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Theoretical docking and antifungal studies of salicylaldehyde derived schiff base

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ABSTRACT

Schiff base was synthesized by the condensation of 4, 4'diamino benzanilide and salicylaldehyde in ethanol using acetic acid as catalyst and characterized by UV, IR and Proton NMR spectral studies. Quantitative structure activity relationship (QSAR) of the Schiff base such as stability, PSA and Clog P values and bond orders of all bonds were calculated by Spartan 14 wave function tool at semi empirical PM3 basis set. Using the same tool HOMO, LUMO energies and gap between them was calculated as well. Docking study of the synthesized Schiff base was done for the following proteins such as SeminaRibonuclease, G.T.paseHRes, Phosphoglycerate Kinase, Glutathione S-transferase and Proto-Oncogene Tyrosine-Protein Kinase through Mcule online tool. Schiff base ligand docking score were also calculated by submitting smiles notation and it showed best docking scores. Preliminary antifungal study of the Schiff base was also done on readymade TLC plate and the study revealed that a concentration of 250 ppm showed minimum inhibition against *Botrytis cinerea*.

Key words: salicylaldehde, DiaminoBenzanilide, QSAR, docking, *B.cinerea* antifungal study

1. INTRODUCTION

Hugo Schiff started the revolution on Schiff bases by the condensation of amine with ketone or aldehyde in the year of 1864. Somany Schiff bases and their applications such as antimicrobial (Mounika, 2010; Venkatesh, 2011), antioxidant (Wei, 2006) and analgesic (Sondhi, 2006) were reported so far. Due to the electron donating tendency of imine functional group, Schiff bases are used in metal complex preparation. This study foot forwarded the synthesis of Schiff base by well-known method and it was characterized by IR and Proton NMR. Molecular formula confirmed by ultimate analysis method approximately. Same Schiff base structure was studied computationally through SPARTAN '14 tool by Semi-Empirical Program PM3 basis set. The same structure docking database calculated through online Mcule tool over five protein data set. This technique mainly combines algorithms like molecular dynamics, Monte Carlo Stimulation, fragment based search methods. These studies are used to define the interaction of two molecules and to find the best orientation of ligand which would form a complex with overall minimum energy. A molecule which is called ligand locked with protein cavity. These protein cavities become dynamic when come in contact with any external compounds and are thus called as energetic sites. Various poses showed different docking scores, from that best score was recorded. The prepared Schiff base preliminary antifungal character against *Botrytis cinerea (B.Cinera)* was studied on readymade silica gelplate.

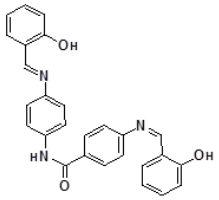

Figure.1.structure of (4Z)-4-(2-hydroxybenzylideneamino)-N-((E)-4-(2-hydroxybenzylideneamino) phenyl)benzamide

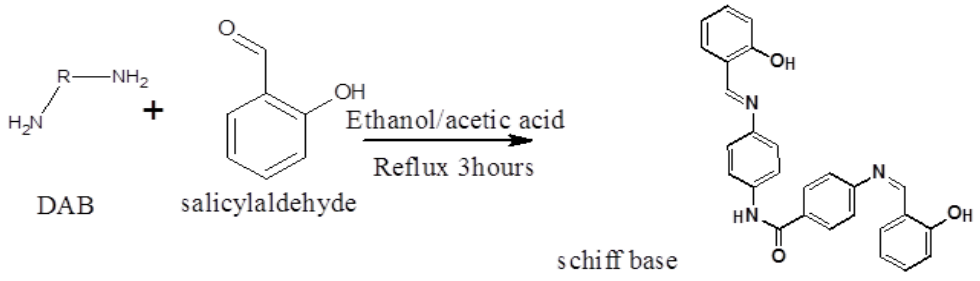

Figure.2.Synthesis of Schiff base

2. EXPERIMENTAL

Reagents And Conditions: Ethanol, Glacial Acetic Acid reflux.

