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Research article

DNA BINDING AND CYTOTOXICITY OF NEWLY SYNTHESIZED SCHIFF BASE (Z)-4-(((2-HYDROXY PHENYL) AMINO) (PHENYL) METHYLENE)-3-METHYL-1-PHENYL-1H-PYRAZOL-5(4H)-ONE AND ITS ANALOGUES.

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ABSTRACT : A novel Schiff base (Z)-4-(((2-hydroxy phenyl)amino)(phenyl)methylene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one and its analogues were synthesized by condensation of 5-methyl-2-phenyl-4-substituted pyrazolin-3-one with 2-amino phenol from alcoholic solution.Schiff base ligands arecharacterizedby IR, UV, NMR, elemental analysis and single crystal X- ray diffraction analysis. These potential ligands are subjected to DNA binding analysis against the Calf thymus DNA, their binding constant values were calculated and compared to the standard ruthenium intercalators. The cytotoxic natureof these Schiff base ligands was also studied with the human breast cancer cell line MCF-7 and their IC_{50} values were determined.

Key Words: Pyrazole Schiff bases, Tautomerism, DNA binding, Cytotoxicity, IC_{50}

INTRODUCTION

Schiff bases appear to be an important intermediates in a number of enzymaticreactionsinvolving interaction of the amino group of an enzyme, usually that of a lysineresidue,with a carbonyl group of the substrate (Lehlinger, 1975). Stereochemicalinvestigations (Otto, et al., 1978) showedthat, Schiff bases formed between methyl glyoxal and the amino group of the lysine side chainproteins can bend back in such a way towards the N-atom of peptide groups that a charge transfer can occur between these groups and the oxygen atoms of the Schiff base. Schiff bases derived frompyridoxal(the active form of Vitamin B_6) and amino acids are considered as very important ligands from biological point of view. The interest in pyrazoles stemmed fromtheir application in drugs, dyes and as anesthetic. Pyrazoles have also beenused as antioxidants in fuels but their major applications have been in medicaland agricultural fields. Transition metal complexes of such ligands are important enzyme model. The rapid development of these ligands resulted in an enhanced research activity in the field of coordination chemistry leading to very interesting conclusions. Many biologically importantSchiff bases have been reported in the literature possessing, antibacterial(Sari, et al., 2003;Karia, et al., 1999; More,et al., 2001; El-masry, et al., 2000;Baseer, et al., 2000; Amir, et al. 2002; More, et al. 2002; Sridhar, et al., 2002), antifungal (Singh, et al., 1988; Rajendran, et al., 2002; Calis, et al., 2002), antimicrobial (Nagatsuka, et al., 2002; Pandeya, et al., 1999;Shaikh, et al., 2001; Deshmukh, et al., 1995) , anticonvulsant (Sridhar, et al., 2002), anti HIV (Sridhar, et al., 2001), antiinflammatory (Sridhar, et al., 2002) and antitumor(Desar, et al., 2001; Pathak, et al., 2000; Tarafder, et al., 2003; Ren, et al., 2002; Sharma, et al., 1998; Kuz'min, et al., 1999; Kuz'min, et al., 2000) activities. Schiff base formation is also involved in the chemistry of vision where the reaction occur between the aldehyde function of 11-cis-retinal and amino group of protein (opsin) (Carry, 1992). The biosynthesis of porphyrin, for which glysine is a precursor is another pathwaywhich involves the intermediate formation of Schiff base between keto group of one molecule of o-amino levulinic acid andamino group of lysine residue of an enzyme. Another importantrole of Schiff base structure is transamination reactions are catalyzed by a class of enzymes called trans-aminases or aminotransferases. Transaminases are found in mitochondria and cytosolof eukaryotic cell. All the transaminases appear to have the same prosthetic group, viz. pyridoxal phosphate, which is covalently attached to them via an imine or Schiff bases.

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By keeping the above points in view, we carried out the syntheses of Schiff bases by the condensation of 5-methyl-2-phenyl-4-substituted pyrazolin-3-one with 2-amino phenol and studied their DNA binding ability and cytotoxic nature against the cancer cells.

Experimental

All chemicals were purchased from commercial sources and used without further purification. Infrared spectra were recorded using KBr pellets on a Perkin Elmer spectrophotometer of RXI model. UV/Visible spectra were recorded in dichloromethane solution on a systronics 2202 double beam spectrophotometer. Elemental analyses were obtained from Sophisticated Test and Instrumentation Centre (STIC), Cochin University of Science and Technology, Kerala. ^{1}H NMR spectra were carried out at Sri Ramachandra University, Chennai using TMS as internal standard. Melting points were recorded with Veego DS model apparatus and are uncorrected. X-ray diffraction analysis was carried out at Indian Institute of Technology, Chennai. Anticancer studies were carried out at the Kovai Medical Centre and Hospital Pharmacy College, Coimbatore, Tamilnadu.

