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Synthesis, Characterization and Biological effect Study of Some New azo and bis (Azo–Schiff) Compounds Derived from 2, 4-Dihydroxybenzaldehyde

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Azo- Schiff, 2, 4-Dihydroxybenzaldehyde , diazonium salt.

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Abstract
The objective of this work was to prepare azo and bis(azo- schiff base) derivatives from 2 , 4-Dihydroxybenzaldehyde which has extensive use in the synthesis of organic compounds. The azo-compound (A_1-A_3) were prepared from the coupling reaction between prepared diazonium salt and with 2, 4-Dihydroxybenzaldehyde . A series of Schiff base have been prepared from prepared compounds (A_1 – A_3) were reacted in acidic condition with different substituted aromatic diamine in ethanol to give fourteen new bis (azo – Schiff base) (A_4-A_{17}) in good yields. The final products were identified by their physical properties besides their FTIR and 1H NMR spectra. Biological activity of the compounds (A_4- A_{17}) were investigated and compounds (A_{13} and A_{14}) showed activity against *E. coli* .

1- Introduction

Azo compounds or dyes constitute one of the largest and most varied groups of synthetic organic dyes in the use today .[1] Azo compounds are highly important and widely used substance in the textile ,paper ,coloring agent for food and cosmetics industries . other application for azo compounds in the field liquid crystals and in the organic photoconductors .Azo compounds serve as important analytical tools by providing a strongly chromophoric label . besides , azo compounds ,are important analytical aid compounds serving as PH indicators and complexometric indicator . The pharmaceutical use of azo compounds, produced from the discovery of the antibacterial action of prontsil on streptococcal infection . Azo compounds were reported as a variety biological activities including antibacterial , antifungal , pesticidal , antiviral and anti-inflammatory activities.[1-6]

Some Schiff bases bearing aryl groups possess excellent biological activities , which has attracted many researchers attention in recent year. They have been reported to

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use as analgesic ,antibacterial , antituberculer , antiverial ,anti-inflammatory , antihypertensive ,antimicrobial and anticancer[7-11].

In this paper we have synthesis new bis (azo - Schiff bases compounds) which derivatives from three different azo- 2,4-dihydroxybenzaldehyde ,when reacted with five different diamino compounds and also study its biological affect ..

2- Experimental

2.1. Materials and Methods

All chemicals were purchased from Merck, Sigma-Aldrich, and Scharlau, and used without further purification. Melting points were determined using melting point apparatus Stuart–SMP11. IR spectra were recorded in department of chemistry, collage of science, University of Kirkuk as KBr discs on a Nicolet 100 FT-IR spectrophotometer. ^{1}H NMR spectra were recorded as solutions in DMSO on a 500 MHz Brucker, in Gazi University, Ankara-Turkey, chemical shifts were referenced to Tetramethylsilane.

2.2. General procedure for the synthesis of azo-2,4-dihydroxybenzaldehyd(A_1-A_3) [14]

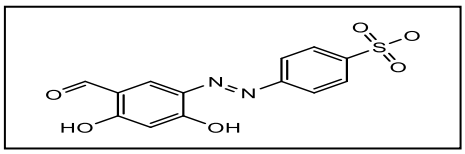
i-Preparation of the Diazonium salt solution ;

50 gm ice , 20ml water and 5ml conc. Hydrochloric acid were filled in a 100ml Erlenmeyer flask with magnetic stirring and internal thermometer. To this mixture (0.01 mol) amino derivatives compounds **(4-amino benzene sulfonic acid , 4-amino benzoic acid, 4- amino naphthalene sulfonic acid)** were added. Under ice cooling and stirring and under internal temperature of (0-5)C. A solution of (0.01 mol) sodium nitrite in 20 ml water was added slowly by using pipette .

