International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.06 pp 48-54, 2016

QSAR, Brine Shrimp Lethal Assay and antimicrobial studies on Synthesized L-Tryptophan-2,4-dihydroxy benzaldehyde Schiff Base

R.Jayaprakash[1], Saroj Kumar Sha[2], S.Hemalatha[2], D.Easwaramoorthy[1]*

[1]Department of Chemistry, B.S.Abdur Rahman University, Vandalur, Chennai-600048, India
[2]School of Life Sciences, B.S.Abdur Rahman University, Vandalur, Chennai-600048, India

Abstract : Schiff base was synthesized by the condensation reaction between L-tryptophan and 2,4-dihydroxy Benzaldehyde in double distilled water. Synthesized Schiff base was characterized by UV, fluorescence FT-IR, mass, ^{1}H-NMR spectral studies and FT-IR, ^{1}H-NMR data were compared with the theoretical Spartan 14 wave function tool values. QSAR properties of the molecule were predicted by MOLSOFT and OSIRIS online tools by submitting smiles notation. Docking affinity of the Schiff base with *E. coli* proteins was studied by online auto dock vina software through mcule server. From the outcomes of the tools, Schiff base was carried for toxicity study by brine shrimp lethal assay test with various concentrations from 25 µg/mL to 250 µg/ mL and antibacterial character against *E. coli*. From the lethal assay test L-tryptophan Schiff base lethal concentration for 50% morality (LC_{50}) value was calculated by regression method and it was found to be 130.32 µg/ mL. Minimum inhibitory concentration (MIC) against *E. coli* was found to be 20µl of the Schiff base, which is lower than the toxicity limit.

Keywords: L-tryptophan, 2, 4-dihydroxy benzaldehyde, Schiff base, QSAR, Docking, Toxicity, Antimicrobial.

Introduction

Amino acid Schiff bases have received large attention due to their biological importance[1,2,3] in medicinal applications. Amino acid Schiff base systems which are the main intermediates in many metabolic reactions. Organometallic complexes derived from amino acid Schiff bases are considered to constitute new kinds of potential antibacterial and anticancer reagents[4,5,6]. L-enantiomers of amino acids are more attention due to the DNA binding, anti-tumor, and various drug activities. Same molecule crystal structure and its anti-HIV character were reported[7] but the QSAR, docking and antibacterial characters have not been reported in detail so far. Present studies are focused on the preparation of L-tryptophan Schiff base with 2, 4-dihydroxy benzaldehyde in water and its spectral characterization, preliminary QSAR studies such as hydrogen bond donor, hydrogen bond acceptor, log p, polar surface area[8] and so on by reported java based software tools such as OSIRIS and MOLSOFT. Similarly, the molecule docking study will be carried for the *E. coli* proteins such as UDP-N-acetyl glucosamine 1-carboxy vinyl transferase, Purine nucleoside phosphorylase, ATP-dependent dethiobiotin synthetase BioD 1, GTP cyclohydrolase 1, Xanthine phosphoribosyltransferase, UDP-glucose 4-epimerase, Formate dehydrogenase-H by online auto dock vina software. From the basic computational studies, the research extended to toxicity study by brine shrimp lethal assay test[9,10,11] and antimicrobial charm on gram-

negative bacteria *E. coli* will be carried. The in-vivo lethality test carried in a simple organism Brine Shrimp and it is used as a simple tool to guide screening and fractionation of physiologically active synthetic and natural products and lethal concentration for 50% mortality (LC_{50}) will be calculated. This bioassay detects a broad range of biological activities and a diversity of chemical structures. Similarly, the compound carried for antibacterial study against *E. coli* pathogens which is causing urinary tract infections on human beings. The outcomes of computational values will be justified with experimental values.

