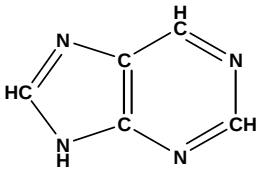
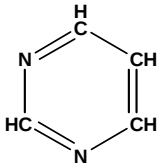
Introduction & Literature Review

1.1 Nitrogen Bases

There are two kinds of nitrogen containing bases – purines and pyrimidines. Purines consist of a six – member and a five – member nitrogen – containing ring, fused together. Adenine and guanine are purines type found in DNA and RNA. pyrimidines have only a six – member nitrogen – containing ring , Cytosine, Uracil and thymine are pyrimidines type . Cytosine is found in both DNA and RNA. Uracil is found only in RNA. while thymine is normally found in DNA. Sometimes tRNA will contain some thymine as well as uracil.

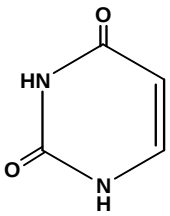


Purine ring

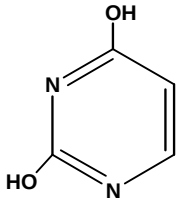


Pyrimidine ring

In fact these bases considered as aromatic molecules forming (lactam) or (lactim) which are Tautomers [1]



Lactam (keto) form

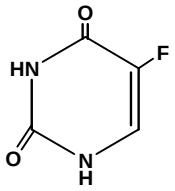


Lactim (enol) form

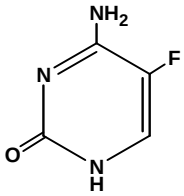
these derivatives and iso-derivatives are processed the nitrogen base are wide used in medical and clinics medical. The most iso-derivatives which

used are purine and pyrimidine rings with modified group could not formed as naturally .

These derivatives and iso derivatives have activity to change the double bases. For example some derivatives[2], 5- Fluoro Uracil (Fura)[3] (1) , 5 - Fluoro Cytocine [4] (2) , 6- Marcapto purine (3)



(1)



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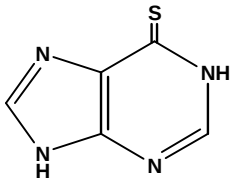
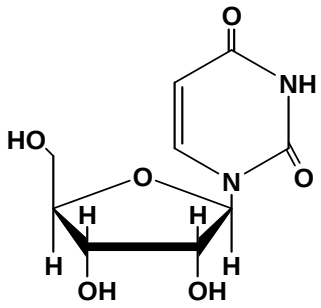


(3)

1.2 Nucleosides

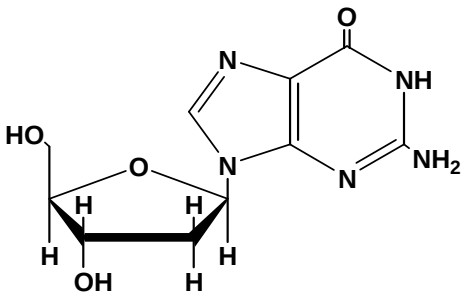
If a sugar, either ribose or 2-deoxyribose, is added to a nitrogen base, the resulting compound is called a nucleoside. Carbon 1 of the sugar is attached to nitrogen 9 of a purine base or to nitrogen 1 of a pyrimidine base. The names of purine nucleosides end in (-osine) and the names of pyrimidine nucleosides end in (-idine). The convention is to number the ring atoms of the base normally and to use l', and soon. to distinguish the ring atoms of the sugar. Unless otherwise specificed, the sugar is assumed to be ribose. To indicate that the sugar is 2'-deoxyribose, a deoxy is placed before the name.[5]

- Adenosine
- Guanosine
- Uridine
- Thymidine



Uridine

(4)



Guanosine

(5)

1.3 Biological activity of nucleoside analogues

The nucleoside is considered as a basic molecule structure of nucleic acid (RNA & DNA) and this today consider which has a very Biological activity in processing many of medicine which used in many tratments as antivirus, bacteria, fungi, anticancer and AIDS because its activity that stops the growthing the pathogenic cells[(6)] . Since discoursed nucleoside and studding its activity in this biological veiled for few years ago.

The derivatives of nucleoside which can used in a wild feild as biological drugs used as antivirus, antibacterial, anticancer and antifungal.[(7)]

The most nucleoside derivatives dideoxy nucleosides (ddNs) which has tested anti biological basically depends on this pyrimidine and purine circle system show in fig (1-1)and (1-2) which examples to show the relationship between the structure and activity for purine and pyrimidine .[8,9]

Nucleoside antibiotics are consider as groups of substances which similar in structure of nucleoside. For example to these anti side is 3-amino-3-deoxyadnosine (6) the biological anti side, this compound has activity for cancer the Adenine arabinoside (7) [10] compound which used also as medicine for cancer and the substance for anti viruses in human which used for brain viruses that consider as neural disorders which caused by the Herpes virus. Another iso nucleoside for anti virus which is used for treating AIDS caused by Human Immunodeficieny Virus (HIV) it 3-azido-3-deoxythymidine(AZT)[11,12] (8)and it's the first treatment used for (AIDS) in USA since 1990.

And also 5-Fluro deoxyuridine (9) has also used as anti cancer, While Puromycin (10) consider as anti bacterial and it's only anti can used to protein synthesis in bacteria.

The iso nucleoside had used as antibiocidal or fungicides (anti fungal) which used the compound (11) as anti fungal which damages the cell wall.[13,14]

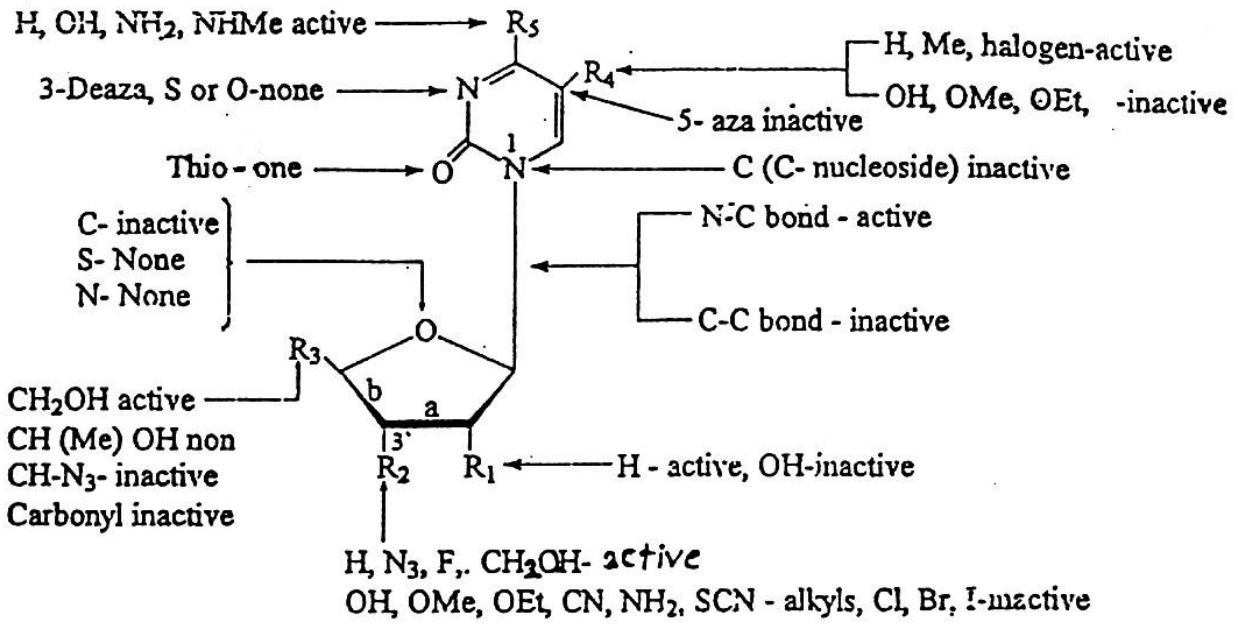


Fig. (1-1): Relationship between structure and activity of iso pyrimidines

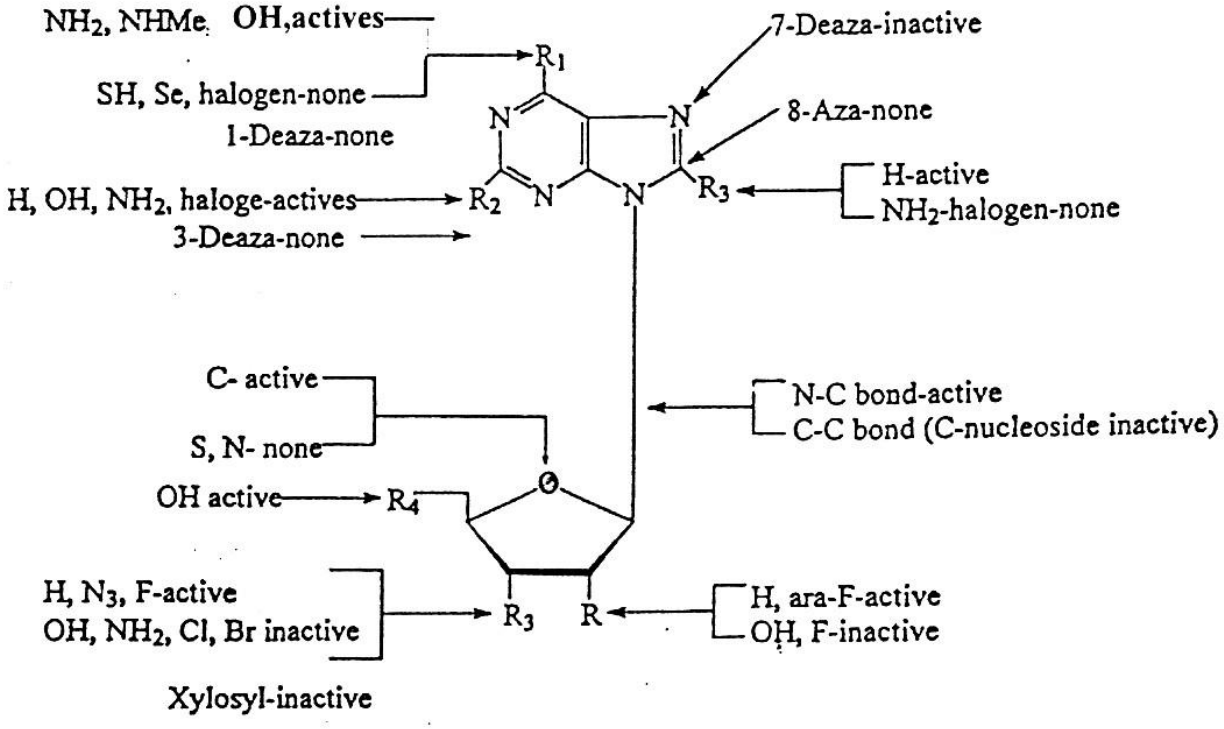
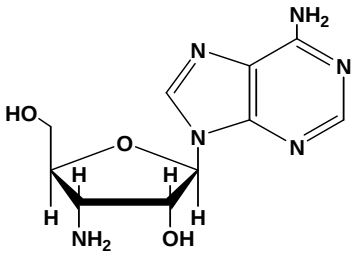


Fig. (1-2): Relationship between structure and activity of iso purines



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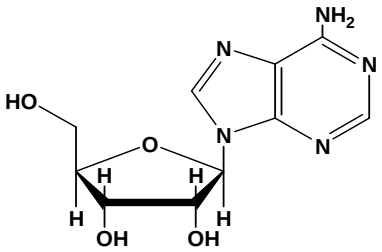

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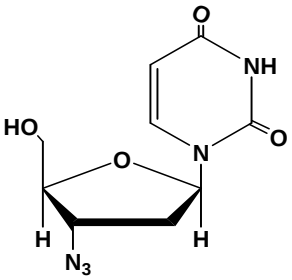

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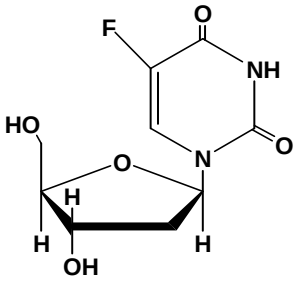

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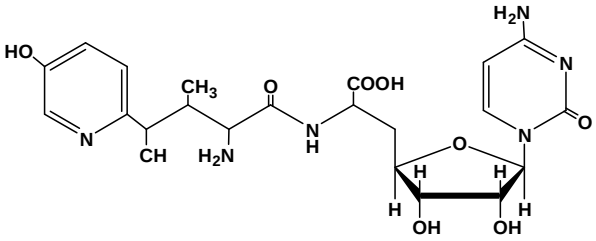

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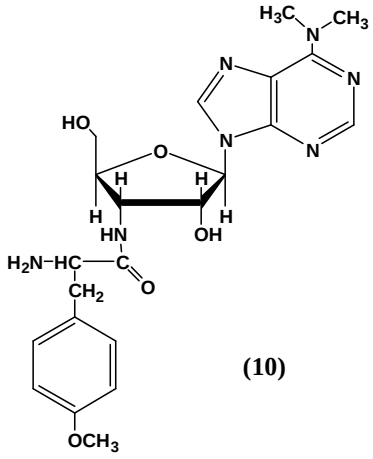


(10)

1.4 AZO Compounds

they are organic compounds consist of two groups of homogenize and non homogenize organic which joined with azo group. They have with gained this group a high stability because it has a double bound and may be joined this groups with multi groups and different aliphatic groups or aromatics[15], since 1858 the chemist scientist Peter Criess[16] had discovered the azo compounds that he had found the primary Aromatic amines are reacting quickly with Nitrous acid (HONO) at low temperature (0-5 ^{0}C) to produce salt which is easy water soluble and had given name of diazonium a salt which are prepared from different ways. The most important way of these is of this ways are coupling for diazonium salt with primary, secondary , tertiary amino aromatic and phenols .

The important method to prepare diazonium salts are :

1. Direct method[17]:

Diazonium salts is prepared by adding $NaNO_2$ to aromatic amine salt with continuous stirring while adding the salt the temperature is kept. between (0-5 ^{0}C)

2. Indirect method:

Soluble aromatic amine in solution base is added to efficient amount of $NaNO_2$ with increasing amount of dilute acid which it cause to have diazonium indirect method ,this method is depending on the amines that have acid group.

3. Witts method[18]:

In this method it has used a weak amino which is insoluble in the dilute acid so it solves in nitric acid meta Di solphate was added to solution (activity of this matter is reduced the some of the conc. Nitric acid and then converted to form Diazonium . This method was also with amines which is not effected by Nitric acid.

4. Knoevenagels method[19]:

In this method the Diazonium salt was prepared for some amines by heating the salt with alcohol solution of Amyl nitrite which is precipitate the Diazonium salt by adding Ether to the reacting solution.

5. Preparation of Diazonium salts for heterocyclic amines[20]:

To preparation of diazonum salt from heterocyclic amines must carried out special condition and high efficiency to control the temperature and degree of acidity,

Azo compounds have strong colors which are easily recognized depending on its structure some of these colours are yellow, red, orange and green which appeared in compounds that have unsaturated group causing the colors. The azo group (-N=N-) is a strong groups donor the color when joined with group that appeared the color as(NH_2, OH, Ar) the other groups which contain the hydrogen atom that able to be replaceto form fixed colors which acting as Chelating Ligand that can

joined with different elements to form azo element complex[21] so the most of these dyes are used in sewing working and other of these compounds are very sensitive to the acids and bases, as they appear a special colors that can recognize one from other clearly so that used as indicators in analytical chemistry[22]

Generall, the azo compounds can be divided into the following[21]:

a. Mono azo compounds:

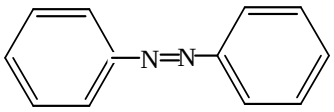
These compounds contain a two groups which joined with azo group such as(12).

b. Bis Azo compounds:

These compounds contain a three groups which joined with each athers by two groups of azo such as (13).

c. Heterocyclic system:

These compounds contain heterocyclic system which joined with azo group such as (14).



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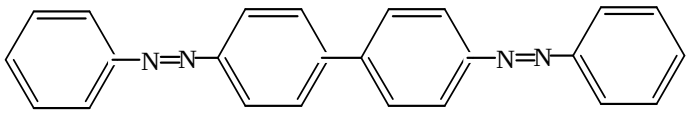

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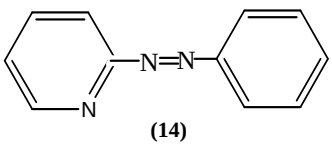
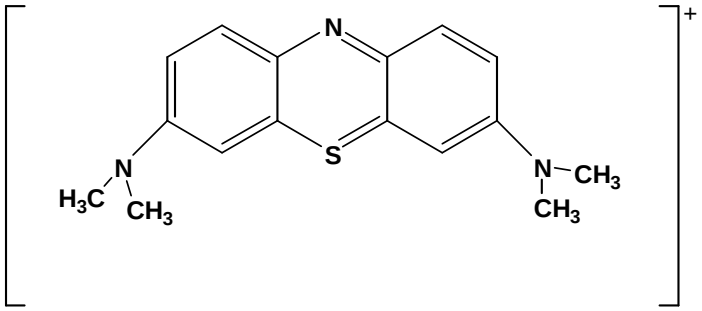


(14)

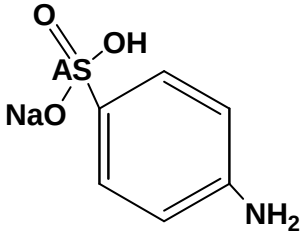
1.5 The Biological Activity of chemical compounds

Paul Ehrich is considered as the pioneer of medical chemistry who had know as first knowledge that range of the biological effects from some pigments and he had studied the effect of methylene blue (15) on the malaria organism as anti malarial



(15)

also he had discovered another anti malaria, for example Trypan red in 1904 which was considered as one of the azo pigment and they knew Atoxyl (16) material as anti malaria when malaria was caused death for hundreds of people in Europe.