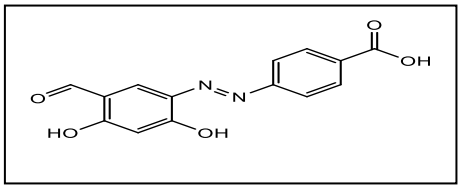
ii- preparation of azo compounds: (A1-A3)

(0.01 mol) 2,4-dihydroxy benzaldehyde is dissolved in (0.1)M sodium hydroxide solution in 250ml Erlenmeyer flask . The solution was cooled .Under strong stirring and ice cooling , the ice cooled diazonium salt solution prepared is added in portions. Towards the end of addition the PH value of the solution is controlled. To keep the solution in the alkaline range . after the addition is finished , the mixture was stirred for 30 min. at (0-5)C.

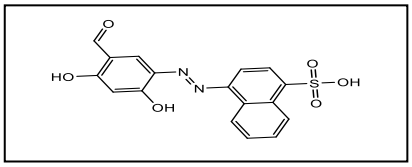
4-((5-formyl-2,4-dihydroxyphenyl)diazenyl)benzenesulfonic acid (A_1): m.p. 177-180 °C,yield: 68%.; IR (KBr) (cm^{-1}): 3344 (O-H, phenolic), 3000 (C-H aromatic), 1655(C=O carbonyl), 1597 (C=C aromatic), 1416 (N=N),).



4-((5-formyl-2,4-dihydroxyphenyl)diazenyl)benzoic acid (**A_2**): m.p. 231-236 °C,yield; 62%; IR (KBr) (cm^{-1}): 3384(O-H, phenolic), 3050 (C-H aromatic),1690 (C=O carbonyl), 1597 (C=C aromatic), 1410(N=N),



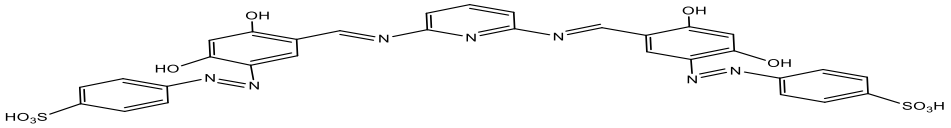
4-((5-formyl-2,4-dihydroxyphenyl)diazenyl)naphthalene-1-sulfonic acid (**A_3**): m.p. 205-209°C,yield; 62%; IR (KBr, cm^{-1}): 3383(O-H,phenolic), 3040 (C-H aromatic), 1667(C=O carbonyl), 1593 (C=C aromatic), 1446 (N=N).



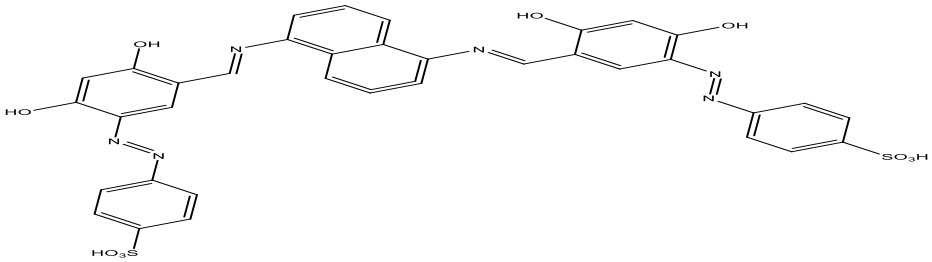
2.3. General procedure for the synthesis of bis(azo- Schiff)(A_4-A_{17}) [15]

(0.002 mol) of the azo compounds (**A_1 – A_3**) was dissolved in (30 mL) of absolute ethanol, the resulting solution was added to (0.001 mol) of different di Amin(**2,6-diaminopyridine, 1,5-diaminonaphthalene, 1,5-diaminoanthracene,benzdiene, urea)** dissolved in (20 mL) of absolute ethanol. The reaction mixture was stirred and reflex for (7 -8) hours. Then the reaction mixture was left in the room temperature to be cool for precipitation. The resulting precipitate was filtrated and recrystallized from the absolute ethanol.

