STUDY OF THE EFFECT OF STIMULATIVE PROPERTIES OF ALLOXAN – THIOSEMICARBAZONE SCHIFF’S BASE LIGAND SYNTHESISED IN AN ECO FRIENDLY MANNER

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Abstract. Ligands are fascinating class of ions or molecule that binds to a central metal ion to form coordination compounds. Ligand ability to donate lone pair of electrons or capacity to act as “Lewis Bases” has created tremendous wave in pharmaceutical industry. Schiff’s bases are multifaceted class of compound formed by condensation of aldehyde or ketone with a primary amine under preliminary condition. The ligands are efficient enough to act as antibacterial, antiviral, anti-inflammatory, antifungal. Recent studies reveal their ability to exhibit antiproliferative, anticancer, anti oxidant properties is a signpost in drug chemistry. The present study focuses on the efficiency of environment friendly synthesized ligand Alloxan thiosemicarbazone which is screened for its antibacterial, antifungal, docking properties.

Keywords: Schiff base-Alloxan Thiosemicarbazone, Antibacterial, Antifungal, Docking.

1. Introduction:
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 synonymous to azomethine (RCH=NR’) where Nitrogen referred as azomethine Nitrogen. Thiosemicarbazones 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 pyrimidinetetrone is a heterocyclic compound with high biological and physiological effect on living organisms [1,2,3,4]. 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. Condensation of the Alloxan (ketone) with thiosemicarbazide (amino) group was carried out using citric acid in place of acetic acid. Its characterization IR, NMR, CHN correlated with the literature data [9,10].
Slightly modified structures of parent Schiff bases leads to enhance activity and reduce side effects relative to the parent molecule. The momentum in utilizing bioactive starting material those have vicinal carbonyl group on either side can provide bio-active sites. Alloxan-2,4,5,6[1H,3H] pyrimidine tetrone is a heterocyclic compound with high biological and physiological effect on living organisms. Alloxan is reported as an agent which selectively destroys pancreatic beta cells of mice which result in inducing permanent diabetes [1]. Chemical compounds that selectively damage pancreatic β cell damage constitute diabetogenic drugs. The defined mechanism of alloxan to induce diabetogenic mechanism is not clearly understood, evidence indicates the pancreatic 13-cell damage of alloxan is due to generation of cytotoxic oxygen free radicals. This property of alloxan is also attributed due to its ability to fragment DNA.

Thiosemicarbazide act as proficient 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. Thiosemicarbazones are of notable interest because of their compatibility to condense with aldehydes or ketones readily. Thiosemicarbazones are effective chemotherapeutic, broad spectrum agents. Conjugated N-N-S tridentate ligand system thiosemicarbazide seems important for anticancer activity[3]; structural alterations that hinder thiosemicarbazone ability to function as a chelating agent tend to destroy or enhance medicinal activity[2,8].

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. Chemists think of sustainable development in terms of preserving environment for the future. Disposal of hazardous chemical waste can be prevented by neutralizing chemicals to appropriate pH or green method of synthesis can be designed leading to sustainable development. In present work we present the synthesis, characterization, biological activity of Schiff’s base ligand synthesized. Acid catalysed condensation reaction of carbonyl compounds with amines is carried out using citric acid (lemon) leading to green technology.

A non classical heating technique using microwaves which is termed as “Bunsen burner of the 21st century” is catering to the needs of the present scenario. This method dramatically reduces reaction times, also reducing the disposal of heat to the environment. The significant outcome of microwave assisted reaction results in development of imitation protocols for drugs. The use of emerging technique in conjunction with greener reaction media dramatically reduces chemical waste and reaction times in organic synthesis and chemical transformations. Synthetic chemistry community are under pressure to produce in an environmentally benign fashion, the multitude 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 [4,5,6]. In our present work the ligand was synthesised through microwave assisted reaction which in the innovative technology compared to classical method using reflux method.

2. Materials/Reagents

The analytical grades Thiosemicarbazide (LOBA) Alloxan (LOBA) Mueller Hilton Agar (HIMEDIA), Potato Dextrose Agar (HIMEDIA), were used as received.

2.1. Methods:
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 efficiency whereas the yield obtained with classical heating under similar conditions did not exceed 50%.

2.2. Antibacterial Activity:
In-vitiro biological activity of the synthesized ligand was investigated for its antibacterial activity. Agar well diffusion method was used to determine bacterial sensitivity test. The sample was assessed for four bacterial cultures Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Staphylococcus. The ligand activity was tested against standard drug streptomycin.

2.3 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.

2.4. Media preparation:
150ml of Mueller Hinton Agar (Composition: Acid Hydrolysate of Casein: 17.50 g, Starch: 1.50 g, Beef Extract: 2.00g, Agar: 17.00 g, 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.

