Open Access
Baghdad Science Journal Vol.13(2)2016

DOI: http://dx.doi.org/10.21123/bsj.2016.13.2.0368

Synthesis and Biological Effectiveness of Some new Azo Compounds as Derivatives of Nitrogen Bases

Noor Khalid Zaidan *Wissam Mahmud Mohamed*

Department of Physiology, Pharmacology & Biochemistry, Veterinary Medicine College, University of Al-Fallujah.

E-mail: Wissamatea@yahoo.com

Received 15/3/ 2015
Accepted 1/7/ 2015

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International Licens

Abstract:

In this study the new azo compounds (3compounds) for nitrogen bases (Adenine and Cytosine) are synthesized through two reaction steps (formation of diazonium salt and coupling reaction). The compounds have been characterized by FTIR, melting point, and ultra-violate (UV) spectra. All synthesized compounds have been estimated *in vitro* for their antimicrobial activities against two species of bacteria(*E.coli*, *S.aureus*)and one kind of fungi (*Aspergillus flavus*) .The results show that these compounds have very good antibacterial and antifungal activities especially compounds 1 and 3.To study the effect of these compounds were making some physiological tests on rats are made ,the results of hematological study showed decreasing level of total hemoglobin concentration in all treatment groups specially in group (1).The values of Packed in cell volume (P.C.V) are within normal blood range of rats . Total leucocytes count (W.B.C) decrease in all groups.

Key words: Azo Compound, Biological Effect, Nitrogen Bases, Antimicrobial activities.

Introduction:

Nitrogen bases are necessary for a large spectrum of metabolic processes, not the lower of which is nucleic acid synthesis. Derivatives of nitrogen bases and nucleotides play probable important roles in cell division, senescence, and protection reactions [1]. Where these derivatives have an important biological effect in the production of many antibacterial drugs, antifungal and antivirus and to treat cancer and AIDS because of their capability to inhibit cell growth pathogenesis [2]. Nowadays, synthetic azo compounds are generally used in different application fields, such as medicines, cosmetics, food, paints, plastics, and in analytical chemistry [3,4]. Biological importance of azo compounds is well known for their usefulness as anti-ceroplastics, anti-diabetics, antiseptics, anti-inflammatory, other useful chemotherapeutic agents [5, 6], and in Photodynamic treatment (PDT) is a new type of treatment for tumors and other diseases [7]. Azo compounds are known to be involved in a number^{o} of biological

reactions such as inhibition of RNA, DNA, protein production, carcinogenesis and nitrogen fixation[8]. Evans blue and Congo Red are being considered as HIV inhibitors. This effect is supposed to be caused by binding of azo dyes to both reverse transcriptase and protease of this virus[o] [9]. The existence of an azo moiety in different types of compounds has caused them to explain pesticidal actions and antibacterial [10, 11]. It has been found that the activity of azo compound increases the incorporation of proper heterocyclic moiety[12]. In the case of anti-herpes-virus agents, the mainly frequently used compounds are derivatives of guanine (acyclovir, ganciclovir) or 5-substituted pyrimidines (brivudin, idoxuridine, trifluridine)[13]. There are a lot of examples of the derivatives that have functional anti-cancer and viral and called nucleosides antibiotics and these examples include compound 5-Iodo-2-deoxy uridine and5-(2-Bromo-2-deoxy) uridine. These two compounds are used as anti-viruses resources where it is used as management for infections caused by one virus types called Herpes simplex virus kind I (HSV-I) [14]. Purine and pyrimidine nucleosides and nucleotides are constituents of essential structures of the cells. In fact, they are constituents of nucleic acids and their formation is present in several coenzymes involved in cellular reduction/oxidation processes, like nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD)[1].For the reason of getting inhibition of cell growth using derivatives or analogues occurs modulating or Replacement[7].This study is conducted including

1- Preparation of new azo compounds for adenine and cytosine.
2- Exhibiting their biological activity by test compounds to inhibit the growth of types of bacteria and fungi.
3- Studying the effect of these compounds on some physiological parameters in rats.

