European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 02, 2020

Identifying the Therapeutic property of the Synthesized Schiff base Ligand and its Metal complex involving Green Technology

G.Padma Priya.*[1,2], C.R.Girija* [3] , Sathish[*4]

1.Research and Development Centre Bharathiyar University, Coimbatore

2. Assistant Professor Jain University School of Sciences, JC. Road, Bengaluru

3. Associate Professor Government Science College, Nrupatunga Road, Bengaluru

4. Assistant Professor ,Department of Science and Humanities, PES University,Bengaluru

Abstract

Schiff bases are aldehyde- or ketone-like compounds in which the carbonyl group is replaced by an imine or azomethine group. Schiff bases are adaptable ligands which are produced from the condensation of primary amines with carbonyl groups. Production of Schiff base transition metal complexes by using Schiff base as ligands seems to be enthralling in view of the opportunity of obtaining coordination compounds of unusual structure and steadiness. They are widely used for industrial purposes and also exhibit a broad range of biological activities. Characterization using UV, IR, NMR, Antibacterial, Docking studies of Schiff base and its metal complex is investigated. The biological activity of the transition metal complexes derived from the Schiff base ligands has been widely studied. This evaluation summarizes the importance, Scope and antimicrobial activities of Schiff base metal complexes.

***Key words** : Alloxan –Thiosemicarbazone ligand, Ni complex, Anti bacterial, Docking*

1. Introduction:

Ligands are enthralling class of ions that binds to a central metal ion to form coordination compounds. Its aptitude to bequeath lone pair of electrons or capacity to act as “Lewis Bases” has created remarkable wave in pharmaceutical industry. Schiff’s bases are widespread class of

compound formed by condensation of aldehyde or ketone with a primary amine under preliminary condition. Schiff base named after the scientist Hugo Schiff base are milder, efficient, less hazardous leading to synthesis of variety of Schiff's base. They are tantamount to azomethine (RCH=NR') Nitrogen referred as azomethine Nitrogen. Thiosemicarbazide are of notable interest because of their compatibility to condense with aldehyde or ketones readily. They are potentially known to exhibit properties like antitumor, antiproliferative, anticancer properties.

Alloxan- 2,4,5,6[1H, 3H] pyrimidinetetrone is a heterocyclic compound with high biological and physiological effect on living organisms [1-4]. It is capable of influencing metabolism of zinc, calcium, phosphorus in organism, also a product of uric acid decomposition. Literature reveals the intense study of the Al- TSC ligands with Au and not much work on d block elements is carried out. The ligand was synthesized in an eco friendly manner leading to sustainable development. Alloxan is reported as an agent which selectively destroys pancreatic beta cells of mice which result in inducing permanent diabetes. Chemical compounds that selectively damage pancreatic β cell damage constitute diabetogenic drugs[1-3]

Thiosemicarbazide act as dexterous ligand because they have better co-ordination tendency and form more stable complexes. They have the ability to produce some new and unique complexes with enhanced biological and analytical properties. Certain thiosemicarbazones are relatively specific inhibitors of ribonucleotide reductase, which is an important metabolic target for the development of chemotherapeutic agents against cancer. Thiosemicarbazone usually act as chelating ligands with transition metal ion bonding through the sulphur and hydrazine nitrogen atom.
According to the reports the coordination mode of thiosemicarbazone is very sensitive towards minor variation in the experimental conditions. The nature of substituent on the carbonyl compound and metal salts. This property of thiosemicarbazone is utilized in designing new methods of synthesis leading to sustainable development.
The momentum of this work was to chemically modify alloxan to produce Schiff base compound that are not diabetogenic, but will have the ability to interact with DNA. A number of authors have been interested in investigating the biological and medicinal properties of transition metal complexes of thiosemicarbazones in recent years.

Sustainable development meets the needs of the present without compromising the ability of future generations to meet their own needs. In present work we present the synthesis, characterization, biological activity of Schiff's base ligand and its metal complex. Acid catalysed condensation reaction of carbonyl compounds with amines is carried out using citric acid (lemon) leading to green technology.

A non classical heating modus operandi using microwaves which is termed as "Bunsen burner of the 21st century" caters to the needs of the present scenario [4-6]. This method dramatically reduces reaction times, also reducing the disposal of huge amount of heat energy to the environment. The significant outcome of microwave assisted reaction results in development of simulation protocols for drugs. The use of promising technique in concurrence with greener reaction media dramatically reduces chemical waste and reaction times in organic synthesis and chemical transformations. Synthetic chemistry community are under pressure to synthesise

compounds in an environmentally benign fashion, the whole host of heterocyclic system required by society in a short span of time.

Microwave- assisted organic synthesis (MAOS) is based on the efficient heat transfer achieved by dielectric heating, which is mainly dependent on the ability of the solvent or reagent to absorb microwave energy .In our present work the ligand was synthesised through microwave assisted reaction which is the innovative technology compared to classical method using reflux method. Characterisation of the synthesised was done using UV, IR, NMR,

2.1. Materials and Methods:

The analytical grades Thiosemicarbazide (LOBA) Alloxan (LOBA) Mueller Hilton Agar (HIMEDIA), Nickel chloride, Diacetyl acetonate, NaOH (Merck).Potato Dextrose Agar (HIMEDIA), were used as received.