Salicyldehyde purchased from Sigma Aldrich-USA. All the solvents purchased from SRL chemicals and used without further purification. Reaction monitoring and impurity profile was carried on Merck silica gel thin layer chromatography plate. Melting point was uncorrected and measured by open capillary method. IR spectra were obtained by Jasco FTIR spectrometer using ATR method.^{1}H NMR spectra was taken on a Bruker NMR400 spectrometer using DMSO as a solvent at a frequency of 500 MHz with TMS as an internal standard. Spartan 14 trial versions installed in dell CPU with 2GB ram500 GB hard disk and program run for4 min 31seconds. Docking study carried through Mcule online tool and poses documented through Molsoft ICM browser.*B. Cinera*collected from Jerusalem College of engineering, Bio Medical Engineering Department.The synthesizedcompoundwasanalyzed for C, H, N and results were found to be within the range of ± 0.4% of the theoretical value.

Synthesis of 4-(2-hydroxybenzylideneamino)-*N*-(2-hydroxybenzylideneamino)henyl)benzamide: 4, 4'-diaminobenzanilide prepared as per the given procedure [Georgeta Maria etal, 2004]. Synthesized diamine recrystallized in methanol (MP-204^{0}C) and dried under vacuum. 2 mm of salicylaldehyde dissolved in 15ml of ethanol and 1 drop of acetic acid added to it. The reaction mixture stirred for 10minutes. 1 mm (0.227g) of synthesized amine dissolved in 15ml of ethyl alcohol and the solution was added drop by drop over a period of 20 minutes to the reaction mixture. After ten minutes stirring at room temperature the reaction mass refluxed for three hours. Reaction completion monitored by 60:40 ethyl acetate, hexane mixture. Un-reacted diamine exists at center of the TLC. Up to the disappearance of the amine, the reaction was continued under reflux. Dark yellow solid filtered at 45^{0}C and washed by hot methanol. The product melting point was checked by open capillary method. (M.P - >250^{0}C).yield: 65%.

3. RESULT AND DISCUSSION

Spectral studies: Ultraviolet maximum absorption λ_{max} showed at 243nm which is the characteristic peak of azomethine group. IR spectrum of the synthesized molecule showed the characteristic frequency at 3235cm^{-1}, 1640^{-1}, 1617^{-1}, for –OH, azomethine and amide peak respectively. Schiff base imine functional group confirmed by UV absorption and IRfrequency. Theoretical IR frequencies and experimental IR frequencies were compared. Almost thesedata's were equal in frequency and showed in Table1.Purified molecule carried for Proton NMR in DMSO solvent at 500 MHz and the15.41ppm,10.59 ppm, 8.31 ppm, 8.07 ppm and 7.98ppm chemical shifts showed for two hydroxyl group ,amide proton and two –CH=N- respectively. yellow solid ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.41 (s, 1H), 10.59 (s, 1H), 8.31 (s, 1H), 8.07 (s, 1H), 7.98 (s, 1H), 7.91 (d, *J* = 7.3 Hz, 2H), 7.83 (d, *J* = 7.4 Hz, 2H), 7.74 (d, *J* = 7.4 Hz, 2H), 7.69 (d, *J* = 7.3 Hz, 2H), 7.45 (m, *J* = 16.3, 7.3, 4.0 Hz, 3H), 7.32 (d, *J* = 7.0 Hz, 1H), 7.27 – 7.20 (m, 2H), 7.18 – 7.02 (m, 2H). Elemental analysis: theoretical: C (74.47%) H (4.86%) N (9.65%) O (11.02%) found: C (74.60%) H (4.56%) N (9.45%) O (11.39%). These spectral and analysis studies confirmed the molecular formula.$C_{27}H_{21}N_3O_3$.

