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ORIGINAL ARTICLE

Green Route Synthesis and *In-Vitro* Anticancer activities of Vanadium Complex with Bidentate Schiff Base

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ABSTRACT
The new VO(II) complex of bidentate Schiff base ligand viz., (E)-N1[(E)-3-phenylallylidene]benzene-1,2-diamine was synthesized and characterized by elemental analysis, metal estimation, molar conductance, magnetic moment, UV-Visible, IR and Far-IR spectral studies. The micro analytical data suggest the stoichiometry of the complex. The molar conductance of complex non-electrolyte nature and thermal stability from melting point were predicted. The electronic spectra and magnetic properties support octahedral geometry of complex. Coordination of ligand was confirmed by the change of FT-IR and metal ligating ability of metal chelates confirmed from Far-IR spectral studies. The antibacterial activity of the Schiff base and VO(II) have been assessed against some gram negative and gram positive microorganisms by agar well diffusion method. The anticancer and cytotoxicity Schiff base and VO(II) complex show its activity against oral cancer cell line. Computational study of the Schiff base and complex were determined by Auto dock method. The docking CDK4 & P-53 protein with complexes exhibited the greatest docking energy.
Key words: Orthophenylenediamine; Cinnamaldehyde; Vanadium; Antibacterial; Molecular docking

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INTRODUCTION

Schiff base is an important class of ligand in the development of coordination chemistry. These ligands are considered as "privileged ligand" because they are easily prepared by the condensation of aldehyde and amine.[1-2] Hugo Schiff was first reported the Schiff base at 1864. The oxygen atom of the phenolic group and nitrogen atom of azomethine group can coordinated with metal ion.[3]Metal ion act as Lewis acid and ligand as Lewis base. Metal complexes of Schiff base have been presented as building block in supramolecular assembly.[4] Schiff base derivative shows special centre of attraction in many areas like medicine, biological, clinical, analytical and pharmacological, *ect.*[5-6] Cinnamaldehyde is the main components of cinnamon from bark extract. It is used in perfume in natural, sweet and fruity scents.[7] They are also used as aroma compounds by the smell of cinnamon in almond, apricot and butterscotch.[8-9]It is a potential anti-obesity drug due to its low toxicity, eco-friendly, food flavoring, medical herb and renowned strong adsorption properties.[10]Orthophenylenediamine (OPD) is one the organic compound it undergoes condensation with carbonyl compounds (aldehyde and ketones) gives Schiff base which is used as antioxidant in rubber product and also used in photo-luminescence, catalytic activity and pharmaceutical with various diketone.[11]Hard- grey color vanadium metal play an important role in biology such as counter ions for protein, DNA and RNA and various biological studies and also enzyme co-factor.[12-13]The present study aims to synthesis of Schiff base vanadium and zinc metal complexes from cinnamaldehyde and Orthophenylenediamine (OPD) and characterized by various physico-chemical, spectral and biological studies.

