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RESEARCH ARTICLE

Synthesis, Physical Characterization and *In Silico* Study of Novel Schiff Base

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ABSTRACT

Now a days treatment of infectious diseases is a challenging issue faced by the medical community. There are bunch of marketed drugs held modified pharmacological and toxicological properties and probably Schiff bases are versatile C=N (Imine) containing compounds possessing broad spectrum of biological activity and showed some degree of antibacterial, cytotoxic effects, antifungal, and antimalarial. In this research work a novel SB's derivative has been designed, synthesized by using green method, and evaluated to explore physical characterization such as purity assessment of synthesized product by using TLC technique, spectral analysis by using IR spectroscopy, melting point determination by conventional method, molecular docking by using various computerized software, solubility determination by using various polar and non-polar solvent. The aim behind this research is to focus on green synthetic procedure used in the synthesis of novel Schiff bases, physical characterization and *in silico* study to find the most efficient method to get high yield in lesser time along with environment friendly.

Keywords: Synthesis; Evaluation; Spectral Analysis; ADMET prediction; Molecular Docking

INTRODUCTION

In 1864 germen chemist Hugo Schiff was introduced the term Schiff base, which is the product obtain by condensation of primary amines with carbonyl compounds. [1] As compared to another chemical compound SBs are easy to synthesized and inexpensive compounds. [2] In recent years, Schiff bases have attracted significant attention because of their wide range of activities, such as antibacterial properties, antiviral properties, antimicrobial properties, and antifungal properties. [3,4] Schiff base is a compound with the general structure R2C=NR', which is also known as a subclass of imines (containing carbon-nitrogen double bond) with either secondary ketimines or secondary aldehydes depending on their structure. [5] The term imine is often used interchangeably with azomethine, which specifically refers to secondary aldimines. These compounds are named as Schiff base after the Italian chemist Hugo Schiff in 19th century and there are many ways to name these compounds. [6] Nowadays, the research field dealing with Schiff base coordination chemistry has expanded enormously. Schiff base complexes possess importance for bioinorganic chemistry, biomedical applications, supramolecular chemistry, catalysis and material science, separation and encapsulation processes, and formation of compounds with unusual properties and structures. [7,8] In this reaction when any primary amine reacts with a ketone or aldehyde a under specific conditions, Schiff's bases are formed. In simple words, SBs are nitrogen analogue of an aldehyde or ketone where the carbonyl group has been replaced by an imine or azomethine group. [9]

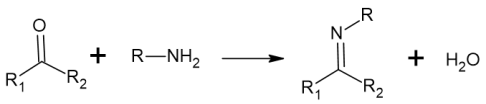

Fig. 1: Schiff base reaction

As compared to traditional approaches, green techniques must improve selectivity, minimize reaction time, and simplify product separation. [10] The primary aim of this research is to concentrate on environmentally friendly synthetic methods for producing Schiff bases, with the goal of identifying the most efficient techniques that offer higher yields in less time while remaining eco-friendly. [11] Also conduct in-silico investigation of synthesized SBs against DHFR to explore the antimicrobial activities. The residual

interaction between ligand and receptor were visualized using Discovery Studio software.[12,13]

EXPERIMENT

All chemicals obtained from Shivajirao Pawar College of Pharmacy, Newasa and used as such without further purification. Melting points were determined using a calibrated thermometer and various polar nonpolar solvent used to determine the solubility of synthesized compound. IR Spectra and purity assessment study were carryout in the Modern College of Pharmacy, S.P.P. University, Pune, as KBr discs on a FT/IR-4100typeA.

- **Chemicals**
  - Acetophenone
  - Sulphanilamide
  - Potassium Hydroxide
  - Ethanol
- **Apparatus and Instrument**
  - Measuring Cylinder
  - Stirrer
  - Beaker
  - Water Bath
  - Mechanical stirrer
  - Hot Plate
- **Softwares**
  - ChemSketch
  - SwissADME
  - Protox 3.0
  - Biovia Studio
  - M-cule Docking
  - Openbabel
  - Molinspiration

Synthesis of Schiff Base (IMINE)

The 4-{N-[4-(methanesulfonyl)phenyl]-3-phenylpropanimidoyl}phenol compound was synthesized according to the method in (V. Koteswara Rao, 13 Oct 2010)[14] and recrystallized from ethanol (m.p. 190~192 °C, Lit 190 °C).