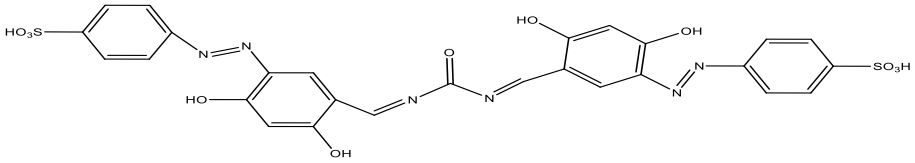
4,4'-(pyridine-2,6-diylbis(azaneylylidene))bis(methaneylylidene))bis(4,6-dihydroxy-3,1-phenylene))bis(diazene-2,1-diyl))dibenzenesulfonic acid (**A_4**): m.p. >250°C, yield :70%; IR (KBr, cm^{-1}):3341 (O-Phenolic), (C-H aromatic),1650 (C=N), 1482(C=C aromatic), 1425 (N=N) ,



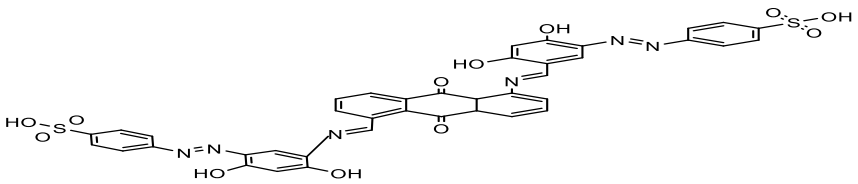
4,4'-(naphthalene-1,5-diylbis(azaneylylidene))bis(methaneylylidene))bis(4,6-dihydroxy-3,1-phenylene))bis(diazene-2,1-diyl))dibenzenesulfonic acid (**A_5**): m. p. 215 - 220 °C, yield; 68%; IR (KBr, cm^{-1}): 3350(O-Phenolic), 3050 (C-H aromatic),1606 (C=N),1557(C=C aromatic), 1410 (N=N),



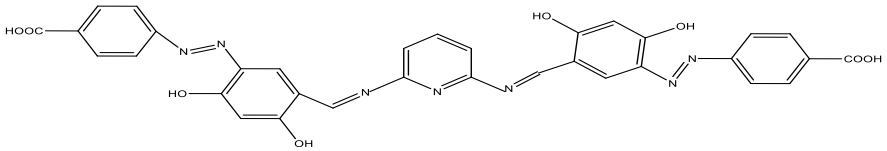
4,4'-(carbonylbis(azaneylylidene))bis(methaneylylidene))bis(4,6-dihydroxy-3,1-phenylene))bis(diazene-2,1-diyl))dibenzenesulfonic acid (**A_6**): m.p. 170- 174°C,yield ; 65 %; IR (KBr, cm^{-1}): 3420 (O-H, phenolic), 2900(C-H aromatic),1652 (C=N), 1494 (C=C aromatic), 1400 (N=N).



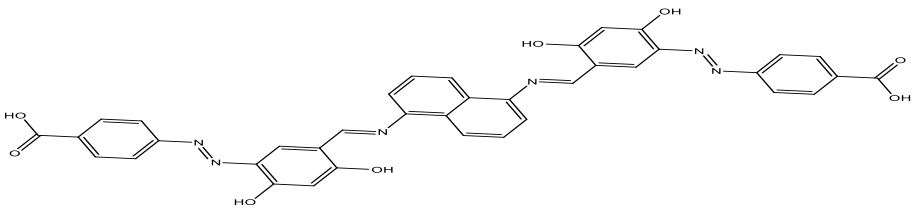
(4)-2,4-dihydroxy-5-(-(4-sulfophenyl)diazenyl)benzylidene)amino)-9,10-dioxo-8a,9,10,10a-tetrahydroanthracen-1-yl)ethyl)-2,4-dihydroxyphenyl)diazenyl)benzenesulfonic acid (**A_7**): m.p. >250 °C,yield; 76%; IR (KBr, cm^{-1}): 3421 (O-H, phenolic), 3090 (C-H aromatic),1610 (C=N), 1542 (C=C aromatic), 1456 (N=N).