Syntheses of 5-methyl-2-phenyl-4-formyl pyrazolin-3-one (Surati, et al., 2006), 5-methyl-2-phenyl-4-acetyl pyrazolin-3-one(Jensen, 1968) and 5-methyl-2-phenyl-4-benzoyl pyrazolin-3-one (Jensen, 1968) were done by the methods cited in the literature.

Synthesis of Schiff base: (Z)-4-(((2-hydroxyphenyl)amino)methylene)-3-methyl-1-phenyl-1H-pyrazol-5-ol(L_1)

5-methyl-2-phenyl-4-formyl pyrazolin-3-one (5mmole, 1.010g) and 2-amino phenol (5mmole,0.545g) were taken in a round bottomed flask in 40 ml of ethanol and refluxed under water bath for about 3 hours. The resulting mixture is tested for the completion of reaction by thin layer chromatography and allowed to stand overnight. The products formed are separated and dried. They were recrystallized twice from ethanol.

Synthesis of Schiff base: (Z)-4-(((2-hydroxyphenyl) amino)(methyl) methylene)-3-methyl-1-phenyl-1H-pyrazol-5-ol (L_2)

5-methyl-2-phenyl-4-acetyl pyrazolin-3-one (5mmole,1.080g)and 2-amino phenol(5mmole,0.545g) were taken in a round bottomed flask in 40 ml of ethanol and refluxed under water bath for about 3 hours. The resulting mixture is tested for the completion of reaction by thin layer chromatography and allowed to stand overnight. The products formed are separated and dried. They were recrystallized twice from ethanol.

Synthesis of (Z)-4-(((2-hydroxy phenyl) amino)(phenyl)methylene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (L_3)

5-methyl-2-phenyl-4-benzoylpyrazolin-3-one (5mmole, 1.39g) and 2-amino phenol (5mmole,0.545g) were taken in a round bottomed flask in 40 ml of ethanol and refluxed under water bath for about 2 hours. The resulting mixture is tested for the completion of reaction by thin layer chromatography and allowed to stand overnight. The products formed are separated and dried. They were recrystallized twice from ethanol.

Schematicrepresentation of the reaction of Schiff base formation and its various possible forms of tautomerism are given below.

DNA binding experiment

The DNA binding studies of the Schiff Bases were carried in Double beam spectrophotometer and UV/ Nano spectrophotometer, Optizen 3220 for Calf-thymus DNA(CT-DNA) in 5mM Tris-HCl, 50 mMNaClbuffer (pH=7.2). Stock solutions were stored at 4°C, and used within 3days. Titration experiments were carried out by varying the concentrations of CT-DNA while keeping the concentration of the Schiff bases constant (30 μ mole). The mixture was allowed to equilibrate for 5 minutes before spectra were recorded.

Antitumor analysis

The human breast cancer cell line (MCF-7) was obtained from National Centre for Cell Sciences (NCCS), Pune, and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). Cells were maintained at 37^{0}C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

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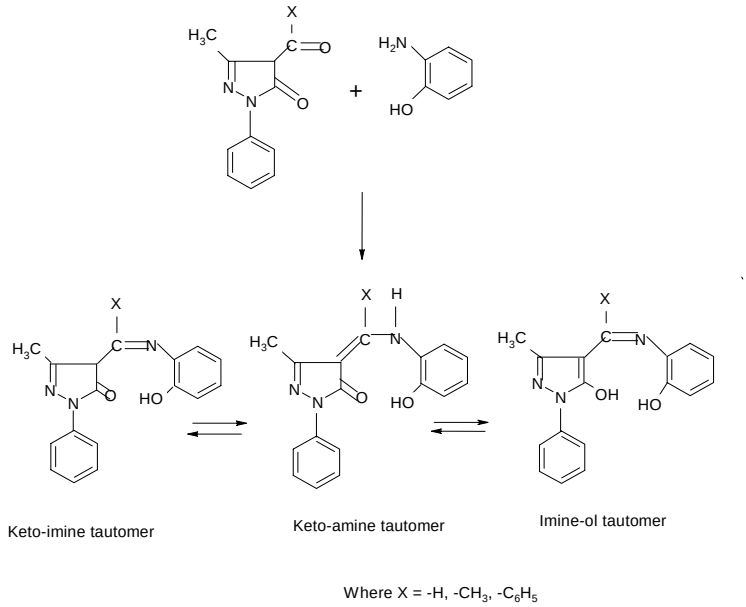

Scheme 1: The syntheses of the Schiff bases and their various possible forms of tautomerism.