Ni $(accac)_2$ Preparation: 0.1 M solution of Nickel chloride is prepared. To this 50 ml of NaOH containing acetyl acetonate (emulsion formed) is added, a green precipitate is formed which filtered , air dried and used as Ni $(accac)_2$

2.2. Instruments:

UV spectrum was recorded in IR spectra were recorded in KBr discs on Brucker FTIR spectrophotometer from 400 to 4000cm^{-1}.^{1}H NMR were recorded using CDCl3 at Brucker 400MHZ.

2.3 Synthesis of Ligand

The ligand Alloxan thiosemicarbazone was synthesized in an eco friendly manner. The acid catalysed reaction was done using citric acid (lemon) instead of acetic acid. 0.01 M solution of Alloxan in methanol was condensed with 0.01 M solution of Thiosemicarbazide in dissolved in ethanol. Filtered buff coloured precipitate is condensed for nearly two hours. The precipitate is then recrystallised using ethanol red orange crystals were obtained. The reaction is carried with microwave irradiation within 4 min. This procedure afforded product yield more with higher competence whereas the yield obtained with classical heating under similar conditions did not exceed 50%.

2.4 Synthesis of metal complexes:

In the preparation of the complex metal and ligands were mixed in the ratio 1:2 molar ratio using required quantities of ethanol. Hot ethanolic solution of ligand 0.001 M and hot ethanolic solution of Ni $(accac)_2$ were mixed together and refluxed in microwave assisted reaction. It is left for evaporation for 3 days black solid metal complex was obtained. The products were filtered, washed with cold ethanol and dried under vacuum.

3. Antibacterial Activity:

In-vitiro biological activity of the synthesized ligand- metal complex was investigated for the antibacterial activity. Agar well diffusion method was used to determine bacterial sensitivity test. The sample was assessed for bacterial cultures Pseudomonas aeruginosa, Escherichia coli, Staphylococcus. The ligand activity was tested against a standard drug streptomycin.

3.1 Sample preparation:

10mg of the ligand was dissolved in 10ml of DMSO (Dimethyl sulphoxide) in eppendorf tube. Aliquots of 100µg, 200µg, 300µg and 400µg of the concentration of the sample was prepared by pipetting out 10µL, 20µL, 30µL and 40µL in sterile eppendorf and the final volume was made upto 50µL by adding DMSO as a blank. Standard streptomycin was dissolved in DMSO and made upto definite volume.

3.2. Media preparation:

150ml of Mueller Hinton Agar (Composition: Acid Hydrolysate of Casein: 17.50 g, Starch: 1.50 g, Beef Extract: 2.00g, Agar: 17.00g, distilled water-1000mL) was prepared by dissolving the respective components in 150ml of distilled water. It was thoroughly mixed such that no particulate components were present. It was then autoclaved at 121°C for 15 minutes.

3.3 Bacterial plate preparation:

Approximately 30ml of the media was poured into the sterile petriplates and it was allowed to solidify. The bacterial cultures were sub-cultured in Nutrient broth at 37°C for 24hours. Later, 100µl of bacteria cultured inoculum of Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus were poured into respective plates. The inoculum was spread throughout using a sterile spreader (via Spread plate technique). On each agar plates, five wells measuring 5.5 mm were punched using a well borer. The wells were filled with aliquots of sample in respective wells and 50µL as the Control in the middle well. The culture plates were incubated at 37°C for 24 hours. The zone of inhibition was measured and recorded in millimeters (mm) formed around the respective wells.

4. **Molecular docking studies:**

4.1 Protein and ligand/ Metal structure preparation

The compound is designed and energy minimized using PRODRG serve[20]. The DNA structures for both groove binding and intercalation studies were retrieved from PDB (www.rcsb.org). In the present study, the docking methodology followed for Benzothienoquinolines for DNA binding studies by Rodrigues et al, 2014 was used[18]. In order to further characterize the interaction of ligand with DNA, docking studies using Autodock4.2[15] was performed using dodecamer *B*-DNA (1HQ7: GCAAACGTTTGC sequence[17] and a structure with nine base pairs intercalated with benzo[*a*]pyrene diol epoxide (B*a*P) (1DXA: GGTC[B*a*P]ACGAG sequence [21] from which the intercalator was removed (1DXA*). The Lamarckian Genetic Algorithm (LGA) was chosen. In the first stage, docking

with the B*a*P was performed with both 1HQ7 and 1DXA* as self docking. Only docking of 1DXA* with BaP is considered as a control for the effectiveness of the docking procedure and as a reference for evaluation of the other docking results.

The docking site for the ligand to DNA was defined using PyRX0.8 interface. A grid box was created with 111 x 57 x 57 points with grid centre 14.9601 x 20.1236 x 7.3199 for 1HQ7, in order to include the entire DNA fragment and 41 x 42 x 47 points with grid centre 0.3137 x -0.0214 x 13.532 for 1DXA* at the intercalation site with a spacing of 0.375 Å. After the grid box was centered in the macromolecule, grid potential maps were calculated using module AutoGrid 4.2. The autodock4.2 was set with 10 runs, 27000 maximum number of generations, 250000 maximum number of energy evaluations, 0.02 mutation rate, 0.8 crossover for both 1HQ7 and 1DXA*. Only the best pose (the one with the lowest binding energy) was considered for the ligand. Visualization of the results was made with the help of the auto dock tools software suite (ADT)(Sanner, 1999). PyMOL (DeLano, 2002) was used for docking conformation representation.