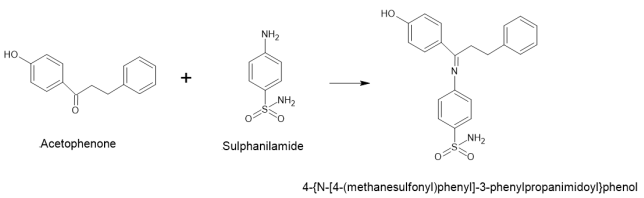

Fig. 2: Synthesis reaction of schiff base

General method for the synthesis of Schiff bases (compounds SB, Figure 1) is Sulphanilamide (10 gm) was dissolved in 30 ml of ethanol. Substituted aromatic ketone (8ml) in 30 ml ethanol containing Sulphanilamide was added dropwise with stirring and the reaction mixture was continue stirred for 4 hours at room temperature. After the completion of the synthesis, the reaction mixture was cooled in an ice bath. The solid precipitate formed was collected by filtration and thoroughly rinsed with distilled water. To further purify the product, it was recrystallized from ethanol. Evaluation result of various physical characteristics of synthesized compounds is given in result and discussion section.

Physical Characterization

- **Physical properties:** Evaluating the physical properties of a novel synthesized Schiff base is necessary for understanding its stability, behavior and potential applications.
- **Solubility:** It is a key physical property that provides insights into how a novel synthesized Schiff base interacts with different solvents. Understanding solubility is essential for applications in solution-phase reactions, formulation in pharmaceuticals, and other practical uses. Solubility test of synthesized compound was carried out by using various polar and non-polar solvents like water, methanol, ethanol, acetone, hexane, chloroform.
- **Melting Point:** Melting point determination is a crucial step in the characterization of a novel synthesized Schiff base. It serves several purposes:
  - **Purity Assessment**: A sharp and narrow melting point range indicates high purity, whereas a broad or depressed melting point range suggests the presence of impurities.
  - **Identification**: It helps in confirming the identity of the synthesized compound by comparing it with known values in the literature.
  - **Thermal Stability**: It provides insights into the thermal stability of the compound, which is essential for its storage and application.
- **Spectral Analysis:** Infrared spectroscopy (IR) is a powerful analytical technique used to identify functional groups and study the molecular structure of compounds. For a novel synthesized Schiff base, IR spectroscopy provides critical information about the presence of the characteristic imine group (C=N) and other functional groups in the molecule.
- **Determination of purity:** First a TLC plate is prepared by spotting the purified unknown and an authentic sample of each possible compound. Next, the TLC plate is developed. For the co-spotting step, an authentic sample with an Rf value closest to the unknown is selected. TLC co-spotting on a second plate helps with the preliminary identification of the compound. Three spots are placed on the baseline of the TLC plate- the purified unknown, the authentic

sample, and a co-spot of both. If only one row of spots appears on the developed plate, it indicates that the synthesized compound has been purified and is possibly the same compound as the authentic sample.

In Silico Study

- **Ligand preparation:** Using the ChemSketch tool, a chemical structure of synthesized SB's derivative was prepared. The theoretical prepared SB's derivative was then converted into SMILES notation and SDF format.
- **Protein preparation:** The 3D crystal structure of the DHPS (1AJ2) protein was downloaded from the RCSB Protein Data Bank in PDB format and opened in Biovia Discovery Studio Visualizer (V16.1.0.15350). During protein preparation, hetero atoms, water molecules, excess chains, and the pre-existing ligand were removed. The file was then saved in MDL MOL/SD format.
- **ADMET and drug-likeness prediction :** The SwissADME tool was used to screen various pharmacokinetic properties of Schiff base derivatives, including gastrointestinal absorption, P-gp substrates, blood-brain barrier permeability, CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 inhibition, bioavailability, and Log Kp. The results were predicted and presented in a tabular format.
- **Prediction of Toxicity:** The Protox 3.0 tool were used to predict the toxicity of SBs derivatives which including organ toxicities like hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity, immunogenicity etc.
- **Molecular Properties and Bioactivity Scores of the ligands:** The SwissADME tool were used to predict the molecular properties like MlogP (partition coefficient between n-octanol and water), TPSA, H-bond donors, H-bond acceptors, molecular weight, and the number of rotatable bonds, molecular volume was calculated and present in tabular format. Bioactivity score of synthesized ligand including GPCR, Ion channels, Nuclear receptors, Kinase inhibitors, Protease inhibitors and Enzyme inhibitors using Molinspiration software.
- **Molecular Docking Studies:** The One-Click Docking tool were used to performed docking studies. The target protein, DHPS enzyme (1AJ2), was downloaded from the Protein Data Bank and prepared by removing water molecules, excess chains, hetero atoms and the pre-existing ligand. Then prepared protein was uploaded to M-cule Docking and docked with the synthesized derivatives. Binding affinity and types of interactions between the ligand and target were analyzed using Discovery Studio Visualizer (V16.1.0.15350).