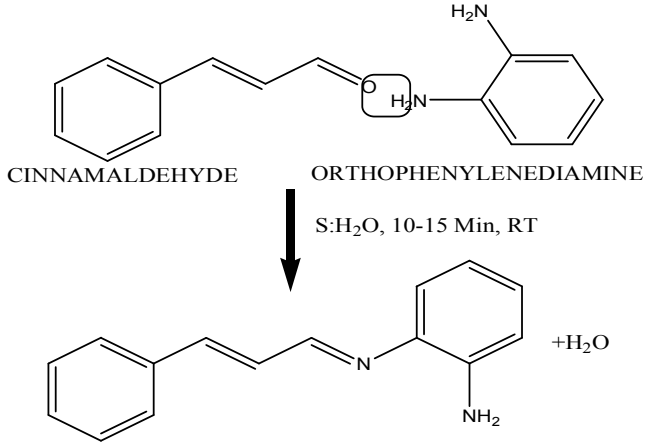
MATERIAL AND METHODS

Experimental: Materials and methods

All the chemicals such as Orthophenylenediamine, cinnamaldehyde, potassium thiocyanate, Vanadylsulphate, solvents and reagents were of AnalaR grade (99% pure) used as such without further purification. The synthesized schiff base and its complex were stable and stored in room temperature. Elemental analysis was carried out using elemental Vario make EL-III model instrument at 950-1200°C temperature. The metal ions were estimated after decomposing a known weight of complex in acids by colorimetric method. Melting point(°C) were recorded on a Ajay melting point instrument in open capillary tube. Molar conductance of complex was measured in acetonitrile solution at 10^{-3}M concentration at room temperature by the use of Systronic Conductivity Bridge. The magnetic moment of VO(II) complex was measured using a Lake Shore 7410 Vibrating Sample Magnetometer (VSM) at room temperature. Electronic spectra were recorded in solid state spectra (DRS method) on JASCO-V650 made spectrometer in the range of 200-800 nm. The IR spectra of schiff base and metal complex recorded by Using Schimadzu, FT-IR-4100 type-A model IR spectrometer in the range of 4000 to 400cm^{-1} as KBr pellet method. The Far IR spectra of the complex were recorded in a Bruker, Germany make, 3000 Hyperion Microscope with Vertex 80 FTIR system model instruments.

Synthesis of Schiff base:

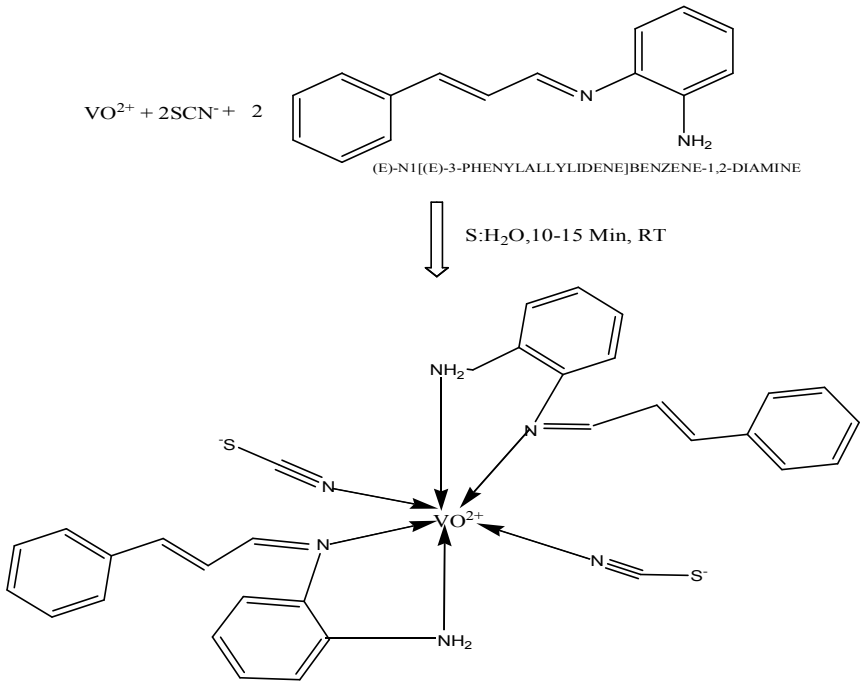
The goal of green chemistry is to develop Eco-friendly synthetic reactions. Green chemistry involves the use of microwave technology, sonochemistry, phase transfer catalysis, ionic liquids, and many other techniques. Green chemical one-pot multi component condensation reaction of 0.486g (4.40 m mol) of Orthophenylenediamine (15 ml ethanol) and 0.624g (4.70 m mol) of cinnamaldehyde (15 ml diethyl ether) using water as a green solvent and stirred continuously for about 10-15 min at room temperature, shiny powdered yellow color precipitate was formed.[14]The product was purified by simple filtration followed by washing with water and drying in desiccators and the yield was (73.33%). This method is experimentally simple, clean, high yielding, and green, with reduced reaction times.