5. Results and Discussion:

5.1 Elemental analysis

The ligand and the metal complex were variously coloured crystalline powders are obtained, air stable insoluble in common organic solvents but soluble in DMSO. The melting point of the ligand L_1 and L_1 Ni (accac) was found to be 278℃ and 330℃ respectively.

5.2 FTIR spectral studies :

The IR spectral bands of ligands and corresponding complex along with assignments are presented in Table 1. The analysis of IR spectrum of pure ligand/ metal complex Figure 1 and Figure 2 reveals the absence of a band in the region 2500-2600cm^{-1} in the ligands which is characteristic of thiol group ν (C-SH) suggests the stable thione form of the ligand. The band at 3440-3200 cm^{-1} is assigned for ν (NH). The thioamide band due to ν (C=S + NH) coupled vibrations appeared at 1419cm^{-1},1432cm^{-1} and the band at 1693 cm^{-1},1604 cm^{-1} is assigned for ν (C=N). Ring ν (C=O) vibration is assigned at 1759 cm^{-1}, 1737cm^{-1}.The bands in the range 759 and 794cm^{-1} are due to ν(C=S) vibrations in the ligands. [11-16]

The IR spectra suggests that in complexes coordination occurs through the azomethine nitrogen and and thioketo sulphur atom.The band at 1693 is lowered to 1604cm^{-1} indicating the coordination of the azomethine Nitrogen atom. The bands at 1076,1045,813and 759cm^{-1} assigned to the ν(c=s) band are shifted towards a lower frequency indicating the sulphur coordination.

Table 1: IR spectral data of ligand and metal complex

S.N	Ligand/complex	Colour	M.P°C	Yield%	Infra red spectral data cm^{-1}				
					ν(N-H)	**ν(C=S + NH)**	**ν (C=N)**	**ν(C=O)**	**ν (C=S)**
1.	$C_5H_5N_5O_3S$	Buff	278	88	3440	1419	1693	1759	759
2.	$[Ni(L_1)accac]$	Black	330	60	3427	1432	1604	1718	695

Figure 1

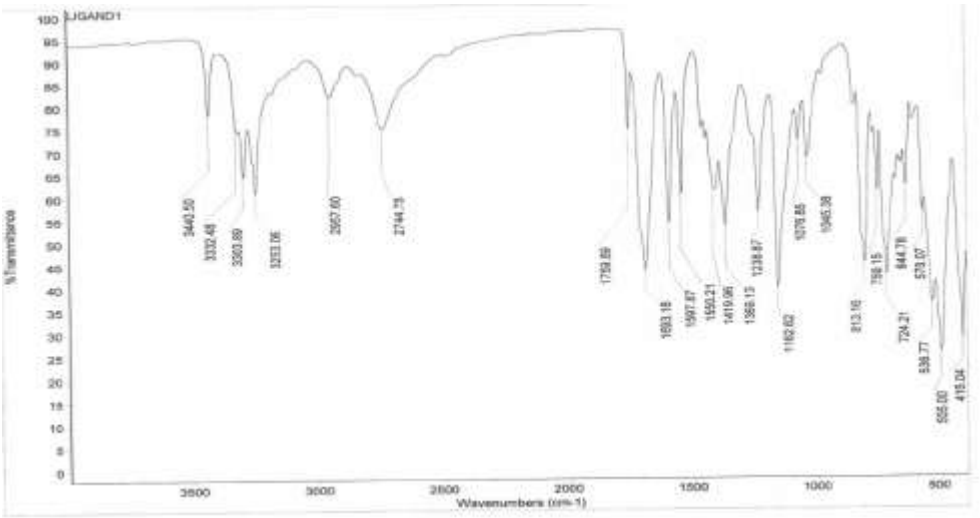
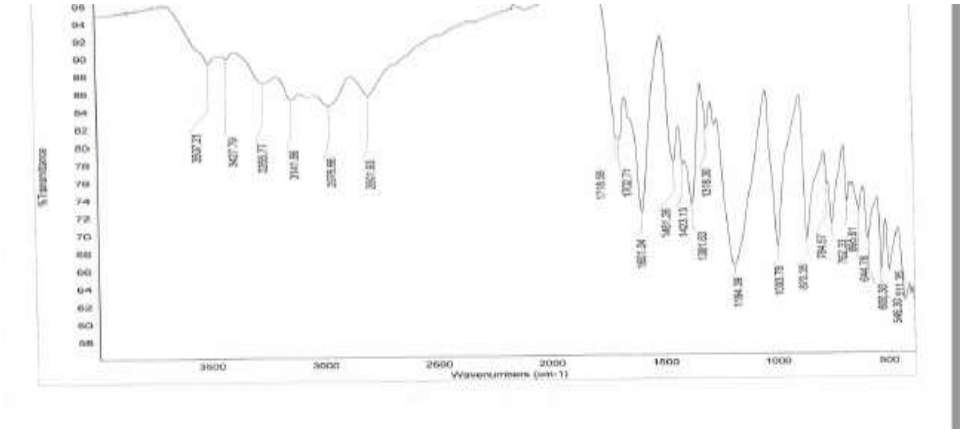


Figure 2 L_1- Ni (accac)