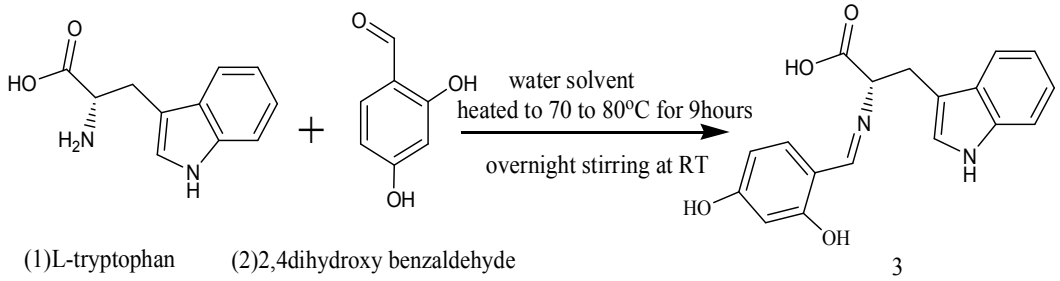
Experimental

Materials and Methods

Double distilled water prepared at lab and methanol obtained from Merck. 2,4-dihydroxy Benzaldehyde , L-tryptophan, and DMSO were purchased from Sigma-Aldrich – USA(Bio corporals India). Brine shrimp egg purchased at aqua marine, Guindy, Chennai, Tamilnadu, India. LYNX-Lawrence& Mayo microscope and progress capture pro 2.8.8 software used for the egg cell damage image capture. Reaction monitoring and impurity profile were carried on Merck silica gel thin layer chromatography plate using CAMAG elution solvent system such as t-butanol: water: acetic acid (7.5:2:0.5).Melting points were uncorrected and were measured by the open capillary method using Sunsim electric melting point apparatus. IR spectra were obtained by Jasco FTIR spectrometer. ^{1}H NMR spectra were taken on a Bruker NMR400 spectrometer using DMSO as a solvent at a frequency of 500 MHz TMS as the internal standard. Mass calculated by Shimadzu. QSAR properties calculated by online tools Osiris and Molsoft. IR, UV, and 1H-NMR values were calculated by Spartan 14 wave. Theoretical UV and ^{1}H- NMR values were calculated by Spartan 14 wave function tool. Docking study carried by using auto dock vina online server. Bacterial strains were purchased from the American type culture collection, *E. coli* ATCC 25922.

Procedure for the synthesis of (2S)-2-[(2, 4-dihydroxybenzylidene) amino]-3-(1H-indol-3-yl) propanoic acid

1 mmol (0.204 g) of L-tryptophan dissolved in 10 mL of water at 50^{o}C and stirred for 10 min. Then 1.1 mmol (0.151 g) of 2, 4-dihydroxy benzaldehyde added four portions in 10 min equal interval. The reaction mass stirred for 30 min at room temperature then gradually heated to 70-80^{o}C. The temperature maintained for 9 h under silver foil closed condition. Then the reaction mass allowed to reach room temperature and stirred for 12 h. Reaction monitored by TLC using t-butanol: water: acetic acid (7.5:2:0.5) system. Starting materials slightly presence in the TLC. The light brown solid filtered and washed by 60^{o}C hot water. The crude product treated with 25 mL of 1:1 methanol, water solvent. The mixture refluxed for one hour and allowed to cool. Solid filtered and washed with hot methanol.



(1)L-tryptophan (2)2,4dihydroxy benzaldehyde 3

Scheme1. Preparaion of (2S)-2-[(2,4-dihydroxybenzylidene)amino]-3-(1H-indol-3-yl) propanoic acid

Characterization of (2S)-2-[(2,4-dihydroxybenzylidene)amino]-3-(1H-indol-3-yl) propanoic acid