(E)-N1[(E)-3-PHENYLALLYLIDENE]BENZENE-1,2-DIAMINE

Synthesis of VO(II) complex: Green route one-pot multi component reactions of an Schiff base, 1.75g, (7.90 mmol) in 50 ml ethanol solution was mixed with Vanadyl sulfate (1g, 3.90 mmol in 10 ml of water) and one more anionic ligand viz., potassium thiocyanate (0.768 g, 7.90 mmol in 10 ml of water) was added. Then water used as a green solvent and stirred continuously for 10-15 min at room temperature green color precipitate was formed. The product was purified by simple filtration followed by washing with water and drying in desiccators and the yield was (82.05%).

Evaluation of Antibacterial Activity: The antibacterial activity of the given compound was assessed against five bacterial species: *Staphylococcus aureus, Escherichia coli, Vibrio parahaemolyticus, Pseudomonas aeruginosa* and, *Enterococcus* and *Candida albicans* by agar well diffusion method. Each bacterial culture was grown in nutrient broth (bacterial) and potato dextrose broth (fungal) medium for 12 hours at 37°C. Then, each grown cultures were swabbed on nutrient agar medium and well were cut about 5 mm using cork borer. Each well was added with 80 µl of sample and incubated at 37°C for 18 hours. After incubation, the plates were observes for zone of inhibition and it was measured.



Bis(E)-N1[(E)-3-phenylallylidene]benzene-1,2-diaminedithiocyanato-KN-vanadium(II)

Anticancer activity: MTT assay: Cancer cell lines were used to determine the cell Cytotoxicity activity obtained from National Centre for Cell Science, Pune, India (NCSS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 µg/ml) and streptomycin (100µg/ml) in a 5% CO_2 at 37^0 C. Cell lines were seeded at 5000 cells/ well in 96-well plates and both were incubated for 48h at 6.25, 12.5, 25, 50,100 and 200 µg/ml concentration for 24h incubation. After the medium are removed it was washed with the phosphate saline solution. Then the sample was placed in a new medium containing 50ul of MTT solution (5mg/ml), to each well incubated for 4h. After the incubation DMSO was added. The viable cells were determined by the absorbance at 570nm by micro plate reader.

2.6 Molecular Docking: Auto Dock (V. 4.0) in the PyRx GUI has been used to confirm the binding abilities of the selected ligand and complex to all of the selected targets. Docking offers useful understanding of the interactions between ligand proteins. During the docking era, ligands were assumed to be flexible and the protein was considered rigid. The configuration file for the grid parameters was created by using Pyrex Auto Grid engine. The implementation has also been used to know / predict amino acids that come into contact with ligands at the active protein site. Results less than 1.0Å in the positional root-mean - square deviation (RMSD) were deemed optimal and grouped together to find a suitable binding. The highest binding energy (most negative) was found to be a ligand with a high binding affinity. Protein and ligand interactions were evaluated and visualized using the PyMol viewer tool. (www.pymol.org). The ADME calculation of the synthesized compounds was executed by Lipinski filter (http:/www.scfbio-iitd.res.in / software / drug design / lipinski.jsp) according to which the oral active drug must comply with at least four of the five established criteria for drug resemblance, namely: molecular weight, cLogP, hydrogen donor and molar refractive index.[15]

RESULTS AND DISSCUSSION

Analytical data: The elemental analysis (C, H, N) of the Schiff base and its complex shown in the Table-1, the data which are good agreement with calculated values. Based on the metal estimation of complexes the stoichiometry and molecular formulae of the schiff base and its complex are assessed as $[V(SB)_2(SCN)_2]$. The molar conductance of 10^{-3} M V^{2+}complex show the non-electrolytic (1:0 type) nature based on the conductance anion and cations are not present on its outside of the coordination sphere.[16]