Cell treatment procedure

The monolayer cells were detached with trypsin- ethylenediaminetetraacetic acid (EDTA) to make single cell suspension and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1 x 10^5 cells/ml. One hundred microliters per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24h the cells were treated with serial concentrations of the test samples. They were initially dissolved in dimethylsulfoxide (DMSO) to prepare stock (200mM) and stored frozen prior to use. At the time of drug addition, the frozen concentration was thawed and an aliquot was diluted to twice the desired final maximum test concentration with serum free medium. Additional three, 10 fold serial dilutions were made to provide a total of four drug concentrations. Aliquots of 100µl of these different drug dilutions were added to the appropriate wells already containing 100µl of medium, resulted the required final drug concentrations. Following drug addition, the plates were incubated for an additional 48h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT assay

MTT is water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0 C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570nm using micro plate reader. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death, or changing metabolism of cells, can be deduced through the production of a dose-response curve.

The % Cell inhibition was determined using the following formula.

% Cell inhibition = 100- Abs (sample)/Abs (control) x 100

Nonlinear regression graph was plotted between % Cell inhibition and Log_{10} concentration and IC_{50} was determined using GraphPad Prism software.

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RESULTS AND DISCUSSION

The Schiff bases synthesized (L_1, L_2, L_3) werestable at room temperature and possessed good keepingqualities. They were soluble in chloroform, dichloromethane, ethylacetateandinsoluble in n-hexane, petroleum ether, toluene. Analytical data of these compounds are in good agreement with their formulation andthe respective data are showed in Table 1.

IR spectroscopy

The resulting compoundL_3 has a strong absorption at 3650 cm^{-1} in the infrared, confirming the presence of N-H bond in the Schiff base. In the infrared spectrum of L_1, a strong absorption at 1659cm^{-1} confirms the presence of a ring C=O group which is a characteristic carbonyl frequency of pyrazolone moiety. However, in the case of L_3, the carbonyl group frequency is observed at 1745 cm^{-1}. This is because the carbonyl group in the pyrazole ring in conjugation with another double bond and substituted phenyl ring at the fourth position of pyrazole ring. So, this may be attributed to the effect of extended conjugation of ring carbonyl group with alternate double bonds. A strong absorption at 1620-1626 cm^{-1} for the compounds L_1, and L_2, confirms the presence of azomethine group which is a characteristic of Schiff bases. For all the three compounds, a strong band due to phenolic C-O was observed at 1271, 1278,1265cm^{-1} respectively. In addition to the phenyl C-O for L_2, there is also another band at 1285 cm^{-1} due to the presence of C-O in the pyrazole ring. All the Schiff bases invariably shows the absorption around 3000-3200 $cm^{-1}$and confirms the presence ofphenolic -OH group .For L_2, the two broad absorptions at 3020 and 3120 cm^{-1} confirms the presence of two O-H groups. With the background of all the above discussions, We may conclude that the compound L_1 exist as keto-imine tautomer, L_2exist as imine-ol tautomer and L_3 exist as keto-amine tautomer in the solid state among the three possible tautomeric forms. The various IR absorptions are given in table 2.

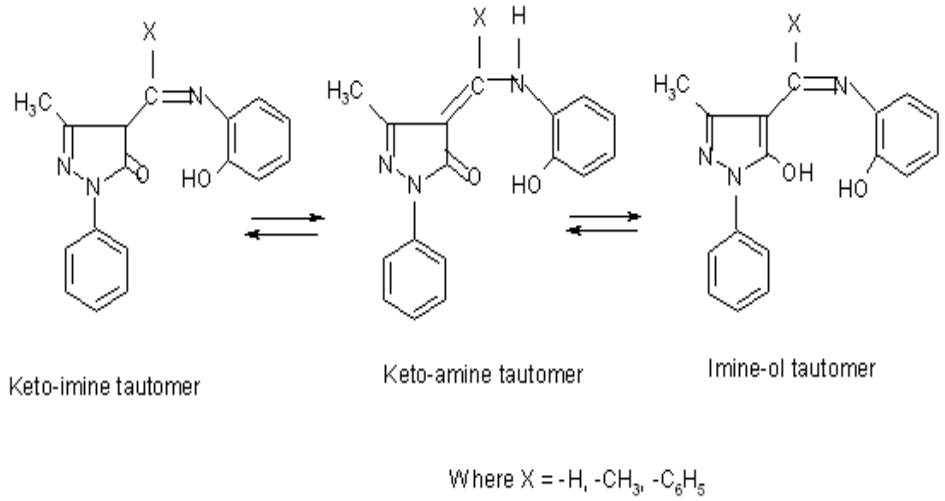

Fig.1 Various possible tautomericforms of Schiff Bases

Electronic spectra

Electronic spectra of these Schiff bases were recorded in dichloromethane solution and the respective spectral details are given in Table 2. The spectra of these, showed four types of transitions and the transitions around 250 and 300nm due to the n-π^*, π-π^*transitions involving the benzene ring, -C=N, phenolic –O-H, and enolic –O-H. The other two transitions near the visible region may be attributed to the presence of extended conjugated π system of various tautomeric forms.

