IN SILICO DOCKING AND ADMET PREDICTION OF A NOVEL ANTIBACTERIAL DERIVATIVES (SCHIFF BASE) FOR DIHYDROFOLATE REDUCTASE (1MVT) INHIBITION

Prerana D. Kalyankar*, Dr. Megha T. Salve

Department of Bachelor in Pharmacy

Shivajirao Pawar College of Pharmacy, Pachegaon, Ahilyanagar-413725.

Email ID- kalyankarprerana31@gmail.com

*Corresponding Author- Prerana D. Kalyankar.

Abstract: Schiff base-containing (imine or azomethine–C=N–) derivatives have been investigated in relation to a broad range of activity, including antibacterial activity, antiviral activity, anticancer activity, polymer technology and in many other areas due to the presence of moiety in their structures. Antibacterial activity of Schiff bases can achieve by various enzyme inhibitory mechanism. Primary target for the antibacterial drugs is inhibiting dihydrofolate Reductase (DHFR) enzyme which result in inhibition of bacterial folate synthesis and act as bactericidal. In this research article pharmacokinetic properties, bioactivity score, in silico docking studies and toxicity prediction of 10 Schiff base compounds i.e. SB1, SB2, SB3, SB4, SB5, SB6, SB7, SB8, SB9, and SB10 were carried out against dihydrofolate Reductase enzyme (1Mvt) was examined and possible probability were recorded.

Keywords: Schiff base, Dihydrofolate reductase Inhibition, Molecular Docking, ADME Prediction, Antibacterial activity.

1. INTRODUCTION-

In 1864 the term Schiff base was introduced by germen chemist Hugo Schiff. [1] SBs are easy to synthesized and inexpensive compounds as compared to another chemical compound. [2] In recent years, SBs gained a lot of attention due to their broad activity including antibacterial activity, antiviral activity, antimicrobial activity, and antifungal activity. [3] Researchers synthesize various new SBs derivatives and explore their potent antibacterial action. [4,5] SBs act as antibacterial agent by showing various mechanism of action they are as follows-

- By inhibiting cell wall synthesis
- By inhibition of ribosome function
- By nucleic acid synthesis inhibition
- By inhibition of folate metabolism

- By change in cell membrane function [6]

In this research article author select a target i.e. dihydrofolate reductase enzyme (1MVT). This enzyme is necessary for the bacterial folate biosynthesis, which catalyzes the the conversion of 7, 8 Dihydrofolate to 5, 6, 7, 8-Tetrahydrofolate using coenzyme NADPH and the proton of water molecule respectively as the donor of hydride ion. As a key step in bacterial folate biosynthesis. DHFR is usually an important target for treatment of variety of microbial infections. This pathway plays crucial role in the synthesis of nucleic acid. [7]

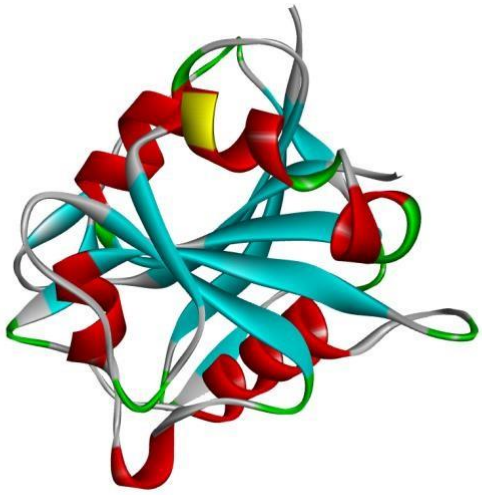
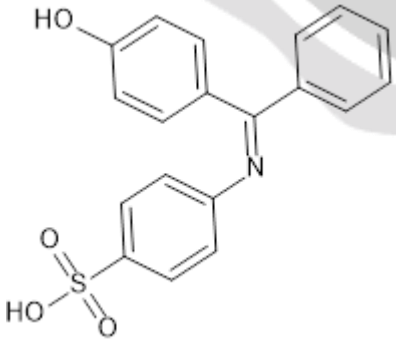


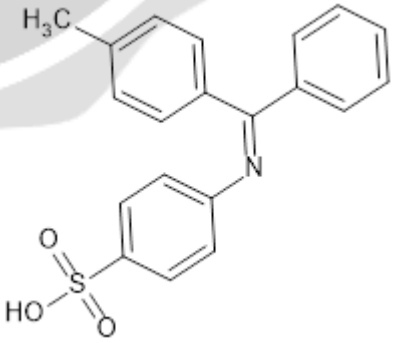
Fig. 1 3D structure of Dihydrofolate reductase enzyme (1mvT). [8]

In bacteria, antibacterial SBs derivatives act as a competitive inhibitor of the dihydrofolate reductase enzyme (1mvt). Hence SBs derivatives exhibit bactericidal effect in bacteria. [9]

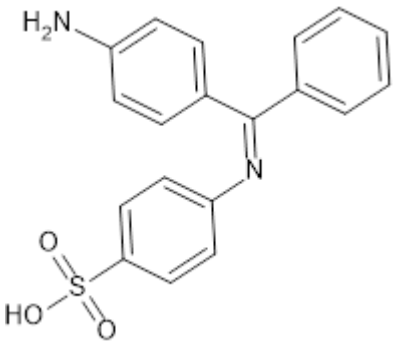
In this study we aim to investigate the antibacterial effect of novel SBs derivatives against **dihydrofolate reductase enzyme** (1mvt). This carryout by various computational techniques like ADME prediction, toxicity prediction, molecular docking etc. [10] Followings are the SBs derivatives which are prepare by substituting various groups on novel Schiff base moiety. [11]