Materials and Methods:

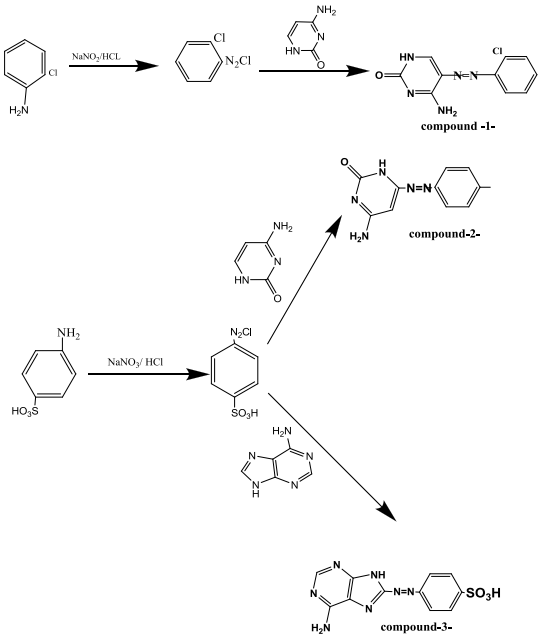
The purity of the synthesized compounds uses absolute ethanol .The melting points are determined in open capillary method by (Gallen Kamp MFB-600) melting point apparatus. The UV spectra of the azo compounds are reported on (CegilCe 302 UV/Vis) Scanning spectrophotometer. In the present investigation the IR spectra of azo compounds were recorded by Perkin-Elmer Paragon Identic hock FT.IR System in DMSO as solvent in antimicrobial studies.

Synthesis of Azo Compounds

Azo compounds are synthesized by depending on the method reported by Jarrahpour and Zarei[15]. There are two steps in the synthesis of azo compounds:

Step I: Diazotization: A mixture of freshly distilled amine and concentrated hydrochloride acid are moved until a clear solution is obtained. This solution is cooled to 0–5^0 C, and a solution of sodium nitrite in water is then added by dropping, maintaining the temperature below 5^0 C. The resulting mixture is moved for an additional 30 min in an ice bath.

Step II: Coupling: The nitrogen bases are dissolved in 10% sodium hydroxide, and cooled to 0–5 C^{o}in an ice bath. This solution is then slowly added to the cooled diazonium salt solution to yield azo compound. Azo compounds are synthesized by depending on Scheme 1.



Scheme (1) Synthesis of Azo Compound

Antimicrobial Studies

The synthesis of azo compounds is screened for their antibacterial action by disc diffusion method [16, 17]. Nutrient agar is used as culture medium for bacterial growth while fungi are sub cultured in dextrose agar medium, where a series of concentrations in DMSO are prepared 0.1 mg/ml, 1 mg/ml, 10 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml and flooded in filter paper discs of 6 mm diameter.^{o}These discs are placed on the seeded plates and incubated at 37C^{o}. The invitro antibacterial activity is carried against 24h old cultures of pathogenic bacteria like gram (+) *S. aureusand gram (-)E. coli* and at 37 C^{o}. Antifungal activity is carried out against 72h old cultures of fungal strains like *Aspergillus flavus* and incubated at 28C^{o},(1 mL) is added to each of the series concentration of compounds prepared to the dextrose agar medium before cultured of fungi to make sure of the effect of these compounds to inhibit the growth of fungus. The compounds are considered active or inactive depending on the fungus growth or no growth .In order to ensure that solvent has no effect on bacteria or fungal growth, a control test is performed with DMSO and found inactive in culture medium. The zones of bacterial inhibition are determined disk surrounding including diameter disk which represents serenaded not accrue where bacterial growth and this area is called zone of Inhibition in diameter, using the ruler.

Hematological Study:

This study includes some physiological tests:

1-Total Hemoglobin Concentration (Hb): The hemoglobin is determined by Cyan methemoglobin method, 20 µl of blood is transferred with the help of Hemoglobin pipette into a test tube containing 5 mL of Drabkin's solution. after adjusting the photoelectric colorimeter at 540 nm with a blank (Drabkin's diluents) [18] .

2-Packed Cell Volume (P.C.V): The blood sample insert in capillary tube, sealed the dry end by pushing it into the sealing clay. Place the tube into one of the micro hematocrit centrifuge plate slots for five minutes. Read PCV in the micro haematocrit reader [18].

3-Total Leucocytes Count (W.B.C): The glacial acetic acid lyses the red cells while the gentian violets lightly stains the nuclei of the leucocyte. The blood sample is diluted to 1:20 in a WBC pipette with the diluting fluid and the cells are counted under low power microscope by using a counter chamber. The number of cells in undiluted blood are reported as the number of white cells/mm^3 of whole blood [18].