Table.1.Comparison of theoretical and experimental IR frequencies

Theoretical IR frequencies (cm^{-1})By SPARTAN 14 TOOL	Experimental IR frequencies (cm^{-1})
670 –OH,747-Aromatic hydrogen, 1306-Aromatic H,1347,1473-OH 1584-Aromatic symmetric stretching, 1629-Aromatic,1674-NH,1780-Aromatic 1862-C=N,1904 CONH,2954 N=CH 3139-Aromatic,3232-CO-NH 3606-OH	3235 ,3050 ,1640 ,1617 ,1596 ,1568 ,1526 , 1490 ,1454,1407,1279,1186,1149,, 825, 740

Theoretical Study by Spartan 14: Spartan 14 (Kevin, 1999; Andrea Colombo, 2008) wave function tool used to study the structure QSAR properties. Structure was drawn through the tool and program run for 45 minutes. HOMO, LUMO energy, bond order between the bond, and gap was calculated AT Semi empirical PM3 basis set. Bond orders were calculate for various bonds and showed in Table.2 and the bond numbers showed in Fig.1. Similarly theoretical HOMO, LUMOenergy, LUMO map and electrostatic potential map structure were drawn.All this three models showed in Fig.2.Same tool provided various QSAR properties. Apart from that polar surface area (PSA) is an important parameter which is less than 90 A^2 and showed good for the absorption. Various properties showed in Table.3.

Table.2. Bond orders calculated by Spartan 14 Tool

Bond	Bond order	S.N	Bond	Bond order	S.N	Bond	Bond order	S.N	Bond	Bond order
C1 C2	0.108	21	N1 C8	1.049	41	N2 C14	1.908	61	C20 H14	0.96
C1 C3	1.432	22	N1 C10	0.037	42	N3 C15	1.912	62	C21 H15	0.965
C1 C4	1.396	23	N1 C11	0.035	43	C14 C16	0.982	63	C22 C23	0.109
C1 H1	0.96	24	N1 O1	0.15	44	C14 H10	0.946	64	C22 C24	1.343
C2 C5	1.441	25	N1 H5	0.949	45	C15 C22	0.98	65	C22 C25	1.392
C2 C6	1.368	26	C8 C9	0.099	46	C15 H11	0.946	66	C22 O3	0.049
C2 H2	0.963	27	C8 C10	1.359	47	C16 C17	0.108	67	C23 C26	1.408
C3 C5	0.11	28	C8 C11	1.372	48	C16 C18	1.39	68	C23 C27	1.436
C3 C6	1.375	29	C9 C12	1.376	49	C16 C19	1.343	69	C23 H16	0.966
C3 C14	0.025	30	C9 C13	1.369	50	C16 O2	0.049	70	C24 C26	0.102
C3 H3	0.961	31	C9 N3	1.034	51	C17 C20	1.436	71	C24 C27	1.367
C4 C5	1.388	32	C10 C12	0.109	52	C17 C21	1.408	72	C24 O3	1.071
C4 C6	0.107	33	C10 C13	1.444	53	C17 H12	0.966	73	C25 C26	1.435
C4 C7	0.936	34	C10 H6	0.961	54	C18 C20	0.112	74	C25 C27	0.112
C4 N1	0.03	35	C11 C12	1.433	55	C18 C21	1.436	75	C25 H17	0.964
C4 O1	0.041	36	C11 C13	0.107	56	C18 H13	0.964	76	C26 H18	0.966
C5 H4	0.962	37	C11 H7	0.951	57	C19 C20	1.367	77	C27 O3	0.038
C6 N2	1.04	38	C12 C15	0.025	58	C19 C21	0.102	78	C27 H19	0.961
C7 N1	1.12	39	C12 H8	0.962	59	C19 O2	1.071	79	O2 H20	0.928
C7 O1	1.762	40	C13 H9	0.963	60	C20 O2	0.038	80	O3 H21	0.927