Yield-60% (0.192g). M.P-220^{o}C. the light brown colour product was freely soluble in DMSO. Anal.Calc. UV-absorption λ max at 243 nm (π→ π* high) and 333 nm (n→π* low). Fluorescence spectrum λ max appeared at 436.65nm (high), 761.29nm (low). IR (KBr pellet , cm^{-1}) 3650 (Indole –NH-), 3472 (Indole aromatic-4H), 3303 (-CH2 stretching), 3262 (-CH2 stretching), 3002 (-CH-), 2486, 1762 (-COOH),1625 (-CH=N-) , 1451 (aromatic), 1379, 1308, 1236, 1145, 1104, 997, 818, 773, 640, 594,538,492.1H-NMR (500.03 MHz, DMSO-d6) δ 17.44 (s, 1H, -COOH), 11.51 (s, 1H,-NH), 9.45 (s, 1H,-OH), 8.11 (d, J = 7.2 Hz, 1H,-CH=N-), 7.84 (s, 1H,-OH), 7.75 (d, J = 7.2 Hz, 1H), 7.56 (t, J = 7.2 Hz, 1H), 7.46 (t, J = 7.2 Hz, 1H), 6.88 (d, J

= 7.3 Hz, 1H), 6.83 (s, 1H), 6.77 – 6.73 (m, 2H,-CH2-), 6.64 (s, 1H), 4.81 (d, J = 10.9 Hz, 1H,-CH-), 3.04 (t, J = 11.7 Hz, 1H), 2.97 (d, J = 11.8 Hz, 1H). Mass m/e [M+] = 324.95.

Spartan 14 wave function tool

Spartan 14 wave function tool[12,13] used to study about the various theoretical parameters of the derived Schiff base. A restricted hybrid HF-DFT SCF calculation will be performed using Pulay DIIS and Geometric Direct Minimization method. The Exchange calculated from 0.2000 Hartree-Fock, 0.0800 Slater and 0.7200 Becke using SG-1 standard quadrature grid. The same tool used to calculate the theoretical UV absorption, IR frequencies and Proton NMR chemical shift values at Job type: Single point. Method: RHF and 3-21G (*) basis set. Predicted data are compared with experimental data and, shown in **Table.1**.

Table.1. Theoretical and experimental IR, ^{1}H-NMR values

Spectra	Theoretical	Experimental
IR (cm^{-1})	3926(-OH),3868(-COOH), 3381(aromatic), 3243(-CH), 1954(-CO), 1868, 1787, 1623 (-CH=N-), 1561, 1468, 1441, 1275, 1208, 1122, 1050, 881,766,709,615	3650(Indole–NH-),3472(Indole Ar-4H) , 3303 (-CH_2 stretching) , 3262 (-CH_2 stretching), 3002 (-CH-) 2486, 1762 (-COOH),1625(-CH=N-), 1451 (aromatic), 1379, 1308, 1236, 1145, 1104, 997, 818, 773, 640, 594, 538, 492.
^{1}H-NMR Chemical Shift(ppm)	8.92,8.46,7.36,7.22,7.18,6.95,6.55,4.83,3.62, 2.59	17.44, 11.51, 9.45, 8.11 , 7.84 , 7.75, 7.56, 7.46, 6.88, 6.83, 6.77, 6.73, 6.64 , 4.81, 3.04, 2.97

QSAR Properties prediction (online tools)

Osiris[14,15,16] and molsoft[14,15,16] tools are freely available online and reported in the recent publication of the drug design combination of various pharmacophore sites by using Spiro-heterocyclic structure. Compound's drug-likeness partially based on topological descriptors, fingerprints of MDL structure keys or other properties as clogp and molecular weights. Apart from the various data, useful data for this research were taken and presented in **Table.2**.