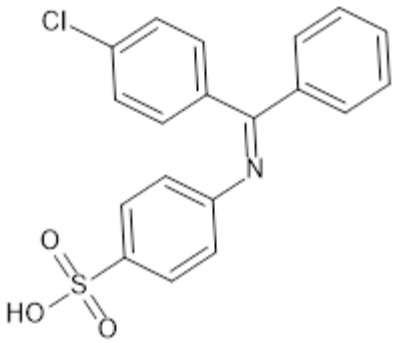
SB1



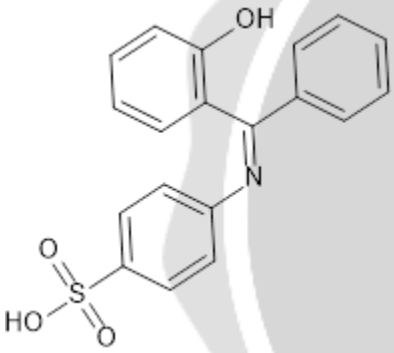
SB2



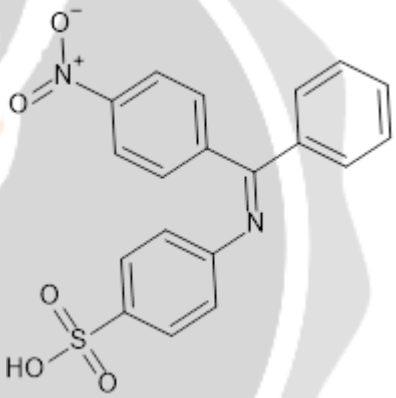
SB3



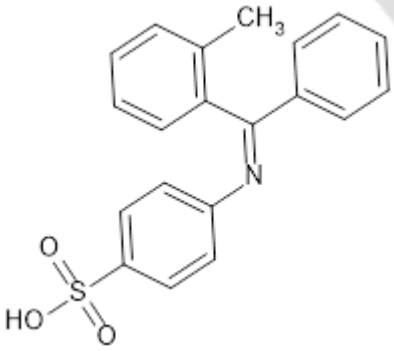
SB4

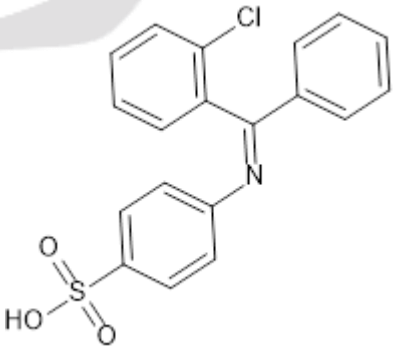


SB5



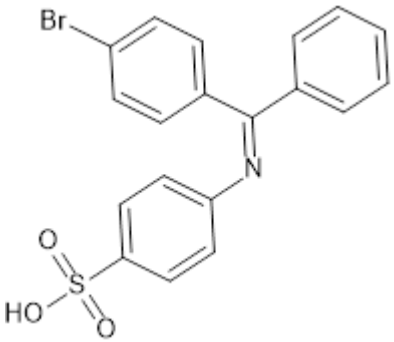
SB6

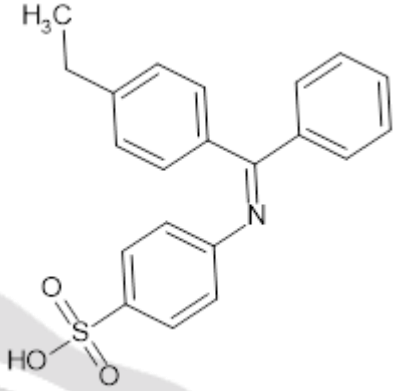




SB8

SB7





SB9 **SB10**

2. MATERIALS AND METHOD-

2.1. Ligand preparation- With the help of ChemSketch tool [12] A set of 10 compounds were prepared by substituting various electron donating group and electron withdrawing group (-OH,Cl, -CH_3, -NH_2, -Br, -C_2H_5) on basic moiety prepare by the Schiff base method. [13] Then SBs derivatives converted into the SDF format. [14]

2.2. Protein preparation- 3D crystal structure of dihydrofolate Reductase (1MVT) protein was download from RCSB, Protein Data Bank as PDB format [8] and open in Biovia Discovery studio Visualizer36 V16.1.0.15350. [15] During the protein preparation process the hetro atom, water molecules, excessive chain and the pre- exist ligand on that protein were removed and file save in the form of MDL MOL/ SD file. [16]

2.3. ADMET and drug-likeness prediction- The SwissADME [17] tool were used to screening of various pharmacokinetic properties of SBs derivatives like Gastrointestinal absorption, Blood Brain Barries permeation, P-gp subs, CYP1A2 inhibitor, CYP2C19 inhibitor, CYP2C9 inhibitor, CYP2D6 inhibitor, CYP3A4 inhibitor, Log Kp, Bioavailability were predicted and present in
tabular format. [18]

2.4. Prediction of Toxicity- The Protox 3.0 tool [19] were used to predict the toxicity of SBs derivatives which including organ toxicities like hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity, immunogenicity etc. [20]

2.5. Molecular Properties and Bioactivity Scores of the ligands- The SwissADME [17] tool were used to predict the molecular properties like MlogP (partition coefficient between n-octanol and water), TPSA, number of hydrogen bond donors and number of hydrogen bond acceptors, molecular weight, and the number of rotatable bonds, molecular volume was calculated and present in tabular format. [21] Another one software i.e. Molinspiration was used to predict ligands modulating GPCR, Ion channels, Nuclear receptors, and also predict the ligands as Kinase inhibitors, Protease inhibitors and Enzyme inhibitors. [22]

2.6. Molecular Docking Studies- Docking studies were carried out by using One click Docking tool. [23] Targeted protein DHFR enzyme (1mvt) was download from Protein Data Bank then prepare a protein by removing the hetro atom, water molecules, excessive chain and the pre- exist ligand. Now all prepared protein upload on M-cule Docking and dock with new derivatives.
Binding affinity and types of interaction present in the ligand and target were examined by using

Discovery studio Visualizer36 V16.1.0.15350. [15,24]

3. RESULTS AND DISCUSSION-

3.1 Screening of designed derivatives through ADMET analysis- Table no.1 evaluation of SBs derivatives on the basis of based on Lipinski's rule of five [25], also known as Pfizer's rule of five or the rule of five (RO5) which specifies that an orally active medication should obey the following rules: less than 5 hydrogen-bond donors, less than 10 hydrogen-bond acceptors, a molecular mass less than 500, and log P less than 5. Other important properties, such as total polar surface area (TPSA), the amount of rotatable bonds, and molar refractivity, were measured as well. A compound's TPSA should be less than 140 Å2, and the number of rotatable bonds should be less than 10. In table no.2 bioactivity scores were calculated for SBs derivative as GPCR ligands, ion channel modulators (ICM), kinase inhibitors (KI), nuclear receptor ligands (NRL), protease inhibitors (PI), and enzyme inhibitors (EI). Values more than 0.00 indicate considerable activity, scores between 0.00 and -0.5 indicate mild activity, and scores less than -0.5 indicate inactivity. [26 While all of the derivatives do not cross the blood-brain barrier (BBB).