Animals:

This study uses (16) rats divided into four groups:

Group 1: Includes four rats given oral solution from 6-(2-chloro phenyl azo)-cytosine a dose of (1 mL/Kg) daily for two weeks.

Group 2: Includes four rats given oral solution from 6-(4-sulfo phenyl azo) -cytosine a dose of (1 mL/Kg) daily for two weeks

Group 3: Includes four rats given oral solution from 8-(4-sulfo phenyl azo)-Adenine a dose of (1 mL/Kg) daily for two weeks.

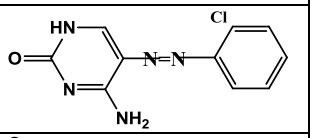
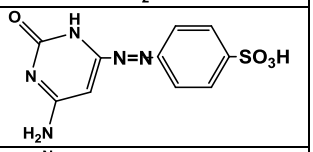
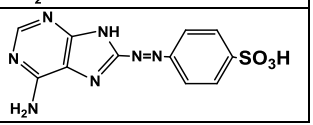
Group4: The control group includes four rats given oral distilled water every 24 hours for two week.

After two days of the last dosage process, blood is pulled by heart puncture and put in tubes container EDTA anticoagulant for physiological tests.

Results and Discussion:

Azo compounds have a wide range of biological effects, so is the preparation of a number of these compounds(1,2,3) by coupling equal amounts of phenylalanine Diazonuim salts substitutes with nitrogen bases purines and pyrimidine, as shown in Scheme 1. Compounds are identified by melting point (Table 1) and ultra violet (Table 2) and infra-red spectroscopy (Table 3).

Table (1) Physical Properties of Compound

Com. No.	Name	Molecular Formula	Color	M.Wt (g/mol)	Yield %	M.P. C°	Structure
1	6-(2-chloro phenyl azo)-cytosine	$C_{10}H_8N_5CLO$	yellow	250	76	216-218	
2	6-(4-sulfo phenyl azo)-Cytosine	$C_{10}H_9N_5SO_4$	Brown	295	68	232-234	
3	8-(4-sulfo phenyl azo)-Adenine	$C_{11}H_9N_7SO_3$	Dark green	319	71	272-274	

Table(2) Major Packs of UV

Compound Number	λ max (nm)		
	Ph-N=N	Purine & pyrimidine	Phenyl
1	302	250	228
2	319	249.6	222
3	370	251.2	237

Table (3) Major Packs of IR

Compound number	v(N-H)	v(C=O)	v(C=N)	v(C=C)	v(N=N)	v(C-N)	Other group
1	3360	1612	1580	1510	1445	1170	760 Ar-CL
2	3310	1565	1618	1517	1473	1363	1080 O=S=O
3	3365	1620	1660	1545	1495	1239	1190 O=S=O

Ultra violet spectra for azo compounds in ethanol expose clearly the presence of (n-Л*) transfer group in concentration(10-4M) showing three packs a major first when λ max = (370-300) nm belonging to the group of azo (ph – N =N-) [19] and the second package at λ max = (251.2-249.6) nm returning to absorb a purine and pyrimidine ring as purines and pyrimidines are units that absorb radiation higher than the required radiation in nuclear acid and their derivatives, either refuse sugar and phosphate shall be transparent on 200nm [20] either package third is shows sharp peak at λ max = (222-237) nm due to π-π* transition of aromatic phenyl ring[18]. Infrared spectra: The glimpse at the structure of azo compounds, one may (O-H, N=N, -N-H, C=C, =C-H, C-N and C-O) vibrations in IR region[21]. suppose the absorption bands due to. Important IR peak values of azo compounds are given in (Table 3).

Below is a breakdown of the packs that appeared in the IR spectrum:
Packs a broad absorption within the range (3365-3310 cm^{-1}) of the spectrum return to vibration the Stretching Vibration to bond N-H. Spectra showed absorption packs azo compounds within the range (1495-1445cm^{-1}) back to the vibration frequencies of the bond N = N and has been suggested in the literature [17] to the absorption site. The azo package depends on the type of compounds connected.

Have been diagnosed with many packs in spectra of azo compounds placed within the range (1367-1172cm^{-1}) return generally to bridge vibration frequencies which is (C-N = N-C,C=N - N=C). also show compounds spectra of (1) packs within the absorption Range in (760 cm^{-1}) back to frequency for bonds Ar - Cl, as stated in sources [22] that a group of Ar-Cl show pack absorption within the range (700-600cm^{-1}). The spectra of compounds (1, 2) show packs within the absorption range (1080-1190cm^{-1}) back to Sulfate group (O=S=O).According to the study carried out by Hadzi that the area between (1700-1400cm^{-1}) is the area which is the probable appearance of (1730-1700cm^{-1}) to the sites (1710-1580 cm^{-1}) [17].

