valine is responsible for the substantial LC_{50} values (Table-5). With its inexpensive cost and high significance for general toxicity and antitumor detection, BSLA is an important tool in antioxidant assays.

TABLE-5
BRINE SHRIMP LETHAL ASSAY
(BSLA) LC_{50} VALUES OF LVDHBSB

Concentrations (µg/mL)	Mortality (%)
31.25	15 ± 0.4
62.5	27 ± 0.8
125	35 ± 0.00
250	60 ± 0.9
500	100 ± 0.00
Reg. equation	y = 0.1763x + 13.25
R^2	0.9932
LC_{50} (µg/mL)	208.45

Antibacterial activity: To further evaluate the *in vitro* antibacterial activity, the agar well diffusion method was employed against the selected pathogens. Initially, the selected pathogens were tested against Schiff base concentrations below

TABLE-4
DRUG DISCOVERY WORKBENCH-3 DOCKING SCORES OF LVDHBSB

Protein ID/ Comp. ID	Docking score (kcal/mol) at active site						
	1shv His112	1a2n Asp123	1hsk Glu144	1s1j Asp35	1xcw Asp147	3k0k Glu50	3fdn Glu135
LVDHBSB	-44.84	-43.21	-43.03	-44.8	-30.49	-42.09	-49.86

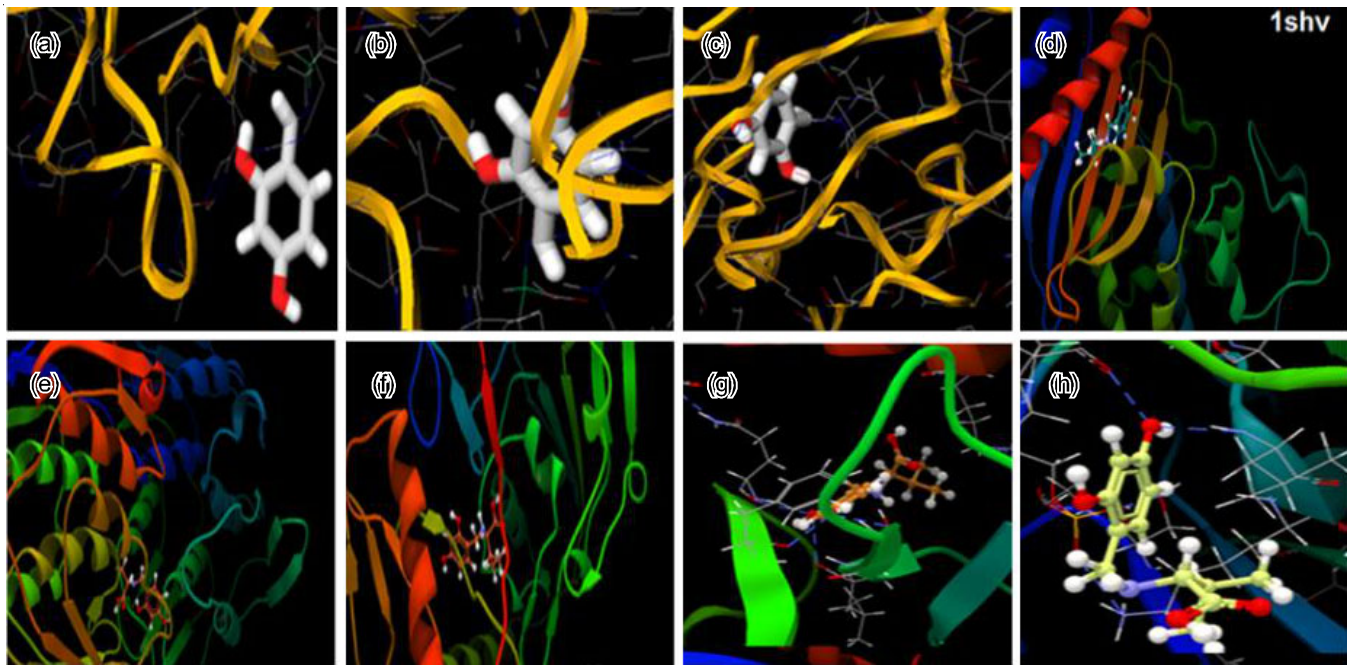


Fig. 5. Docking images of LVDHBSB against the targets such as (a) 1shv, (b) 1a2n, (c) 1hsk, (d) CLC offline docking against 1shv, (e) 1a2n, (f) 1hsk, (g) 1s1j, (h) 3fdn proteins

TABLE-7
α-AMYLASE INHIBITION AND DPPH ASSAY RESULTS OF LVDHBSB

α-Amylase inhibition assay				DPPH assay	
Std. acarbose conc. (µg/mL)	Inhibition (%)	Conc. (µg/mL)	Schiff base	Conc. (µg/mL)	Inhibition (%)
20	4.1	100	-9.52	100	6.15
40	18	200	8	200	9.23
60	32.3	300	25.81	300	10.77
80	45.2	400	37.84	400	13.85
100	61	500	45.24	500	16.92
IC_{50} µg/mL	85.38 ± 0.34		504.54 ± 0.29		1773.33 ± 0.32

the LC_{50}, which is 200 µg/mL. The results demonstrated that the LVDHBSB had superior antibacterial activity, with a zone of inhibition ranging from 9 mm to 11 mm (Table-6). Moreover, the toxicity LC_{50} is lower than the minimal inhibitory concentration. When it comes to Gram-negative bacteria, the Schiff base is more effective as compared to Gram-positive bacteria. Meanwhile, as compared to the standard, the LVDHBSB compound tendency is moderate.

TABLE-6