Table 1. Calculations of Lipinski rule of five for the designed derivatives SB1-SB10)

Ligand	Molecular weight	TPSA	Molar refractivity	MlogP	Rotatable bonds	Hbond donors	H-bond acceptors
SB1	353.39	95.34	96.51	3.05	4	2	5
SB2	351.42	75.11	99.45	3.84	4	1	4
SB3	352.41	101.13	98.89	3.05	4	4	2
SB4	371.84	75.11	99.49	4.11	4	1	4
SB5	353.39	95.34	96.51	3.05	4	2	4
SB6	382.39	120.93	103.31	2.65	5	1	6
SB7	351.42	75.11	99.45	3.84	4	1	4
SB8	371.84	75.11	99.49	4.11	4	1	4
SB9	416.29	75.11	102.18	4.22	4	1	4
SB10	365.45	75.11	104.26	4.06	5	1	4

Table 2: Bioactivity Scores of designed derivatives (SB1-SB10)

Ligands	GPCR	ICM	KI	NRL	PI	EI
SB1	0.27	0.16	-0.05	0.04	0.16	0.21
SB2	0.19	0.06	-0.13	-0.10	0.11	0.12
SB3	0.29	0.20	0.01	-0.12	0.26	0.28
SB4	0.24	0.13	-0.10	-0.09	0.13	0.15
SB5	0.22	0.17	-0.09	-0.01	0.13	0.23

SB6	0.08	0.08	-0.22	-0.15	0.03	0.07
SB7	0.15	0.12	-0.17	-0.03	0.11	0.15
SB8	0.34	0.24	-0.11	-0.08	0.12	0.17
SB9	0.14	0.06	-0.13	-0.18	0.06	0.11
SB10	0.24	0.13	-0.14	-0.04	0.17	0.18

Table 3. The pharmacokinetic properties of the designed derivatives (SB1-SB10)

Codes	GI abs.	BBB perm.	CYP 1A2	CYP2 C19	CYP2 C9	CYP2 D6	CYP3A4	Log Kp (cm/s)	Bioavailability
			inhibitor						
SB1	High	No	No	No	No	No	No	-5.98	0.56
SB2	High	No	No	No	No	No	No	-5.45	0.56
SB3	High	No	No	No	No	No	No	-6.20	0.56
SB4	High	No	No	Yes	Yes	No	No	-5.39	0.56
SB5	High	No	No	No	No	No	No	-5.98	0.56
SB6	Low	No	No	Yes	Yes	No	No	-6.02	0.56
SB7	High	No	No	Yes	Yes	Yes	Yes	-6.11	0.55
SB8	High	No	No	Yes	Yes	No	No	-5.39	0.56
SB9	High	No	No	Yes	Yes	No	No	-5.58	0.55
SB10	High	No	No	No	No	No	No	-5.23	0.56

3.2 Molecular docking- From the initial screening through Lipinski rule, ADME calculations, and bioactivity score, molecules SB1-SB10 successfully passed all the filters and displayed most drug-likeness nature. In table no.4 SBs derivatives selected for docking against dihydrofolate reductase enzyme (1mvt) had exhibited more potent interactions and binding affinity with the target. Binding affinities (kcal/mol), and the types of interaction of the docked molecules are examined and the molecules' 2D and 3D docking postures are represented. More the negative docking score show the higher affinity of ligand towards the target. SB7 derivative shows highest negative docking score (-10.4 Kcal/mol) and SB9 shows lowest negative docking score (-8.0 Kcal/mol).

Table 4. The binding interactions of all the designed derivatives (SB1-SB10) with DHPS enzyme (1AJ2)

Comp Code	Binding affinity (Kcal/mol)	Type of interaction

SB1	-9.3	Van der Waals, Pi-Alkyl, Carbon Hydrogen Bond, Amide Pi-stacked
SB2	-9.1	Van der Waals, Conventional Hydrogen bond, Pi-Pi T-shaped, Pi-Alkyl, Pi sigma.
SB3	-9.5	Van der Waals, Pi-Alkyl, Pi stigma.
SB4	-9.3	Van der Waals, Conventional Hydrogen bond, amide -pi stacked, Pi-Alkyl
SB5	-9.8	Van der Waals, Pi-Alkyl, Pi sigma, AmidePi stacked.
SB6	-8.2	Van der Waals, Pi-Alkyl, salt bridge, pi-pi stacked, unfavourable +ve-+ve,
SB7	-10.4	Van der Waals, Carbon Hydrogen bond, Alkyl, Pi-Alkyl, Amide Pi –stacked, Pi sigma.
SB8	-9.8	Van der Waals, Carbon Hydrogen bond, PiAlkyl, Amide Pi stacked.
SB9	-8.0	Van der Waals, Alkyl, Pi-Alkyl, Pi-Pi
		stacked.
SB10	-9.7	Van der Waals, Conventional Hydrogen bond, Pi-Pi stacked, Pi-Alkyl, Carbon Hydrogen Bond, Alkyl, Pi-Pi-shaped.

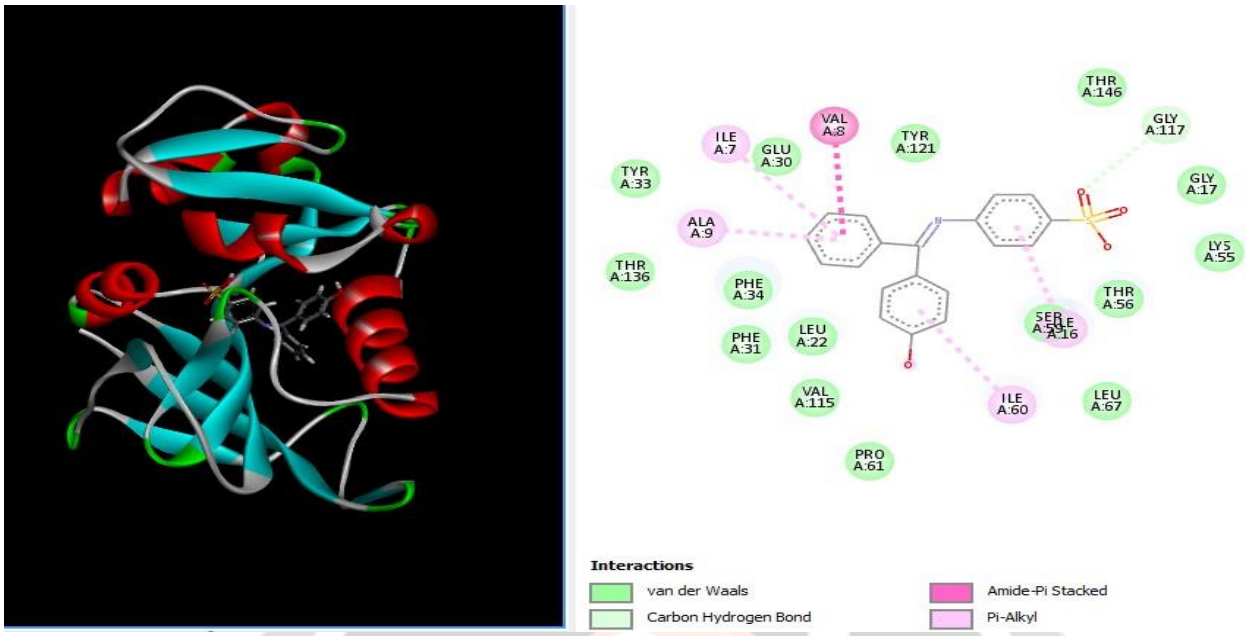


Figure 2. 3D and 2D docking poses of ligand SB1 with dihydrofolate reductase enzyme (1MVt).