RESULT AND DISCUSSION

The novel Schiff base was synthesized using a sustainable chemistry approach, specifically through continuous stirring under solvent-free conditions. This method aims to minimize environmental impact by eliminating the use of harmful solvents and reducing energy consumption.

Table 1: Physical evaluation of schiff base

Compound	Molecular Formula	Molecular Weight	Colour	Odour	% yield
SB	$C_{22}H_{21}NO_3S$	379.47	Yellow	Pleasant	86

Table 2: Solubility test of schiff base

Compound	Methanol	Ethanol	Water	Chloroform	Acetone
SB	Freely Soluble	Slightly Soluble	Soluble	Insoluble	Soluble

- **Melting Point:** The synthesized Schiff base had a melting point of 170°C, suggesting high purity and successful formation of the desired product. It was compared with no literature value available for direct comparison, but similar Schiff bases typically melt around 178-183°C, indicating our result is plausible.
- **Thin Layer Chromatography:** Thin Layer Chromatography (TLC) is a quick way to check if a substance is pure and it require very little sample. If compound is pure, you'll only see one spot developed on the TLC plate.

Fig. 3: TLC plate

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum exhibited characteristic peaks:

- A strong band at 1636 cm^{-1} corresponding to the C=N stretching vibration, confirming the formation of the imine group.
- The absence of a peak around 1700 cm^{-1} indicated the complete consumption of the aldehyde.

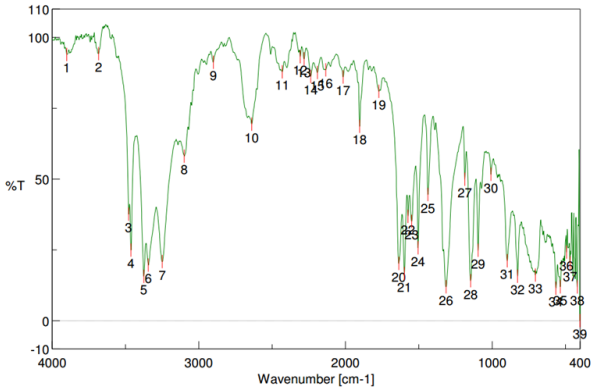

Fig. 4: IR spectra

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3900.32	93.9432	2	3683.37	94.2642
3	3477.99	37.594	4	3462.56	24.9084
5	3374.82	15.6126	6	3343.96	19.6118
7	3249.47	20.861	8	3099.05	58.0731
9	2901.38	91.2102	10	2639.11	69.3538
11	2430.83	87.8011	12	2308.37	93.1828
13	2282.34	92.398	14	2237.02	86.2555
15	2191.7	87.6026	16	2134.81	88.54
17	2016.21	86.0432	18	1903.4	68.5927
19	1772.26	80.9564	20	1636.3	20.191
21	1598.7	16.5854	22	1573.63	36.8336
23	1548.56	35.0762	24	1505.17	25.6955
25	1437.67	44.4994	26	1314.25	11.9439
27	1186.01	49.9252	28	1146.47	14.0991
29	1095.37	24.8487	30	1007.62	51.6928

In Silico Study

1. Screening of the designed derivatives was conducted through ADMET analysis: In Table 3, the synthesized Schiff base derivatives were evaluated based on Lipinski's Rule of Five (RO5), also known as Pfizer's Rule of Five. This rule specifies that for a compound to be orally active, it should meet certain criteria.

- Less than 5 hydrogen-bond donors,
- Less than 10 hydrogen-bond acceptors,
- A molecular mass less than 500,
- Log p less than 5
- Total polar surface area (TPSA)

A compound's TPSA should be less than 140 Å2, and the number of rotatable bonds should be less than 10. In Table 4, bioactivity scores were calculated for Schiff base derivatives as GPCR ligands, ion channel modulators (ICM), kinase inhibitors (KI), nuclear receptor ligands (NRL), protease inhibitors (PI), and enzyme inhibitors (EI). A score greater than 0.00 indicates significant activity, scores between 0.00 and -0.5 suggest moderate activity, while scores below -0.5 indicate inactivity.

2. Molecular docking: From the initial screening through Lipinski rule, ADME calculations, and bioactivity score, were successfully passed all the filters and displayed most drug-likeness nature. In Table 6 SBs derivative selected for docking against dihydropteroate synthase enzyme (1AJ2) had exhibited more potent interactions and binding affinity with the target. Binding affinities, and the types of interaction of the docked molecules are examined and the molecules' 2D & 3D docking postures are represented. More the negative docking score show the higher affinity of ligand towards the target.