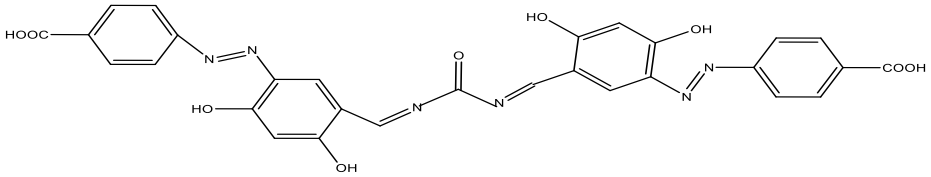
4,4'-(pyridine-2,6-diylbis(azaneylylidene))bis(methaneylylidene))bis(4,6-dihydroxy-3,1-phenylene))bis(diazene-2,1-diyl))dibenzoic acid (**A_8**): m.p. 118- 120Cdec.,yield: 75 %; IR (KBr, cm^{-1}) 3354- 3450 (O-H, phenolic), ---- (C-H aromatic),1600 (C=N), 1534 (C=C aromatic),1425(N=N) ,



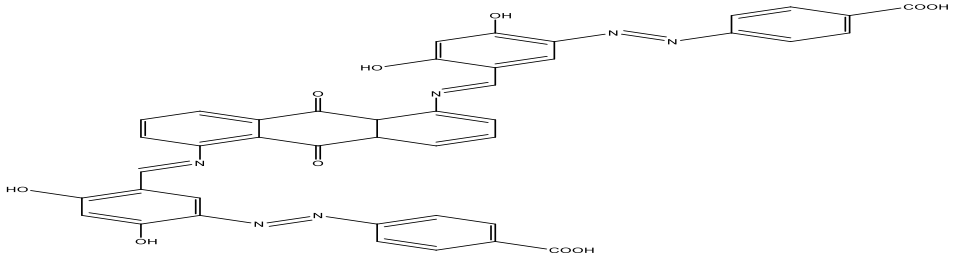
4,4'-(naphthalene-1,5-diylbis(azaneylylidene))bis(methaneylylidene))bis(4,6-dihydroxy-3,1-phenylene))bis(diazene-2,1-diyl))dibenzoic acid (A_9): m.p. 166- 170 °C, yield: 72%; IR (KBr, cm^{-1}): 3377- 3420 (O-H, phenolic), 2971 -3090 (C-H aromatic),1597 (C=N), 1514 (C=C aromatic), 1416 (N=N). **C=O:1699**



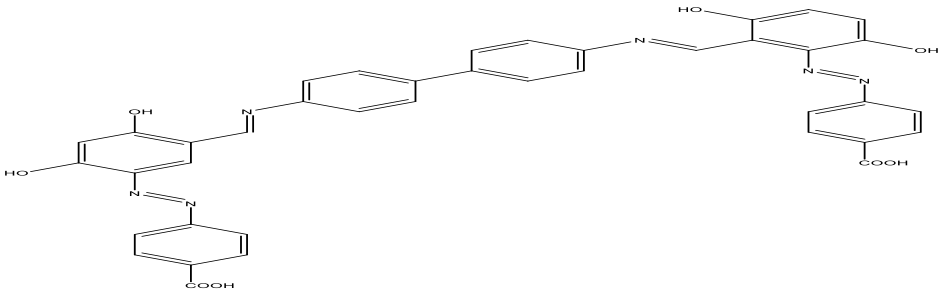
4,4'-(carbonylbis(azaneylylidene))bis(methaneylylidene))bis(4,6-dihydroxy-3,1-phenylene))bis(diazene-2,1-diyl))dibenzoic acid (**A_{10}**): m.p. 138- 140 °C,yield ; 69 %; IR (KBr, cm^{-1}): 3181- 3385 (O-H, phenolic), 3000 - 3100 (C-H aromatic),1602 (C=N), 1509 (C=C aromatic), 1418 (N=N). C=O:1690,:



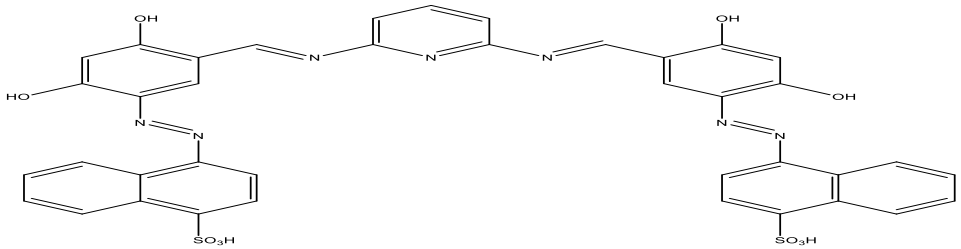
4,4'-((9,10-dioxo-4a,9,9a,10-tetrahydroanthracene-1,5-diyl)bis(azaneylylidene))bis(methaneylylidene))bis(4,6-dihydroxy-3,1-phenylene))bis(diazene-2,1-diyl))dibenzoic acid (**A_{11}**): m.p. 142- 145.°C,yield: 70%; IR (KBr, cm^{-1}): 3419m – 3490sh (O-H, phenolic), 3100 (C-H aromatic),1618 (C=N), 1543 (C=C aromatic), 1410 (N=N). **C=O:** acid 1699, C=O carbonyl anthraquinone:1611



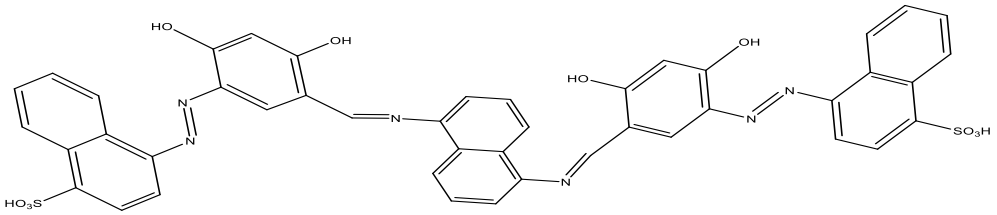
4,4'-([1,1'-biphenyl]-4,4'-diylbis(azaneylylidene))bis(methaneylylidene))bis(4,6-dihydroxy-3,1-phenylene))bis(diazene-2,1-diyl))dibenzoic acid (**A_{12}**): m.p. 150- 152 °C,yield: 71%; IR (KBr, cm-1): 3414 – 3480sh (O-H, phenolic), 3020 (C-H aromatic),1614 (C=N), 1500 (C=C aromatic), 1430 (N=N).



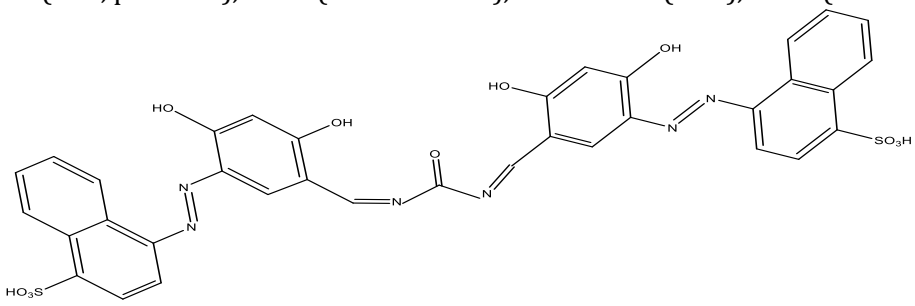
4,4'-pyridine-2,6-diylbis(azaneylylidene))bis(methaneylylidene))bis(4,6-dihydroxy-3,1-phenylene))bis(diazene-2,1-diyl))bis(naphthalene-1-sulfonic acid) (A_{13}): m.p. > 250 °C, 68 %; IR (KBr, cm^{-1}): 3354- 3400 (O-H, phenolic), 3000 (C-H aromatic),1603 (C=N), 1500 (C=C aromatic),1450 (N=N).