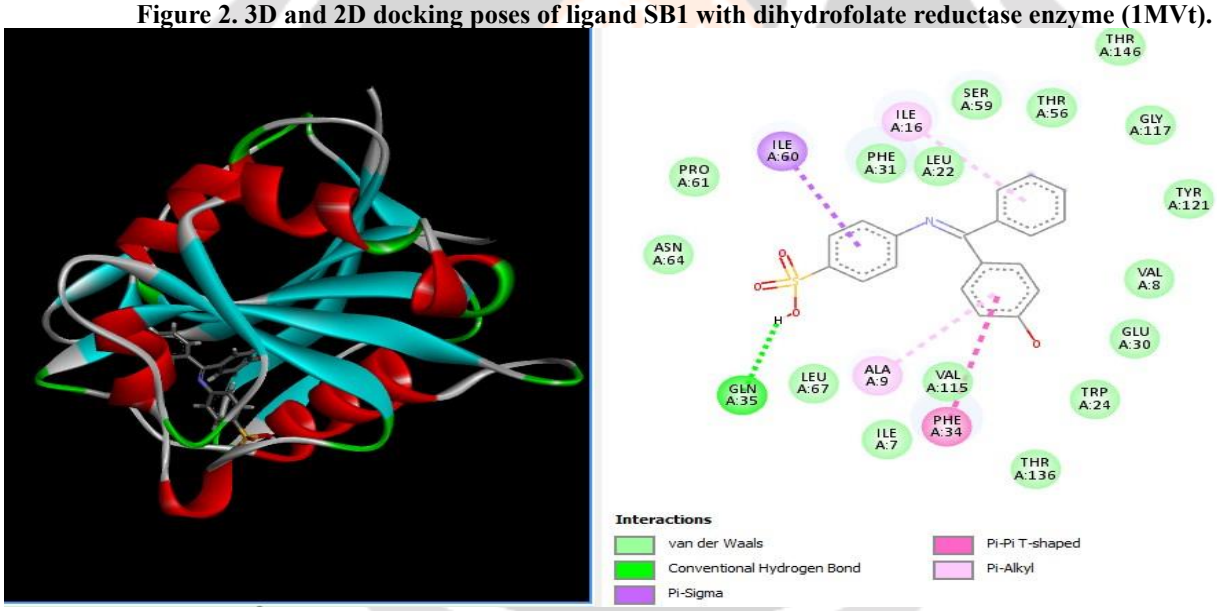


Figure 3. 3D and 2D docking poses of ligand SB2 with dihydrofolate reductase enzyme (1MVt).

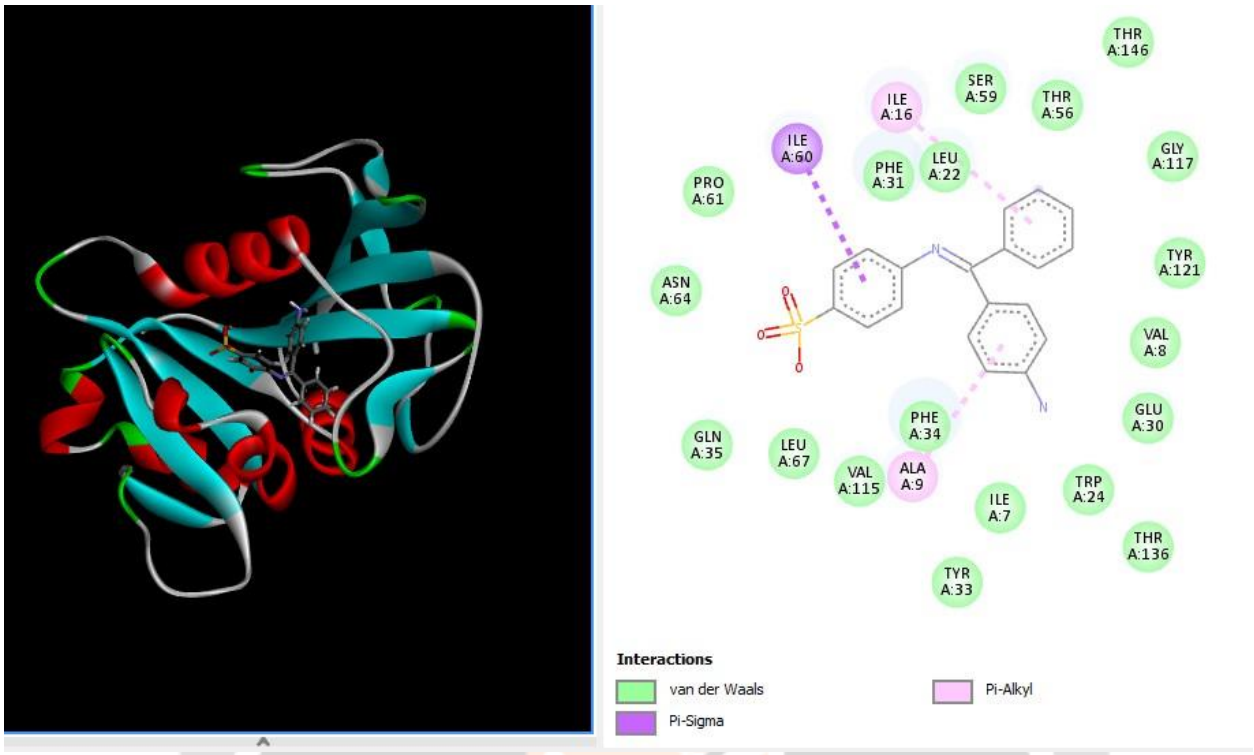


Figure 4. 3D and 2D docking poses of ligand SB3 with dihydrofolate reductase enzyme (1MVt).