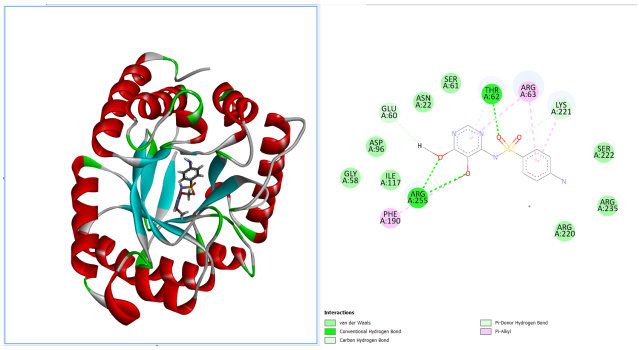

Fig. 5: 3D and 2D docking poses of SB ligand with dihydropteroate synthase enzyme (1AJ2)

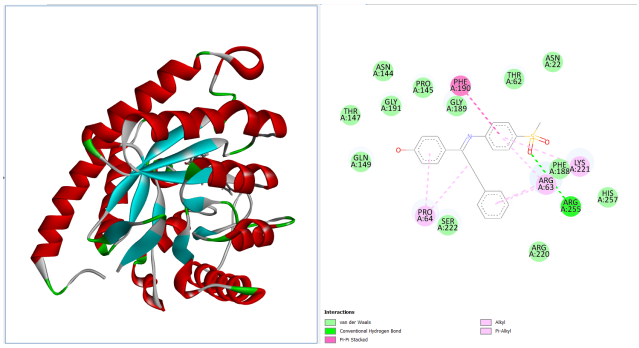

Fig. 6: 3D and 2D docking poses of Sulfadoxine with dihydropteroate synthase enzyme (1AJ2)

3. Prediction of Toxicity: In this study, the toxicity of Schiff base (SB) derivatives was evaluated using various toxicological endpoints, including hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity, and immunogenicity. The toxicity prediction results were presented in a binary format, indicating whether each derivative was classified as

Table 3: Calculation of Lipinski rule of five for the synthesized derivative

Ligand	Molecular weight	TPSA	Molar refractivity	MlogP	Rotatable bonds	H-bond donors	H-bond acceptors
SB1	379.47	75.11	109.36	4.22	6	1	4

Table 4: Bioactivity Score of synthesized derivative

Ligands	GPCR	ICM	KI	NRL	PI	EI
SB	0.12	-0.30	-0.04	0.14	0.04	0.14

Table 5: The pharmacokinetic properties of the synthesized derivative

Codes	GI abs.	BBB perm.	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	Log Kp (cm/s)	Bioavailability
			inhibitor						
SB1	High	No	Yes	Yes	Yes	No	Yes	-5.62	0.55

Table 6: The binding interactions of synthesized derivative with DHPS enzyme

Ligand	Binding affinity (Kcal/mol)	Type of interaction
SB	**-7.9**	Van der Waals, Pi-Alkyl, Alkyl, Conventional Hydrogen Bond, Pi-Pi Stacked
Sulfadoxine	**-7.5**	Van der Waals, Pi-Alkyl, Carbon Hydrogen Bond, Pi-Donor Hydrogen Bond

Table 7: The toxicity profile of the synthesized derivative

Ligand	Hepato-toxicity	Carcino-genicity	Immuno-toxicity	Muta-genicity	Cyto-toxicity
SB	Inactive	Inactive	Inactive	Inactive	Inactive

active or inactive for each endpoint.

DISCUSSION

The synthesis of the novel Schiff base using continuous stirring under solvent-free conditions proved to be an efficient and environmentally friendly method. The physical characterization confirmed the structure and purity of the synthesized Schiff base. The in-silico studies indicated promising biological relevance, showing strong binding affinity to the target protein and favorable ADME properties.

CONCLUSION

The study titled "Synthesis, Physical Characterization, and *In Silico* Study of Novel Schiff Base" successfully presented an environmentally friendly method for synthesizing a new Schiff base. The process was followed by detailed physical characterization and computational analysis, demonstrating both practical and theoretical insights into the compound. The synthesis process employed environmentally benign methods, minimizing the use of hazardous reagents and solvents. This approach not only aligns with sustainable chemistry principles but also reduces the environmental impact, making the synthesis process more appealing for industrial and pharmaceutical applications. Physical Characterization include the determination of the melting point provided insights into the purity and thermal stability of the synthesized Schiff base. The solubility profile in various solvents was essential for understanding the compound's interaction with different media, which is crucial for both its practical use and potential biological applications. IR spectral analysis confirmed the presence of the characteristic imine (C=N) group along with other functional groups, validating the successful synthesis of the Schiff base. Computational modeling and *in silico* analyses provided valuable insights into the Schiff base's molecular geometry, electronic properties, and potential reactivity. These studies complemented the physical characterization, offering a deeper understanding of the compound's behavior at the molecular level and predicting its interactions with biological targets or other chemical entities.

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