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Table 2: IR and electronic spectroscopic data of the Schiff bases

Schiff Base	FT-IR cm^{-1}					UV-Vis
	ν_{N-H}	ν_{O-H}	$\nu_{C=O}$	$\nu_{C=N}$	ν_{C-O}	λ_{max}(nm)
L_1	-	3150	1659	1626	1271	240, 330, 368, 420
L_2	-	3020, 3120	-	1620	1278, 1285	257, 300, 344, 424
L_3	3650	3200	1745	-	1265	255, 298, 368, 440

Table 3: Crystal data and structure refinement of the new Schiff base L_3

CCDC entry no.	835665
Empirical formula	$C_{23}H_{19}N_3O_2$
Formula weight	369.41
Color and habit	Colorless, cubical
Crystal size(mm)	0.30 x 0.25 x 0.20
Temperature(K)	293(2)
Space group	p-1
a (Å)	7.1822(3)
b (Å)	11.0015(4)
c (Å)	13.6352(5)
α (º)	111.7150(10)
β (º)	92.255(2)
γ (º)	106.002(2)
Volume ($Å^3$)	950.02(6)
Z	2
D_{calc} (Mg/m^3)	1.291
Absorption Coeff.(mm^{-1})	0.084
Absorption correction	Semi-empirical from equivalents
Max. and Min. transmission	0.9833 and 0.9752
F (000)	388
R indices[I>2σ(I)]	R1 = 0.0391, wR2 = 0.1054
R indices (all data)	R1 = 0.0440, wR2 = 0.1102
Largest peak/hole(e.$Å^{-3}$)	0.193 and 0.201

Table 4: Binding constant values for Schiff Bases

Schiff base	Binding constant
L_1	7.777×10^3
L_2	1.450×10^4
L_3	4.320×10^5

Table 5: Absorption intensities of the samples at various concentrations

Schiff bases	Absorption intensities at various Conc. (µM)				
	0.1	1.0	10	100	Control
L_1	0.309	0.294	0.239	0.016	0.307
L_2	0.308	0.293	0.271	0.232	0.307
L_3	0.299	0.289	0.259	0.248	0.301

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^{1}H NMR spectroscopy

The tautomerisms of these compounds in solution and solid state have been the subject of studies. From ^{1}H NMR spectra, it was inferred that the resulted Schiff bases exist as a mixture of tautomers (II) and (III) (Scheme 1)in $CDCl_3$ solution. The phenolic proton leads to a singlet of integration intensity equivalent to one hydrogen at 9.8-12.8 ppm. This leads to the conclusion that there is no existence of imine-ol tautomer in $CDCl_3$. The aromatic protons give multiplet around 6.3-8ppm. The singlet at 1.3 -2.1ppm indicates the presence of methyl protons. The two singlets at 2.1ppm and 2.4 ppm can be assigned to the presence of two methyl groups of acyl pyrazole (L_2).The two singlets at 0.8-1.2ppm and 3.5-3.8 ppm are assigned to N-H proton of keto-amine tautomer, C-H proton of the keto- imine tautomer respectively. Moreover, the equal intensities of these two tautomersfor the compounds L_2 and L_3conclude that they present in equal ratio in CDCl3 solution. In addition to theC-H proton of the keto-imine tautomer for L_1, there is a singlet at 4.1 ppm which may be assigned to the formyl substitution at the fourth position of the pyrazole ring. On comparision of the intensities of N-H proton of keto-amine tautomer and C-H proton of the keto- iminetautomer(0.33: 0.24), concludes that the Schiff base L_1 exist as keto-amine tautomer in more percentage than keto-imine tautomer.