[1]HNMR spectra:[1]H NMR (DMSO-d6) spectrum of the ligands**(figure3)** displays the following signals Hydrazinic proton signal at 13.5 ppm (L_1) is seen in the ligands shows a strongly bonded proton (proton A). There is a downfield shift at 11.78- (L_1due to (proton B) bound to pyramidine ring integrate as single proton. Further more signal at 11.5 (L_1 (proton C) in pyramidine (alloxan ring proton).Interestingly thiosemicarbazone –amide proton resonate at 9.51ppm (protonD), 8.60 ppm (proton E) in case of L_1. Two separate and distinct resonance are seen for two amide protons. Ni metal complex shows a downfield shift to 15.1ppm of hydrazinic proton to a downfield shift indicating the participation of NNH proton in metal complexation. Alloxan ring proton signal also shifted to a down fiels to 10.76,Semicarbazone amide proton remains undetectable due to exchageable proton. This is evident from figure 4. [11-16]

Table 2: [1] H NMR for the ligands

		[1] H NMR signals ppm			
S.No	Ligand Formula	Hydrazinic N-NH proton	Alloxan ring N-H proton	Alloxan ring N-H proton	Semicarbazone amide N-H proton
1.	$C_5H_5N_5O_3S$	**13.4**	**11.78**	**11.56**	**9.58,8.60**
2.	[Ni(L_1)accac]	**15.1**	**10.76**	**-**	**Not detectable**

Figure 3 NMR spectra L1

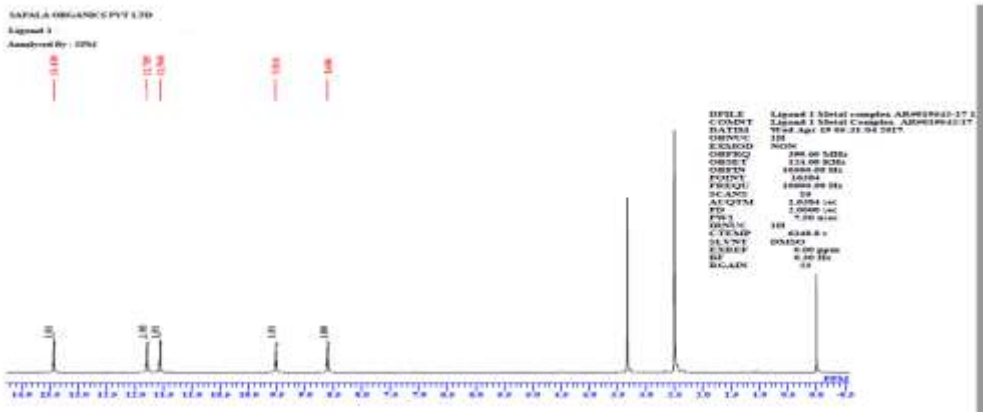
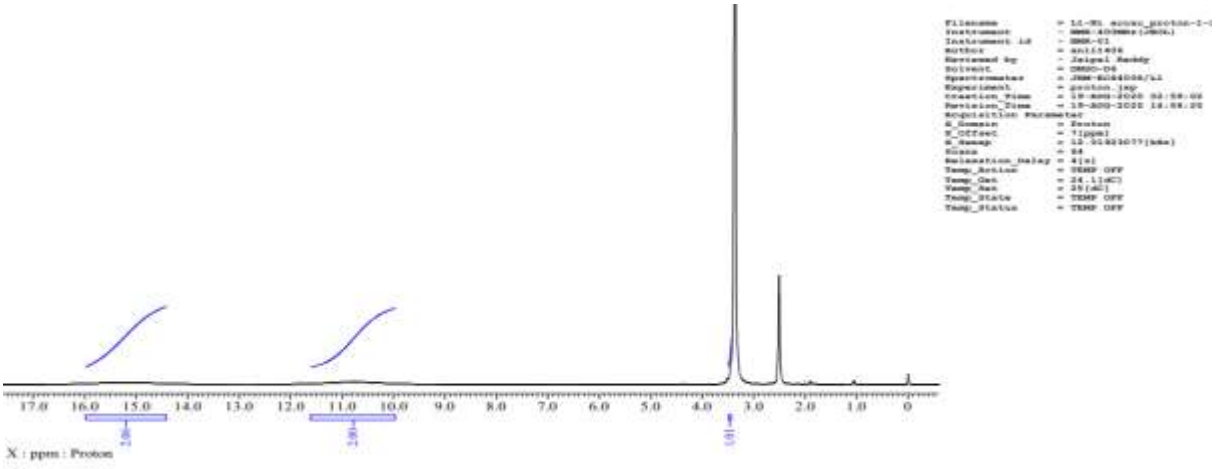


Figure 4 NMR spectra Ni - Li (accac)complex



5.1 Antibacterial Activity:

The basic principle to quantify the antimicrobial assay of the compound is to identify and scrutinize the growth of inhibition of untailored microbes without any side effects. Theoretically if ionizing disinfecting molecule are absorbed or replaced by electrical charges at the initial contact and absorption stage growth can be inhibited (bacteristatic). The antibacterial property of the ligand is due to its ability to penetrate into the lipophilic membrane of the bacteria to be tested.[7-11] The lipid membrane surrounding the cell favours the passage of only lipid soluble materials. Thus liphophilicity is an important factor to determine bacterial activity. The inhibitory activity of these known analogous systems is conceivably attributed to the hydrogen bonding through the azomethine group or imine group (C=N-), with active centers of cell constituents ensuing in interference with normal cell processes It is worth mentioning the hydroxyl group (hydrogen bonding) adjacent to imine group a common feature in Schiff bases participates to be stabilized by a hydrogen bond. Therefore, the hydroxyl substituent may be important group for structure stability.