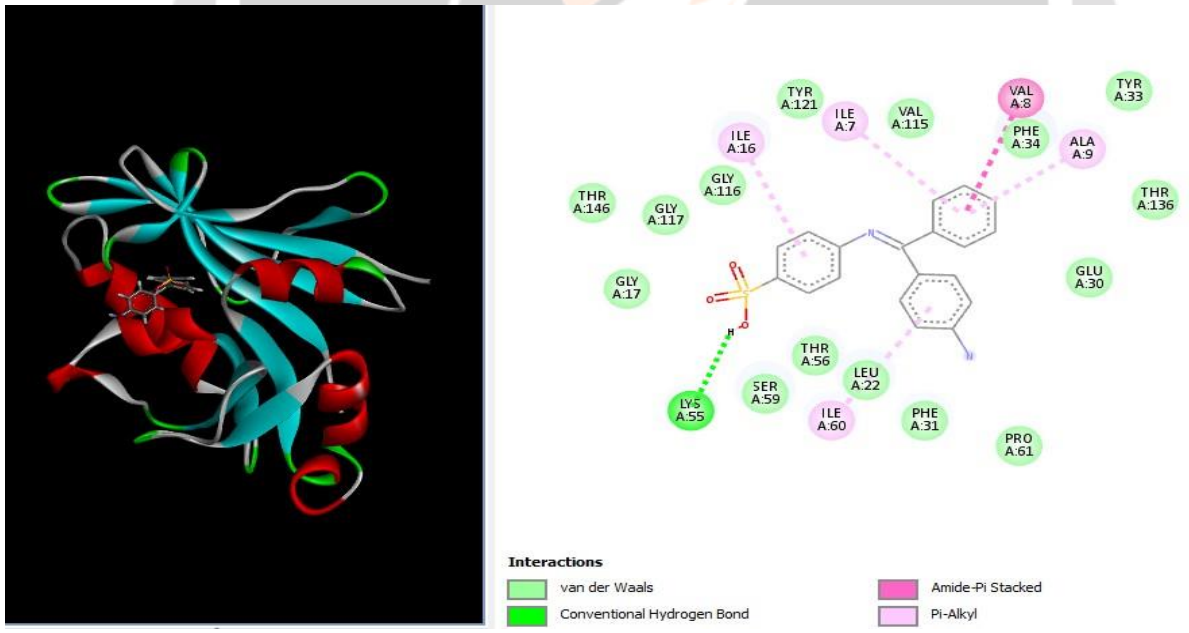


Figure 5. 3D and 2D docking poses of ligand SB4 with dihydrofolate reductase enzyme (1MVt).

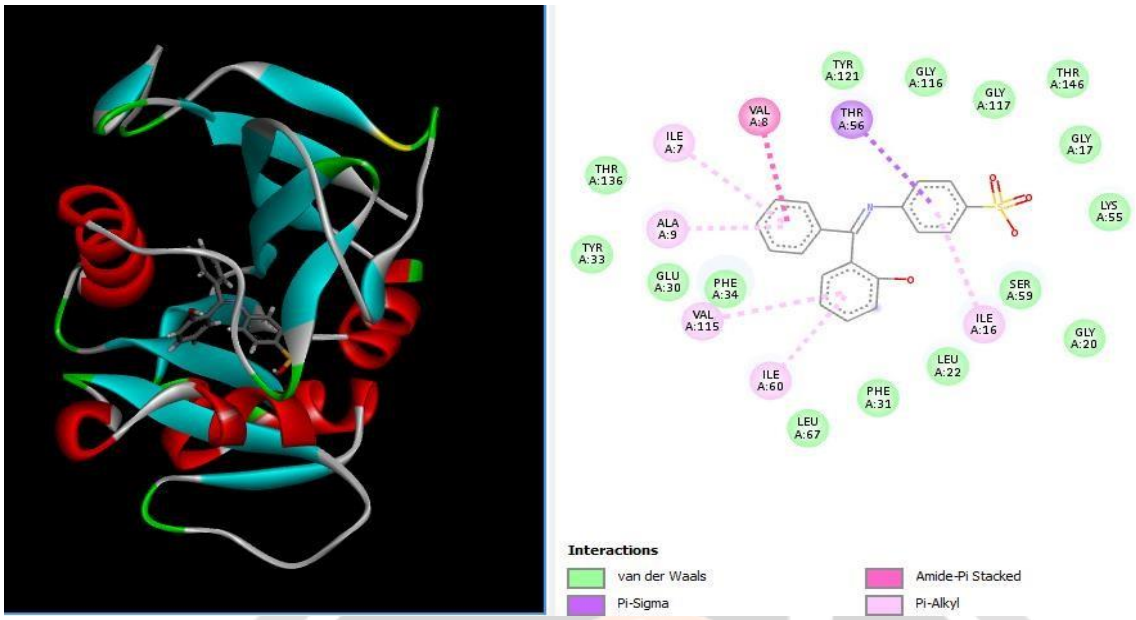


Figure 6. 3D and 2D docking poses of ligand SB5 with dihydrofolate reductase enzyme (1MVt).

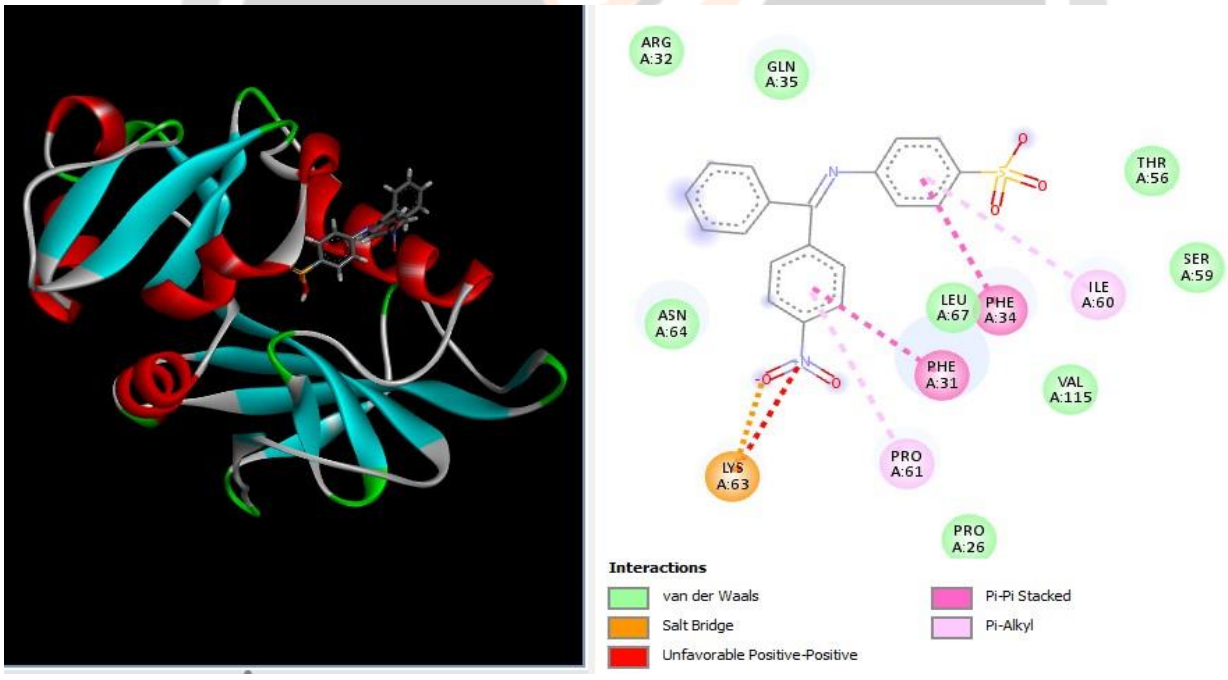


Figure 7. 3D and 2D docking poses of ligand SB6 with dihydrofolate reductase enzyme (1MVt).