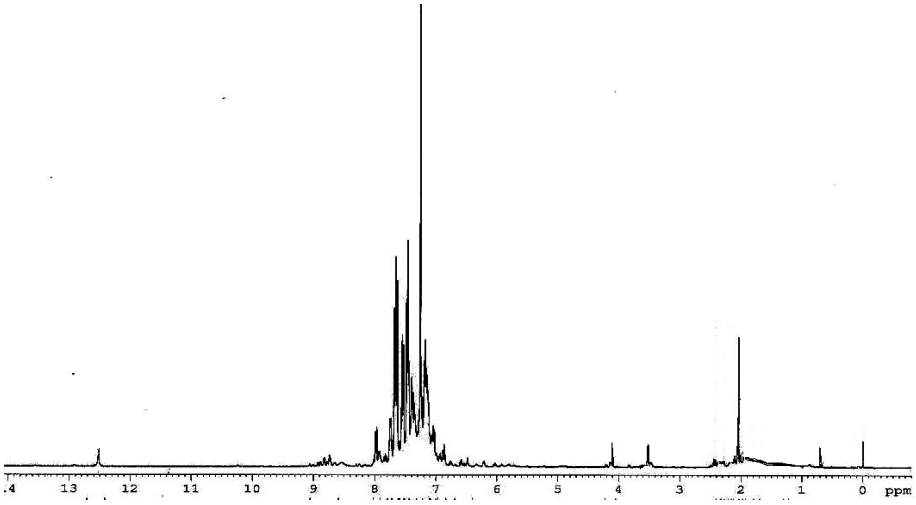

Fig.2 H^1 NMR spectrum of Schiff base L_1

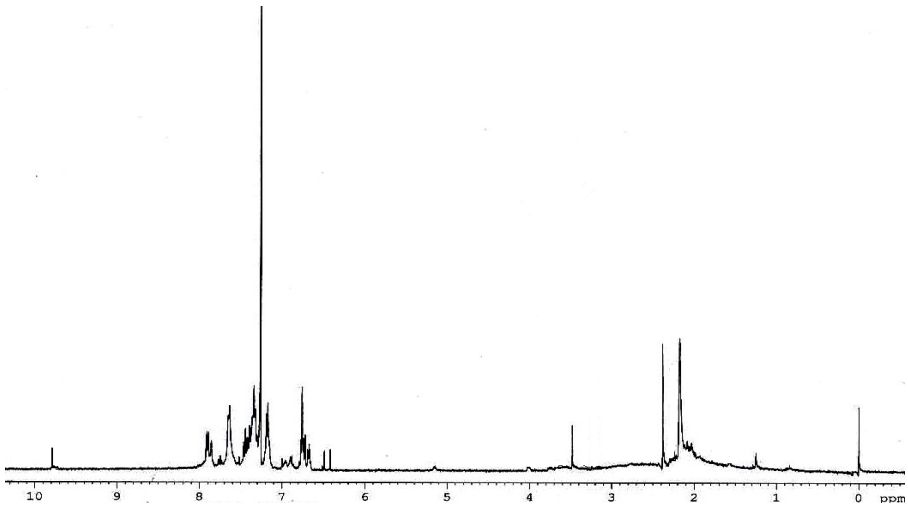

Fig.3 H^1 NMR spectrum of Schiff base L_2

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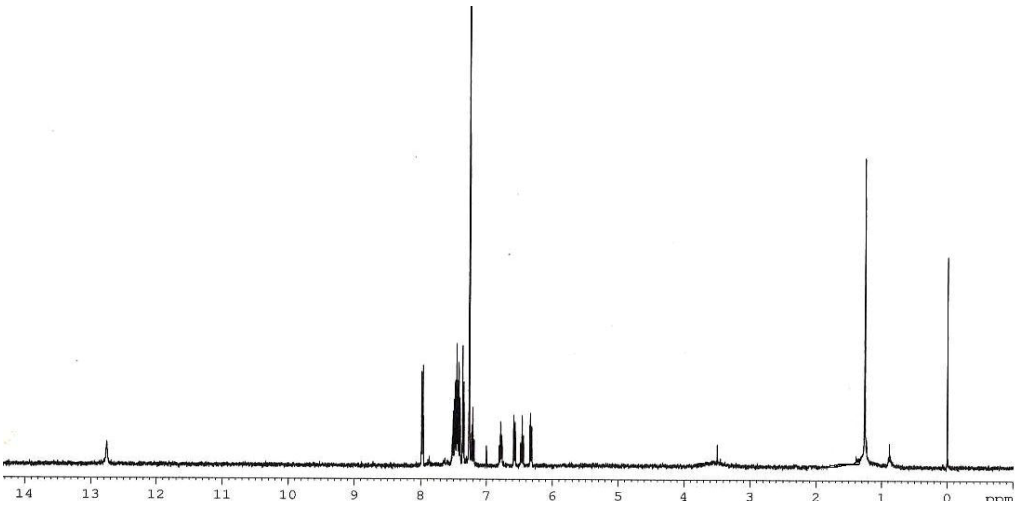

Fig.4 H^1 NMR spectrum of Schiff base L_3

X-ray diffraction studies

The molecular structure of the compound was determined using single crystal X-ray diffraction analysis for the Schiff base L_3. Data was collected on SADABS (Bruker, 1999). Data collection and cell refinement was performed using APEX2/SAINT (Bruker, 2004). The structure was solved by direct methods using SIR929 (Altornare et al. 1993). The refinements were completed using the program SHELXL-97(Sheldrick, 1997). The Schiff base belongs to the triclinic crystal system with space group p-1 with two molecules in the unit cell (half of the molecule in the asymmetric unit). The lattice parameters obtained are a=7.1822(3) Aº, b=11.0015(4) Aº, c=13.6353(5) Aº, α= 111.715(10) º, β = 92.255(2) º, γ =106.002(2) º and volume = 950.02 (6) A^3. The crystal structure of the compound with atom numbering scheme is shown in Fig.5. The diffraction data reveals that the nitrogen atom from 2-aminophenol exist as N-H group, the bond between the C8-C11 carbon atoms is double bond and it further concludes that the resulting Schiff base (L_3) present as keto-amine tautomeric form rather than the keto-imine in the solid state. The crystallographic data has been deposited in the Cambridge structure database (CCDC - 835665).