(16)

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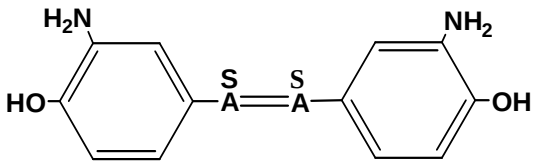
So Eurich was continued in his researches on active compound as different organisms and in 1909 he has found anti Salvarsan (17) which knew as one of Zaranach compound.



(17)

Many researches in chemotherapy which Ehrich has founded in 1953 and the Pronsil material as one of azo compounds were used to anti malaria and antibacteria[23].

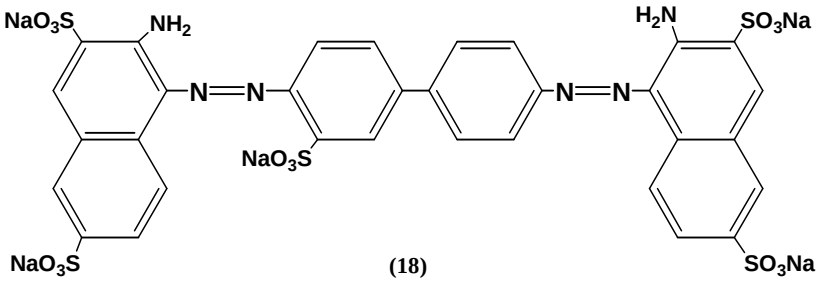
1.6 The biological activity of AZO compounds

The Ligands of azo compound rings are Hetero cyclic compounds and identified in its importance in biological[24] because these compounds contains of different atoms such as oxygen , nitrogen and sulphate which make it to join with different elements, also there compounds were used in pigments of tissues for this knowledge made it very important in industrial processing.[25]

The properties and activity of these compounds were studied by many researchers which Hetero cyclic compounds were gained the big importance in the decades. The Biological effects of these compounds

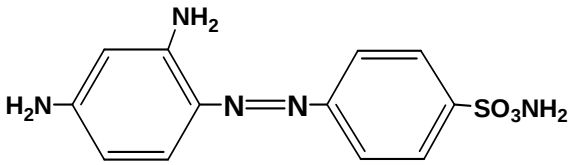
were used in the researches of cancer[26,27] in addition to the multi types of these compounds which were used as anti organisms[28] .

The azo compounds have many medical effects and used as medicine such as (18) which is used as anti malaria



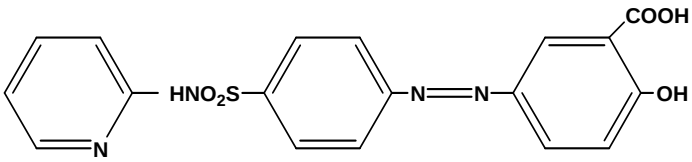
(18)

Also the substance of one azo compounds the prontosil (19) is used for anti malaria and anti bacteria.



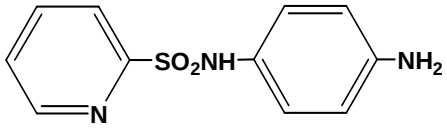
(19)

Another azo compounds which medical effects was Salicyl azo sulfopyridine (Azulfidine) (20) which is used to ulcerative colitic.



(20)

The other that is slowly absorbent in digestive system was sulfapyridine (21) which form an amino salicylic acid (22) after broking azo bonded .



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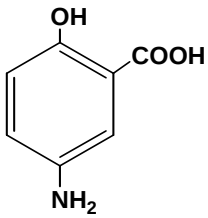
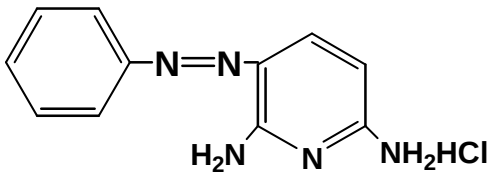


(22)

2,6-diamino-3-(phenyl azo)pyridine monohdrochloride (23) is medical known as Pyridium which is soluble in water and used as a drug for uric system as anticeptic and antibacterial[17]

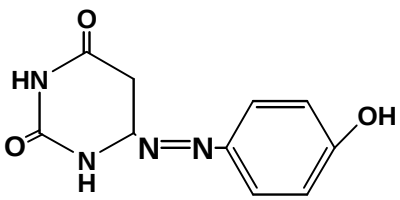


(23)

Azo compounds were also used (2,2dioxy- azo benzene dicarboxylic acid and 2,2dioxy-3,3dicardoxy- 5,5azo benzene – disulphonate to study the its effects on killing of some organisms such as *Staphlo cocc*us and *Sterpto coccus* and its found these compounds have effect on these organism[29].

For another examples that used in medical valid is 6-(p-hydroxy phenyl azo)-uracil (24) and 6-(p-hydroxy phenyl azo)- cytosine (25) which are used as antibacterial degrading on its Biological activity which inhibites the processing the DNA in bacteria's cell gram positive after reducing the

medical compound to Hydrazine (26)in biosynthesis in the bacteria cell [30]. Before act as an inhibition substance which is happened by azo reductase that is produced by the bacteria found in human intestine to reduce the azo compound to be more activity [31].



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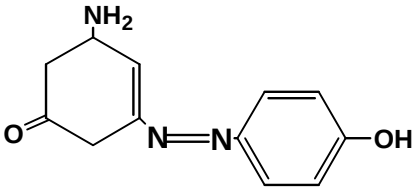

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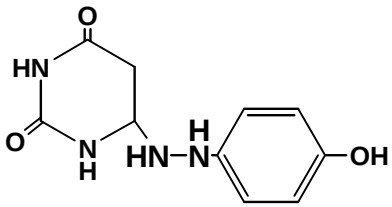
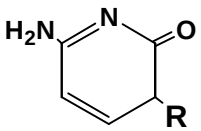
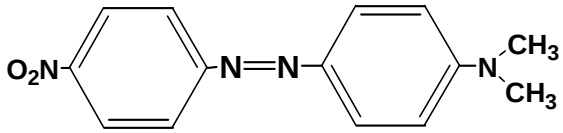




R: the nucleotide residue

(26)

In 1994 had prepared the N,N-4Dimethyle -4-nitro azo benzene (27) and that year had studied the genetic toxic to this compound outside of body (invitro) on the four bacteria of Salmonella and it' s found this compound which is made a mutation on all bacteria dynasties which tested and also was studied the effect of this compound inside the human body (invivo) on genetic material and inside mice liver and these studies did not indicate any impact of this compound on the genetic materials[32].



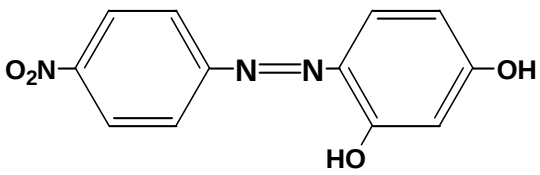
(27)

In 1994 A.Kalski [33] and his group had studied the effect of toxicity of these azo compounds derivative from thiadizole with 2-naphtol and p-chloro phenol in the in vivo on the mice bone marrow and in vitro human blood while this study was not pointed to any toxic effects of these compounds, so we can used these compounds in medicine valid.

In 1994 two compound of organic azo had prepared:

p-nitro phenyl azo resorcinol (28) , m-nitro phenyl azo salicylic acid (29) and its new complexes with trance elements: (Cu , Zn , Co , Ni , Cd , Mn , Cr) and were studied the biological activity for anti organisms.

These compounds and there complexes have high activity for *S. aureus* bacteria, while didn't give any activity for *E.coli*[34] .



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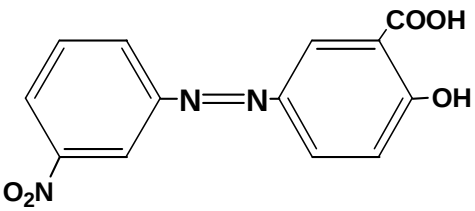


(29)

1.7 Mechanism of AZO compounds and other antibacterial activity

The antibacterial is divided, according to its acts on the organism's cell [35], into the following:

1. Inhibitor of cell wall

Including compounds that have **β**_lactam such as Penicillin, monobactam, cycloserine and bacitracin

2. Inhibitor of protein synthesis

Includes the same compounds of Amino glycosides such as tetracycline ,chloramphenicol and fusidic acid.

3. Inhibitor of cytoplasmic membranes

Includes Polymixins such as Amphotercin and Nystain.

4. Inhibitor of Nucleic Acid

Includes three types of inhibiter:

A- Inhibitors of starting material;

Such as sluphonamide and trimethoprin .

B- Inhibitor of RNA Polymerase;

Such as Rifampicin .

C- DNA replication inhibitors:

They includes many compounds such as azo compounds. The vital action of azo compounds depend on its ability to inhibit DNA circle sensitive bacterial cells, and the inhibiting action of these compounds is performed by two stages:

1- Azo compound must be reduced to hydrazine before it works as an inhibitor, and that occurs as metabolize action in bacteria cells [(36)]. The studied point to that bacteria in human intestine have the ability to reduce azo compounds by azo reductase enzyme which is produced by these bacteria to reduce azo compounds to become more active.

2- Three DNA polymerases, I, II, and III are isolated from *Bacillus subtilis*. It was found that one of the enzymes DNA Polymerases III can only be inhibited the reducing of azo compounds, where the interpenetration of these medicines (Azo compounds) with DNA figure (1-3) depends on the abnormal organization of the nitrogen bases pairs. This order of nitrogen bases prevents (purine DNA) to settle in their right position the created compound between treatment and DNA is the main factor to make treatment, for it reacts with the primer stand by making basic pairs with 3-OH and this connection gives the compound a stability. The compound also reacts with unpaired pyrimidine in the template strand to make un-natural pairing, and the aromatic part of treatment is thought that it deposits with aromatic amino acids DNA-polymerases III in Hydrophobic which is next to the active part of that enzyme[(37)].

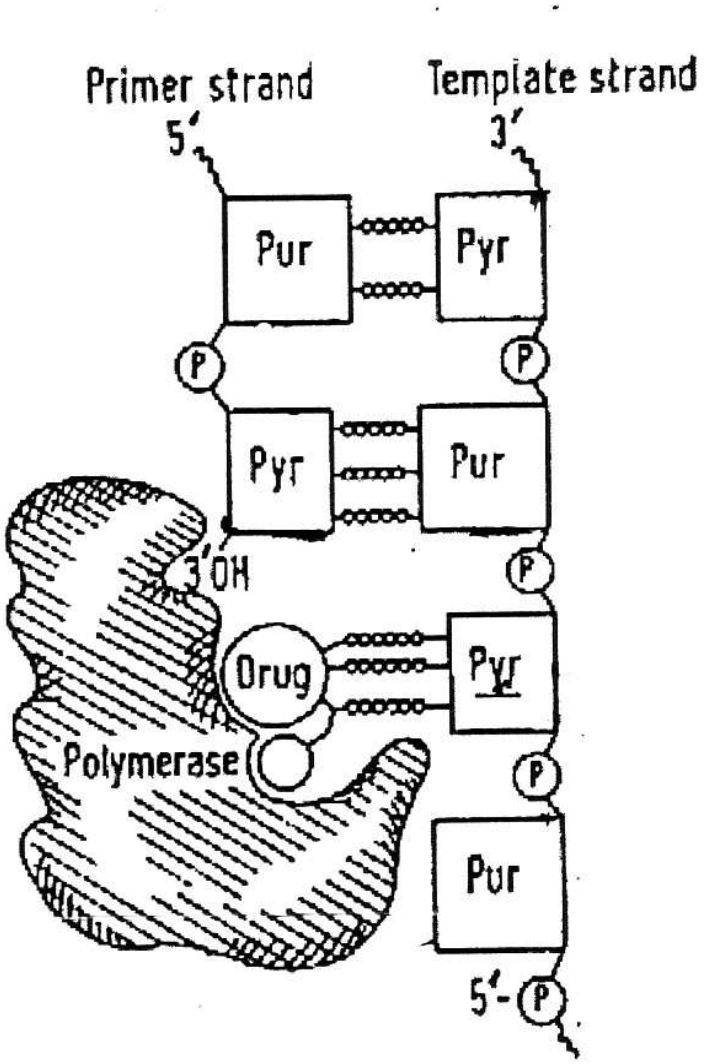


Fig. (1-3): Assumed Mechanism of treatment in contact with the DNA and inhibition of the (Polymerase) enzyme action[37]

1.8 Pathogenic bacteria

The pathogenesis of bacterial infection includes initiation of the infectious process and the mechanisms that lead to development of signs and symptoms of disease .Characteristics of bacteria that are pathogens include transmissibility, adherence to host cell invasion of host cells and tissues. Toxigenicity, and ability to evade the host's immune system. Many infections caused by bacteria that are commonly considered to be pathogens are in apparent or asymptomatic. Disease occurs if the bacteria or immunologic reactions to their presence cause sufficient harm to the person[45].

Human and animals have abundant normal flora that usually do not produce disease but achieve a balance that ensures the survival growth and propagation that are of both the bacteria and the host. Some bacteria that are important causes of disease are cultured commonly with the normal flora *(Streptococcus pneumoniae, Staphylococcus aureus*). Sometimes bacteria that are clearly pathogens (*Salmonella typhi*) are present but infection remains latent or sub clinical and host is a carrier of the bacteria[45].

1.8.1 Staphylococci

They are gram positive spherical cell. Usually arranged in grape like irregular clusters. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of normal flora of skin and mucous membranes of humans, a variety of pyogenic infections. And even fatal septicemia. The pathogenic *staphylococci* often hemolyze blood. Coagulate plasma and produce a variety of extra cellular en-zymes and toxins. The most common type of food poisoning is caused by a heat-stable *staphylococci* rapidly develop resistance to many antimicrobial agents and present difficult therapeutic problem.

The genus *Staphylococcus* has at least 30 species. The three main species of clinical important are *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*.

Staphylococcus aureus is coagulase – positive which different it from the other species. S aureus is a major pathogen for human. Almost every person will have some type of *S aureus* Infection during a lifetime[(45)].

1.8.2 Escherichia coli

They are gram negative cells, part of normal flora and incidentally caused disease, while others, the salmonellae and shigellae, are regularly pathogenic for humans. *E. coli* cause diarrhea are extremely common world wide. These *E. coli* are classified by characteristics of their virulence properties and each group caused disease by different mechanism. The small or large bowel epithelial cell adherence properties are encoded by genes on plasmids. Similarly, the toxins often are plsmid or phage-mediated. Some clinical aspects of diarrheal diseases.

Enteropathogenic *E. coli* (EPEC*)* is an important cause of diarrhea in infants especially in developing countries. EPCE adhere to the mucosal cell small bowel [(45)].

1.9 Pathogenic Fungi:

Fungi cause direct harm to man and animals by means of toxins or inducing allergic reactions, or by progressive infection (mycoses).About one – fifth of the world's population, suffer or have suffered from mycoses. In man, most infection involve the skin, hair and nails (Superficial mycoses). These mycoses are unpleasant and can be difficult to cure, but will not normally be lethal. Infections inside the body (deep – seated mycoses) are much more dangerous. They may become generalized and be fatal unless treated. The agents of most superficial mycoses are a group known as dermatophyton, belonging to genera. *Microsporium, Epidermophyton and trichophyton*. The various different dermatophytes usually cause virtually identical lesion if the infection involve the same part of the body [47].

Dermatophytoses refers to infections of the skin, nail or hair that are caused by classified as dermatophytes.

Normally, it is not considered that dermatophytic fungi can systemic disease, in fact, with only one minor exception; none of these fungi can grow at 37^0C .

The dermatophytic fungi include numerous species of fungi which contained in three major genera. These organisms occur world wide, mainly in soil and on certain animals. Within a given locale one never sees all of these organisms causing diseases. Dermatophytoses are some

of the most common diseases of man. Although incidences of infection vary greatly, at least 10 – 20% of the world's population may be infected with these organisms. An outstanding feature of these organism is that they are all keratinophilic which means that love keratin [48].