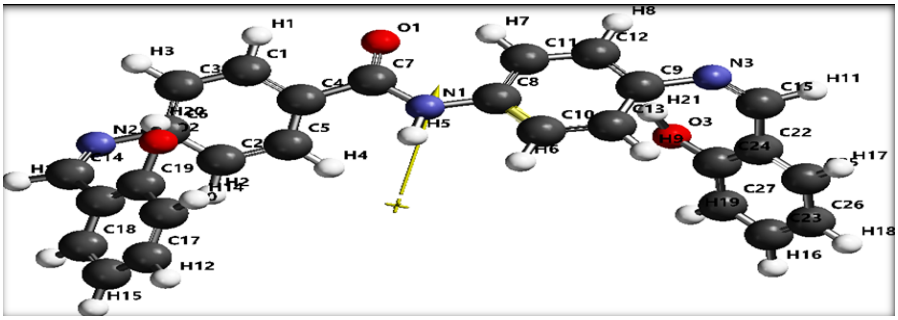

Figure.3.Bonds with Number

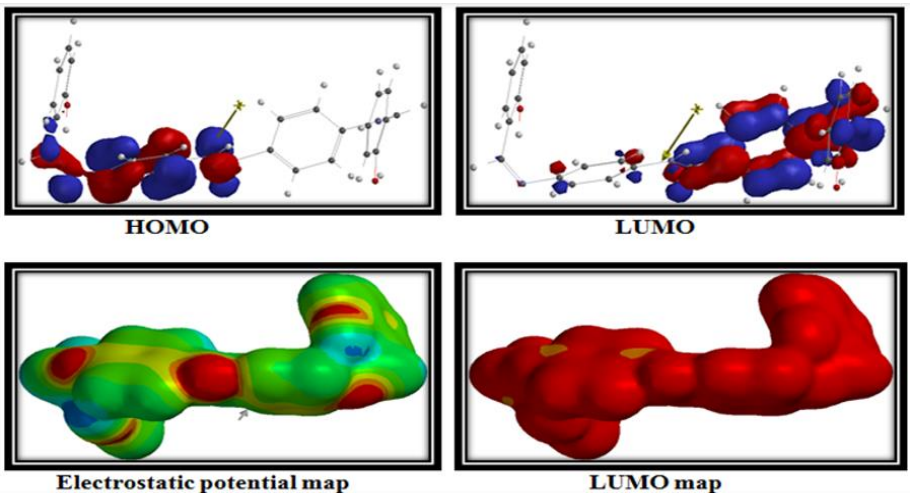

Figure.4.3D Models For HOMO, LUMO, Electrostatic, LUMO Map

Table.3.Spartan 14 wave function calculated properties

Properties	Values	S.N	Properties	Values
Energy	123.82 kj/mol	16	HBD	3
Energy (aq)	41.49 kj/mol	17	ZPE	1106.32kj/mol
Solvation	-82.33 kj/mol	18	H^0	0.492073224au
HOMO	-8.64ev	19	Cv	339.42J/mol^0
Dipole moment	5.78 debye	20	S^0	633.99 J/mol^0
Tautomers	9	21	G^o	0.420077478au
LUMO	-0.80ev	22	Temperature	298.15 k
Point group	C_1	23	PSA	68.686 A^2
Conformers	128	24	Ovality	1.62
Area	458.11A2	25	Area	73.20A2
Volume	447.81A3	26	Maximum electro potential	127.11 kj/mol
Accessible area based on electron density	350.40A2	27	polarizability	75.85
Minimum value of the electrostatic potential	-289.02 kj/mol	28	HBA count	6
Minimum value of the local ionization potential	45.54kj/mol	29	η = LUMO –HOMO gap	7.84ev
Log p	-1.47			

Docking Study: Molecular docking (Anitha, 2013, PawanKaushik, 2014) study carried for the synthesized structure and its binding tendency in terms of docking score was studied by using online docking tool. Various proteins 3D structures obtained from NCBI and confirmed. Semina Ribonuclease, G.T.pase H Res, Phosphoglycerate Kinase, Glutathione S-transferase and Proto-Oncogene Tyrosine-Protein Kinase structures docked with Schiff base molecule. Then docking scores were recorded and tabulated. Various docking scores of the proteins with various poses were recorded and best score mentioned in Table.4. Best docking images of the proteins showed in Figure.3.