Table.2. QSAR properties of starting material and Schiff base

Properties	Tool	Tryptophan	2,4-dihydroxy Benzaldehyde	Schiff base
MlogP	MOLSOFT	-0.09	1.01	2.75
HBDH		4	2	4
T_PSA A^2		59.31	48.56	81.76
HBA		3	3	5
Drug - likeness		-0.64	-0.98	-0.36
ClogP	OSIRIS	-1.51	0.9	1.77
Drug score		0.48	0.3	0.74
T_PSA A^2		79.11	57.53	105.9
Drug likeness		-8.93	-3.81	0.91

Docking Study

Schiff base affinity tendency with *E. coli* proteins such as UDP-N-acetylglucosamine 1-carboxyvinyltransferase, Purine nucleoside phosphorylase, ATP-dependent dethiobiotin synthetase BioD, GTP cyclohydrolase 1, Xanthine phosphoribosyltransferase, UDP-glucose 4-epimerase and Formate dehydrogenase - H respectively. Schiff base java based structure was converted to word based smiles notation (O=C(O)[C@@H] (\N=C/c1ccc(O)cc1O)Cc3cnc 2ccccc23) and used for docking study using auto dock vina software through mcule 1-click docking server. This server provides highest and lowest affinity in the negative score and its poses. Output score differed in each docking. Therefore, the three to four trials were carried and the highest docking score was noted. From these values, highest affinity negative score is tabulated for an each

protein and shown in **Table.3**. Apart from that, few images are shown in **Fig.1** and used for the antimicrobial study.

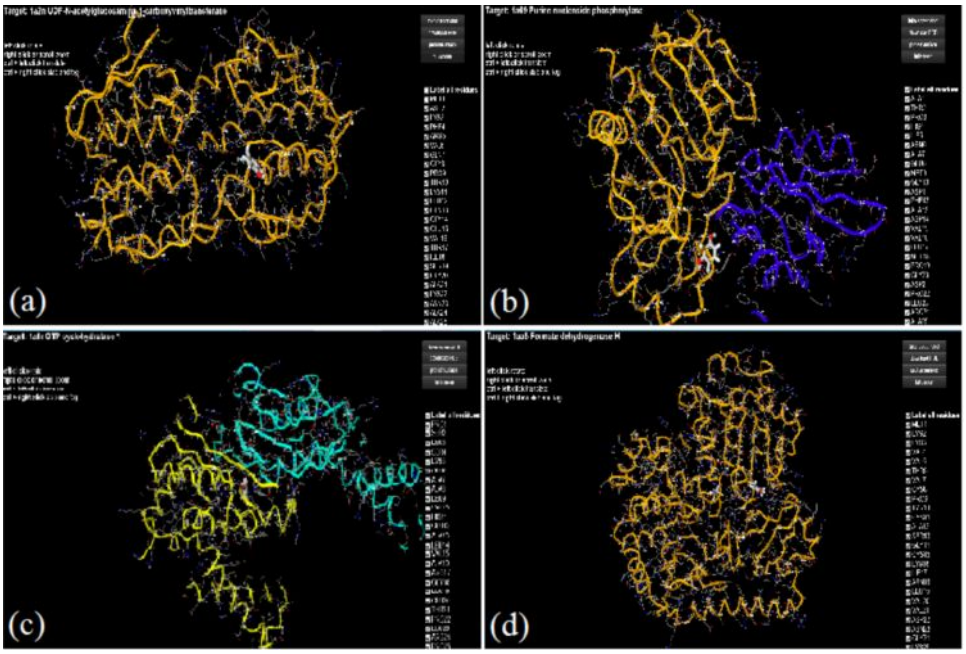


Fig .1. (a)Dock image for a (b) Dock image for b (c) Dock image for d (d) Dock image for g

Table.3. Docking score against *E. coli* protein

S.N	Target *E. coli* protein name	Score1	Score2	Score3	Score4	Best score
1	UDP-N-acetylglucosamine 1-carboxy vinyl transferase (a)	-8.7	-8.2	-8.0	-7.9	-8.7
2	Purine nucleoside phosphorylase (b)	-7.5	-7.1	-6.3	-6.1	-7.5
3	ATP-dependent dethiobiotin synthetase BioD 1 (c)	-7.3	-7.2	-6.8	-6.4	-7.3
4	GTP cyclohydrolase 1(d)	-8.2	-7.5	-7.3	-7.1	-8.2
5	Xanthine phosphoribosyltransferase (e)	-7.4	-7.1	-6.8	-6.7	-7.4
6	UDP-glucose 4-epimerase (f)	-8.7	-8.5	-8.3	-8.0	-8.7
7	Formate dehydrogenase-H (g)	-9.9	-9.5	-8.7	-8.6	-9.9