1.9.1 Candida albicans:

Candida albicans is ubiquitous, dimorphous yeast; it has been known for many centuries and has the potential to cause human diseases under specific circumstances and condition . the actual taxonomy of Candida albicans and related species has only been confirmed within the twentieth century . The oral carriage rate for the organism is high, with nearly one half of the healthy population harboring the organism. Numerous predisposing factors for oral condidiasis have been recognized including metabolic, dietary, mechanical and iatrogenic factors. Multiple clinical forms of the disease including acute, chronic and mucocutaneous presentation. Although rarely fatal in the absence of other serious underlying disease, oral candidiasis may serve as a useful clinical marker for the presence of significant predisposing condition [49] .

Candida albicans is a component of the normal skin flora and also the chief cause of the mucocutaneous fungal disease in humans [50]. Candida can also infect fingernail, producing onychomycosis and paronychia and is more common with advanced HIV disease [51].

Candida albicans is the yeast pathogen most frequently isolated from patients with fungemia : Oropharyngeal candidiasis is the most common opportunistic infection immunodeficiency virus infected patients[52] .

1.9.2 Trichophyton rubrum:

Trichophyton rubrum occurs world wide because of the serverity and longevity of the disease and its refractivity to therapy, this organism. causes many problems. Very often *T. rubrum* is the cause of long – established foot and nail infection. *T. rubrum* many also cause tinea corporis. Skin lesions caused by *T. rumbum* often have a red margin; the central portion to be relatively clear, although scaling may be apparent. On rare occasions, this organism may cause tinea capitis. Infected hairs do not fluoresce and hair invasion is endothrix. Primarily, these organisms are anthropophilic. Because cultures of *T. rubrum* are quite variable, identification can be frustrating.

This fungus is white and very fluffy and exhibits many aerial hyphae, this downy from is most is most commonly isolated from cases of chronic tinea pedis and tinea corporis. The other from of T. rubrum is called the granular form. This white colony is very flat, lacks aerial and has a pronounced granular appearance [48].

Trichophyton rubrum is the most common isolated from all region except the scalp. *Tinea* pedis usually of the *moccasin* type is the most frequently seen form of disease [53].

1.9.3 Trichophyton mentagrophytes:

This fungus a world wide cause of althlete's foot. It also can cause tinea capitis, tinea corporis, tinea barbae cruris. In many instances these infection are sporadic. According to many authorities infection by *Trichophyton mentagrophytes* are not difficult to cure. There are several different variants of this organism; some are anthropophilic, while other are zoophilic. If hair is infected, the infection is endothrix [48].

Trichophyton mentagrophytes is a cosmopolitan and is the most commonly isolated dermatophyte from man and animal.

Typically colonies of *Trichophyton mentagrophytes* tends to produce deeply pigment colonies [54].

Trichophyton mentagrophytes has several different colonial forms. One major form is called " downy " because the culture is white, very fluffy and grossly has downy appearance. Usually this form is anthropophilic.

The other major form of Trichophyton metagrophytes is " granular " ; usually this colonial type is zoophilic in origin and when it infected man, may induce considerable inflammation [48].

The reverse side of the colony is rose brown, occasionally orange to deep red in color [55].

Trichophyton mentagrophates is the species of *Trichophytom* most commonly isolated in clinical laboratories and its produce a positive urease reaction within two or three days [56].

The aim of the study

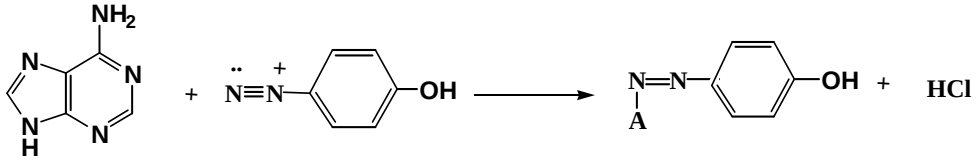
1- Using one of the devices or known procedures in organic preparation, for the Diazonium salts basically in preparing several kinds of azo compounds as derivatives of purine & pyrimidine.

2- Studying effect of compounds on several types of bacteria and fungi to estimate their biological activities.

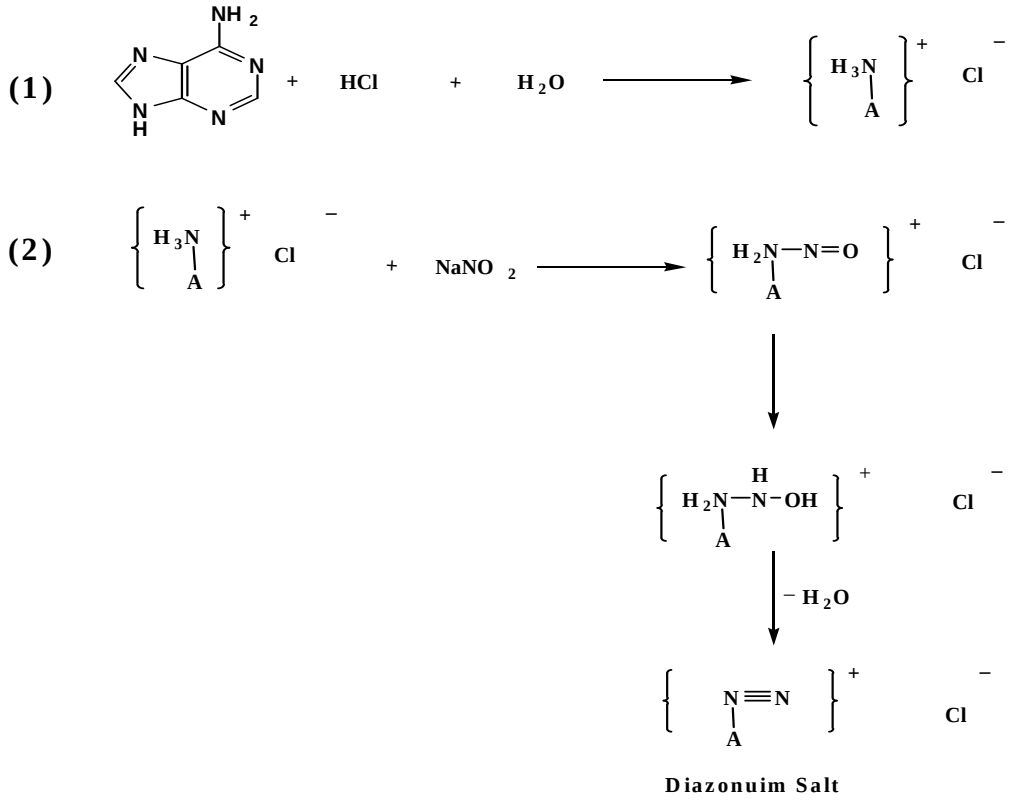
Result and Discussion

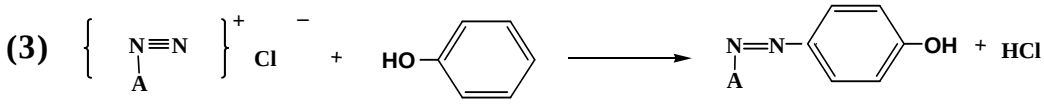
3. 1 Preparation of Azo Compounds

Azo compounds have a wide range of biological effect. Thus several compounds were made by coupling equivalent amounts of phenyl diazonium salts with nitrogen bases (purines , pyrimidines) as in the following equation.



The reaction includes an electrophilic attack of diazonium ion on carbon atom of nitrogen base, followed by a hydrogen atom loss to make stable compounds. In general, coupling reaction represents electrophilic aromatic substitution [61]





3. 2 The results of characterization of prepared compounds

Prepared compounds were characterized by using of ultra violet and visible spectrum (Figure (3-1) to (3-7) and Table (3-1)), infrared spectrum (Figure (3-8)to(3-17)1, Table 3-2), and the ratios of C.H.N were calculated by specific analysis of the elements (table (3-3)).

3.2.1 Ultraviolet and visible spectrum UV-Vis for prepared compounds

Ultra violet and visible spectrum for the prepared compounds (1-10) in absolute DMSO are show in figures (3-1) to (3-7) and table (3-1). Three bands were appeared, first at λ max = (300-370nm) belongs to azo[62] group, second band at λ max = (251.2 – 260.6m) belongs to purines and pyrimidines, they have a bond system that absorb ultraviolet in nucleic acids and their derivatives[63]. while the third band is at λ max = (222-229nm) belongs to Phenyl group or may be electronic transition represents (π-π*) belongs to bonded rings through azo (-N=N-), of charge transfer and these transition occurs from benzene cycle to heterocyclic . Ultraviolet spectrums of aromatic compounds of homogenous ring that attach with non homogenous ring, the hetero atom causes a drastic

changes the absorption the attached groups depending on the nature and position of the group (donating and withdrawing)[64,65].

Table (3-1) ***Absorption ultra violet bands for the prepared compounds***

Compound Number	$\lambda max\,(nm)$		
1	**230**	**259**	**302**
2	**225**	**260**	**300**
3	**229**	**252**	**348**
4	**230**	**249**	**326**
5	**228**	**251**	**299.1**
6	**229**	**259**	**335**
7	**222.4**	**249.8**	**312**
8	**226.4**	**255**	**367**
9	**225.2**	**260**	**357**
10	**229**	**250**	**345**

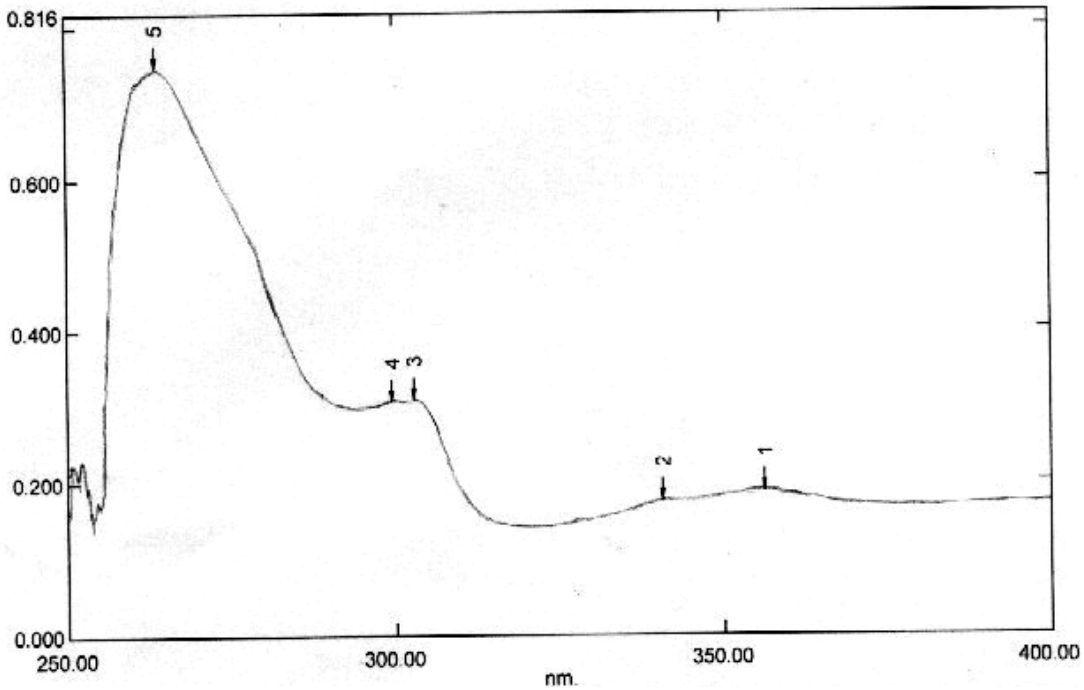


Figure (3-1): UV-Vis spectrum of
8-(phenyl azo)-Adenine.

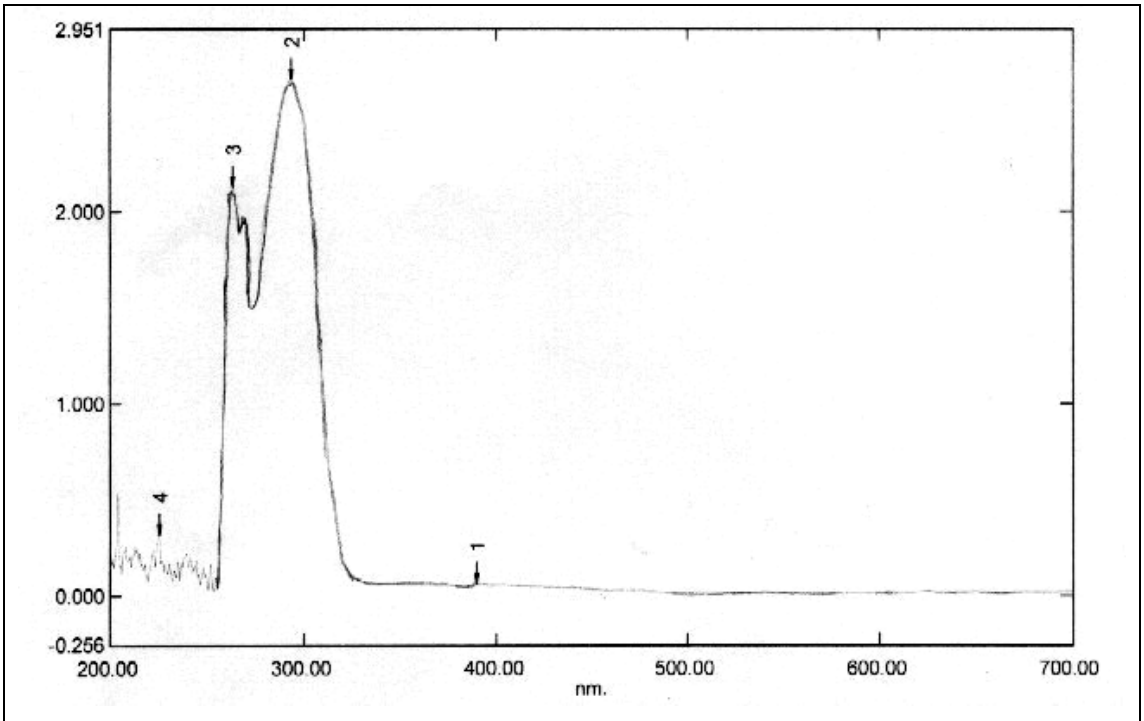


Figure (3-2): UV-Vis ***spectrum of.***

6-(3-Methyl -4-Amino phenyl azo)-Adenine

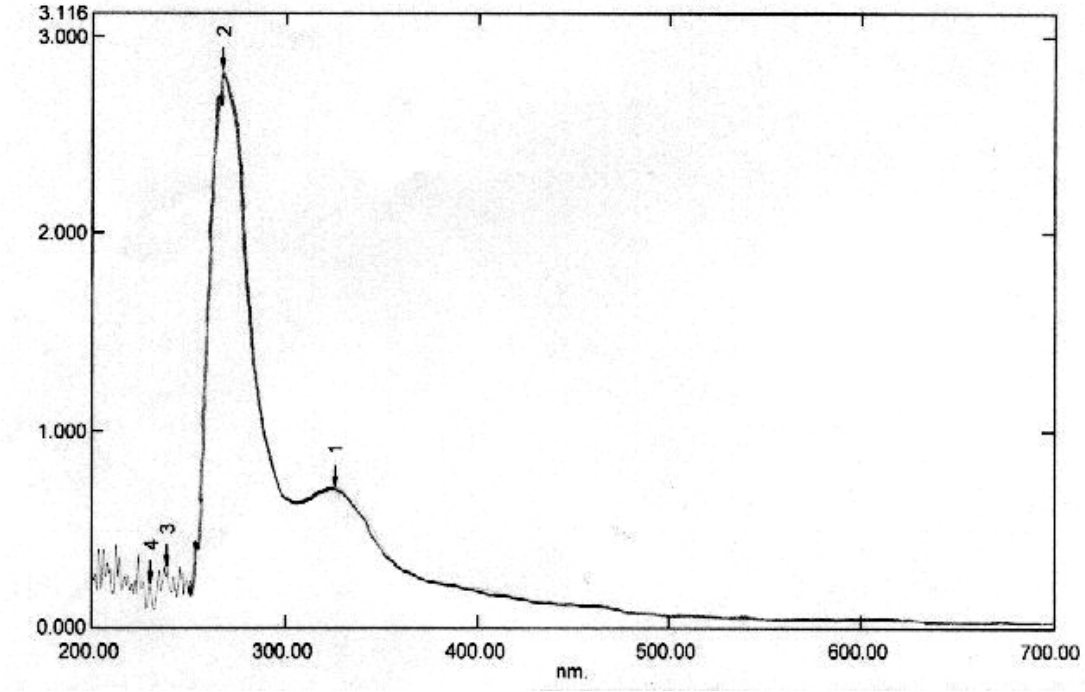


Figure (3-3): UV-Vis spectrum of.
6-(2-Amino - anthrachinionyl azo)-Adenine

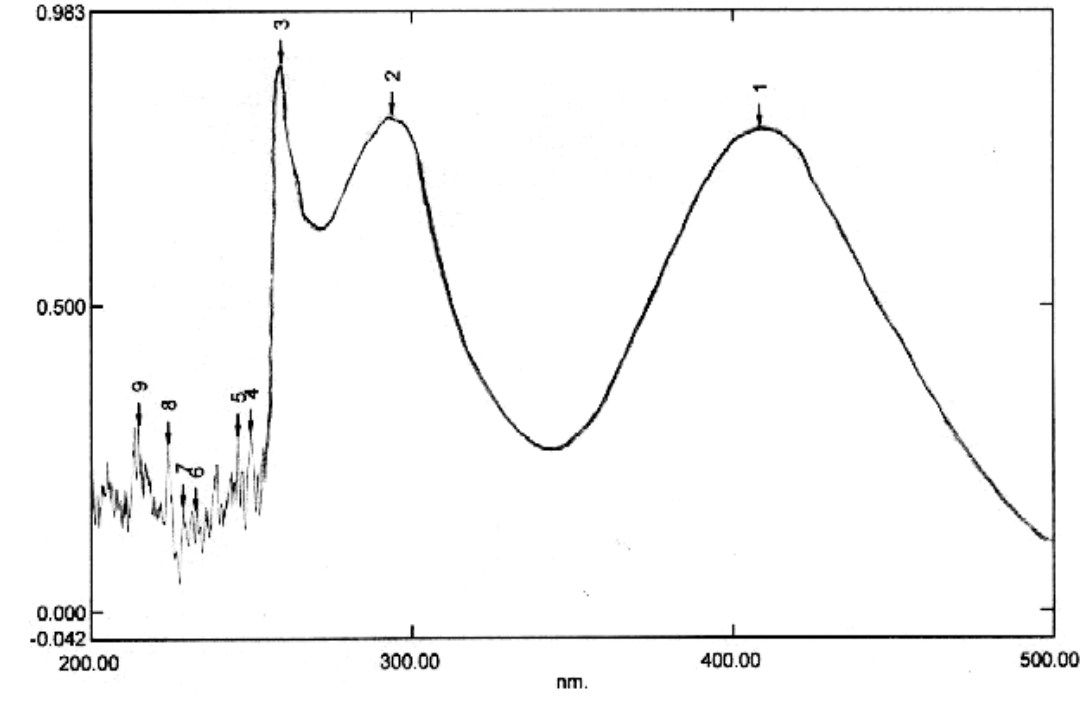


Figure (3-4): UV-Vis spectrum of.
6-(3-Methyl -4-Amino phenyl azo)-1,3dimethyl Uracil