Table.4.Docking scores with various proteins and various poses

Name of the protein	Pose1	Pose2	Pose3	Pose4	Best docking score
Semina Ribonuclease	-7.6	-7.6	-7.5	-7.3	-7.6
G.T.paseHRes	-8.3	8.0	-7.9	-9.4	-9.4
Phosphoglycerate Kinase	-9.7	-8.8	-8.3	-8.1	-9.7
Glutathione S-transferase	-9.9	-9.3	-8.6	-8.4	-9.9
Proto-Oncogene Tyrosine-Protein Kinase	-7.7	-7.3	-7.0	-6.7	-7.7

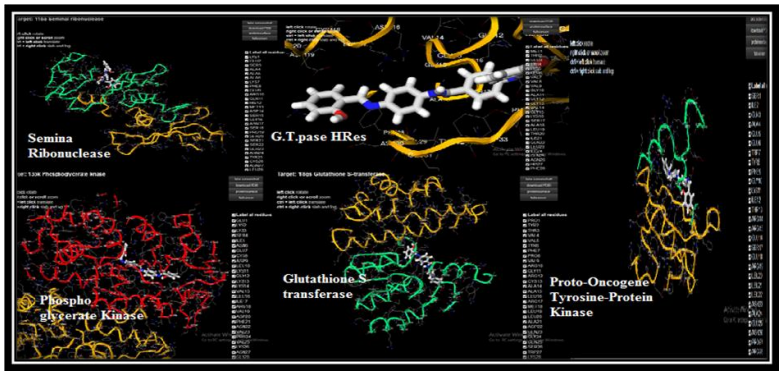

Figure.5.Ligand-Protein Docking Images

Antifungal activity: Synthesized molecule was carried for antifungal activity (HarisonMasih, 2014) and it was serially diluted such as 50µg/ml, 100µg/ml, 250µg/ml, 500µg/ml, 750µg/ml, 1000µg/ml by using DMSO and water mixture. The TLC plate cut into 6x1 cm dimension and it was divided in to 1x1 square cm equal dimensions by pencil. The center of the each square was spotted by 1ml of each solution by using capillary. Then the *Botrytis cinerea* fungus sprayed on the TLC plate inside the incubator under controlled condition and incubator cleaned by acetone and water. After the completion, TLC plate kept in tray under calcium chloride atmosphere and closed by

glass plate. Every day growth was monitored and observed. After a weak plate showed the antifungal activity above 100µg/ml and showed good result at 250µg/ml.

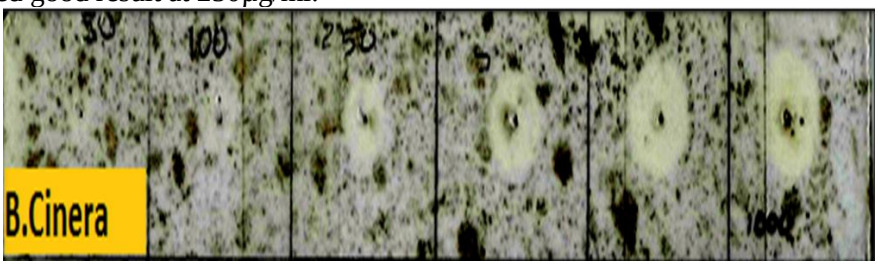

Figure.4.concentrations of Schiff base 25, 50, 100, 250, 500, 750, 1000 ppm against B. Cinera

4. CONCLUSION

This research successfully synthesized and confirmed the salicylaldehyde derived Schiff base. Various useful computational studies carried for the structure and activity against fungi *B. Cinera* and observed that 250ppm showed good result against the fungus. Based on the theoretical data experimental study will be carried in future.

5. ACKNOWLEDGEMENT

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