Table-1: Analytical data of schiff base and V(II) complex

S.No.	Schiff Base/ Complex	Mol. Wt. (g/Mol)	Mp (°C)	Color	Elemental Analysis					Molar Conductance ($Ohm^{-1}cm^2mol^{-1}$)
					%C	%H	%N	%S	%M	
1	Schiff base	222.30	117	Shiny yellow	80.97 (80.19)	6.29 (6.30)	12.59 (12.52)	-	-	20.00
2	$[V(SB)_2(SCN)_2]$	611.73	130	Green	62.77 (62.10)	4.57 (4.80)	13.73 (13.00)	10.46 (10.12)	08.32 (08.59)	07.69

ESI Mass spectra: ESI mass spectrum of Schiff base recorded at room temperature. It is proposed that the empirical formula of the Schiff base and also support the stability of them. From the data of m/z the value at 222 confirmed the molecular formulae of schiff base ($C_{15}H_{14}N_2$) but the fragments at 105and 117 corresponds to the $C_6H_6N_2^-$ and $C_9H_8^+$ respectively.[17]

Electronic spectra: The electronic spectra of Schiff base exhibit one broad band at the transition of 25,575cm^{-1} shows the $\pi \rightarrow \pi^*$ whereas in complex this transition present with related values confirming the imine bond of the Schiff base, VO(II) complex exhibits three broad absorption band's in the region at 15385 cm^{-1}, 24038 cm^{-1} and 26954 cm^{-1} corresponding to the three spin allowed transitions $^4T_{2g} \leftarrow {}^4A_{2g}$, $^4T_{1g} \leftarrow {}^4A_{2g}$ and $^4T_{1g}$ (P)$\leftarrow {}^4A_{2g}$ respectively which suggest the VO(II) complex have octahedral geometry, the magnetic moment at 3.70 BM shows the three unpaired electrons in the complex also indicating their high spin octahedral nature of the complex which is triply degenerate ground state of $^4A_{2g}$.[18-19]

IR and Far-IR spectra: IR spectra provided the valuable information regarding the nature of functional group in the ligand attached to the metal atom/ion. Determine the binding site of Schiff base to the metal ion in the complex. IR spectrum of the free ligand was compared with the spectra of metal complex (Fig-1). The spectral data of the ligand showed the appearance of new band at 1595cm^{-1}due to the Schiff base azomethine linkage formation. In VO(II) complex the ν(C=N) stretching frequency shifted to higher values at 1617 indicate the metal ion can coordinate with free Schiff base through azomethine nitrogen atom. There are two aromatic rings ν(C-H) are present in the Schiff base at 3371 and 3142 cm^{-1}. In complex, one of the value are shifted to lower region at 3360 cm^{-1} but the other frequencies was also changed at 3142 cm^{-1} due to the effective coordination through the imine nitrogen atom which is attached in one aromatic ring, the other amine group coordination to the metal ions is confirmed by the ν(N-H) at 3500 cm^{-1} while in complex these are shifted to 3490 cm^{-1}. The C-N stretching frequency of Schiff base is at 1398cm^{-1} which is also shifted to higher frequency at 1407 cm^{-1} in metal complex which is the further evidence of coordination of metal ion through imine nitrogen.[20-21]The mixed anionic ligand thiocyanate ion coordinated through nitrogen or sulphur to the metal ion. If it is coordinated through "N" it is N-bonded at below 2100 cm^{-1} where as through "S" it is above 2100 cm^{-1}. In metal complexes the ν(SCN) stretching frequency at 2066 cm^{-1} was confirmed by the coordination of metal ion through 'N' atom of thiocyanate ion.[22]In far-IR spectra, the low frequencies at 423 $cm^{-1}$and 540 cm^{-1} corresponding to the M-N coordination bonds of imine and amino nitrogen atoms of schiff base to the metal complex. The frequency at 491 cm^{-1} shows the M-N coordination bond of thiocyanate additional ligand in the complexes.[23]