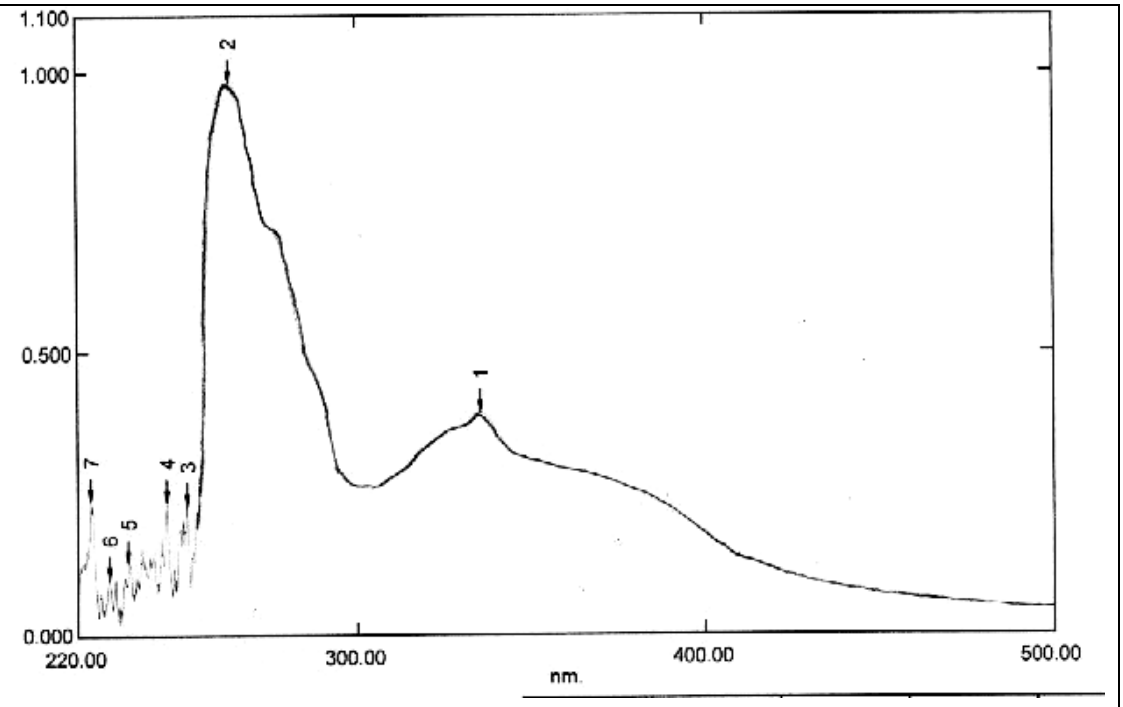


Figure (3-5): UV-Vis spectrum of
6-(2-α-Naphtolyl azo)- 1,3dimethyl Uracil

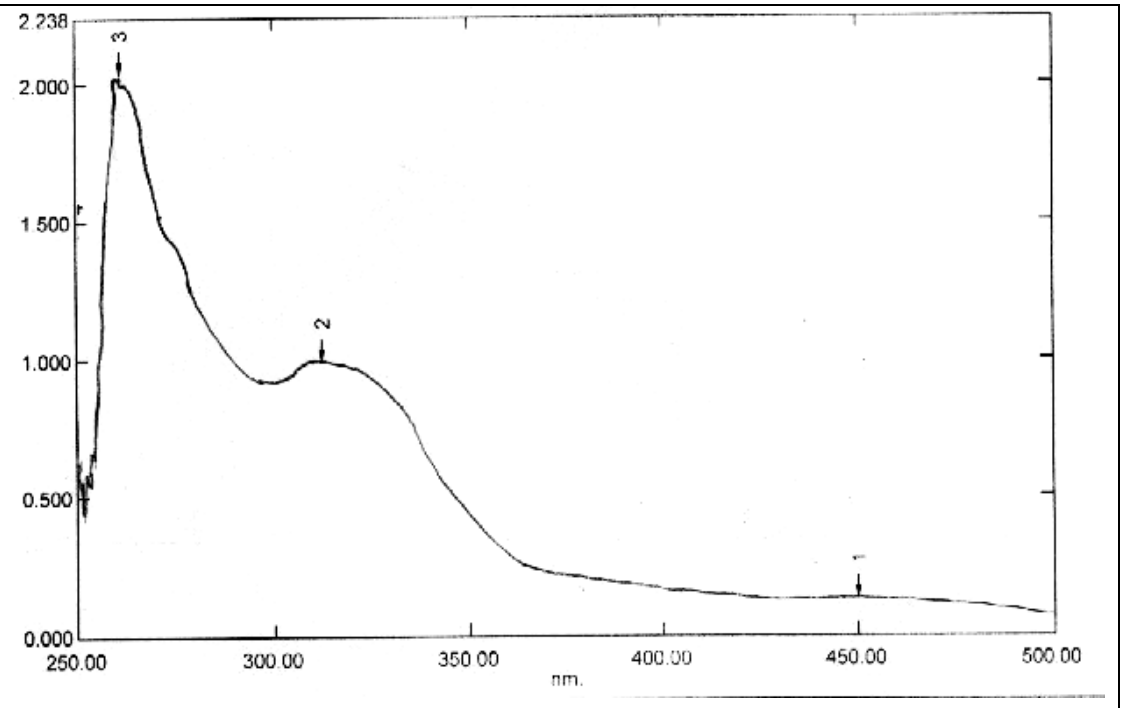


Figure (3-6): ***UV-Vis spectrum of***
6-(2-Amino - anthrachinionyl azo)- 1,3dimethyl Uracil

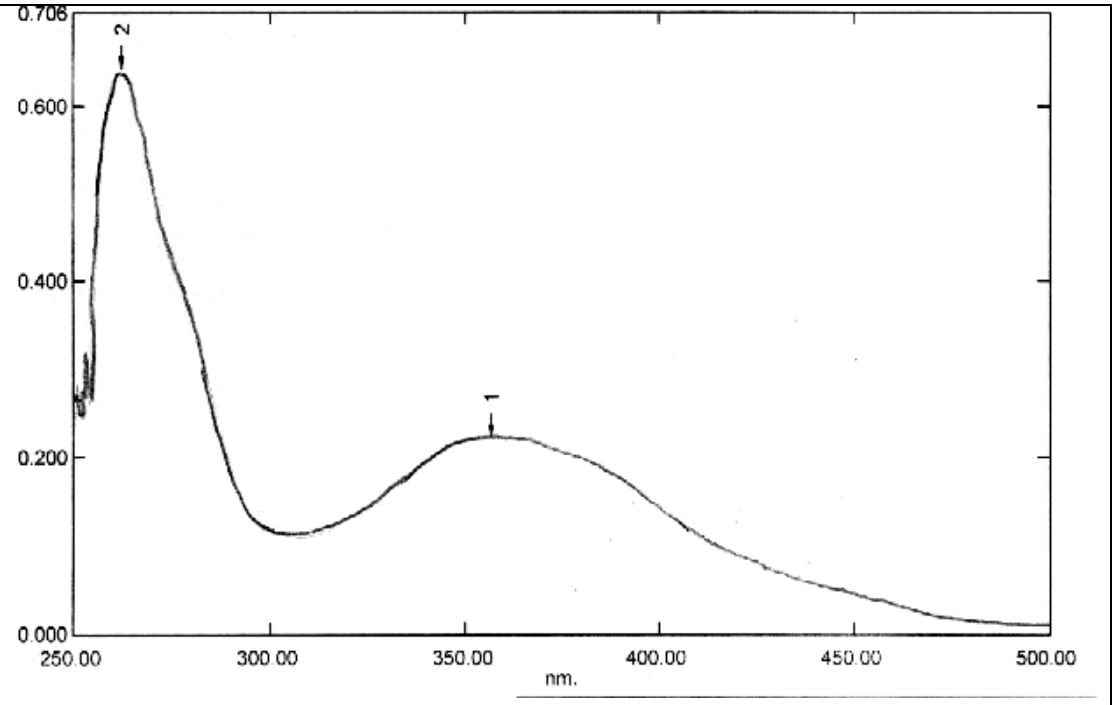


Figure (3-7): UV-Vis spectrum of.
6-(2-α-Naphtolyl azo)- Guanine

3. 2. 2 Spectrum of Fourier transforms infrared (FTIR) for prepared compounds

Interference among bands belongs to homogeneous and non homogeneous rings and their relation with azo group in azo derivatives, made studying of frequency spectrums of these compounds very difficult which made it unsuitable with spread and use of the colors. They are also not suitable with the literature of spectrum studies such as electronic spectrum[66,62] and NMR[67,68]. We observe appearance of absorption band of these colors such as C=C, C=N and N=N and else, by examining the spectrums of IR for azo compounds prepared and shown in figures(3-8) -(3-17), table(3-2).

For the purpose of discussing these spectra, it was divided into the following regions:

1- Spectrum region between (1700 – 4000 cm^{-1})

Infrared spectrums of azo compounds (2, 5, 7) absorption bands in the region ((3310-3365 cm^{-1}) from spectrum resulted from stretch vibration of the band N-H, meanwhile compounds (3, 6, 9) show strong absorption in (3020-3180 cm^{-1}) result to stretch vibration of OH and NH. Reference [69] show interference of absorption bands which belong to N-H and O-H and give wide range at (3000-3600cm^{-1}). Literatures[70,71] mentioned absence of OH from the spectrum of some organic exposes containing hydrogen bond. OH group attached by hydrogen bond with N-H group in case that OH will fall in ortho position to NH group in the same compound. It is confirmed that the absence of OH bond is related to hydrogen bond created. Its strength increases as resonance stability of the tautomeric formulas increase. That band indicates hydrogen bond in infrared band spectrum and it is weak and relatively wide[72,73].

Siroki[74] has proved that difficulty in characterization and identification bands position of OH and NH groups in spectrum of compounds containing hydrogen bonds as a result of tautomeric formula, which their spectrum led to wide absorption in the region (2500-3500 cm^{-1}) in the range that CH, NH, and OH are aromatic. We also couldn't distinguish these bands that belong to frequencies NH and OH in spectrum of this colors because of weakness and width of band nearby absorption region.

Spectrum of several colors showed narrow absorption band limit in (3000 – 3080 cm^{-1}) belong to the stretching vibration for C-H bond, meanwhile this band does not appear in many spectra because of absorption width which belongs to NH, OH.

2- Spectrum region between (666 -1700 cm^{-1})

This region is distinguished by its importance because it shows several absorptions at various intensities.

1. Azo compounds spectra of the prepared compounds showed absorption band in region (1565-1660 cm^{-1}) resulted from frequencies of stretching vibration C=N. also this spectrum shows three absorption bands in the region (1500-1590 cm^{-1}) belong to frequency of stretch vibration C=C, where absorption positions in bands C=C and C=N depend on the nature and position of substitute groups in the ring (withdrawing or donating group)[75],as literatures indicates[76,77] the C=N group shows stretching band of (1689-1471 cm^{-1}) location

The study of Hadzi[78] indicates that the position between (1400-1700 cm^{-1}) is the region which predicts the appearance of bands which cause frequency of stretching resulted from compact vibration of C=N and C=O which emphasis the tautomeric formula and as a result to existence of hydrogen bonds including resonance formula that change bonds length and occur of ring formula that on sequently occur formulas that make

removal of vibration frequency of the carbonyl on expected at region (1700-1730 cm^{-1}) to (1580-1710 cm^{-1}) region.

2. Azo compounds spectrum shows absorption band in range (1400-1495 cm^{-1}) resulted from vibration frequencies of N=N bond.

The literatures[74] indicates that absorption region of azo band depends on the terminal group attached.

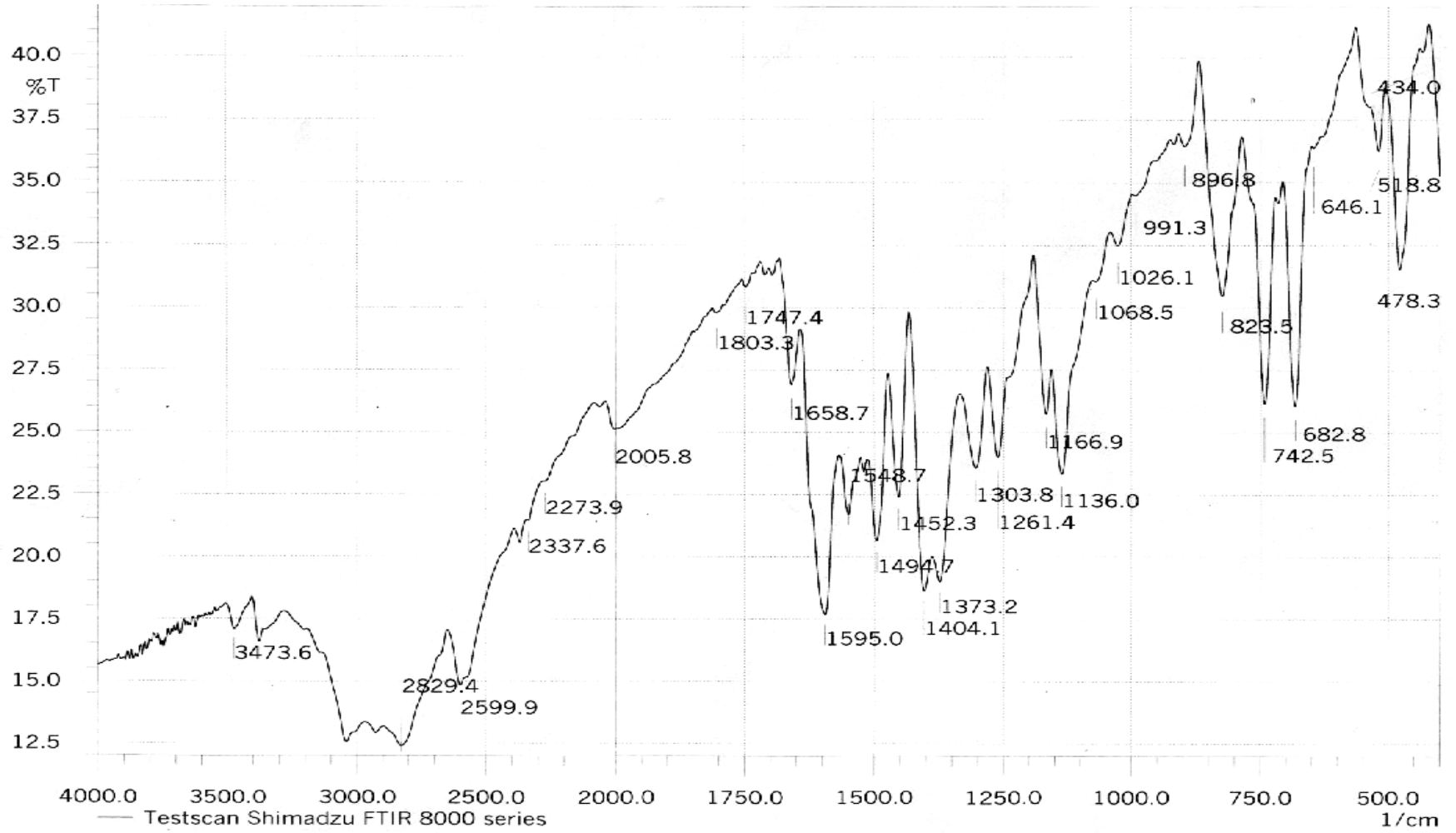
Characterization of this band in Raman spectrums is performed more easily than characterization by infrared spectrum. This band appears in aromatic azo compounds at Raman spectrum[79] about (1406 cm^{-1}). By infrared spectrum bonds for azo dyes which two researchers have made, where they emphasized that absorption band in the region(1400-1510cm^{1}) belong to azo group N=N and they are identical to absorption bands of prepared compounds. Several bands are characterized in spectrum of azo compounds which are located in range (1172-1367 cm^{-1}) belong mostly to vibration frequencies of (C-N=N-C), (C=N, N=C)[80,81].