2.5. 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.

3. Antifungal Activity:
Minimum Inhibition Concentration (MIC) technique was followed to check the Antifungal activity for the synthesized ligand. The technique was followed by Agar diffusion method. The sample was
assessed for anti-fungal activity against three fungal strains-\textit{Aspergillus niger}, \textit{Aspergillus flavus} and \textit{Candida albicans}.

3.1 Sample preparation:
10mg of the synthesized Al- TSC ligand was dissolved in 1mL of DMSO (Dimethyl sulfoxide) in sterilized 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, 50μL Control (DMSO) in the middle well. The ligand activity was tested against a standard drug clotrimazole which is used as a control.

3.2 Media preparation:
Potato Dextrose Agar (PDA composition: Potato-200g, dextrose-20g, agar-20g, distilled water-1000ml) was prepared by boiling 200g of potato in 500ml of distilled water and it was filtered. The remaining components were added into the filtrate and the volume was made upto 1000ml with distilled water. The content was then autoclaved at 121°C for 15 minutes.

3.3 Fungal plate preparation:
Approximately 30ml of media was poured into the sterile petri plates and it was allowed to solidify. Later, fungal cultures of 100μL inoculums of \textit{Aspergillus niger}, \textit{Aspergillus flavus} and \textit{Candida albicans} was poured into respective plates and it was spread thoroughly using a plate spreader. On the agar plates, five wells measuring 5.5 mm was made using well borer in respective plates. The wells were filled with aliquots of sample in respective wells and 50μL as the Control DMSO blank was added in the middle well. The culture plates were incubated at 25°C for 72 hours. The zone of inhibition was measured and recorded in millimeters (mm) formed around the wells. Fluconazole is the standard antifungal used.

4. Molecular docking studies:
4.1 Protein and ligand structure preparation
The compound is designed and energy minimised 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 \textit{B}-DNA (1HQ7: GCAAAACGTTTGC sequence[17] and a structure with nine base pairs intercalated with benzo[\textit{a}]pyrene diol epoxide (BaP) (1DXA: GGTC[BaP]ACGAG sequence [21] from which the intercalator was removed (1DXA*). The Lamarckian Genetic Algorithm (LGA) was chosen. In the first stage, docking with the BaP were 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 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. Bacteria is protected against its surrounding by a membrane, integrity of that membrane is essential for its survival. It is made up of basic components like lipo polysaccharides, phospholipids and is more stable because of Ca$^{2+}$, Mg$^{2+}$ ions. 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. The lipid membrane surrounding the cell favours the passage of only lipid soluble materials. Thus lipophilicity 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.

| Concentration | Escherichia coli | Pseudomonas aeruginosa | Bacillus subtilus | Staphylococcus |
|---------------|------------------|------------------------|------------------|---------------|
| 100 μg        | 14               | 19                     | 15               | 12            |
| 200 μg        | 16               | 23                     | 18               | 16            |
| 300 μg        | 19               | 28                     | 20               | 22            |
| 400 μg        | 22               | 30                     | 22               | 25            |

Table 1: Antibacterial Property Ligand

| Concentration | Escherichia coli | Pseudomonas aeruginosa | Bacillus subtilus | Staphylococcus |
|---------------|------------------|------------------------|------------------|---------------|
| 100 μg        | 16               | 22                     | 17               | 11            |
| 200 μg        | 17               | 24                     | 19               | 18            |
| 300 μg        | 19               | 29                     | 22               | 24            |
| 400 μg        | 21               | 31                     | 23               | 26            |

Table 2: Antibacterial Property Standard Streptomycin
Table 1. Results show that the antibacterial activity of the ligand was screened using the 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 Pseudomonas aeruginosa. It is a rod-shaped bacterium that can cause disease in plants and animals, including humans. This gram negative bacteria is a multi drug resistant pathogen associated with serious illnesses like hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes. It has a capacity of extensive colonization, and can aggregate into enduring biofilms.

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. 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 [12]. Literature studies reveals metal complexes to be more potent antibiotic than the ligand itself in most of the investigations. Future study may be carried on the metal complexes to test the potency. [11,12,13,14 15,16]. Graph 6 also indicates the same observation.