Table 3: Antibacterial Property Ligand

	Micro organism zone of inhibition (in cm)		
Concentration	Bacillus cereus	Escherichia coli	Pseudomonas aeruginosa
100 µg	-	-	-
200 µg	-	1.0	-
300 µg	-	1.2	-
400 µg	0.9	1.3	1.2

Table 4: Antibacterial Property [Ni (accac) L]

	Micro organism zone of inhibition (in cm)		
Concentration	Bacillus cereus	Escherichia coli	Pseudomonas aeruginosa
100 µg	-	-	`1.6
200 µg	-	1.0	2.5
300 µg	-	1.0	2.6
400 µg	0.9	1.1	2.8

Table 3 results show that the antibacterial activity of the ligand was screened using well diffusion method. Ligand exhibits inhibitory activity in proportion to standard streptomycin with the same efficiency. It also clearly indicates that with the increased concentration of the ligand shows greater inhibition in the growth of the organism. The ligand shows enhanced activity against gram negative bacteria E.coli. This gram negative bacteria are harmless some serotypes cause serious food poisoning in humans, even life threatening complications hemolytic –uremic syndrome. E.Coli are the main causative bacteria for UTIs often leading to lysis of urinary tract cells.

Treatment of *P.* aeruginosa infections can be difficult due to its natural resistance to antibiotics. When more advanced antibiotic drug regimens are needed adverse effects may result. Nickel complex prepared is found to potent bactericide against E.coli as well as P.aeruginosa. Alloxan thiosemicarbazone ligand synthesized is found to be a potent bactericide. Streptomycin is an effective broad spectrum antibiotic that inhibits growth of bacteria by preventing protein synthesis. This heterocycle ligand which contain –NH, C=O group might mimic streptomycin amino glycoside linkage which act as potent inhibitor. The interaction of the ligand enhances permeability through the lipid layers of the bacterial cell membrane and cause cell death of the bacterial strain.

Although no define structure-activity relationship could be determined, some conclusions on structural changes that may influence the anti bacterial activity can be drawn by the comparison among the structure of compound with structure of the standard. The biological activity is depending on hydrogen bonding present in that molecule.Literature studies reveals metal complexes to be more potent antibiotic than the ligand itself in most of the investigations.

Figure 5.1: Zone of inhibition Ligand

Figure 5.2: Zone of inhibition [Ni (accac) L]

5.3. Molecular docking studies

Table 5: Molecular docking interaction details of Alloxan thiosemicarbazone and the reference ligand / Metal - BaP at the minor/major groove binding (1HQ7) and intercalation study (1DXA).

Compounds	Docking	Nucleic acid	Type of binding	Binding Energy (Kcal/mol)	IC_{50} (nM)	Hydrogen Bonds
Ligand-1	A	1HQ7	Minor groove	-5.86	50.31 µM	1hq7_1:B: DG23:H21 Ligand: N
						1hq7_1:A: DA4:H3 Ligand: N
						1hq7_1:A: DA4:H3 Ligand: O
						1hq7_1:A: DA3:H3 Ligand: N
						Ligand: H 1hq7_1:A: DA3:O4'
	B	1DXA*	Intercalation	-4.68	0.33µM	Ligand:N3 1dxa_1:A: DA5:N6
						Ligand: N5 1dxa_1:A: DA5:N6
Metal	A	1HQ7	Minor groove	-5.21	150 µM	Ligand: H 1hq7_1:B: DA17:O4'
						1hq7_1:B: DA15:H3 Ligand: O
	B	1DXA*	No intercalation	-4.65	387.38 µM	1dxa_1:A: DG7:H22 Ligand: O
						Ligand: H 1dxa_1:B: DC3:O3'
						Ligand: H 1dxa_1:A: DA8:O3'
BaP	A	1HQ7	Minor groove	-9.55	99.67nM	no hydrogen bonds formed
	B	1DXA*	Intercalation	-9.22	174.77nM	BAP:A:BAP10:HO2 : 1dxa_1:A: DC6:O4'
						BAP:A:BAP10:HO3 : 1dxa_1:A: DC6:O2

Figure 6 .1 Ligand- Nucleic acid interactions at 1HQ7

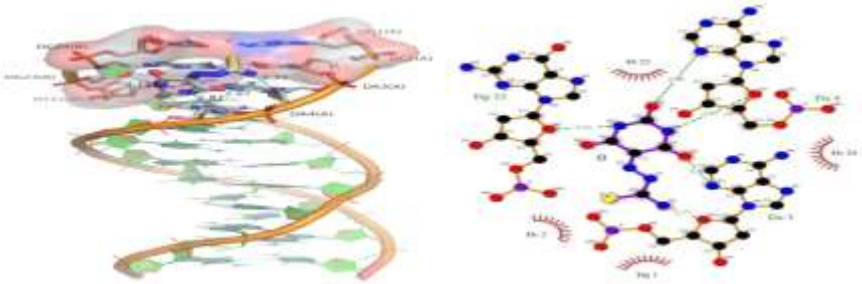


Figure 6.1: Interaction of Alloxan thiosemicarbazone with 1HQ7 at the minor groove binding site. a) 3D representation of the ligand with 1HQ7. Nucleotides are represented in lines and interacting nucleotides are in stick representation covered under surface. Green line represents hydrogen bonds with bond length. B) 2D Ligplot representation where arcs represent the hydrophobic interaction and green line represents the Hydrogen bonding with bond length.