Table (3-2) ***Absorption Fourier transforms infrared spectrophotometer (FTIR) for the prepared compounds using KBr discs***

Compounds Number	V(N-H)	V(O-H) + V(N-H)	V(C=O)	V(C=N)	V(C=C)	V(N=N)	V(C-N)	Other group
1	3473.6	--------	------	1658.7	1595.5 1548.7 1494.7	1452.3 1404.1	1373.2 1303.8 1261.4	------
2	3454.3	------	------	1587.3	1496.7	1454.2	1377.1 1298.0 1230.5	------
3	3460.1	3064.7 3024.0	1616.2	1560.3	1523.7	1450.4 1400.2	1326.9 1254.2 1212.1	------
4	3253.7 3070.5	------	1676.0	1600	1585.4	1463.9	1326.9 1292.2 1247.9	------
5	3371.3	------	1587.3	1625.0	1496.7	1456.2	1303.8 1228.6	------

Table (3-2) (continued)

Compound Number	V(N-H)	V(O-H) + V(N-H)	V(C=O)	V(C=N)	V(C=C)	V(N=N)	V(C-N)	Other group
6	**3282.6**	**3057.0**	**1687.3**	**1593.1**	**1521.7**	**1458.1 1396.4**	**1326.9 1259.4 1209.0**	**-----**
7	**3230.5 3072.4**	**------**	**1675.0**	**1628.0**	**1585.4**	**1452.3**	**1328.9 1290.3 1238.2**	**------**
8	**3340.5 3111.0**	**------**	**1678.0**	**1554.5**	**1519.8**	**1467.7**	**1373.2 1261.4**	**------**
9	**3402.2**	**3025.0**	**1613.7**	**1570.0**	**1537.2**	**1469.7 1427.2**	**1340.4 1294.1 1247.9 1211.2**	**------**
10	**3377.1 3230.5 3072.4**	**------**	**1674.1 1631.7**	**1585.4**	**1537.2 1519.8**	**1463.9**	**1388.7 1328.9 1290.3 1203.5**	**------**



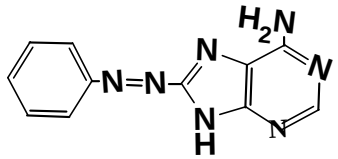
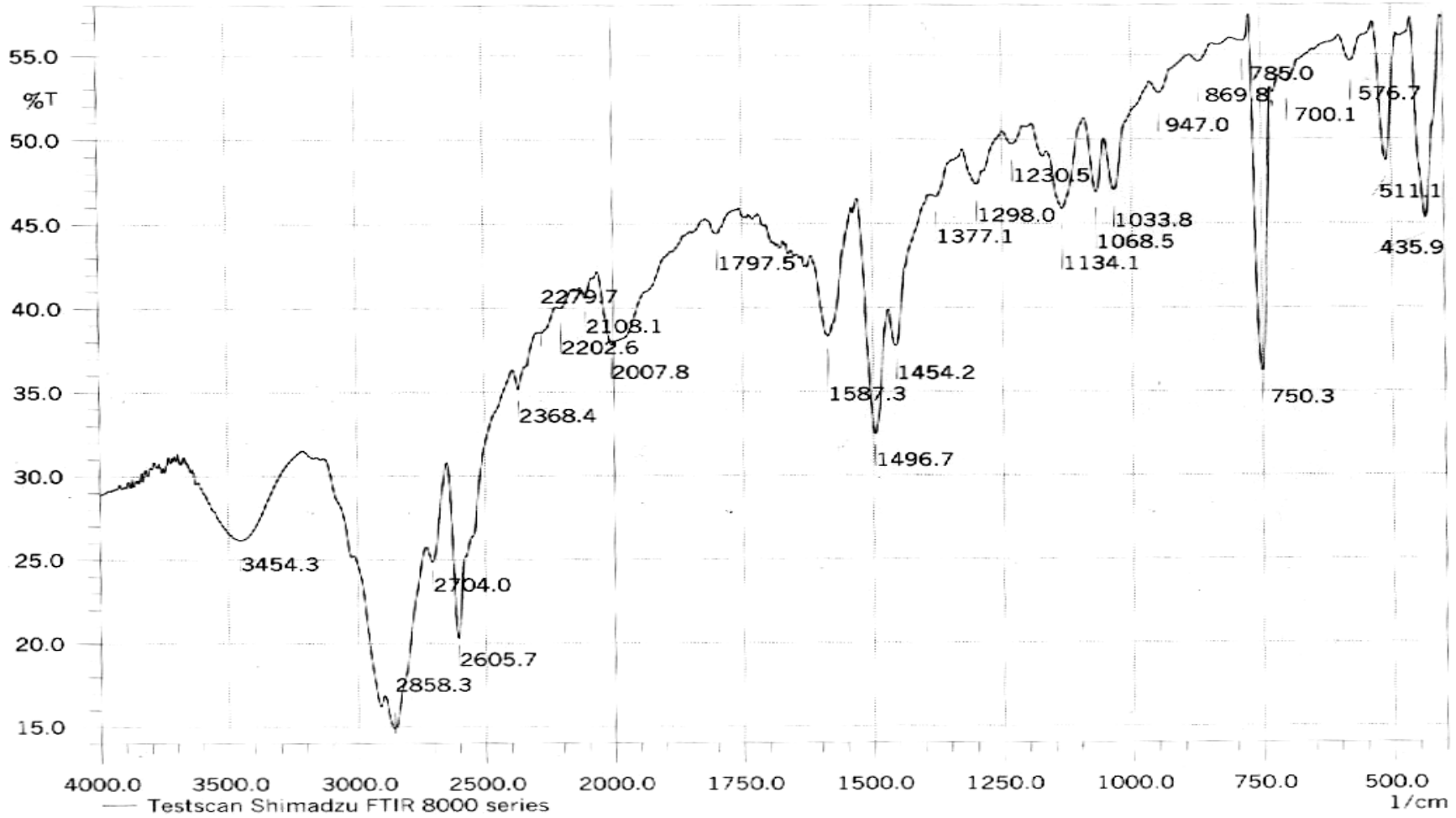
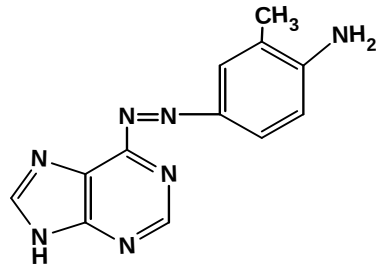
	**M.P. : 100 - 1020°C**	**Color: yellow**

Figure (3-8): structure, physical properties and F.T.IR spectrum of
8-(phenyl azo)-Adenine.

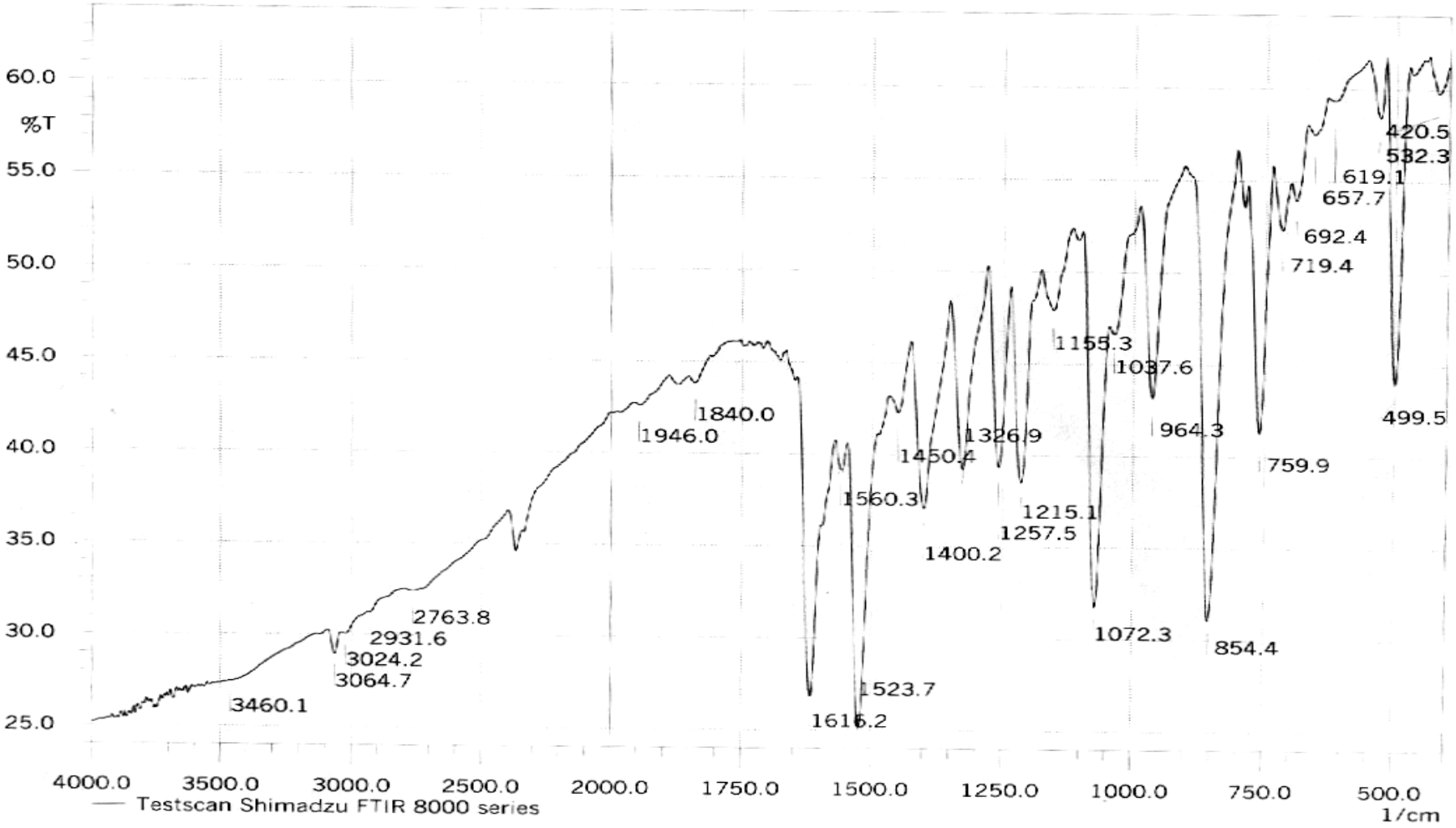


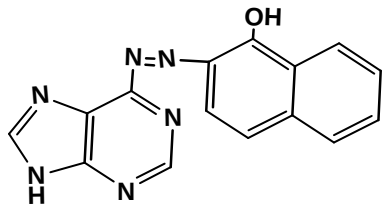


M.P. : 128 - 130°C	**Color: : green**

Figure (3-9): structure, physical properties and F.T.IR spectrum of.

6-(3-Methyl -4-Amino phenyl azo)-Adenine

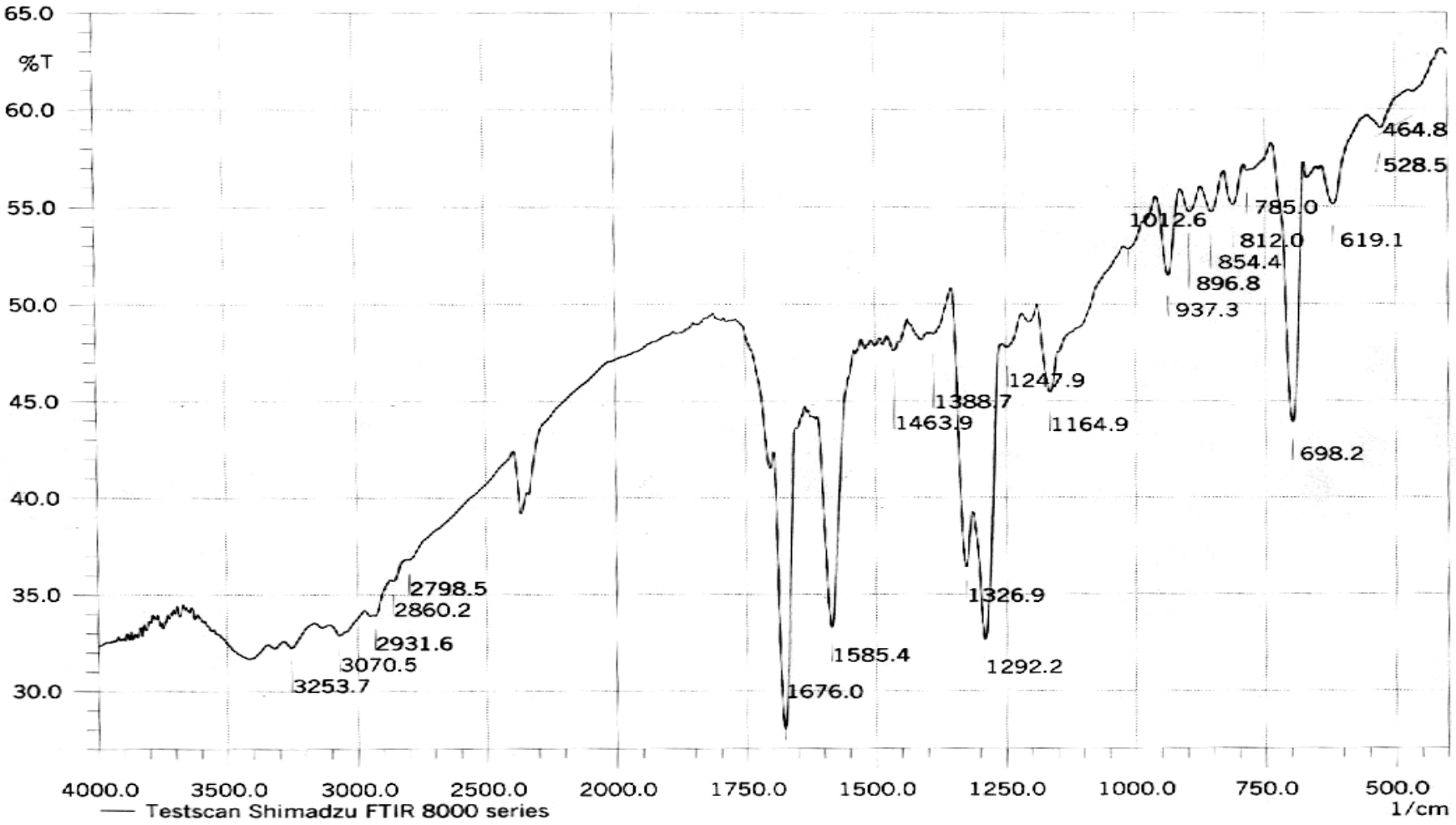


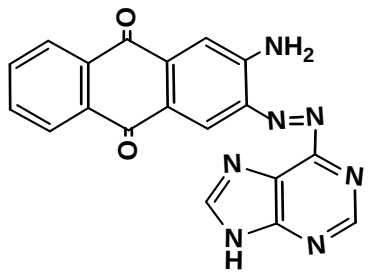


M.P. : 103 – 105°C	**Color: red**

Figure (3-10): structure, physical properties and F.T.IR spectrum of.