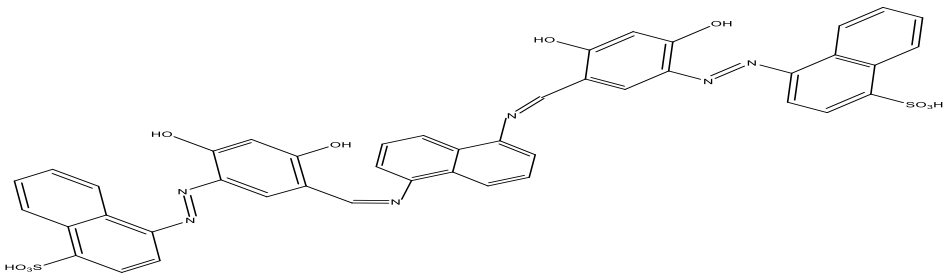
4,4'-(naphthalene-1,5-diylbis(azaneylylidene))bis(methaneylylidene))bis(4,6-dihydroxy-3,1-phenylene))bis(diazene-2,1-diyl))bis(naphthalene-1-sulfonic acid) (A_{14}): m.p. 136 - 140 °C,yield; 69 %; IR (KBr, cm^{-1}): 3300- 3413 (O-H, phenolic), 3030 (C-H aromatic),1625 (C=N), 1517 (C=C aromatic), 1410- 1424(N=N).



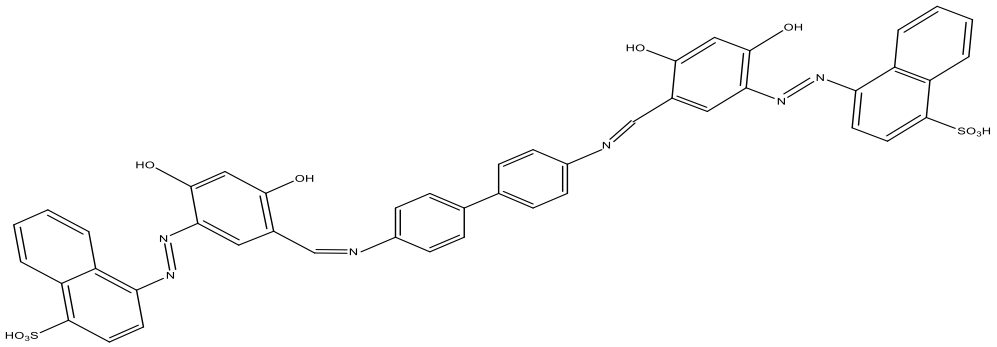
4,4'-((carbonylbis(azaneylylidene))bis(methaneylylidene))bis(4,6-dihydroxy-3,1-phenylene))bis(diazene-2,1-diyl))bis(naphthalene-1-sulfonic acid) (A_{15}): m.p. > 250°Cyield:, 68 %; IR (KBr, cm^{-1}): 3480- 3550 (O-H, phenolic), 2980 (C-H aromatic),1617- 1641 (C=N), 1509 (C=C aromatic), 1420 (N=N).