Figure 6.2 Interaction of Ligand at Intercalation site at 1DXA*

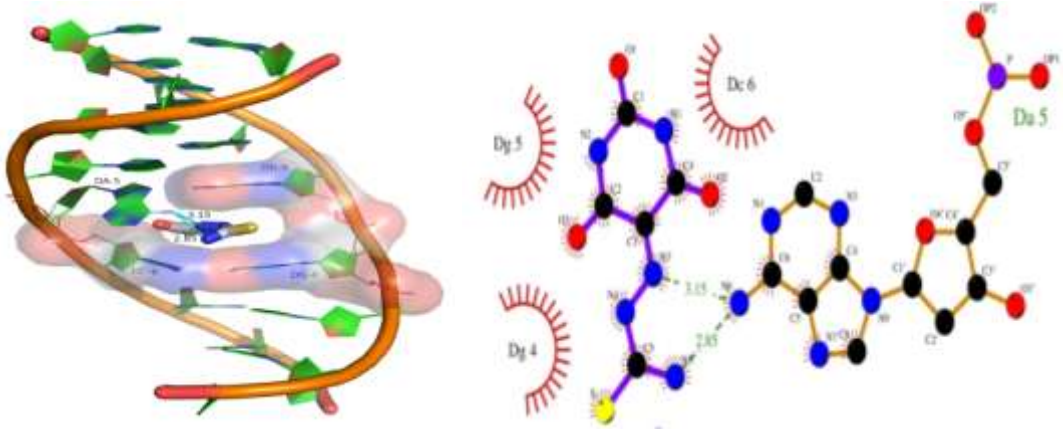


Figure 6.2: Interaction of Alloxan thiosemicarbazone with 1DXA* at the intercalation site. a) 3D representation of ligand with 1DXA*. Nucleotides are represented in lines and interacting nucleotides are in stick representation covered under surface. Green line represents hydrogen bonds with bond length. b) 2D Ligplot representation where arcs represent the hydrophobic interaction and green line represents the Hydrogen bonding with bond length.

Figure 6 .3 Metal - Nucleic acid interactions at 1HQ7

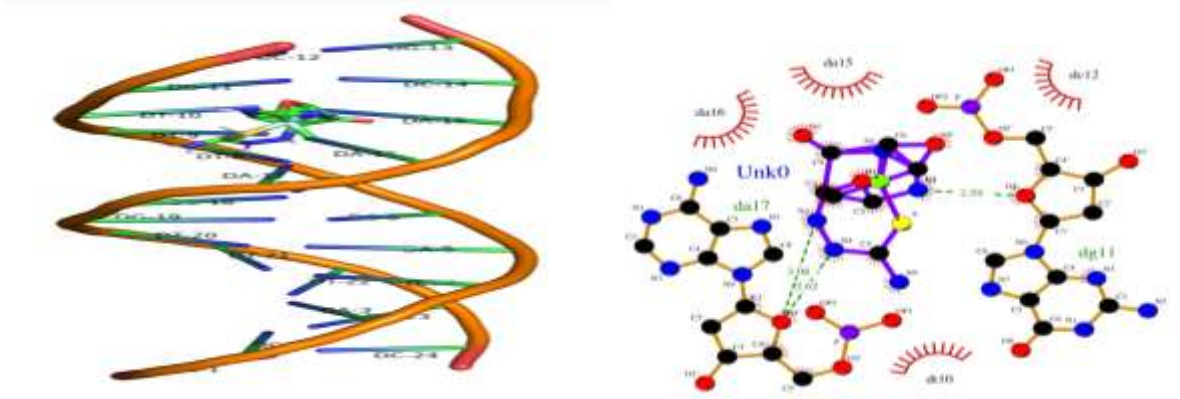


Figure 6. 3: Interaction of metal with 1HQ7 at the minor groove binding site. a) 3D representation of ligand with 1HQ7. Nucleotides are represented in lines and interacting nucleotides are in stick representation covered under surface. Green line represents hydrogen bonds with bond length. B) 2D Ligplot representation where arcs represent the hydrophobic interaction and green line represents the Hydrogen bonding with bond length.