6-(2-α-Naphtolyl azo)- Adenine

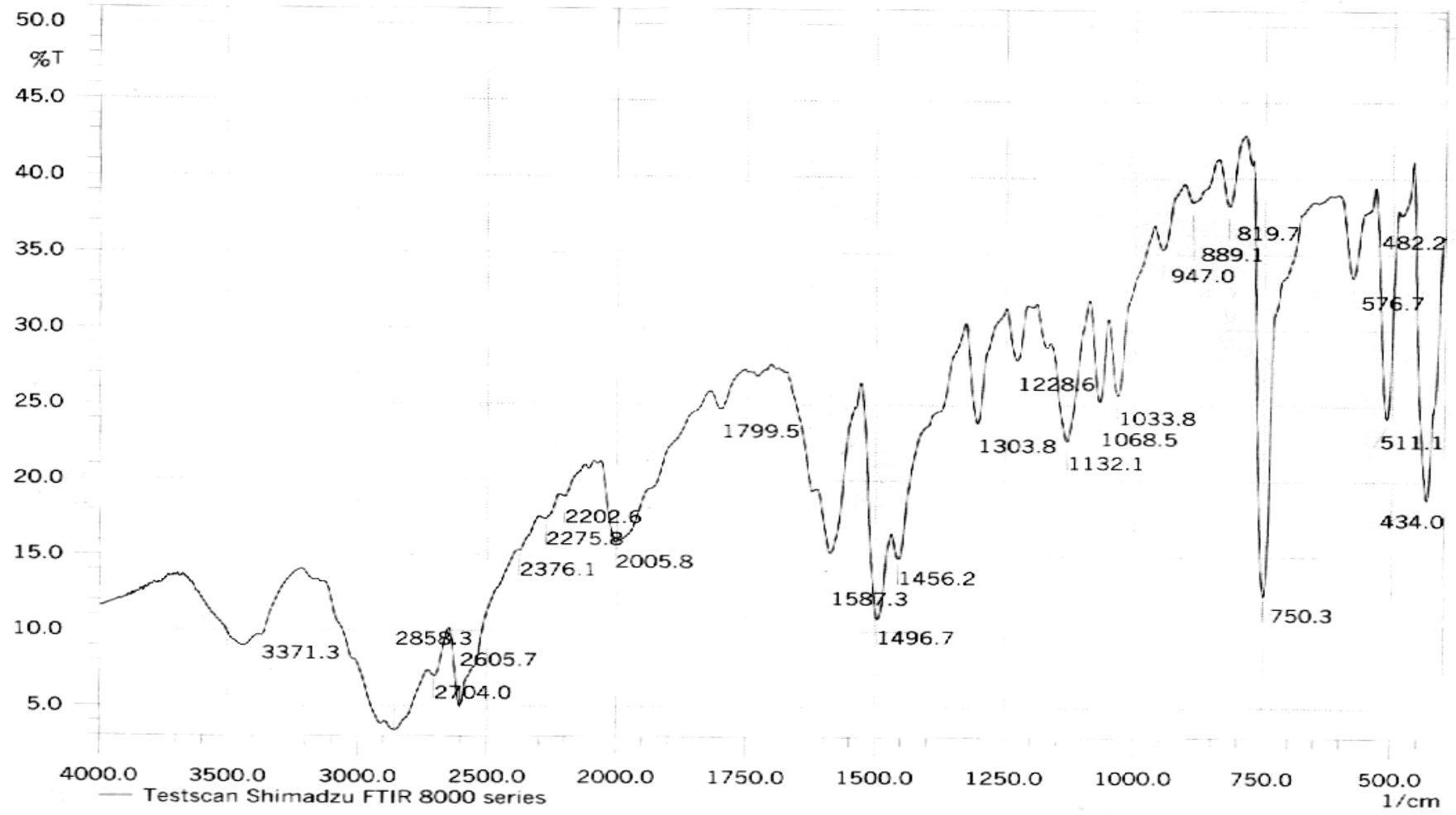


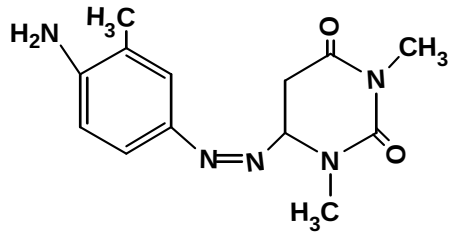


M.P. : 220 - 222°C	**Color: orange**

Figure (3-11): structure, physical properties and F.T.IR spectrum of.

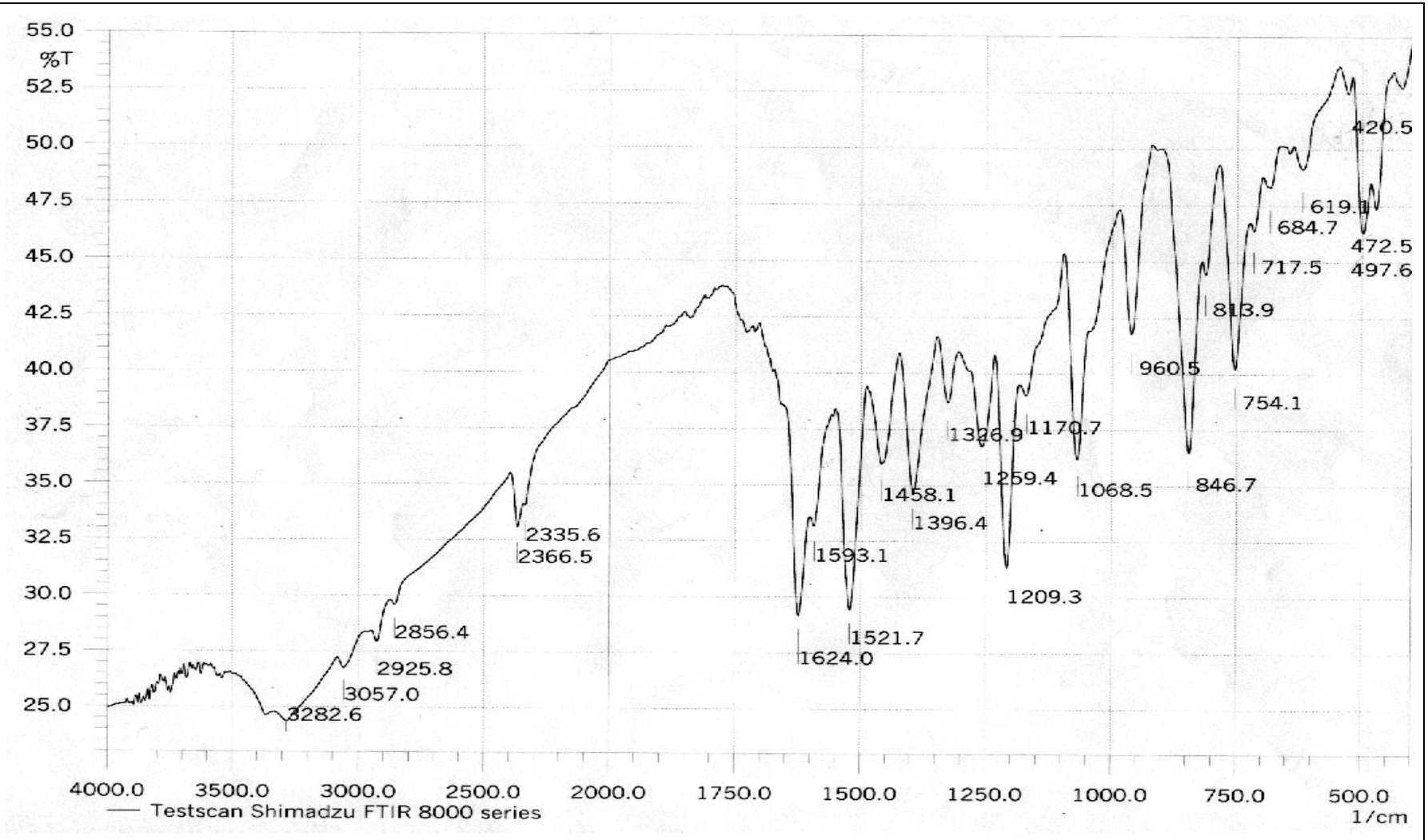
6-(2-Amino - anthrachinionyl azo)-Adenine

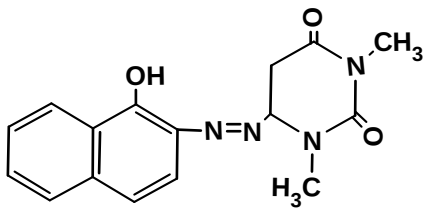




M.P. : 185 - 187 °C	**Color: : green**

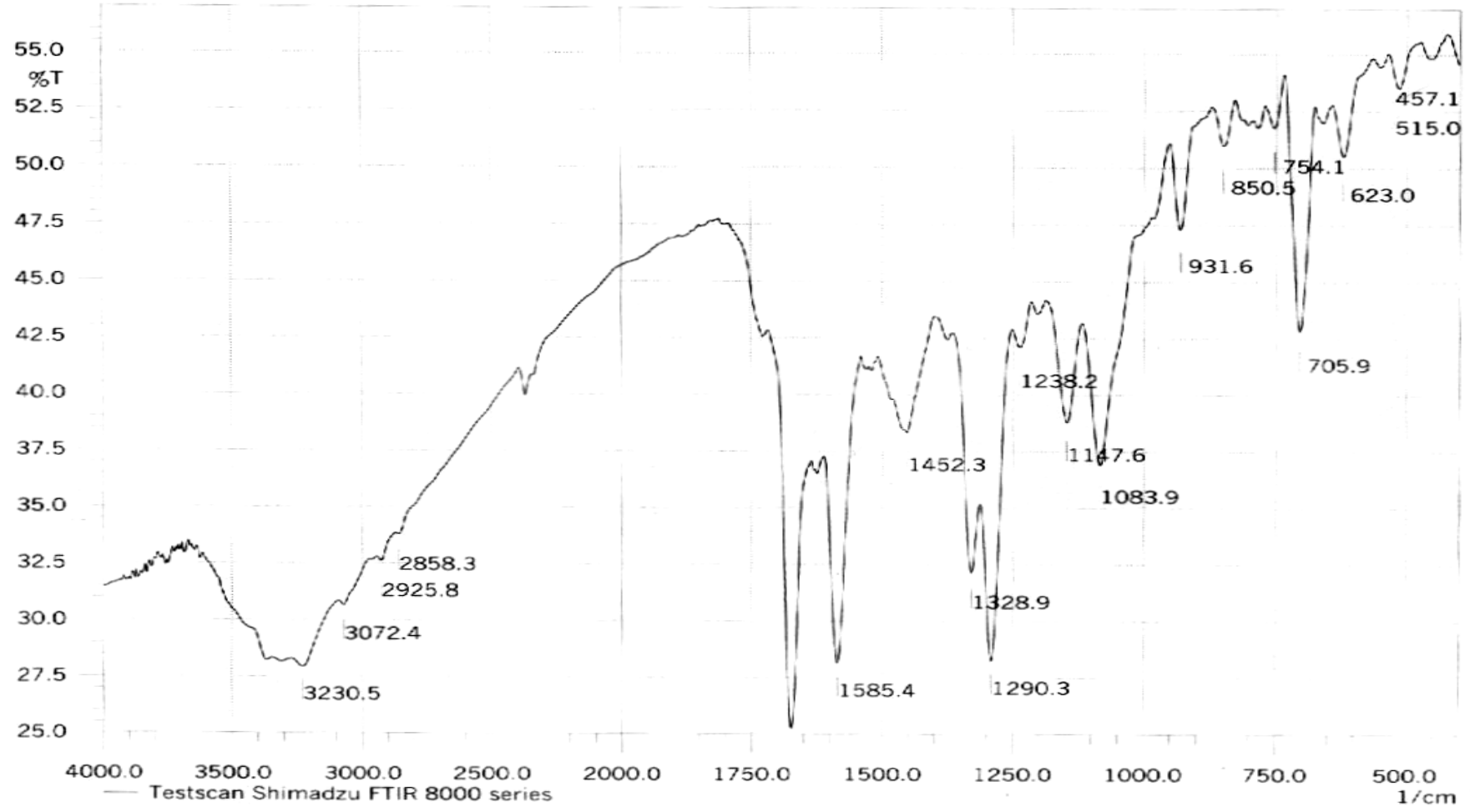
Figure (3-12): structure, physical properties and F.T.IR spectrum of.
6-(3-Methyl -4-Amino phenyl azo)-1,3dimethyl Uracil.

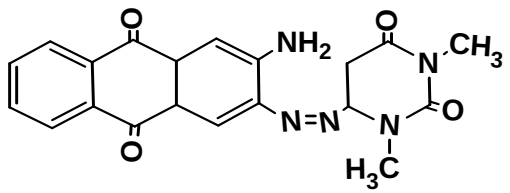




M.P. : 153 - 155 °C	**Color: red**

Figure (3-13): structure, physical properties and F.T.IR spectrum of
6-(2-α-Naphtolyl azo)- 1,3dimethyl Uracil