**Figure 1. Zone of inhibition Ligand**

**Figure 2. Zone of inhibition Standard Streptomycin**
Figure 3. Alloxan Thiosemicarbazone

Figure 4. Alloxan Thiosemicarbazone/ Streptomycin

5.2 Anti fungal activity:
In vitro antifungal activity was studied for the ligand Alloxan thiosemicarbazone where Table 7 indicates for some species with increase in concentration it shows almost same inhibitory action. This kind of observation is also seen in Table 8 where increases in concentration almost shows same zone of inhibition for the standard fluconazole used.
Standard fluconazole is used to treat and prevent candidiasis, infection caused due to fungus Candida. It is known to cause infection of lungs, throat, esophagus and blood. Pharmaceutical fungicide or fungistatic used to treat and prevent mycosis such as athlete's foot, ringworm, also used to treat meningitis. The standard drug works by blocking the ability of fungi to reproduce and helps to get rid
of the infection. The synthesised ligand exhibits inhibition towards all the three organism Aspergillus niger, Aspergillus flavus, Candida albicans. The organism which effectively showed greater zone of inhibition was Aspergillus niger which is known to effect humans to lesser effect. The increased lipophilicity of the ligand leads to breakdown of the cell permeability barrier.

Table 3. Antifungal Property Ligand

| Concentration | Aspergillus Niger | Aspergillus flavus | Candida albicans |
|---------------|------------------|-------------------|-----------------|
| 100 μg        | 13               | 15                | 9               |
| 200 μg        | 15               | 11                | 14              |
| 300 μg        | 17               | 11                | 14              |
| 400 μg        | 17               | 12                | 15              |

Table 4. Antifungal Property Standard Fluconazole

| Concentration | Aspergillus Niger | Aspergillus flavus | Candida albicans |
|---------------|------------------|-------------------|-----------------|
| 100 μg        | 18               | 20                | 29              |
| 200 μg        | 20               | 23                | 30              |
| 300 μg        | 23               | 25                | 35              |
| 400 μg        | 23               | 25                | 35              |

Figure 5. Zone of inhibition Ligand
Figure 6. Zone of inhibition Standard Flucanazole

Figure 7. Alloxan Thiosemicarbazone / Standard Flucanazole

Ligand acts as a bidendate ligand coordinating through azomethine nitrogen and contains thiol- thione form which confers fungicidal property[22]. Literature study reveals that complexes show more activity than the ligand. Further studies need to be carried to find the efficacy of the metal complex.
5.3. Molecular docking studies

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

| Compounds | Docking | Nucleic acid | Type of binding | Binding Energy (Kcal/mol) | IC$_{50}$ | Hydrogen Bonds |
|-----------|---------|--------------|-----------------|--------------------------|----------|----------------|
| Ligand    |         | A 1HQ7       | Minor groove    | -5.86                    | 50.31 μM | 1hq7_1:B: DG23:H21 Ligand: N |
|           |         | B 1DXA*      | Intercalation   | -4.68                    | 0.33 μM  | 1hq7_1:A: DA4:H3 Ligand: O |
|           |         |             |                 |                          |          | 1hq7_1:A: DA3:H3 Ligand: O |
|           |         |             |                 |                          |          | Ligand: H |
|           |         |             |                 |                          |          | 1hq7_1:A: DA3:O4' |
|           |         |             |                 |                          |          | Ligand: H |
|           |         |             |                 |                          |          | 1dx_1:A: DA5:N6 |
|           |         |             |                 |                          |          | Ligand: H |
|           |         |             |                 |                          |          | 1dx_1:A: DC6:O4' |
| BaP       |         | A 1HQ7       | Minor groove    | -9.55                    | 99.67nM  | no hydrogen bonds formed |
|           |         | B 1DXA*      | Intercalation   | -9.22                    | 174.77nM | BAP:A:BAP10:HO2: 1dx_1:A: DC6:O4' |
|           |         |             |                 |                          |          | BAP:A:BAP10:HO3: 1dx_1:A: DC6:O2 |
Figure 8. Ligand- Nucleic acid interactions at 1HQ7

Figure 8: 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 9. Interaction of Ligand at Intercalation site at 1DXA*

Figure 9: 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.
5.4: Molecular docking interaction details of ligand and the reference ligand BaP

Docking is a molecular modeling technique that is used to predict interaction of protein small molecules like ligands. The associations between biologically relevant molecules such as proteins, peptides, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Docking is useful for predicting both the strength and type of signal produced.

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.

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 BaP, 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.

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). 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 is 5'-D(* GP * GP * TP * CP * AP * CP * GP * AP * G) - 3'5' - D ( * CP * TP * CP * 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 -4.68Kcal/mol. From the above data ligand had more efficiency of association through hydrogen bonding at the minor groove than at the intercalation site.

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. This value proves to be potent drugs which can bring inhibition of multiplication of cells.

6. Conclusions:

Schiff bases are treated as significant class of compound because of their ability to form complex with transition metal ions and of their pharmacological properties. The increasing therapeutic value of these potential Schiff’s base complexes urges chemists to find new methods that lead to sustainable development.

Alloxan Thiosemicarbazone ligands was synthesized in 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.

Future work in this lab entails the synthesis of transition metal complexes series using metal (acac)$_2$ 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.

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