Brine Shrimp Lethal Assay

In one liter beaker, 19g of rock salt dissolved in 500 mL of water. 0.5 g of Artemia cyst egg was added and kept under 60 watts lamp for 36 hours[17,18]. After 36 hours, Artemia cyst larvae ready for toxicity study. 35 mg of Synthesized Schiff base dissolved in 5 mL of DMSO solvent and made to 100 mL by using artificial sea water and carried for Brine shrimp lethal test. This stock solution serially diluted to 25 µg/mL, 50 µg/mL, 75 µg/mL, 100 µg/mL µg/mL, 125 µg/mL, 150 µg/mL, 200 µg/mL, 250 µg/mL, 300 µg/mL and 350 µg/mL. 10 mL of each solution filled in vials and ten nauplii of brine shrimp were added to each vial. Test vials were kept in the incubator at 27^{o}C in the presence of 40 watts tungsten lamp. After 24 hours, numbers of dead shrimp were calculated and the same trials were triplicated and shown in **Table.4**. Similarly, vincristine sulphate (positive control) and DMSO used as negative control. Positive control showed LC_{50}= 0.754 µg/mL and DMSO showed higher LC_{50} against Artemia cyst. LC_{50} of synthesized Schiff base was calculated by regression graph method.

Table 4. Brine Shrimp Assay Test Trials for Schiff base

Concentration µg/ mL	Brine Shrimps Out Of 10 nos			Total Number Of Live	Total Number Of Dead	% Of Mortality	LC_{50} µg /mL
	Trial1	Trial2	Trial3				
25	9	9	9	27	3	10	130.32
50	8	9	8	25	5	16.6	
75	8	8	8	24	6	20	
100	8	7	8	23	7	23.3	
125	6	7	7	20	10	33.3	
150	5	6	5	16	14	46.66	
200	4	3	3	10	20	66.66	
250	2	3	2	7	23	76.66	
300	1	1	1	3	27	90	
350	0	0	0	0	30	100	
DMSO 5 mL and 95 mL artificial salt water (-ve)	10	10	10	30	0	0(-ve)	

Agar well diffusion antibacterial activity

Antimicrobial sensitivity tests were performed on Mueller-Hinton agar (Hi -media -Mumbai) by Kirby-Bauer agar diffusion method and interpreted according to CLSI (Clinical and Laboratory Standards Institute) standard tables. Antimicrobial activity of B3 compound was performed against Gram- negative (*E. coli* ATCC 25922) bacteria. In brief, the pure cultures of organisms were the subculture in Nutrient broth at 37° C ±2 rotary shaker at 150 rpm. For bacterial growth, a lawn of culture was prepared by spreading the 0.1 mL fresh culture having 106 colony-forming units (CFU)/ mL of each test organism on Mueller-Hinton agar (MHA) plates with the help of a sterile L-rod spreader. Plates were left standing for 5 min to let the culture get absorbed. Then 6 mm wells were punched into the MHA plates for testing B3 compound, antimicrobial activity. Wells were conserved with one drop of molten agar (0.8% agar) to stop the outflow of the compound from the bottom of the wells. The Chemical compounds B3 approx. 5mg/ mL was dissolved in 50% DMSO and vortex 30 s. Using a micropipette, 10µl-50µl of the sample of Schiff base (B3) compound was loaded onto each of five wells on a plate and solvent 50% DMSO was used as a negative control. After overnight incubation at 37°C, the zone of inhibition was measured using zone measuring scale (Hi-media Mumbai India).