Antimicrobial Activity

Bactericidal activities of the synthesized azo compounds contra pathogenic bacteria are recorded by disc diffusion method [23] and the results are given in (Table 4) and (Table 5).Both azo compound 1, 2 and azo compound 3 exhibit varying degree of antibacterial activity against the test packets caused by frequency resulting by the combination groups vibrations C=O and C=N, which confirm the presence of formulas as a result of the presence of hydrogen ties underlying that work on change lengths ties and the formulas occurs which leads to a displacement of red for the vibration frequency of the carbonyl group for its pure anticipation at frequency region organisms. The azo compound 3 shows the highest antibacterial activity with the inhibition zone of 29 mm against *S.aureus* at 100 mg concentration. Both azo compound 1 and 3 shows varying degree of antibacterial activity in all concentration against *E.coli*, while azo compound 2 shows antibacterial activity in highest concentration only.

Table (4) Diameter of Inhibition Zone (mm) against *E .coli*

Diameter of Inhibition Zone (mm) against *E. coli*						
Compound Number	100 mg/mL	50 mg/mL	25 mg/mL	10 mg/mL	1 mg/mL	0.1 mg/mL
1	16	13.5	11	10.5	8	7
2	9	7.5	6.5	--	--	--
3	14	11	9	8.5	7	6.5

Table (5) Diameter of Inhibition Zone (mm) against *S. aureus*

Diameter of Inhibition Zone (mm) against *S. aureus*						
Compound Number	100 mg/mL	50 mg/mL	25 mg/mL	10 mg/mL	1 mg/mL	0.1 mg/mL
1	9	7	7	--	--	--
2	8	6.5	--	--	--	--
3	29	24	19	9	7	--

The results of fungicidal screening (Table 6) shows that azo compounds (1) and (3) are active, while (2) are inactive against fungal kind. The difference in the activity of different compounds against different organisms depends either on the impermeability of the cells variation or microbes in ribosome of microbial cells [24]. Pyrimidine complexes compound are effective in the control of fungi and microorganisms such as bacteria and viruses [25]. Synthetic antimicrobial agents include sulfonamides, diamino pyrimidine derivatives and N- chloro compounds represented by amides. This explains why effective biological large compound 1 and 3 and their inhibitory to bacterial and fungal in among concentrations compared with compound 2, are a highly effective at inhibiting the growth of bacteria in concentrations of 100, 50 and 25 but it inactive in inhibiting the growth of fungal. The compound 3 is effective in inhibiting the growth of bacteria in all concentrations, but in fungal growth is effective in high concentrations 100, 50 and 25 only .This compound does not have an impact on low concentrations. Organic dyes have been extensively used as antibacterial agents. The process of action of anti microbials may either kill microorganisms perfectly or simply. The exact mechanism is not understood biochemically, antimicrobials may affect various targets in microorganisms [26].

Table (6) Antifungal activity against *Aspergillus flavus*

Antifungal activity against *Aspergillus flavus*						
Compound Number	100 mg/mL	50 mg/mL	25 mg/mL	10 mg/mL	1 mg/mL	0.1 mg/mL
1	Active	Active	Active	Moderate	Inactive	Inactive
2	Inactive	inactive	Inactive	Inactive	Inactive	Inactive
3	Active	moderate	Inactive	Inactive	Inactive	Inactive
Control (DMSO)	Inactive	inactive	inactive	inactive	Inactive	Inactive

Hematological Study:

The results of physiological study (Table 7) show decrease in the level of total hemoglobin concentration in all groups especially in group (1) where the value is 9.3 g/dl compared with the control group when the value is 13.2 g/dl. These results coincided with Sharma, K.P et.al; all decrease the erythrocyte count and haemoglobin content in the exposed lab animals signify the declined haematopoiesis followed by anaemic condition of lab animals due to haemo dilution. Dye might decrease haemoglobin's affinity towards oxygen making the erythrocytes more weak and porous, which results in cell inflammation, deformation and damage [27]. While the results of packed cell volume PCV show that no significant change in the dosing group compared to the control group. These results are within normal blood range of rats whose range is (30-40%) [28]. Total leucocytes count (W.B.C) decrease in all groups, having the values 2565, 2980, 2724 cell/mm^3 while the value in the control group is 3430 cell/mm$^{3.}$ This decrease in (W.B.C) may be due to the influence of the preparation compounds which probably cause the lack of balance in (W.B.C).