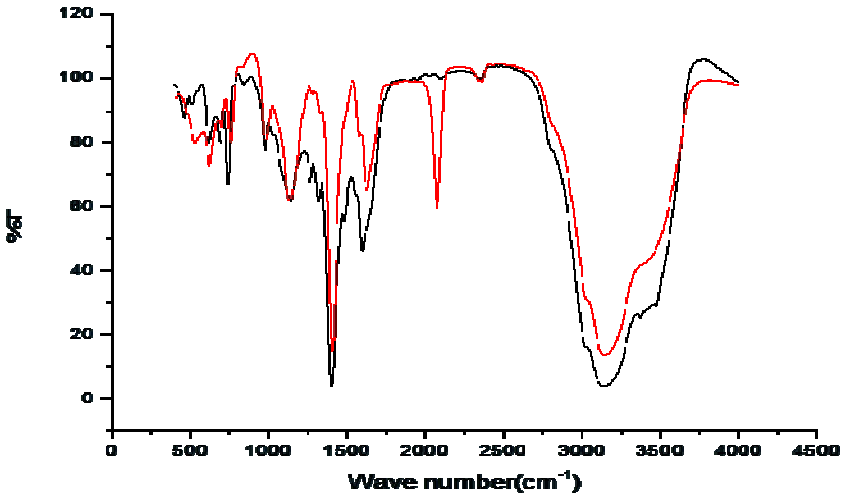


Fig-1: IR spectra of Schiff base and V(II)complex

Antibacterial and antifungal activities: The synthesized Schiff base and metal(II) chelates have been subjected for the screening of their *in-vitro* antibacterial and antifungal activities against two gram positive bacteria *viz., S. Aureus* and *Enterococcus,* three gram negative bacteria such as *E.coli, Vibrio parahaemolyticus* and *P.aeruginosa* and one fungus *viz., C.albicans* by Agar well diffusion method. The MIC values (Table-2) of the microorganisms predicted which indicating the Schiff base and its V(II) complex exhibit bio-potential activity (Fig-2). It is noted that the antibacterial activities of V(II) complex show higher activity than the Schiff base for tested microorganismnamely *S. Aureus* and *P.aeruginosa* whereas other microorganisms are moderately active to the complexes. The increased activity of the metal chelates may be explained on basis of Tweedy chelation theory. The chelation of metal complexes acts as powerful and bactericidal agent. That chelation killing more bacteria, the positive charge on the metal ion partially shared with the ligand. There may be a π electron delocalization in the chelates ring is present which increase the lipophilicity which increase the enhanced bio-potential activities, solubility, conductivity and bond parameters are also increase the activity of metal complexes.[24]