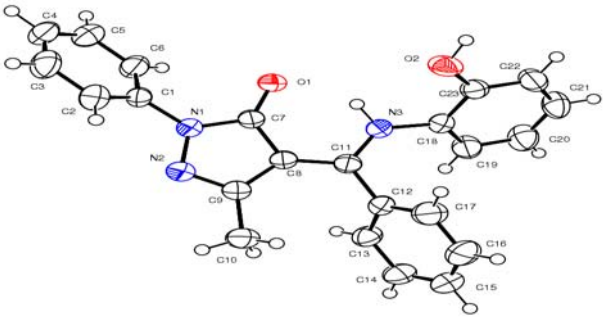

Fig.5 The crystal structure of L_3 with atom numbering scheme

DNA binding studies

The electronic absorption titrations of the Schiff base ligands L_1, L_2, and L_3 with CT-DNA were carried out. The spectra were shown in Fig.6a-c.The intensity of the bands of the Schiff bases around 230 and 260nm were found to increase with increasing the concentrations of DNA.

The Schiff base L_1 shows the increase in wavelength about 3nm (252.8-255.8), $L_2$and L_3 shows the decrease in wavelength of 8.5nm (266.5- 258) and 14.4nm (269.6- 255.2) respectivelyandincrease of intensity in all these cases shows hypo chromism. The blue shift in L_2 and L_3 and red shift in L_1 with hypochromism invariably indicates the interaction of these Schiff bases with Calf- thymus DNA. The binding constant for these ligands have been determined from the plot of [DNA] / (ε_a-ε_f) versus [DNA] and were given in table 4.

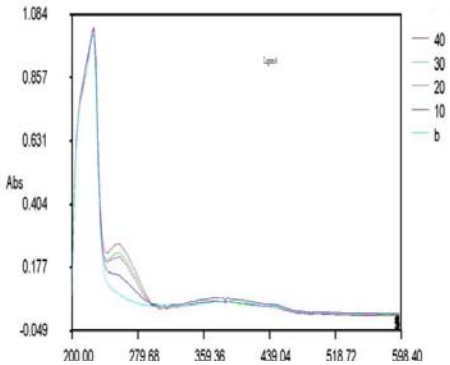

Fig. 6a [L_1]

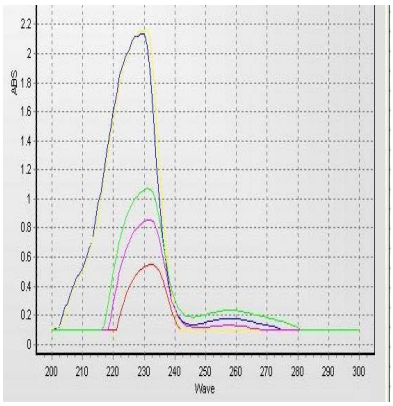

Fig. 6b[L_2]

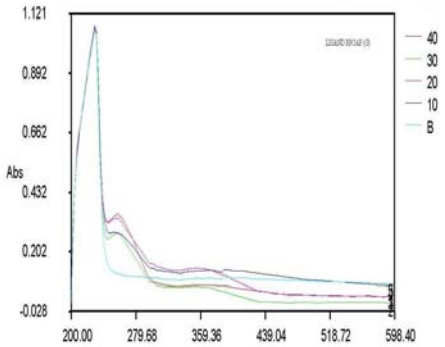

Fig. 6c [L_3]

Fig. 6a- c Absorption spectral traces with increasing concentration of CT-DNA in a TrisHCl – NaCl buffer (pH 7.2)

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The calculated binding constants (Table.4) on comparison with those of the so-called DNA intercalative ruthenium complexes (1.1 x 10^4 – 4.8 x 10^4 M^{-1}) (El-Shahawi, et al., 2004) suggest that L_2 and L_3bind to the DNA through intercalation and they are found to have more affinity than the classical intercalators, whereas L_1 may bind to the metal by partial intercalation.

Cytotoxicity

The Schiff base ligands L_1, L_2, and L_3 were tested for antitumor activity. The cytotoxicity against the human breast cancer cell line (MCF-7) was studied. MTT assay was carried out for each sample at four different concentrations (0.1µM, 1µM, 10µM and 100µM) and the medium without the samples were served as control. The photography of the cell treatment of these three Schiff bases at four different concentrations was given in fig. 7. The absorbance at 570nm using micro plate reader are determined and their average values given in table 5. The % Cell inhibition was determined using the following formula given below. The % Cell inhibition values are given in table 6.