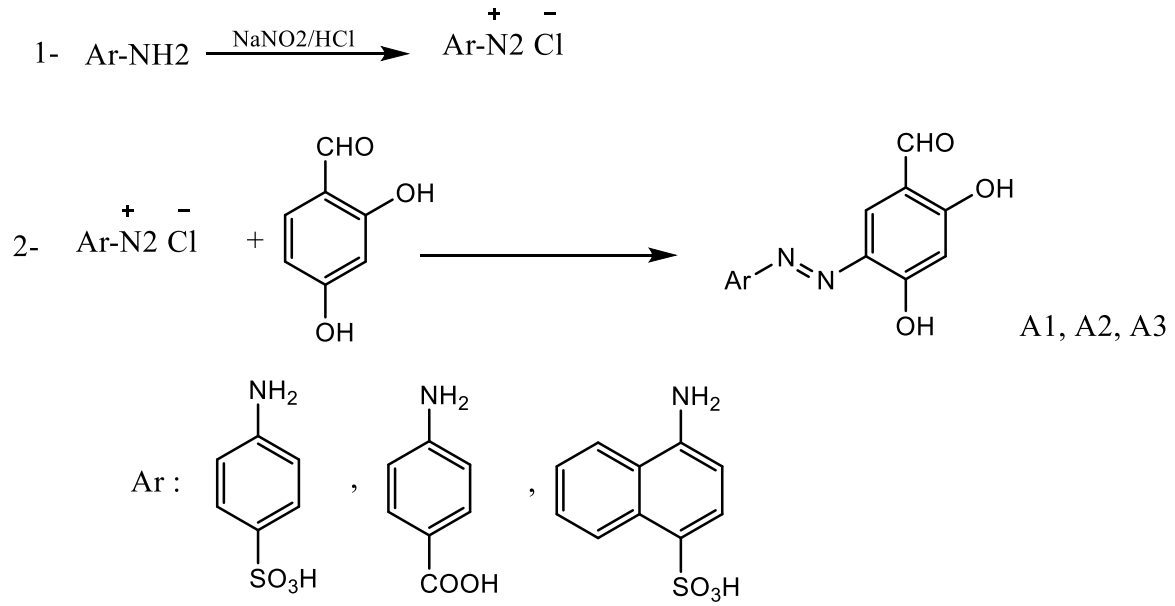
4,4'-(naphthalene-1,5-diylbis(azaneylylidene))bis(methaneylylidene))bis(4,6-dihydroxy-3,1-phenylene))bis(diazene-2,1-diyl))bis(naphthalene-1-sulfonic acid) (A_{16}): m.p. **>** 250°C,yield: 65 %; IR (KBr, cm^{-1}): 3314- 3421 (O-H, phenolic), 3100(C-H aromatic),1620 (C=N), 1542 (C=C aromatic), 1420 (N=N). 1609 (C=O)



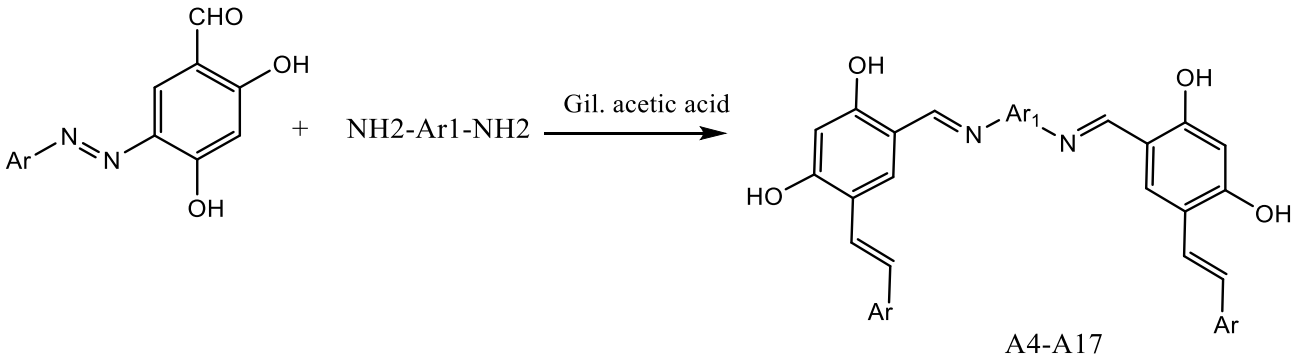
4,4'-([1,1'-biphenyl]-4,4'-diylbis(azaneylylidene))bis(methaneylylidene))bis(4,6-dihydroxy-3,1-phenylene))bis(diazene-2,1-diyl))bis(naphthalene-1-sulfonic acid) (A_{17}): m.p. < 250°Cyield:, 68 %; IR (KBr, cm^{-1}): 3400 (O-H, phenolic), 3068 (C-H aromatic),1655 (C=N), 1513 (C=C aromatic), 1437 (N=N).