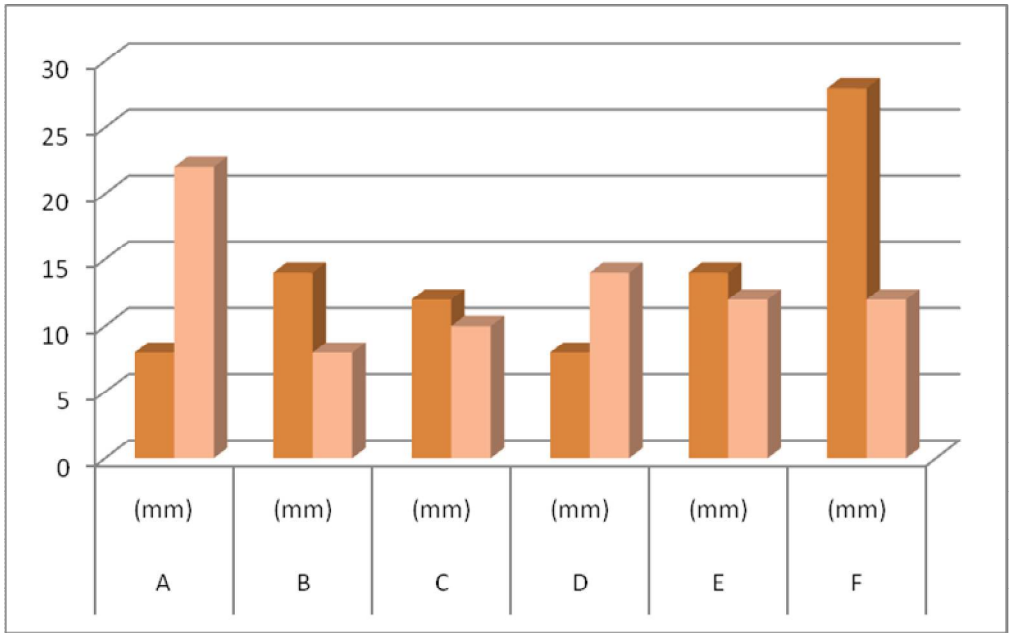
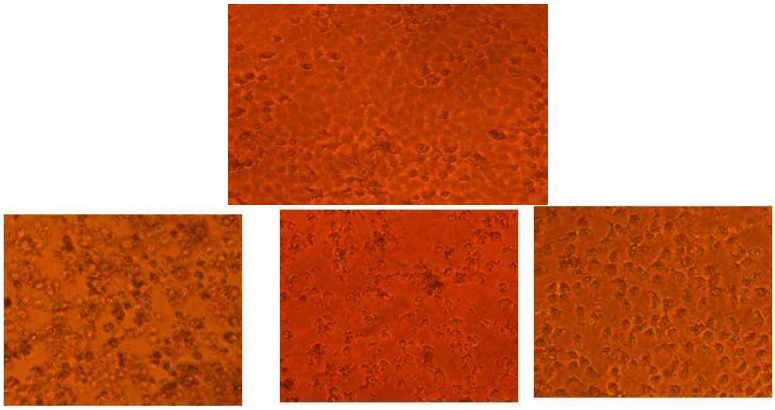


Fig-2: comparative Bio-potential activities of Schiff base and V(II) complex

Table-2: Antibacterial and antifungal activities of Schiff base and its complex

S.No	*Compound*	*S.aureus (mm)*	*E.coli (mm)*	*Vibrio parahaemolyticus (mm)*	*P.aeruginosa (mm)*	*Enterococcus (mm)*	*C. albicans (mm)*
1	SCHIFF BASE	08	14	12	08	14	28
2	VO(II) COMPLEX	22	08	10	14	12	12

Anticancer activity: Chemotherapy is the method to treat the cancer. Cisplatin is anticancer drug. *In-vitro* oral cancer activity (antiproliferation) of Schiff base and V(II) complex against KB oral cancer cell line was measured using MTT assay. The cytotoxicity of cancer cell line was measured to find out median growth inhibitory concentration (IC_{50}) at different concentrations after 48 h exposure to the tested compounds. The I_{C50} value of Schiff base is at 13.24 and the complex is at 34.56, at higher concentration the percentage cell death for the tested compounds is about 83.92% and 83.63% (Table-3&4) which shows higher anticancer activity schiff base and VO(II) complex.[25](Fig-3).



200µg/ml 100µg/ml 50µg/ml

Fig-3 cancer cell line of Schiff base & Vo(II) complex

Molecular docking

Molecular docking is a computational technique used to assess non-covalent binding between a protein (receptor) and Schiff base and VO(II) complex (inhibitor). Docking determines the mode of interaction between both the target protein and the Schiff base at the identified binding site. Binding energy indicates the affinity of the specific ligand and strength by which compound comes into contact and attaches to the pocket of the target protein. A compound with lower interaction affinity is chosen as a potential drug candidate.