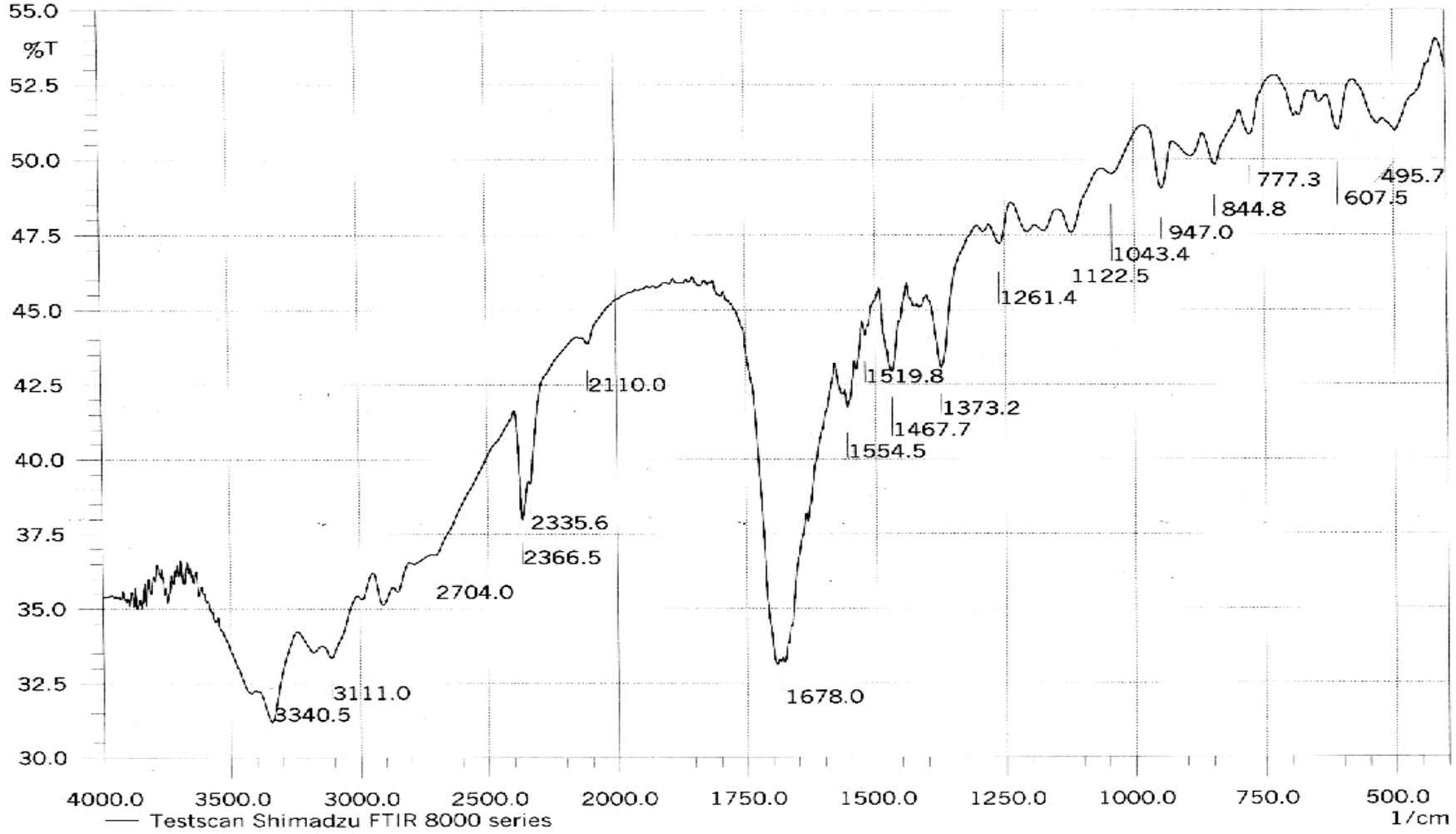


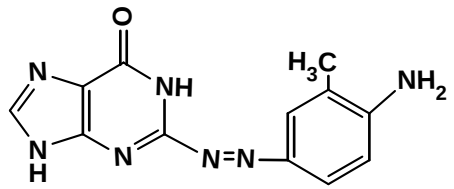
M.P. : 258 - 260 °C

Color: orange

Figure (3-14): structure, physical properties and F.T.IR spectrum of

6-(2-Amino - anthrachinionyl azo)- 1,3dimethyl Uracil



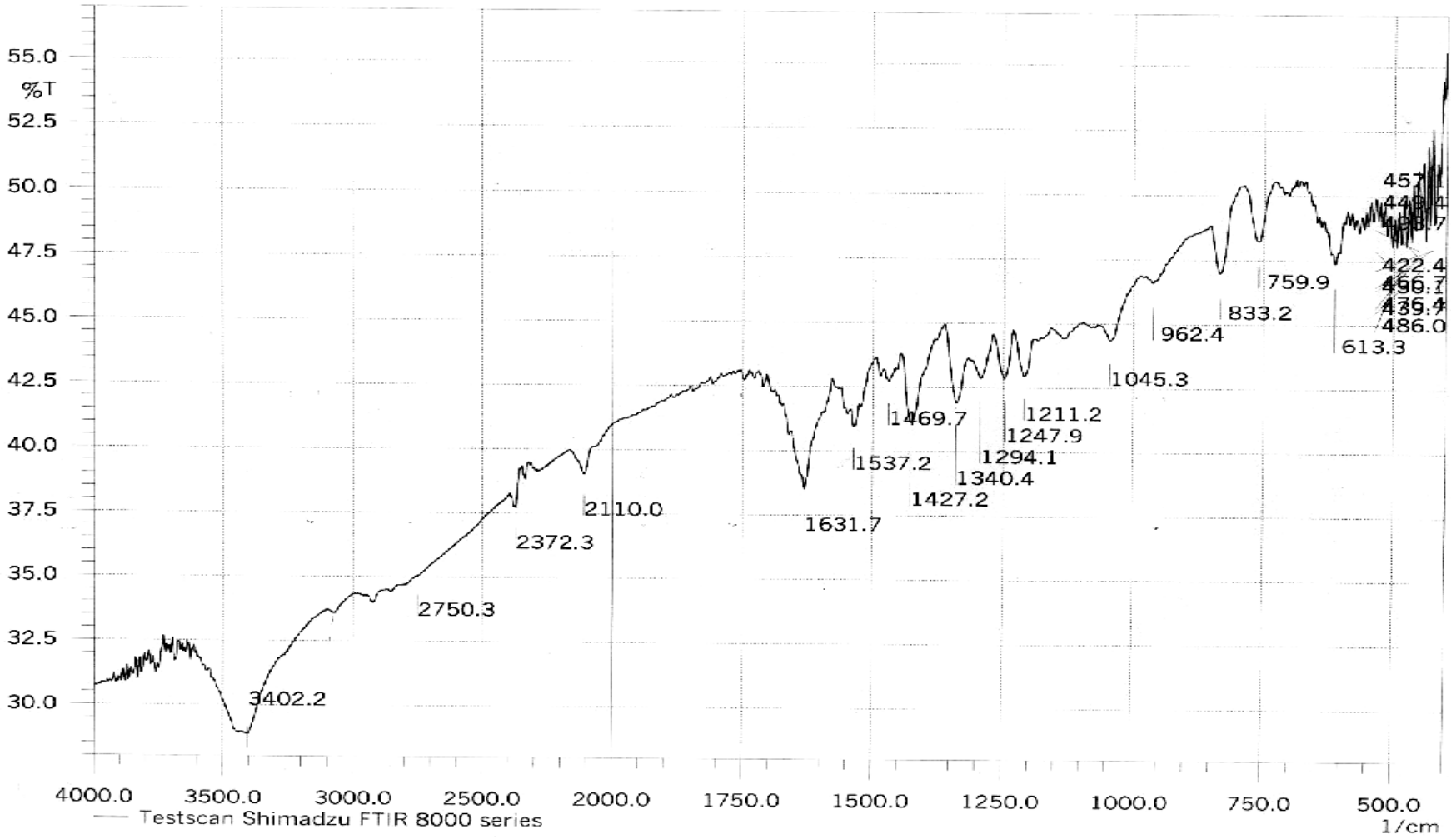


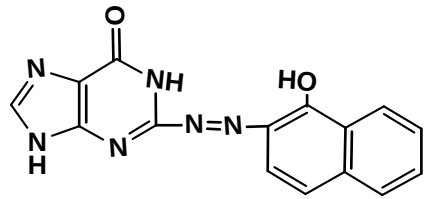
M.P. : 166 - 168 °C

Color: green

Figure (3-15): structure, physical properties and F.T.IR spectrum of.

6-(3-Methyl -4-Amino phenyl azo)-Guanine

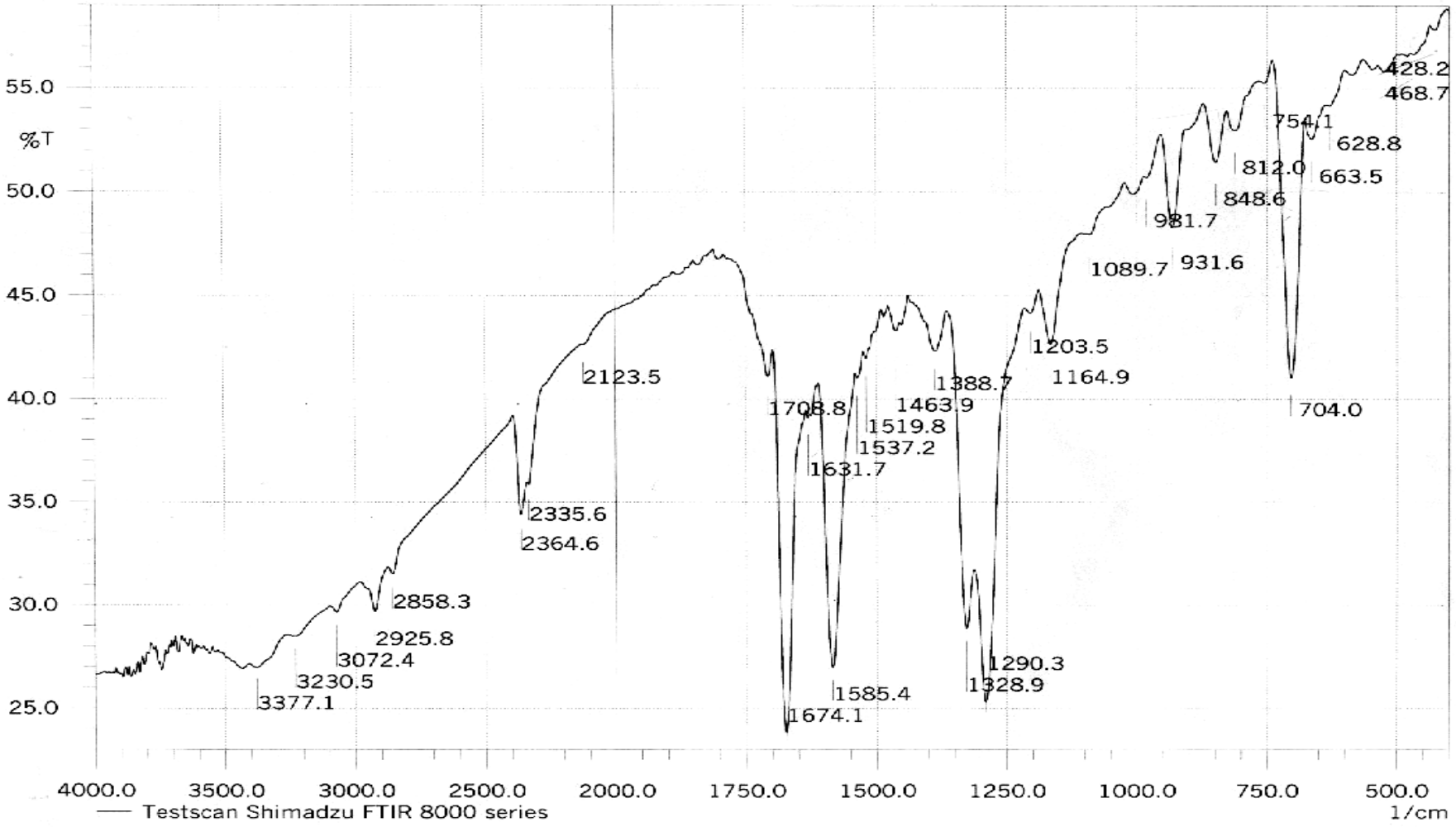


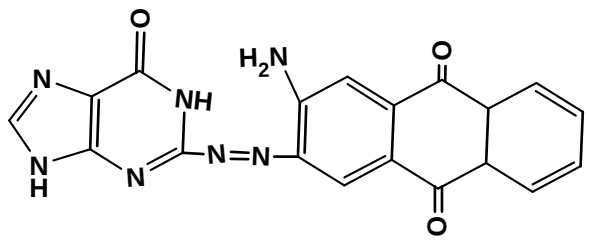


M.P. : 122 - 124°C	**Color: red**

Figure (3-16): structure, physical properties and F.T.IR spectrum of.

6-(2-α-Naphtolyl azo)- Guanine





M.P. : 235 - 237 °C

Color: orange

Figure (3-17): structure, physical properties and F.T.IR spectrum of.

6-(2-Amino - anthrachinionyl azo)- Guanine

Specific analysis of elements (C.H.N)

A specific analysis was done for Carbon, Hydrogen ad Nitrogen (C.H.N) for the prepared compounds (1 – 10). The results are registered in table (3-3) which shows the practical and theoretical ratios for these elements, which leads to that the compounds were nearly prepared to its real compounds.

Table (3-3) ***Results of the Specific Analysis for prepared compounds (C.H.N)***

Compound Number	*Analysis % calculated (found)*		
	% C	*% H*	*% N*
1	**54.56 (55.22)**	**2.89 (3.79)**	**39.75 (40.98)**
2	**55.98 (56.91)**	**3.65 (4.38)**	**37.88 (38.71)**
3	**61.22 (62.06)**	**2.50 (3.47)**	**27.85 (28.95)**
4	**61.44 (62.50)**	**2.73 (3.67)**	**24.66 (25.51)**
5	**57.35 (58.12)**	**5.49 (6.62)**	**23.28 (24.21)**
6	**60.62 (61.53)**	**4.79 (5.16)**	**16.53 (17.94)**
7	**60.74 (61.06)**	**3.51 (4.87)**	**17.23 (17.80)**
8	**52.75 (53.53)**	**3.26 (4.12)**	**35.45 (36.41)**
9	**27.90 (58.82)**	**2.40 (3.29)**	**26.82 (27.44)**
10	**57.99 (58.91)**	**2.60 (3.38)**	**24.44 (25.31)**

3. 3 Results of the Biological Study

From different studies and researches, it was found that nitrogen bases derivatives have serious effect on Gram negative and Gram positive bacteria and fungi. Pharmalogically, most of active medium are amines[82]. In this research, several azo compounds were prepared as derivatives of nitrogen bases and have various groups: amines groups, azo groups, and non-homogeneous ring groups. The studies indicate to great variation to non-homogeneous ring groups as anti-microorganisms. Amino groups were used as antibiotics for a long time to eliminate bacterial pollution especially in hospitals.

In this study, two bacteria species isolated from different diseases cases in either Gram positive and negative and considering that one of the Gram negative type, It high resistant to many antibiotics. Grow and reproduction process were made, and these bacteria are:

Escherichia coli , *Staphylococcus aureus,*

Also three fungal species isolated from different diseases, grow and reproduction process made, and these fungi are:

Trichophyton rubrum , *Trichophyton mentagrophytes*

Candida albicans

DMSO was used as a solvent by using Kirby-Bauer [58]method in calculating the inhibition or fatal effect for the compounds by measuring the surrounding area of the discs which had no growth of

bacteria. The compounds show positive results as antibacterial for separators used in the test.

3. 3. 1 Inhibition ability of azo compounds for fungi

The examined fungi were *Candida albicans, Trichophyton rubrum, Trichophyton mentagrophytes;*

. In *Candida abicans*, compound (6) table (3-4) fig.(3-19) shows highest inhibition at (500 µg/Disc) where inhibition radius rate was (20 mm). Two compounds (3,10) showed lowest inhibition at highest concentration and with radius (10 mm). Other compounds showed different inhibition abilities (14-8mm).

In_*Trichophyton rubrum*, compound (5) table (3-5) fig (3-21) gave highest inhibition ability at the highest concentration with radius of (30mm). Compounds (2,3) gave lowest ability at the highest concentration of (10 mm), other did not show any activity towards this fungi at low concentration (10 µg/Disc).

The affection of *Trichophyton mentagrophytes* table (3-6) fig. (3-23) was less than its affection in the previous types of fungi. For compound (5) gave the highest inhibition ability at the highest concentration reaching a radius of (20 mm), while compounds (3,7) had a moderate effect with radiuses of (12, 15 mm) respectively. Other did not show any activity towards this fungi at concentration lower than (100, 1٠µg/Disc)

3.3.2 Inhibition ability of azo compounds for gram positive bacteria

Prepared compound shows a various abilities of inhibition to *Staphylococcus aureus* as shown in table (3-7) fig (3-24). Compound affection in these bacteria (1,3,9) shows high activities on (500 µg/Disc). inhibition zone for these compounds between (32-35 mm). Compound (1, 3, 6, 9) shows highest activity at (250 µg/Disc) and the inhibition radius rate was (27-19) mm. compounds (7) shows little activity at high concentration (13 mm). Other compounds showed various activities, (7.5-24 mm). In general, all compounds showed high inhibition ability in higher concentrations than in low concentration.

3. 3. 3 Inhibition ability of azo compounds for gram negative bacteria

The examined bacteria was *E. coli*,. In *E. coli*, compound (3,9) table (3-8) fig.(3-25) shows highest inhibition at (500 µg/Disc)where inhibition radius rate was (22-25mm). Two compounds (1,6) showed lowest inhibition at highest concentration and with radius (12 mm). Compounds (2, 4, 5, 7, 10) did not show any activity towards these bacteria. Other compounds showed different inhibition abilities (15-10 mm).

Generally, all prepared compounds have no inhibition affection on Gram negative bacteria in low concentration (100, 1٠µg/Disc). highest activity was in highest concentration.

In comparing the results, it is concluded that the affection of chemical prepared compounds on two types of bacteria, the affection was high on Gram positive *S. aureus* and moderate on *E. coli*. This bacteria is known by its resistance for many chemical compounds[83]. Those Gram negative bacteria, especially *E. coli* have the ability to resist heavy elements and antisepticim. It is concluded that many bacteria resist heavy materials. Also the resistance of any bacterial species to chemical materals is because[84] of resistance resulted from mutation or plasma for inexistence of suitable transporter of these compounds and consequently inability to reach their target in the cell or because of cell membrane thickness because it contains high concentration of fats[39].

Gram negative has three distigtive layers; Cytoplasmic membrane, peptidglycon layer and external membrane[85]. In general, *E. coli* had multi resistance for chemical compounds and was registered in many studies as it has defensive factors and its resistance reflects the risk of these separators in making infection and leading to difficulty in treatment.[86,87]

Several diseases caused by microorganisms were the cause to prepare chemicals to resist these creatures in many mechanisms[88] such as:

1- Destroying cellular membrane; or prevention to made it.

2- Failure in absorbency of cytoplasmic membrane.

3- Failure in cellular enzyme activity.

4- Stop protein and nucleic acids production, in addition to failure in chemical and physical compositions.

From the above discussion, it is explained that prepared azo compounds have good activity and inhibition towards bacteria and fungi species used. This property is due to the following reasons:

1- Catching characteristics of azo compounds which makes it able to create consistent complexes with mineral ions in bacteria's cell body such as potassium, calcium, and iron where tiny creatures are needed, where these elements are deprived from necessary elements and then fail leading to fatality.

2- These compounds have the ability to dissolve fatty layer of barrier in gram negative bacteria which cause leaking of cell liquids outwards and destroy it.

3- Ability to inhibit DNA creation in sensitive bacteria cells and fungi cell by its ability to inhibit DNA polymerase.

4- Ability to make hydrogen bond (NH or OH) in prepared compounds and water moles in bacteria cells. That causes failure of biological activities of the cell and destroys it.