Figure 6.4 Interaction of Metal at Intercalation site at 1DXA*

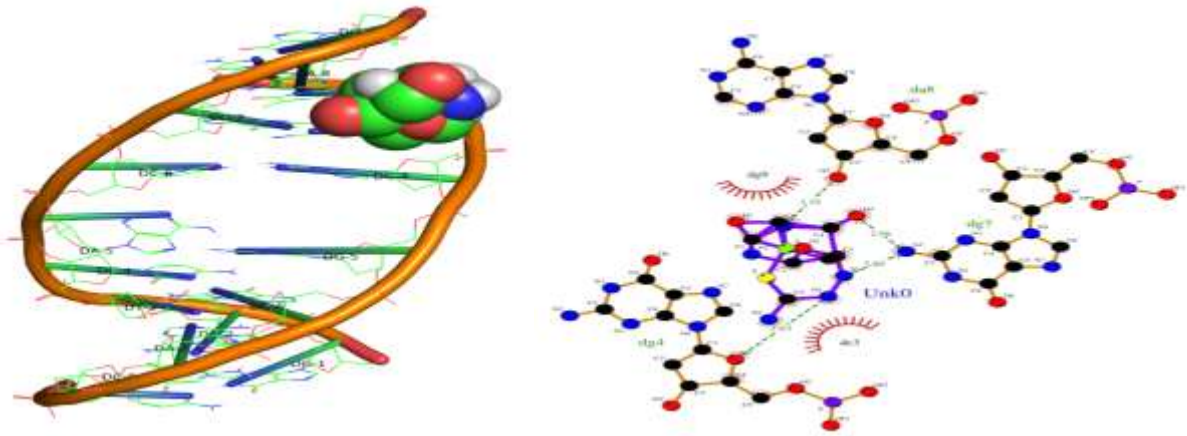


Figure 6.4: Interaction of metal with 1DXA* at the intercalation site. a) 3D representation of metal with 1DXA*. Nucleotides are represented in lines and interacting nucleotides are in stick representation covered under surface. Green line represents hydrogen bonds with bond length. B) 2D Ligplot representation where arcs represent the hydrophobic interaction and green line represents the Hydrogen bonding with bond length.

5.4: Molecular docking interaction details of ligand and the reference ligand BaP

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Characterisation of the binding behaviour plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes.[17-21]

Molecular docking interaction details of ligand and the reference ligand BaP at the minor groove and intercalation site. As observed by [19] in their studies on Benzothienoquinolines (BaP), similar interaction result was observed for the reference intercalating molecule B*a*P, where the intercalation in 1DXA* forms with two hydrogen bonds and hydrophobic binding at minor groove in 1HQ7 is also equally favourable with -9.55kcal/mol. Intercalation of BaP happens with the 1DXA* with -9.22kcal/mol free energy and accommodates itself at the intercalating sites. With reference to BaP, from the Table 3 it can be observed that ligand binds to 1HQ7 with binding energy of -5.86kcal/mol at the minor groove region. Though the binding energy is high as compared to the reference ligand BaP, but ligand forms four hydrogen bonds at the minor groove and brings the specificity to the interaction as depicted in figure 3.

The interaction of ligand in the intercalating region 1DXA* forms two hydrogen bonds with DA5. BaP forms only one hydrogen bonds with DC6 whereas in ligand forms two hydrogen bonds with DA5. The atoms N3 and N5 of ligand forms hydrogen with N6 of the DA5 which brings more specificity and stability for the complex as observed in figure 4.

Binding energy or binding affinity is an important factor in docking studies. It is the energy released when a molecule associates with a target, leading to lowering of overall energy of the complex. The release in binding energy also compensates for any transformation of the ligand from its energy minimum to its bound conformation with the protein. Greater the energy on binding of a ligand to the protein greater will be the predilection of the ligand to associate with that protein.

The synthesised Ligand with binding energy of -5.86Kcal/mol in minor groove is found to associate with 1HQ7 of nucleic acid in found in Protein data bank (PDB) .-5.21 Kcal/mol for metal comple.The do decamer is D- (GCAAACGTTTGC)2 B-DNA is resolved in PDB file 1HQ7. N, O, donor atoms from the ligand along with azomethine nitrogen was found to associate with minor groove of this nucleic acid through Hydrogen bonding.

DXA*- BENZO[A]PYRENE DIOL EPOXIDE ADDUCT OF DA IN DUPLEX DNA.
Molecular description is5'-D(*GP*GP*TP*CP*AP*CP*GP*AP*G)-
3'5'-D(*CP*TP*CP*GP*GP*GP*AP*CP*C)-3'· Intercalation is the insertion of molecules between the planar bases of deoxyribonucleic acid (DNA). In this Docking studing DXA* DNA was used for ligand insertion. The ligand had a binding energy release of -4.68 Kcal/mol. From the above data ligand had more efficiency of association through hydrogen bonding at the minor groove than at the intercalation site. In metals there is little intercalation of association.

Inhibition constant is the change on the electrostatic non bounded energy of ligand or protein upon binding. Torsion energy is related to dihedral term of internal energy. Inhibition constant is an indication of how potent an inhibitor is, It is concentration required to produce half maximum inhibition. Lower the inhibition value greater is the efficiency for inhibition. IC_{50} for the ligand is 0.5µM for HQ7 association and 0.3µM for 1DXA* interaction site. Thisvalue prove

to be potent drugs which can bring inhibition of multiplication of cells. Also the amount of metal concentration required to inhibit the activity to 50% is found to more than the ligand. This may be due to the bulkiness of the metal complex.

6. Conclusions:

Alloxan Thiosemicarbazone ligands was synthesized in a green methodology. The antibacterial activity of the ligand efficiently proved to be more active than its antifungal effect. From the docking studies, it can be concluded that the bioactive compound can be used as a potent inhibitor to block the action of DNA by its activity of minor groove binding and also potentiality of intercalation. The bioactive compound can be analyzed by molecular dynamics studies and then in vivo studies for detailed investigations. Further co-crystallization of DNA-ligand and animal trials followed by biopharmaceutical scale up feasibilities could be encouraged.
The synthesized metal transition complex using metal $(accac)_2$ using microwave assisted reaction (MPAS). Alloxan was condensed with various series of amines and its effect on binding capacity, cell line studies on DNA will follow a line of investigations.