Table (7) Results of Physiological Tests (mean ±SD)

Groups	Hb %	PCV%	WBC cell/mm^3
G1	9.3±0.2	32±1	2565
G2	11.8± 0.5	33±2	2980
G3	10.9±0.4	31±2	2724
G4	13.2±0.7	32±1.8	3430

References:

[1]Bernd, G. and Lukas, B. 2000. A New Family of High-Affinity Transporters for Adenine, Cytosine, and Purine Derivatives in Arabidopsis. Amer. Soc. of Plant Phys, 12(2): 291–300.

[2]Art, J.and Weinberg, M. 1996. Mechanism of nucleoside analog

antiviral activity and resitance during human immunodeficiency virus reverse transcription. Amer .Soc for microbiology,40(3):527-540

[3]Wainwright, M. 2001.Acridine-aforgotten antimicrobial chromophore. J. Antimicrob. Chem, 47(1): 1-13.

[4]Simu, G. M.; Hora, S. G.; Grad, M. E. and Sisu, E. N. V. 2005.Azo compound with antimicrobial activity Rev. Roum. Chim, 50(2): 113.

[5]Bae, J. S.; Freeman, H. S. and El-Shafei, A. 2003. Dyes and Pigments, 57(2): 121-129.

[6]Sourabh, S. and Shrikant, T. 2012. Synthesis of some new azo compound and their antimicrobial screening. Int. J. of Pharm. & Techn, 4(3):4600-4606.

[7]Anitha, K. R and Venugopala, R. 2011. Synthesis and antimicrobial evaluation of metal (II) complexes. J. Chem. Pharm. Res, 3(3):511-519

[8]Rajendra, N.; Madhu. S.; Verma, and Naveen, K. 1998. Croatica-chemicaacta, 71(3): 715-726.

[9]Fatma, K. and Eser, K. 2007. Spectroscopic and thermal studies on solid complexes of 4-(2-pyridylazo) resorcinol with some transition metals. Acta Chim. Slov, 54(2): 242–247.

[10] Pandey, G. and Narang, K. K. 2005. Synthesis, Characterization, Spectral Studies and Antifungal Activity of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) Complexes with 2-(4- Sulphophenylazo)-1,8-Dihydroxy-3,6-Napthalene Disulphonic Acid Trisodium Salt. Bioinorganic Chem. and Ap, 3(3-4): 217-238.

[11] Swati, A. and Manu, G. 2011. Synthesis, Spectroscopic haracterization and Antibacterial Activity of 2-[2- Hydroxyphenylazo]-1-naphthol-4-sulphonicacid and its Fe (III) Co (II) and Cu (II) complexes, Journal of Pharmaceutical, Biological and Chemical Sciences,2(2):809

[12] Shridhar, A. H; Keshavayya, J.; Hoskeri, H. 2011. Synthesis of Some Novel Bis 1,3,4-Oxadiazole Fused Azo Dye Derivatives as Potent Antimicrobial Agents. Int. Res. J. of Pure & Applied,1(3): 119-129.

[13] De Clercq, E. 2001. Antiviral drugs J. Clin. Virol, 22(1): 73-89.

[14] Hocek, M. 2005. Nucleic Acids symposium series, 49(1):29-30.

[15] Jarrahpour, A. A. and Zarei, M. 2004. Synthesis of novel azo Schiff base bis[5-(4-methoxyphenylazo)-2-hydroxy -3-methoxy benzaldehyde]-1,2-phenylene diimine. Molebank, 377.

[16] Oakes, J. and Gratton, H. 1998. Kinetic investigations of the oxidation of arylazonaphthol dyes in hypochlorite solutions as a function of pH. J. Chem. Soc., Perkin Trans, 2: 2201-2206.

[17] Swati, T. and Ginni, G. 2011. Synthesis characterization and antimicrobial screening of some azo compounds. Int. J. of Basic & Ap. S., 2: 332-338.

[18] Sood, R. 1992. Medical laboratory Technology methods and interpretation.6thed, New Delhi-India, Jaypee Brothers.

[19] Gopala, S.; krishnan, N. and Nevaditha, T. 2011. Antibacterial activity of azo compounds synthesized from the natural renewable source, cardanol. J. Chem. Pharm. Res., 3(4):490-497.