PyRx was used to measure the effects of selected compounds on cell cycle proteins in oral cancer through molecular docking. The docking tests of CDK4 & P-53 protein with synthesized compounds exhibited the greatest docking energy and had been considered to be the best molecules at the protein target site. The least binding energy implied that the chosen molecules effectively docked with the target protein CDK4 & P-53. The amino acid residues GLN-71, ARG-72, GLU-115, GLU-258, GLY-262, ARG-106, LYS-147 and TYR-205 formed the H-bond interaction with the synthesized compounds (Fig-4&5)& (Table-5). The distance of the H-bonds was less than three, confirming the formation of desirable interactions between ligand and receptor. Both complexes have a distance of less than three H-bonds. This also proves that the chosen compounds showed good activity against CD4 & p-53. Lipinski's rule of five is a key criterion for assessing the possibility of a drug and if a specific chemical compound of a certain biological and pharmacological activity has chemical and physical properties which would make it a probably orally active drug in humans. Lipinski's rule sets out the molecular properties are very important for the pharmacokinetics of a drug in the human body, such as absorption, distribution, metabolism and excretion (ADME).[26-27]

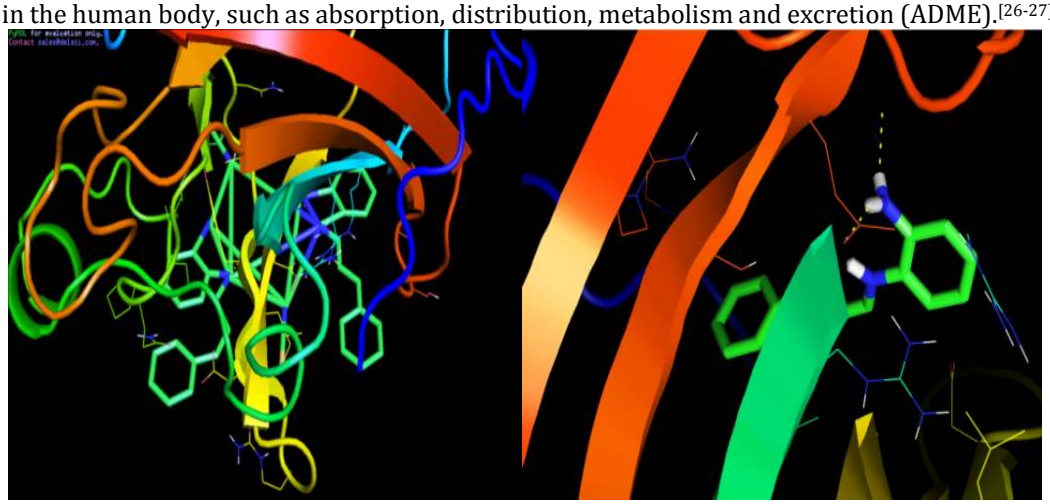


Fig-4: Docking of Schiff base and V(II) complex of p53

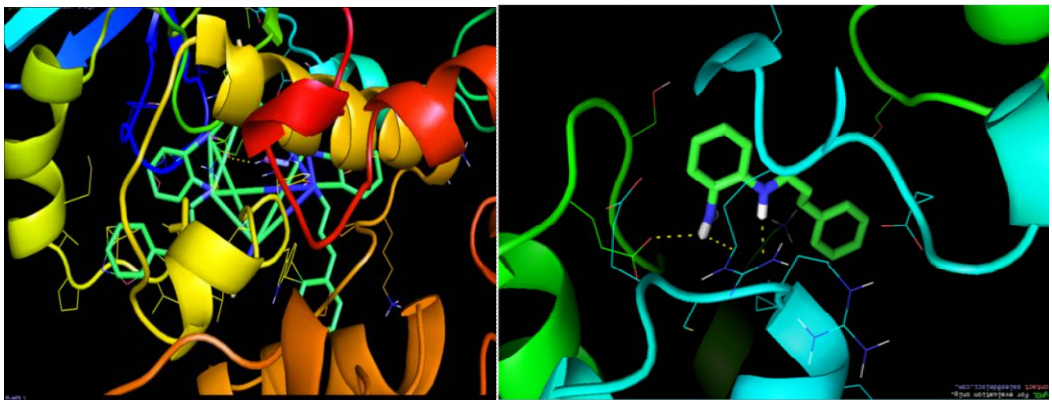


Fig-5: Docking of Schiff base and V(II) complex of CDK4

Table-3: Anticancer activity: optical density and % cell death of Schiff base

Concentration (µg/ml)-KB cell line	OD 1	OD 2	OD 3	% of cell death			Mean	SD	SEM	% Live cell
250	0.109	0.122	0.198	83.92	82.01	70.80	78.91	7.09	4.09	21.09
100	0.312	0.333	0.343	53.98	50.88	49.41	51.43	2.33	1.35	48.57
50	0.378	0.388	0.37	44.25	42.77	45.43	44.15	1.33	0.77	55.85
25	0.389	0.389	0.398	42.63	42.63	41.30	42.18	0.77	0.44	57.82
12.5	0.413	0.409	0.433	39.09	39.68	36.14	38.30	1.90	1.09	61.70
6.25	0.433	0.434	0.437	36.14	35.99	35.55	35.89	0.31	0.18	64.11
3.125	0.456	0.466	0.467	32.74	31.27	31.12	31.71	0.90	0.52	68.29