Antibacterial activity

Schiff base (B3) was tested for their in-vitro antibacterial activity against gram-negative bacteria *E. coli*[19] ATCC 25922 10µl-50µl concentration (Fig.2). The in-vitro antimicrobial activity of the tested compounds was assessed by minimum inhibitory concentration (MIC) using the broth dilution method (National Committee for Clinical Laboratory Standards, 1982). Amoxicillin was used as a control. The results of antibacterial screening (**Table 5**) make known that 50 µl concentration high bactericidal effect compares than decreasing concentration and concentration against the zone of inhibition graph shown in Graph.1. Normally antibacterial outcome of an antibacterial agent will be superior against Gram-negative bacteria than Gram-positive bacteria due to the occurrence of multi-layered thick peptidoglycan layer in the cell wall of gram-negative bacteria *E. coli* at the minimum inhibitory concentration (MIC) of 20 µl in comparison to Amoxicillin, which was taken as a standard drug.

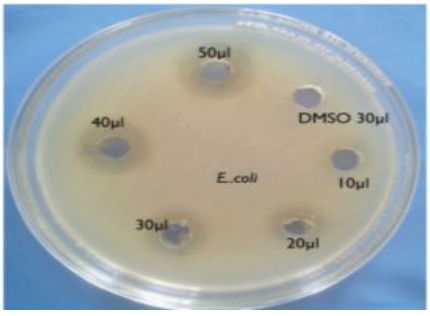
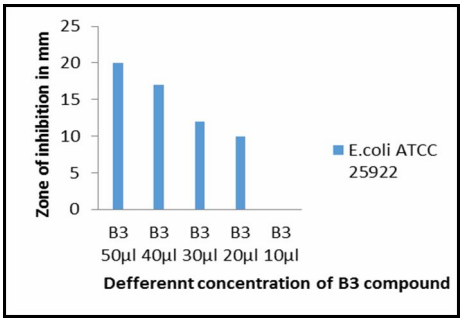


Fig.2.Antibacterial test on *E.coli*



Graph1.conc Vs zone of inhibition

Table.5. Concentration and zone of inhibition of Schiff base against *E. coli*

Antimicrobial testing against *E. coli* ATCC 25922					
Compound Schiff Base (B3)	50µl	40 µl	30 µl	20 µl	10 µl
Zone of inhibition in mm	20	17	12	10	0

Result and Discussion

This research work was successfully synthesized the chiral Schiff base as per the **Scheme. 1** from L-tryptophan and 2,4-dihydroxy benzaldehyde in eco-friendly water solvent. The product characterized by UV, fluorescence, FT-IR and ^{1}H-NMR spectral studies. Product showed UV λ max characteristic absorption for azomethine functional group at 243 nm and fluorescence showed the maximum intensity at 436 nm. From this quantum yield Ø (abs/emis)= 0.56 was calculated and it exists below 1 and confirmed the azomethine group $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ excitations. IR spectrum showed characteristic frequency at 1625 cm^{-1} for azomethine group, broad peak at 2500-2600 cm^{-1} for hydroxyl and 1762 cm^{-1} for carboxyl group. ^{1}H-NMR spectrum was confirmed sixteen protons of Schiff base and the chemical shifts 8.11 ppm for imine proton -CH=N- and chiral center proton at 4.81 ppm. Further, the molecule was confirmed by mass spectrum and the molecular ion peak was found [M+] at 324.95. Experimental FT-IR, ^{1}H-NMR values were compared with theoretical Spartan 14 wave function tool predicted values which were obtained by the energy at ground state with Hartree-Fock 3-21G in a vacuum , B3LYP basis set. The theoretically calculated analytical data were compared with experimental data (**Table 1)** and the experimental data almost coincidence with the theoretical data.