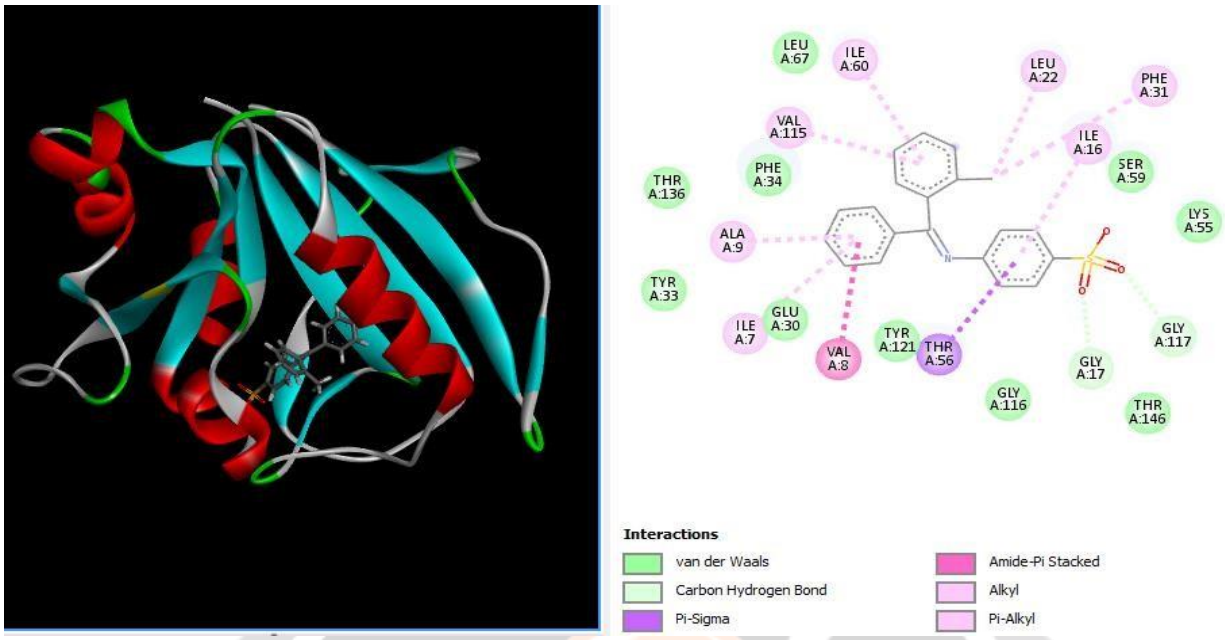


Figure 8. 3D and 2D docking poses of ligand SB7 with dihydrofolate reductase enzyme (1MVt).

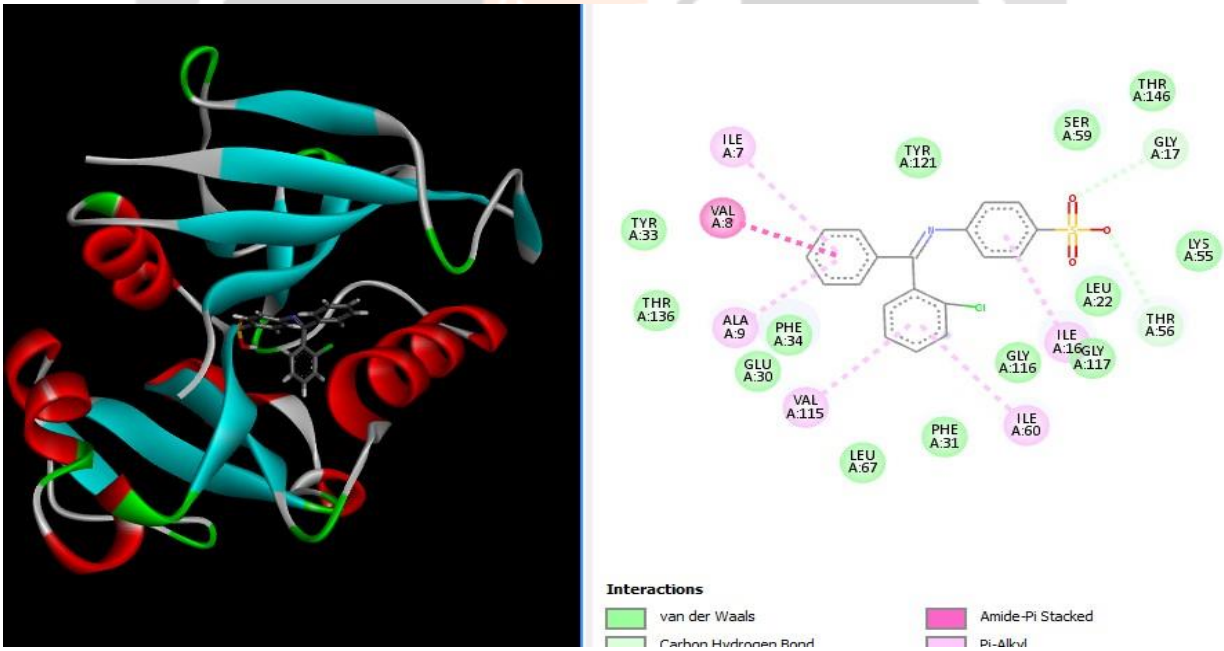


Figure 9. 3D and 2D docking poses of ligand SB8 with dihydrofolate reductase enzyme (1MVt).