Table (3-4) ***The effect of azo compound on Candida albicans grown on sabouraud dextrose agar at (30^{0}C) measured in (mm)***

Compound Number	(500 µg/Disc) *(mm)*	(250 µg/Disc) *(mm)*	(100 µg/Disc *(mm)*	(10 µg/Disc) *(mm)*
1	**15**	**10**	**------**	**------**
2	**12**	**10**	**------**	**------**
3	**10**	**------**	**------**	**------**
4	**15**	**14**	**10**	**-----**
5	**15**	**12**	**8**	**------**
6	**20**	**19**	**10**	**-----**
7	**14**	**10**	**------**	**------**
8	**11**	**7**	**-----**	**------**
9	**15**	**12**	**------**	**------**
10	**10**	**6.5**	**------**	**------**

Fig. (3-18): *Candida albicans* grown on sabouraud dextrose agar at (30^0C)

Fig. (3-19): Inhibited *Candida albicans* by different concentration of 8-(phenyl azo)-Adenine

Table (3-5) ***The effect of azo compound on Trichophyton rubrum grown on Sabouraud's dextrose agar at (30^{0}C) measured in (mm)***

Compound Number	(500 µg/Disc) *(mm)*	(250 µg/Disc) *(mm)*	(100 µg/Disc *(mm)*	(10 µg/Disc) *(mm)*
1	**20**	**13**	**10**	**------**
2	**10**	**------**	**------**	**------**
3	**10**	**8**	**------**	**------**
4	**14**	**------**	**------**	**-----**
5	**30**	**12**	**------**	**------**
6	**18**	**6.5**	**------**	**-----**
7	**15**	**7**	**------**	**------**
8	**20**	**14**	**-----**	**------**
9	**14**	**8**	**------**	**------**
10	**12**	**6.5**	**------**	**------**

Fig. (3-20) *Trichophyton rubrum* grown on Sabouraud's dextrose agar at (30^0C)

Fig. (3-21) inhibited *Trichophyton rubrum* by different concentration of 8-(phenyl azo)-Adenine

Table (3-6) ***The effect of azo compound on Trichophyton mentagrophytes grown on Sabouraud's dextrose agar at (30^{0}C) measured in (mm)***

Compound Number	(500 µg/Disc) *(mm)*	(250 µg/Disc) *(mm)*	(100 µg/Disc *(mm)*	(10 µg/Disc) *(mm)*
1	**18**	**15**	**------**	**------**
2	**------**	**------**	**------**	**------**
3	**15**	**-----**	**------**	**------**
4	**------**	**------**	**-----**	**-----**
5	**20**	**14**	**------**	**------**
6	**16**	**------**	**------**	**-----**
7	**12**	**7**	**------**	**------**
8	**10**	**-----**	**-----**	**------**
9	**10**	**6.5**	**-----**	**------**
10	**15**	**6.5**	**------**	**------**

Fig. (3-22):*Trichophyton mentagrophytes* grown on Sabouraud's dextrose agar at (30^0C*)*

Fig. (3-23) : Inhibited *Trichophyton mentagrophytes* by different concentration of 8-(phenyl azo)-Adenine

Table (3-7) ***The effect of azo compounds on (Staph. aureus) grown on nutrient agar at (37^{0}C) measured in (mm)***

Compound Number	(500 µg/Disc) *(mm)*	(250 µg/Disc) *(mm)*	(100 µg/Disc *(mm)*	(10 µg/Disc) *(mm)*
1	35	25	15	------
2	14	8	------	------
3	30	24	10	------
4	18	10	------	------
5	16	8	------	-----
6	21	19	15	------
7	13	7.5	------	------
8	22	10	------	------
9	32	27	15	8
10	14	10	-----	------

Table (3-8) ***The effect of azo compounds on (E. coli) grown on nutrient agar at (37^{0}C) measured in (mm)***

Compound Number	(500 µg/Disc) *(mm)*	(250 µg/Disc) *(mm)*	(100 µg/Disc *(mm)*	(10 µg/Disc) *(mm)*
1	**12**	**8**	**------**	**------**
2	**------**	**------**	**------**	**------**
3	**25**	**15**	**10**	**------**
4	**------**	**------**	**------**	**------**
5	**------**	**------**	**------**	**-----**
6	**12**	**7**	**------**	**------**
7	**------**	**------**	**------**	**------**
8	**14**	**10**	**------**	**------**
9	**22**	**15**	**8**	**------**
10	**------**	**------**	**-----**	**------**

Fig. (3-24) : Inhibited *Staphylococcus aureus* by different concentration of 8-(phenyl azo)-Adenine

Fig (3-25) : Inhibited *Esherichia coli* by different concentration of 8-(phenyl azo)-Adenine

3.4 Conclusion & Recommendations:-

First: Conclusions:

From the gained results of the current study, we can conclude the following:

1- Most of the azo compounds have, as derivatives for the nitrogen bases, a biological activity anti bacteria and fungi, which differ by the difference of their concentrations.

2- The prepared compounds had a large effect on the gram positive bacteria ***(Staphylococcus aureus)*** by comparison with *E.coli* under studied.

Gram negative bacteria (***Esherichia coli)*** was the most resistible for the prepared compounds from other species studied.

3- The prepared compounds had a high effect on the fungi ***(Trichophyton rubrum)*** by comparison with other species under studied

Second: Recommendations:

1- Studying the possibility of using the organic prepared compounds in creating coordinated complexes with several kinds of metallic ions.

2- Continuing studies on these active compounds with different attach group to studying the effect of these groups on the compound activity.

3- Studying the biological effects of these compounds on different kinds of fungal, or viruses and the ability of improving this study to show its cytotoxicity and mutagenrcity carcinogenic, and if it succeeds it can be used as medical compounds in the future.

Expeirmetal part

2.1 Instruments:

- Melting points were measured by using ***Gallen Kamp*** melting point.

- Infrared spectra were recorded on F.T.IR-8300 Fourier transforms infrared spectrophotometer ***SHIMADZU*** as potassium bromide disc in the (600-4000) cm^{-1} spectral range.

- The electronic spectra of the compounds were obtained using (***SHIMADZU*** UV-Vis. 160A) ultraviolet spectrophotometer.

- Elemental Analysis were calculated (C.H.N) ratio for prepared compounds using (***Perkin Elmer*** 240B) college of science_ Jordan university

2.2 Materials

Number	*materials*	*Company*
1	**Adenine**	**B.D.H**
2	**Uracil**	**B.D.H**
3	**Guanine**	**B.D.H**
4	**Aniline**	**B.D.H**
5	**α-naphtol**	**B.D.H**
6	**o- Tolidine**	**B.D.H**
7	**2-amino anthraquinone**	**B.D.H**
8	**Sodium nitrite**	**Fluka**
9	**Ethanol \ absolute**	**B.D.H**
10	**Sodium hydroxide**	**B.D.H**
11	**Hydrochloric acid**	**B.D.H**
13	**Potassium bromide for infra – red spectroscopy**	**B.D.H**

2.3 The preparation of the 8-(phenyl azo)-Adenine

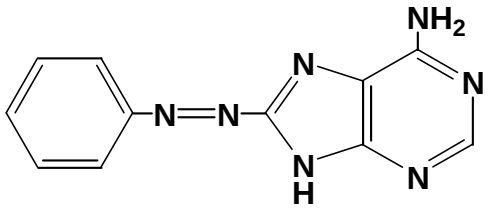
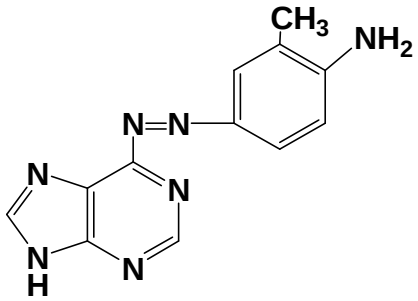
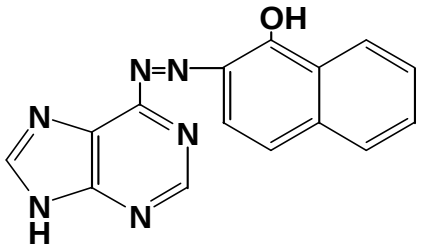
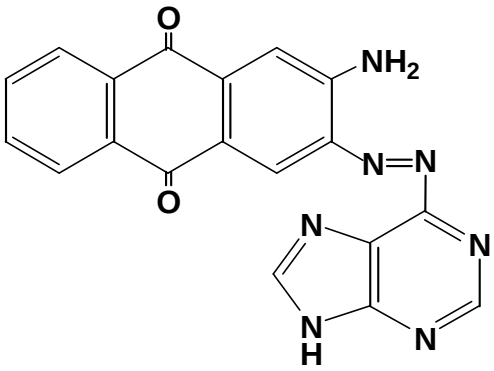
Used 0.002 mole adenine (0.215g dissolved in 1 ml of HCl and 3 ml of H_2O) (0.155 g) $NaNO_2$ dissolved in 4 ml of water then added. The mixture was stirred and the temperature should not exceed 5^0C. After 15 minutes the diazonium salt was added to a solution 0.04mole aniline (0.25g which was dissolved in 7.5 ml NaOH).

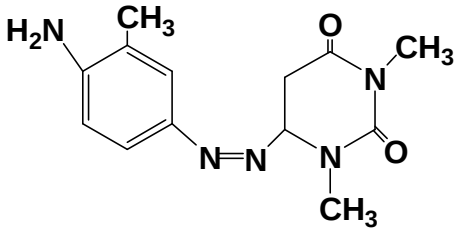
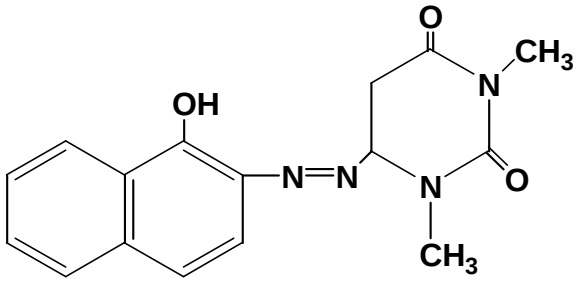
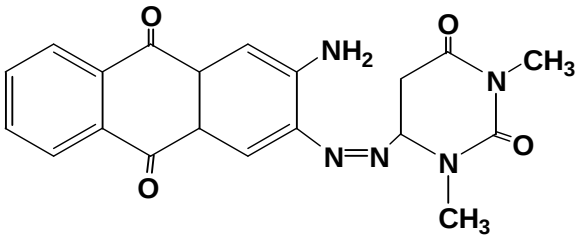
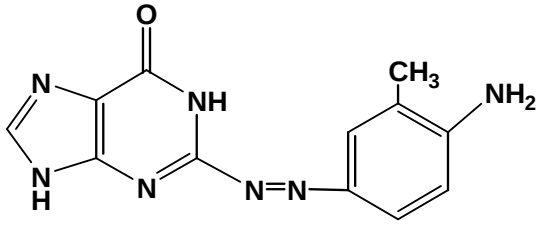
The mixture was cooled for 15 minutes which produced in ice bath until the precipitation of product is complete , filtered and dried the produce crystals under vacuum and by using mixture of ethanol – water (1:1) solution for recrystallization process .

By this procedure preparation of azo compounds (1-10) was shown in table (2-1) which also shows the deducted ultraviolet spectrophotometer (U.V) and Fourier transforms infrared spectrophotometer (FTIR) and the ratios of (C.H.N) were calculated by specific analysis of the elements.

Table (2-2) that appear the Chemical and physical properties for prepared compounds by calculated the molecular formula , Mol.wt , M.P. and the yield %

Table (2-1) the Name and Structure of prepared compounds

Compound Number	*Structures*	*Name*
1		**8-(phenyl azo)-Adenine**
2		**6-(3-Methyl -4-Amino phenyl azo)-Adenine**
3		**6-(2-α-Naphtolyl azo)-Adenine**
4		**6-(2-Amino -anthraquinionyl azo)-Adenine**

Compound Number	*Structures*	*Name*
5		**6-(3-Methyl -4-Amino phenyl azo)-1,3dimethyl Uracil**
6		**6-(2-α-Naphtolyl azo)- 1,3dimethyl Uracil**
7		**6-(2-Amino - anthraquinionyl azo)- 1,3dimethyl Uracil**
8		**2-(3-Methyl -4-Amino phenyl azo)-Guanine**

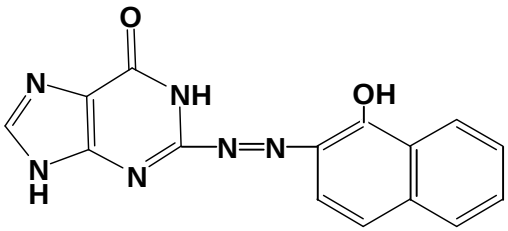
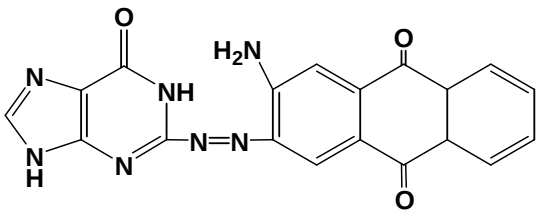
Compound Number	*Structures*	*Name*
9		**2-(2-α-Naphtolyl azo)- Guanine**
10		**2-(2-Amino - anthraquinionyl azo)- Guanine**

Table (2-2) Chemical and physical properties for prepared compounds

Compounds Number	*Molecular formula*	*Mol. Wt (gm/mol)*	*M.P (^{0}C)*	*yield %*
1	$C_{11}H_9N_7$	**239.24**	**100 - 1020**	**85**
2	$C_{12}H_{11}N_7$	**235.26**	**128 - 130**	**70**
3	$C_{15}H_{10}N_6O$	**290.1**	**103 – 105**	**87**
4	$C_{20}H_{14}N_7O_2$	**384.37**	**220 - 222**	**55**
5	$C_{14}H_{19}N_5O_2$	**289.33**	**185 - 187**	**68**
6	$C_{16}H_{16}N_4O_3$	**312.32**	**153 - 155**	**80**
7	$C_{20}H_{19}N_5O_4$	**393.40**	**258 - 260**	**59**
8	$C_{12}H_{11}N_7O$	**269.26**	**166 - 168**	**78**
9	$C_{15}H_{10}N_6O_2$	**306.28**	**122 - 124**	**64**
10	$C_{19}H_{13}N_7O_3$	**387.35**	**235 - 237**	**57**

2.4 Biological Study

2.4.1 Equipment and Apparatus

The following equipments and apparatus were used during the study:

Equipment	*Company (origin)*
Autoclave	*Express (West-Germany)*
Incubator	*Termaks (U.K.)*
Oven	*Gallenkamp Sanyo – (U.K.)*
Cooled incubator	*Sanyo incubator (USA)*

2.4.2 Chemical and media

The following chemical and media used during this study:

Media and materials	**Company (origin)**
Nutrient broth	Difco
Nutrient agar	Difco
Sabouraud's dextrose agar	Difco

Ethanol \ absolute	Difco
Dimethyl Sulfoxide	Fluka

2.4.2.1 Culture Media

Some liquid and solid media are used and prepared according to methods. These media are:

1- Nutrient agar for bacteria

2- Nutrient broth for bacteria by dissolved 38 gm for each liter of distil water.

3-Sabouraud 's dextrose agar for fungi by dissolved 63 gm for each liter of distil water.

And then Sterilization by autoclave under pressure of (1.5 atm) and temperature of (115^{o}C) for (15-20 minutes) .

And the medium was cooled to 50^{o}C. After that it was poured into the plates and left in room temperature to dry so that the plantation medium will be solid, were a semi-solid gelatinous layer was generated

2.4.3 Microorganisms:

In this study, two bacterial species were used: one was gram positive bacteria which was *Staphylococcus aureus* and the other was gram negative bacteria, which was *Escherichia coli* and more type of fungi there explain by it table:

Microorganisms	Sources
Candida albicans, *Trichophyton rubrum,* *Trichophyton mentagrophytes*	AL _ Nahrain university , College of Science, Department of biotechnology
Escherichia coli *Staphylococcus aureus*	University of Baghdad , college of Science, Department of biology .

2.4.3.1 Activation of Bacteria

3 – 5 colonies of studied bacteria were transported by using a loop to a test tube containing (5 ml) of sterilized nutrient broth. The tube was shaken well and incubated in 37^{o}C for two hours. The loop was sterilized by a flame before using in order not to kill the planted bacteria.

And medium Inoculated bacteria suspension was diluted by 1/100 by using normal-saline liquid with concentration of (0.85%) to prevent crowded growth. (0.1 ml) of bacteria diluted suspension was transported to each plate and spread by using sterilized cotton spreader on test medium surface. The plates were left for 15-20 minutes in 37^{o}C to make absorption.

2.4.4 Preparation of discs

The discs were prepared from filter paper type (Whatman No.2) using the drill and we obtain 6 mm discs. The DMSO solvent was used to prepare four concentrations for each prepared material (10 µg/Disc) (100 µg/Disc), (250 µg/Disc) and (500 µg/Disc). for each concentration, 50 discs from filter paper were prepared , then sterilized in oven for (15 minutes) and (0.01 ml) from each solution was applied to the discs, then the discs were dried at (40^{o}C) for (30 minutes). A control sample was made for the DMSO solvent by adding (0.01 ml) from the solution to each disc from the sterilized filter paper.

The above mentioned discs were fixed by a sterilized forceps with a tiny end in addition to light pressure on the disc surface. 25 mm distance was left between each disc and 1 cm between the disc and the edge of plate to prevent interference of inhibition zone

2.4.5Evaluation of the biological activity of the prepared compounds

To study effect of the chemical compounds prepared on the species of bacteria and fungi mentioned before, Kirby-Baur's[58] method was selected to measure the effect of the prepared compounds [59]on bacteria .

There are two common methods to study anti bacterial activity, these are:

1- Dilution method

2- Diffusion method

Method no.2 depends on the known concentration spread of exposer tested to measure fatal or inhibitor for chemical compounds by taking discs from paper filter type (Whatman No.2) with a (6mm) diameter in clean glass tubes with spiral plugs sterilized by an Autoclave for (15 minutes), then sinking it into chemical compounds solution for (5 minutes) and then transporting it into solid agar plate maculated with (0.1 ml) of a bacterial culture of age 18 hours which was planted on the plate by spreading. The plates were than incubated at (37^{o}C) for 24 hours, and the inhibition zone for the area that had no bacteria growth was the measured reflects the influence of the compounds and depend upon many physical and chemical factors.

And for fungi were prepared the agar media and drilled the media as hauls which far form other (2-3 cm) .Then fixed apices of isolated

fungus on the agar and the discs for prepared compounds with different concentrations with (2 cm) distance between the haul fungi and disc.

The plates were than incubated at (30^0C) for 24 hours,

Then measurement of Inhibition Zone the transparency area which surrounds the disc, including the radius of disc which represents the area which had no bacterial or fungal growth, was measured. This area is called inhibition zone by using a mm ruler. The bacteria and fungi were considered sensitive, mean-sensitive or resistant depending on inhibition zone see tables (3-4) to (3-8).

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