% Cell inhibition = 100- Abs (sample)/Abs (control) x 100

Table 6: The % inhibition of the samples at various concentrations

Schiff Bases	% inhibition at various Conc. (µM)			
	0.1	1.0	10	100
L_1	-0.542	4.229	22.23	94.90
L_2	-0.433	4.664	11.71	24.51
L_3	0.442	3.756	13.84	17.39

Table 7: IC_{50} values for the Ligand and the Complexes

Schiff bases	IC_{50}
L_1	20.08
L_2	>100
L_3	>100

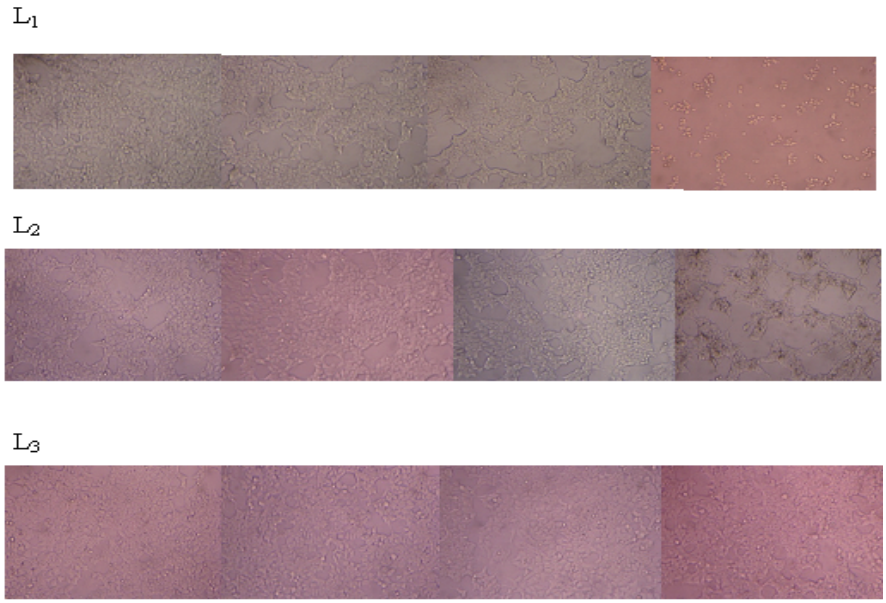

Fig.7The photographs of the cell treatment of Schiff bases at four different (0.1, 1, 10, 100µM) concentrations

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Nonlinear regression graph (fig.8) was plotted between % Cell inhibition and Log_{10} concentration and IC_{50} was determined using Graph Pad Prism software. The $IC_{50}$for the Schiff bases are given in table 7. The formyl derivative of the Schiff base is found to possess greater antitumor activity than the acetyl and benzoyl derivatives. The IC_{50} for the formyl Schiff base concludes that it is found to be effective and comparable with the standard anticancer drug cis-platin.

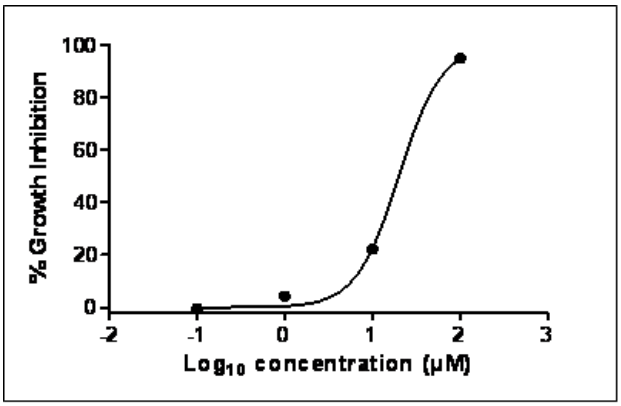

Fig. 8 A plot of % inhibition Vs Log_{10} Conc. of L_1

CONCLUSIONS

The pyrazole Schiff bases of formyl, acetyl and benzoyl substitutions with 2-aminophenol were synthesized, characterized by IR, NMR, elemental and X-ray diffraction studies.The ^1H NMR spectra reveals that thesecompounds exist as mixture of keto-amineand keto-imine tautomer in $CDCl_3$ solution. The spectral characterization and the XRD data confirms that the Schiff base L_1 present as Keto-imine tautomer and Schiff bases L_2 and L_3 present as keto-aminetautomer in the solid state among the three possible tautomers.DNA binding ability of these ligands shows that the Schiff bases L_2 and L_3 were foundto bind by intercalation and L_1 by partial intercalation. Moreover, the benzoyl derivative has higher binding constant value than the classical intercalators.Information obtained from our study will be helpful to understand the mechanism of interactions of Schiff bases with nucleic acids and should be useful in the development of potential probes of DNA structure and conformation.These potential ligands were also tested for their antitumor activity and the formyl derivative of pyrazole Schiff bases, found to have low value of IC_{50} than the acetyl and benzoyl derivatives.