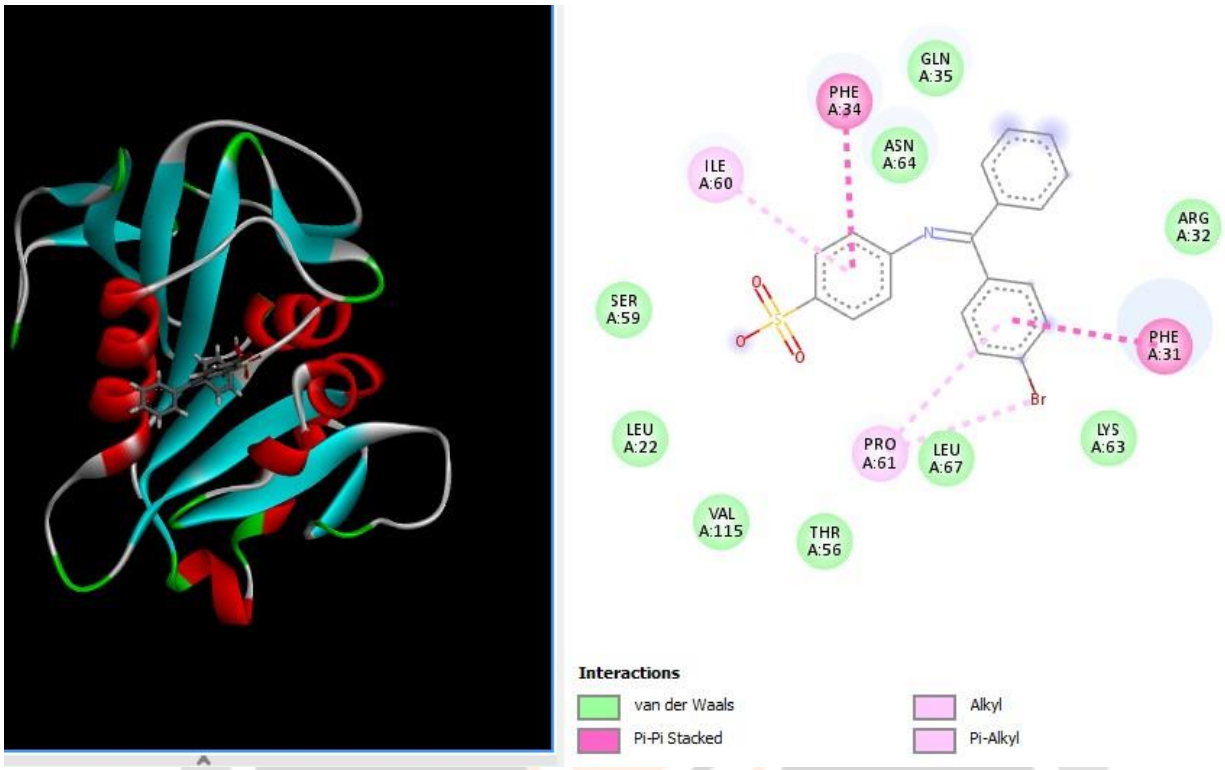


Figure 10. 3D and 2D docking poses of ligand SB9 with dihydrofolate reductase enzyme (1MVt).

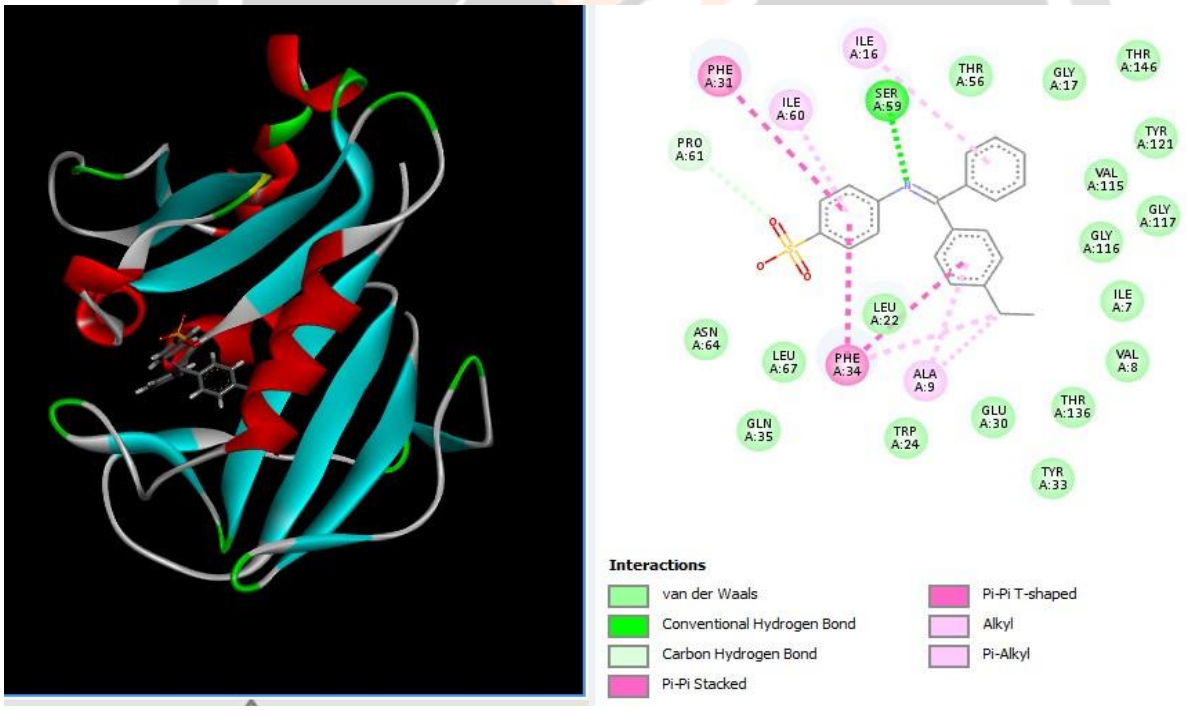


Figure 11. 3D and 2D docking poses of ligand SB10 with dihydrofolate reductase enzyme (1MVt).

3.3 Prediction of Toxicity- In this study toxicity of SBs derivatives was assessed by using various toxicological endpoints such as hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity, immunogenicity. The results of toxicity prediction were quantified in binary form
i.e. active/ inactive. [27] Most of the SB's derivatives shows no carcinogenicity like SB1, SB2, SB3, SB5, SB4, SB6, SB7, SB8, SB9, SB10.

Table 3.1. The toxicity profiles of the designed derivatives (SB1-SB10)

Ligand	Hepato-toxicity	Carcinogenicity	Immunotoxicity	Muta-genicity	Cyto-toxicity
SB1	Inactive	Inactive	Inactive	Inactive	Inactive
SB2	Inactive	Inactive	Inactive	Inactive	Inactive
SB3	Inactive	Inactive	Inactive	Inactive	Inactive
SB4	Inactive	Inactive	Inactive	Inactive	Inactive
SB5	Inactive	Inactive	Inactive	Inactive	Inactive
SB6	Inactive	Inactive	Inactive	Inactive	Inactive
SB7	Inactive	Inactive	Inactive	Inactive	Inactive
SB8	Inactive	Inactive	Inactive	Inactive	Inactive
SB9	Inactive	Inactive	Inactive	Inactive	Inactive
SB10	Inactive	Inactive	Inactive	Inactive	Inactive

3. CONCLUSION-

According to the results of docking analysis Schiff base compound SB3, SB5, SB7 and SB8 had highest binding affinity score i.e. --9.5 Kcal/mol, -9.8- Kcal/mol, --10.4 Kcal/mol and --9.8 Kcal/mol respectively. In silico toxicity prediction study suggest that compound SB1, SB2, SB3, SB4, SB7, SB9 exhibits low or negligible toxicity in hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity. Based on above conclusion compound SB and SB can be a promising ligand for antibacterial activity. This in silico results suggest to researcher for further investigation through in vitro and in vivo studies to confirm the predicted properties and explore the therapeutic action of compound SB7 and SB8. The computational data from this study will guide further development and optimization of the Schiff base (bearing- imine) derivatives for a dihydrofolate reductase (1mvt) Inhibition.