7. Acknowledgements:

The author is thankful to Government Science College- Nrupatunga Road Bengaluru for carrying out experiments for this present work. PES University, Bangalore, Assyme Bio science Bangalore.

References:

1. L. S. Shebaldina, O. V. Kovalchukova,S. B. Strashnova, B. E. Zaitsev & T. M. Ivanova in Russian Journal of Coordination Chemistry 2004,38–42.
2. S. Padhye, and G.B. Kauffman. Coordination Chemistry Reviews, 1985, 63, 127 -160.
3. J.N.Brown, K.C. Agarwal, Acta Crystallogr., Sect. B 34 , 2038, 1978.
4. Vivek Polshettiwar and Rajender S. Varma .Acc. Chem. Res. 2008, 41, 5,629–639 – 2008.
5. Pranab Chandra Saha, Rabi Sankar Das, Tanima Chatterjee, Maitree Bhattacharyya, Samit Guha.Acs. Bioconjugate Chemistry 2020**,** *31* (5) , 1301-1306.
6. Dandan Hou, Joshua E. Bostwick, Jeffrey R. Shallenberger, Everett S. Zofchak, Ralph H. Colby, Qinfu Liu, ACS Applied Nano Materials 2020**,** *3* (2) , 962-968.
7. S. Padhye, Z. Afrasiabi, E. Sinn, J. Fok, K. Mehta; N. Rath. Inorg. Chern., 2005, 44 (5), 1154-1156.
8. Kannan.S and Ramesh.R, Polyhedron.2006; 25:3095.
9. Neelakantan.M.A, Rusalraj F, Dharmaraja Johnsonraja S,Jayakumar. T, Pillai.MS Spectralcharacterization,cyclic voltametry, morphology, biological activities and DNA cleavage studies of amino acid Schiff base metal (II) complexes. Spectrochim Acta 2008;71A: 1599-609.
10. N Raman, JD Raja, A Sakthivel - Russian Journal of Coordination ,2008 – Springer34, pages400–406.
11. Dharmaraj,N, Viswanathamurthi. P & Natarajan.K. Ruthenium(II) complexes containing bidentate Schiff bases and their antifungal activity. *Transition Metal Chemistry* **26,** 105–109 (2001)
12. Vhanale, B.T. & Deshmukh, N.J. & Shinde, A.T.. (2019). Synthesis, characterization, spectroscopic studies and biological evaluation of Schiff bases derived from 1–hydroxy-2-acetonapthanone. Heliyon. 5. e02774. 10.1016/j.heliyon.2019.e02774.

13. Abu-Dief AM, Nassr LAE. Tailoring, physicochemical characterization, antibacterial and DNA binding mode studies of Cu(II) Schiff bases amino acid bioactive agents incorporating 5-bromo-2-hydroxybenzaldehyde. J Iran Chem Soc 2015;12:943.
14. Emara AAA, Ali AM, El-Asmy AF, Ragab EM. Investigation of the oxygen affinity of manganese(II), cobalt(II) and nickel(II) complexes with some tetradentate Schiff bases. J Saud Chem Soc 2014;18:862.
15. G. Ayhan, N. Altanlar, Il Farmaco 58 (2003) 1345.
16. Y. He, B. Wu, J. Yang, Bioorg. Med. Chem. Lett. 13 (2003) 3253. 20. V.K. Limesova, J. Koci, K. Waisser,
17. Locasale, J.W., A.A. Napoli, S. Chen, H.M. Berman, and C.L. Lawson, 2009. Signatures of protein-DNA recognition in free DNA binding sites. Journal of molecular biology 386: 1054-1065.
18. Morris, G.M., D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew, and A.J. Olson, 1998. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. Journal of Computational Chemistry 19: 1639-1662.
19. Rodrigues, A.R.O., M.S.D. Carvalho, J.A.V. Cardoso, R.C. Calhelha, M.-J.o.R.P. Queiroz, P.J.G. Coutinho, and E.M.S. Castanheira, 2014. Benzothienoquinolines: New one-pot synthesis and fluorescence studies of their interaction with DNA and polynucleotides. Journal of Photochemistry and Photobiology A: Chemistry 294: 20-30.
20. SchuÃˆttelkopf, A.W., and D.M.F. Van Aalten, 2004. PRODRG: a tool for high-throughput crystallography of proteinâ€“ligand complexes. Acta Crystallographica Section D: Biological Crystallography 60: 1355-1363.
21. Schurter, E.J., H.J.C. Yeh, J.M. Sayer, M.K. Lakshman, H. Yagi, D.M. Jerina, and D.G. Gorenstein, 1995. NMR solution structure of a nonanucleotide duplex with a dG mismatch opposite a 10R adduct derived from trans addition of a deoxyadenosine N6-amino group to (-)-(7S, 8R, 9R, 10S)-7, 8-dihydroxy-9, 10-epoxy-7, 8, 9, 10-tetrahydrobenzo [a] pyrene. Biochemistry 34: 1364-1375.