Spectrally confirmed Schiff base was used for virtual screening and it was successfully calculated using smiles notation. From the screening, useful drug-likeness, drug score, total polar surface area (TPSA), the number of hydrogen bond donor (HBD), and the acceptor (HBA) were calculated based on the Lipinski five rules. Virtual screening tools Osiris and molsoft were showed drug score, drug-likeness were 0.74,-0.36 respectively. The Schiff base has higher drug-likeness and drug score than the starting materials. Calculated polar surface area (PSA) of the molecule exist in between 80$^oA^2$ and 90 $^oA^2$, this PSA less than 90 $^oA^2$ is usually needed for molecules to penetrate the blood–brain barrier and thus, act on receptors in the central nervous system. Similarly the docking affinity against *E. coli* was measured by autodock vina tool through mcule server and highest affinity was showed against various proteins UDP-N-acetylglucosamine 1-carboxyvinyltransferase = -8.7, Purine nucleoside phosphorylase = -7.5, ATP-dependent dethiobiotin synthetase BioD 1 = -7.3, GTP cyclohydrolase 1 = -8.2, Xanthine phosphoribosyltransferase = -7.4, UDP-glucose 4-epimerase = -8.7 and Formate dehydrogenase -H = -9.9 respectively.

From the usefulness of the software data, isolated product carried for brine shrimp lethal assay test (BSLA) and LC_{50} = 130.32 µg/mL was calculated by regression graph method. Based on the toxicity limit Schiff base (B3) was tested for its in-vitro antibacterial activity against gram-negative bacteria *E. coli* using 10 µL to 50 µL concentrations (Fig.2). The in-vitro antimicrobial activity of the tested compound was assessed by minimum inhibitory concentration (MIC) using the broth dilution method (National Committee for Clinical Laboratory Standards, 1982). Amoxicillin was used as a control. The results of antibacterial screening (Table 5) make known that 50 µl concentration high bactericidal effect compares than decreasing concentration. Normally antibacterial outcome of the antibacterial agent will be superior against gram-negative bacteria than gram-positive bacteria due to the occurrence of multi-layered thick peptidoglycan layer in the cell wall of gram-negative bacteria. The Schiff base showed the minimum inhibitory concentration (MIC) of 20 µL against *E. coli* in comparison to amoxicillin, which was taken as a standard drug. These experimental data showed the low toxic higher drug character of our molecule and justified the virtual screening, QSAR data. Further, the research reveals that our molecule fulfilled the Lipinski five rules which are useful for further medicinal study using our molecule and also the result, showed the antibacterial character below the toxic limit and justified the binding affinity of docking study against the *E. coli* proteins.

Conclusion

In this research summary, Schiff base was successfully synthesized by the eco-friendly method with good purity. We have used simple mcule tool for docking study for the basic drug activity. This research further revealed that 2, 4-dihydroxy benzaldehyde condensed Schiff base showed good drug activity due to hydrogen bonding character of two substituted hydroxyl groups and more stable.

Acknowledgments

R.J thanks the authorities of Jerusalem College of Engineering for their encouragement to do research and providing the necessary facilities. Dr.Vidyarani Shyam Sundar, Center for oral cancer prevention awareness and research,.Sree Balaji Dental College, Pallikaranai.