[20] Yousi, H. K. 2008. Synthesis of number of azo compound derivative of guanine. J. of al-anbar University for pure science, 2:3.

[21] Hameed, A. K.; Hassan, A., k. and Sattar, S. A. 2014. Preparation and diagnosis of new types of Schiff bases one-and two- claw and complexes to study the effectiveness of chemical. Baghdad Science Journal, 2(11):311-316.

[22] Balint, G. and Andoni, M. 2010. Azo compound and anti-microbial activity. International electronic conference on synthetic organic chemistry, 1(30): 14.

[23] Jorgensen, J. H. and Turnidge, J. D. 2003. Susceptibility test methods: dilution and disk diffusion methods. Manual of Clinical Microbiology, 8th edn (Murray PR, Baron EJ, Jorgensen JH, Pfaller MA & Yolken RH, eds). Am. Soc. for Microbiology, Washington, DC, 1108-1127.

[24] Masoud, M. S.; Khalil, E. A.; Hindawy, A. M.; Ali, A. E. and Mohamed, E. F. 2004. Spectrochimical Acta Part A. Molecular and Bimolecular Spectroscopy, 60: 2807-2817.

[25] Machhi, J. K. 2000. Studies on some aspects of toxicology of Ayurvedic drug. Ph. D. Thesis, University of Veer Narmad South Gujarat, Surat, 66.

[26] Yarovenko, V. N.; Lysenko, O. V. and Krayushkin, M. M. 1993. Chemistry of heterocyclic compounds, 29(4): 452-54.

[27] Sharma, K. P.; Sharma, S.; Sharma, S.; Singh, P. K.; Kumar, S.; Grover, R. and Sharma, P. K. 2007. A comparative study on characterization of textile wastewaters (untreated and treated) toxicity by chemical and biological tests. Chemosphere, 69: 48-54

[28] Tites, N.W. 1982. Fundamental of clinical chemistry. 3rd ed. philadiliphai. U.S.A. 1100.

تحضير ودراسة الفعالية الحيوية لبعض مركبات الآزو الجديدة كمشتقات للقواعد النايتروجينية

نور خالد زيدان **وسام محمود محمد**

فرع الفسلجة والأدوية والكيمياء الحياتية، كلية الطب البيطري، جامعة الفلوجة.

الخلاصة :

تضمنت الدراسة الحالية تحضير عدد من مركبات الازو(3 مركبات) كمشتقات للقواعد النتروجينية (الادينين و السايتوسين). تم تحضير هذه المشتقات بخطوتين الأولى تحضير أملاح الفنيل دايزونيوم المعوضة والخطوة الثانية تفاعل الازدواج Coupling مع القواعد النتروجينية.

تم تشخيص التراكيب الكيميائية للمركبات المحضرة بقياس درجة الانصهار كما أجريت التحليلات الطيفية المتمثلة بطيف الأشعة فوق البنفسجية UV وطيف الأشعة تحت الحمراء I.R .ولإظهار أهمية هذه المركبات من الناحية التطبيقية أجريت اختبارات على كائنات حية مجهريه (بكتريا وفطريات) كما أجريت بعض الاختبارات الفسلجية على الجرذان البيضاء . استخدمت طريقة الانتشار حول القرص على إطباق وسط الاكار Plate agar disc diffusion method في حالة البكتريا واستعمل نوعيين من البكتريا وهي(*S.aureus* و*E.Coli*) فضلا عن نوع واحد من الفطريات وهو(*Aspergillus flavus*) حيث تم إضافة تراكيز مختلفة من المركبات المحضرة إلى الوسط الزرعي للفطر فضلا عن اوساط السيطرة control. أعطت هذه الاختبارات نتائج جيدة في تثبيط نمو الأنواع المذكورة من البكتريا و الفطر و خاصة المركب 1 و3 .من جانب اخر أوضحت نتائج الدراسة الفسلجية انخفاض في مستوى صبغين الدم (الهيموكلوبين) (Hb) في كل المجاميع وخصوصا في المجموعة 1، بينما كانت قيم مكداس الدم(PCV) ضمن المستوى الطبيعي في دم الجرذان البيض. اما مجموع كريات الدم البيض (NBC) فقد انخفض في كل المجاميع مقارنة بمجموعة السيطرة.

الكلمات المفتاحية : مركبات آزو ، قواعد نايتروجينيه ، فعالية حيوية ، ادنين وسايتوسين.