ANTIBACTERIAL SUSCEPTIBILITY TEST OF LVDHBSB AT 200 µg AGAINST GRAM-NEGATIVE AND POSITIVE BACTERIA

	Std. gentamycin 50 µg/mL	Zone of inhibition (mm) for 200 µg/mL	MIC (µg/mL)
E. coli	17 ± 0.22	11 ± 0.32	100
K. pneumoniae	14 ± 0.19	10 ± 0.29	100
S. aureus	14 ± 0.21	9 ± 0.36	150

Antidiabetic activity: The α-amylase inhibition assay was used to determine the antidiabetic activity of L-valine based Schiff base and the outcomes are shown in Table-7. The regression method was used to compute the results of the α-amylase inhibitory activity for 50% inhibition when exposed to standard acarbose. The IC_{50} value of 504.54 ± 0.29 µg/mL is found to be seven times higher than the standard's IC_{50} value of 85.38 ± 0.34 µg/mL.

Antioxidant activity: The observed data showed that the DPPH radical scavenging was more for 50% inhibition by the chiral valine Schiff base. The chiral valine Schiff base exposed the scavenging of the DPPH radical by more than 50% and the calculated values are summarized in Table-7. From the triplicated results, this research identified LVDHBSB has moderate antioxidant activity at higher concentrations due to the hydroxy group, -COOH and imine groups.

Conclusion

The eco-friendly solvent water and a small amount of highly pure methanol were used to synthesize the L-valine based Schiff base. Theoretically expected data supports the characterization, antibacterial, antidiabetic and antioxidant effects of chiral L-amino acid Schiff bases in this research. The chemical reactivity and QSAR were computed using two distinct DFT methods. Docking studies were performed using online mcule tool in conjunction with the offline CLC drug discovery work bench. In addition, the study found that compared to its antidiabetic and antioxidant properties, the L-valine based Schiff base exhibited good antibacterial character. The Schiff base has moderate antioxidant activity, which are due to the presence of chiral imines (-CH=N-), carboxylic (-COOH) and hydroxyl (-OH) groups.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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