Supplementary Material

CCDC – 835665 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or emailing data_request@ccdc.cam.ac.uk. or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 IEZ, UK.

REFERENCES

Altornare A., G. Cascarano, C. Giacovazzo, and A. Guagliardi, (1993). J. Appl. Cryst.: Vol.26, 343.

Amir M., S.M. Hasan and A.Wadood(2002), Orient. J. chem.: Vol 18(351) 204981 y.

Baseer M.A., V.D. Jadhav, R.M. phule, Y.V. Archana and Y.B. vibhute (2000), Orient. J.chem.: Vol.16, 553.

Calis U., M. Yasim, M. Koksal and M. Ozalp (2002), Arzneimittel-Forsthung: Vol. 52, 778.

Carry F.A (1992), 'Organic Chemistry', McGraw- Hill, Ed, 2nd, 675.

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Desar B., P.B. Desai and K. R. Desai(2001), Heterocycl.,communi.: Vol.7, 83.

Deshmukh M.D. and A.G. Doshi (1995), Orient. J. Chem.: Vol.11, 85

El-masry A.H., H.H. Fahrney and S.H.A. Abdelwahed (2000), Molecules: Vol.5, 1429.

El-Shahawiand M.S A.F.Shoair (2004), Spectrochem. Acta.A: Vol. 60, 121.

Jensen B.S. (1968), Acta chem. Scand.: Vol. 13, 1668.

Karia F.D.and P.H. Parsania (1999), Asian J. Chem.:Vol. 11, 991.

Kuz'min, V.E.R.N.Lezitskaya ,V.PLezitskii, A.I. Zheltvai, A.S.Fedchuk and G.L.Kamalov (1999), Dopovidi National NoiAkademiiNauk. Ukraini :Vol.12, 143.

Kuz'min V.E., V.P.Lozitsky, G.L.Kamalov, R.N.Lozitskaya, A.I. Zheltray, A.S. Fedtchouk and D.N.Kryzhanovsky (2000), ActaBiochimicaPolonica :47

Lehlinger A.L. (1975), 'Biochemisrty' Worth publisher, Ed. 2nd, p.84, 85, 220, 563.

More S.V., D.V.Dongerkhadekar, R.N.Chavan, W.N.Jadhav, S.R. Bhusare and R.P. Pawar (2002), J. Indian Chem. Soc.: Vol. 79,768.

Nagatsuka M., H.Ishida and S. Tanaka (2002), Jpn. KokaiTokkyaKohoJP :Vol. 128, 610.

Otto P., J. Ladik and A. Szent-Gyorgyi (1978), Proc. Nau. Acad.Su., USA.:Vol. 75, 3548.

Pandeya S. N. , D. Sriram, G. Nath and E. QeClereq (1999), Farmaco: Vol. 54, 624.

Pathak P., V.S. Jolly and K.P. Sharma (2000), Orient. J. Chem.: Vol. 16, 161.

Rajendran S.P. and R. Karvembu(2002) , Indian J. Chem., Sect.B : Vol.41B, 222.

Ren S., R.Wang, K.Komatsu, P.Bonaz-Krause, Y.Zyrianov, C.E.Mekenna, C.Cripke, Z.A.Tokesand E.J.Lien (2002), J.Med.chem, : Vol. 45, 410.

Sari, N. S. Arslan, E. Logoglu and I. Sakiyan (2003), G.U. Journal of Science: 283.

Shaikh K.A., M.A. Baseer and N.A. Mote (2001), Ashian J. Chem.: Vol.13, 496.

Sharma K.P., V.S.Jolly and P.Phatak (1998), Ultra Scientist of Physical Sciences: Vol.10, 263.

Sheldrick G.M (1997), SHELXL, Program for Crystal Structure Refinement, University

Singh W. M and B.C. Dash (1988), Pesticides :Vol.22, 33.

Sridhar S.K., M. Saravanan and A.Ramesh (2001) , Eur. J. Med. Chem.: Vol.36, 615.

Sridhar S.K., S.N. Pandeya, J.P. Stables and A. Ramesh (2002), Eur. J. Pharm. Sci.: Vol. 16, 129

Sridhar S. K., S.N. Pandeyaand E.DeClereq (2001), BollettinochimicoFarmaceutico: Vol. 140, 302,

Sridhar S.K., M. Saravanan and A.Ramesh (2001) , Eur. J. Med. Chem.: Vol.36, 615.

Sridhar S.K and A. Ramesh (2001), Indian Drugs: Vol. 38, 174S.

Surati, R.Kiran, B.T.Thaker (2006), J. Coordination Chemistry: Vol. 59 (11) 1191-1202.

Tarafder M.T.H., A.Kasbollah, N.Saravanan, K.A.Crouse, A.M.Ali and K.T.Oo (2002), J.Biochem.Mol.Biol. Biophys.: Vol. 6,85.