1.562	0.49	0.49	0.488	27.73	27.73	28.02	27.83	0.17	0.10	72.17
0.781	0.567	0.554	0.578	16.37	18.29	14.75	16.47	1.77	1.02	83.53
CONTROL	0.678	0.677	0.679	-	-	-	0.678	0.001	0.00058	-
IC_{50}	13.24	-	-	-	-	-	-	-	-	-

Table-4: Anticancer activity: optical density and % cell death of VO(II) complex

CONCENTRATION (MG/ML)	OD 1	OD 2	OD 3	% OF CELL DEATH			MEAN	SD	SEM	% LIVE CELL
250	0.111	0.121	0.165	83.63	82.15	75.66	80.48	4.24	2.45	19.52
100	0.232	0.243	0.212	65.78	64.16	68.73	66.22	2.32	1.34	33.78
050	0.353	0.365	0.259	47.94	46.17	61.80	51.97	8.56	4.94	48.03
025	0.474	0.487	0.455	30.09	28.17	32.89	30.38	2.37	1.37	69.62
12.5	0.489	0.492	0.488	27.88	27.43	28.02	27.78	0.31	0.18	72.22
6.25	0.523	0.52	0.522	22.86	23.30	23.01	23.06	0.23	0.13	76.94
3.125	0.577	0.565	0.577	14.90	16.67	14.90	15.49	1.02	0.59	84.51
1.562	0.59	0.592	0.59	12.98	12.68	12.98	12.88	0.17	0.10	87.12
0.781	0.609	0.613	0.622	10.18	09.59	08.26	09.34	0.98	0.57	90.66
CONTROL	0.678	0.677	0.679	-	-	-	00.68	0.00	0.00	-
IC_{50}	34.56	-	-	-	-	-	-	-	-	-

Table-5: H-bonding details of schiff base and VO(II) complex

PROTEIN NAME	BINDING AFFINITY K/MOL	H-BOND DETAILS	H-BOND DISTANCE
COMPOUND-RS			
CDK4	-5.5	GLN-71 ARG-72 GLU-115	2.1 2.7 2.4
P-53	-5.5	GLY-262 GLU-258	2.3 1.9
COMPOUND –RS1			
CDK4	-5.5	ARG-106 LYS-147	2.6 2.8
P-53	-5.6	TYR-205	2.0

CONCLUSION

The Schiff base (E)-N1[(E)-3-phenylallylidene]benzene-1,2-diamine and V(II) complex was synthesized by high efficient green route method with higher yield. The Schiff base coordinated to metal ion through its imine and amino nitrogen by bidentate mode to form metal chelates, based on the analytical and

spectral data (UV-Visible, IR, Far-IR and Mass spectra) reports confirmed the mononuclear, non-electrolytic octahedral geometry of V(II) complex. The schiff base is high potential to the screened microorganisms but the complexes are highly active for some microorganisms and moderately active against the growth other microorganisms. The oral cancer studies of Schiff base and V(II) complex show the anticancer activity which is further confirmed by the molecular docking studies of selected targets P53-3 and CDK4-2.

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