ACKNOWLEDGMENTS-

The corresponding author would like to express their sincere gratitude to Dr. Megha T. Salve for their aspiring guidance, valuable contributions and support throughout the preparation of this research work.

REFERENCE-

1. Da Silva, C.M., da Silva, D.L., Modolo, L.V., Alves, R.B., de Resende, M.A., Martins, C.V. and de Fátima, Â., 2011. Schiff bases: A short review of their antimicrobial activities. Journal of Advanced research, 2(1), pp.1-8.
2. Ceramella, J., Iacopetta, D., Catalano, A., Cirillo, F., Lappano, R. and Sinicropi, M.S., 2022. A review on the antimicrobial activity of Schiff bases: Data collection and recent studies. Antibiotics, 11(2), p.191.
3. Mushtaq, I., Ahmad, M., Saleem, M. and Ahmed, A., 2024. Pharmaceutical significance of Schiff bases: an overview. Future Journal of Pharmaceutical Sciences, 10(1), p.16.
4. Imran, S., Taha, M., Ismail, N.H., Khan, K.M., Naz, F., Hussain, M. and Tauseef, S., 2014. Synthesis of novel bisindolylmethane Schiff bases and their antibacterial activity. Molecules, 19(8), pp.11722-11740.
5. Matar, S.A., Talib, W.H., Mustafa, M.S., Mubarak, M.S. and AlDamen, M.A., 2015. Synthesis, characterization, and antimicrobial activity of Schiff bases derived from benzaldehydes and 3, 3′-diaminodipropylamine. Arabian Journal of Chemistry, 8(6), pp.850-857.
6. Rao, A.S. and Tapale, S.R., 2013. A study on dihydrofolate reductase and its inhibitors: A review. International Journal of Pharmaceutical Sciences and Research, 4(7), p.2535.
7. Bank, R.P.D. (no date) RCSB PDB – 1MVT: CRYSTAL STRUCTURE OF a BINARY COMPLEX OF E. COLI DIHYDROFOLATE SYNTHASE. https://www.rcsb.org/structure/1MVT.
8. Ommenya, F.K., Nyawade, E.A., Andala, D.M. and Kinyua, J., 2020. Synthesis, characterization and antibacterial activity of Schiff base, 4-Chloro-2-{(E)-[(4-fluorophenyl) imino] methyl} phenol metal (II) complexes. Journal of Chemistry, 2020, pp.1-8.
9. Chang, Y., Hawkins, B.A., Du, J.J., Groundwater, P.W., Hibbs, D.E. and Lai, F., 2023. A guide to in silico drug design. Pharmaceutics, 15(1), p.49.
10. Raczuk, E., Dmochowska, B., Samaszko-Fiertek, J. and Madaj, J., 2022. Different Schiff bases—structure, importance and classification. Molecules, 27(3), p.787.
11. Free chemical drawing software for students | ChemSketch | ACD/Labs (2024). https://www.acdlabs.com/resources/free-chemistry-software-apps/chemsketch-freeware/.
12. Raczuk, E. et al. (2022) 'Different Schiff Bases—Structure, Importance and Classification,' Molecules, 27(3), p. 787. https://doi.org/10.3390/molecules27030787.
13. SMILES to 2D or 3D SDF/MOL converter. http://biotech.fyicenter.com/1000068_JSME_SMILES_to_SDF_Mol_Online_Converter.ht ml.
14. Ds (2024) Free download: BIOVIA Discovery Studio Visualizer. https://discover.3ds.com/discovery-studio-visualizer-download.
15. Agu, P.C., Afiukwa, C.A., Orji, O.U., Ezeh, E.M., Ofoke, I.H., Ogbu, C.O., Ugwuja, E.I. and Aja, P.M., 2023. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Scientific Reports, 13(1), p.13398.
16. SwissADME. http://www.swissadme.ch/.
17. Cheng, F., Li, W., Liu, G. and Tang, Y., 2013. In silico ADMET prediction: recent advances, current challenges and future trends. *Current topics in medicinal chemistry*, *13*(11), pp.1273-1289.
18. ProTox-3.0 - Prediction of TOXicity of chemicals (no date). https://comptox.charite.de/protox3/index.php?site=compound_search_similarity.

19. Marin, D.E. and Taranu, I., 2023. Using In Silico Approach for Metabolomic and Toxicity Prediction of Alternariol. Toxins, 15(7), p.421.
20. Kuchana, M. and Kambala, L.B., 2021. Design, synthesis and in silico prediction of druglikeness properties of new ortho, meta and para-(2-cyano-3-(3, 5-di-tert-butyl-4hydroxyphenyl) acrylamido) benzoic acids. Journal of Applied Pharmaceutical Science, 11(8), pp.031-035.
21. Hussein, Y.T. and Azeez, Y.H., 2023. DFT analysis and in silico exploration of druglikeness, toxicity prediction, bioactivity score, and chemical reactivity properties of the urolithins. Journal of Biomolecular Structure and Dynamics, 41(4), pp.1168-1177.
22. 1-Click-Docking. https://mcule.com/apps/1-click-docking/.
23. Pullagura, P., Vallabhaneni, M.R., Addanki, H.R., Chennamsetty, S. and Yenisetty, R., 2021. A simple, efficient synthesis and molecular docking studies of 2-styrylchromones.
Organic Communications, 14(2).
24. Lipinski, C.A., 2004. Lead-and drug-like compounds: the rule-of-five revolution. Drug discovery today: Technologies, 1(4), pp.337-341.
25. Husain, A., Ahmad, A., Khan, S.A., Asif, M., Bhutani, R. and Al-Abbasi, F.A., 2016. Synthesis, molecular properties, toxicity and biological evaluation of some new substituted imidazolidine derivatives in search of potent anti-inflammatory agents. Saudi Pharmaceutical Journal, 24(1), pp.104-114.
26. Raies, A.B. and Bajic, V.B., 2016. In silico toxicology: computational methods for the prediction of chemical toxicity. Wiley Interdisciplinary Reviews: Computational Molecular
Science, 6(2), pp.147-172.