References

1. A.E. Martell, B. Szpoganicz, "Zinc (II), Aluminum (III), and Gallium (II1) Complexes of Schiff Bases of 3-Amino-3-phosphonopropionic Acid and 5'-Deoxypyridoxal" Inorg. Chem.28 (1989)4199.
2. I.I. Mathews, P.A. Joy, S. Vasudevan, H. Manohar "Synthetic, Spectroscopic, Magnetic, and X- ray Structural Studies on a Vitamin B,-Amino Acid Schiff Base Complex, Aqua (5'-Phosphor pyridoxal dene tyrosinato) copper(II) Tetrahydrate "Inorg. Chem. 30 (1991) 2181.
3. J.W. Pyrz, A.L. Roe, L.J. Stern, L. Que Jr., Model Studies of Iron-Tyrosinate Proteins J. Am.Chem. Soc. 107 (1985) 614.
4. T.M. Aminabhavi, N.S. Biradar, S.B. Patil, V.L. Roddabasanagoudar,W.E. Rudzinski,Amino Acid Schiff Base Complexes of Dimethyldichlorosilane Inorg. Chim. Acta 107 (1985) 231.
5. G. Plesch, C. Friebel, O. Svajlenova, J. Kratsmar-Smogrovic, D. Mlynarcik, EPR, Ligand Field Spectra and Antimicrobial Activity of some N-Pyruvidene-glycinatocopper(I1) Complexes Inorg.Chim. Acta 151 (1988) 139.
6. D. Sattari, E. Alipour, S. Shriani, J. Amighian, Metal Complexes of N-Salicylideneamino Acids J. Inorg. Biochem. 45 (1992) 115.
7. Salah Ahmed Ba-Salamah, Naser Eltaher Eltayeb,Siang Guan Teoh and Kong Mun Lo.(E) - 2-{[1-Carboxy-2-(1H-indol-3-yl)ethyl-iminio]methyl}-6-hydroxyphenolate Acta Cryst.2011). E67, 02113-02114
8. Hitchcock SA, Pennington LD (May 2006). "Structure - Brain Exposure Relationships". J Med. Chem. 49 (26): 7559–7583
9. Md. Asaduzzaman et al, cytotoxic (brine shrimp lethality bioassay) and antioxidant investigation of barringtonia acutangula (l). International Journal of Pharma Sciences and Research IJPSR, Vol. 6 No.8 Aug 2015
10. Md. Jakaria et.al. Bioassay of brine shrimp lethality and thrombolytic activity of methanolic extract of macaranga denticulate leaves. Journal of Pharmacognosy and Phytochemistry 2015;3(6): 43-46
11. Ahmed Taha1, Hashim Alsayed. Brine Shrimp Bioassay of Ethanol Extracts of Sesuvium verrucosum, Salsola baryosma and Zygophyllum quatarense Medicinal Plants from Bahrain Phytother. Res. 14, 48–50 (2000)
12. M. S. A. Abdel-Mottaleb , , DFT Studies of Caffeic Acid Antioxidant:Molecular Orbitals and Composite Reactivity Maps Correlation with Photophysical Characteristics and Photochemical \ Stability, Journal of Chemistry , Volume 2016, Article ID 8727130, 8 pages
13. Kieran Alden, Mark Read, Jon Timmis , Paul S. Andrews, Henrique Veiga-Fernandes , Mark Coles, PLOS Computational Biology, Correction: Spartan:A Comprehensive Tool for Understanding Uncertainty in Simulations of Biological Systems February 2013, Volume 9,Is- 2
14. Parvez, A.; Jyotsna, M.; Tiwari V. Microwave Mediated Cyclo condensation of 2-amino thiazoleinto β-lactam Derivatives: Virtual Screening and In Vitro Antimicrobial Activity with Various Microorganisms, Vol.2, No.2, pp 956-964, April-June 2010.
15. Parvez, A.; Jyotsna, M.; Taibi Ben H. 2009, Manuscript accepted for publication in Phosphorus, sulphur, silicon and related elements. (GPSS-2009-0124.R1).
16. Shefali Agnihotri, Ranjana Narula, Kaishiv Joshi, Sandeep Rana & Maneet Singh.In silico modeling of ligand molecule for non-structural 3 (NS3) protein target of Flaviviruses Bioinformation 8 (3) :123-127 (2012).
17. Vidotto, C. et al,Biosci. J., Brine Shrimp lethality test as a biological model for Preliminary selection of pediculicidal components from Natural source Uberlândia, v. 29, n. 1, p. 255-263, Jan. /Feb. 2013.
18. Mohammed Rayees Ahmad et al , Microwave Assisted Synthesis Of Novel A,B–Unsaturated Carbonyl Derivatives And Their Activity.Int.J.PharmTech Res.2012,4(4)
19. Dharamraj, N., Viswanathanmurthi, P. and Natarajan, K. (2001) Ruthenium (II) complexes containing bidentate Schiff bases and their antifungal activity. Transition Metal Chemistry, 26, 105-109.

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