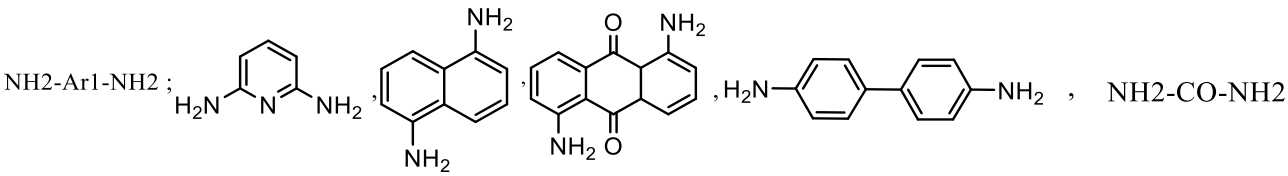
Scheme-1-synthesis of azo compounds



Scheme-2-synthesis of bis(azo - schiff base) compounds



A4-A17



3. Results and Discussions

The present work involve two lines.

First line ; included preparation of new three azo –compounds (A_1-A_3) were prepared by Coupling of diazonuim salts prepared from various substituted aromatic amines (shown in the scheme -1-) with (2,4-dihydroxy benzaldehyde) were held to synthesize the azo -compounds in the active position 5 of the benzene ring (A_1-A_3), scheme 1. The physical properties for azo – compounds including melting point rang of (180-240)C and yield were rang of (60-70)% and these compounds were identified by FT-IR spectroscopy and proton NMR .

Second line; include preparation of new bis(azo-schiff base) derivatives (A_4-A_{17}) which prepared by reaction of azo-compounds(in the first line) with different diamine in ethanol.The synthesis of this compounds were carried out line in scheme-2- and the physical properties for bis (azo – Schiff base) derivatives A_4 including melting point is (215-220)C and percentage yield is 68% . This compound is identified by Ft-IR , HNMR and . Ft-IR spectrum of compound A4 showed absorption band for N=N is 1425cm^{-1} and band for C=N is 1650 cm^{-1}

Proton NMR spectrum of compound A4 showed multiplet signals at (6.5-8) ppm due to aromatic protons and singlet signal at 9.3 ppm due to(CH=N) group proton and signal at 11.1 ppm due to OH group proton at position 4 and other signal at 9.8 ppm due to OH group proton at position 2 in the dihydroxy benzene ring ,

Microbiological investigations

The synthesized compounds were screened for their *in vitro* antimicrobial activity against Gram (+) strain, *Bacillus subtilis* and Gram (-ve) strain, *Escherichia coli* using the Agar dilution method, DMSO as solvent and control [14]. The zone of inhibition around the disc was measured (in mm) as explained beneath the Table 1. The examination of antimicrobial activity of the synthesized compounds reveal that compound A_{13} has high activity against the Gram (-ve) strain, *Escherichia coli* of bacteria; with moderate activity towards the Gram negative bacteria and none activity towards the Gram (+) strain, *Bacillus subtilis* .But that compound A_{14} moderately activity against the Gram (-ve) strain, *Escherichia coli* of bacteria ; and none activity towards the Gram (+) strain, *Bacillus subtilis* . But none of the other compounds (A_4 - A_5 - A_6 - A_7 -

A_8 - A_9 -A_{10} - A_{11} - A_{12} - A_{15} - A_{16} - A_{17}) had any antibacterial activities when compared with the control.

.

Table 1: Antibacterial activity data of the synthesized compounds (A_4-A_{17})

Compd. No.	*B. subtilis* G + ve	*E. coli* G – ve
A_4	-	-
A_5	-	-
A_6	-	-
A_7	-	-
A_8	-	-
A_9	-	-
A_{10}	-	-
A_{11}	-	-
A_{12}	-	-
A_{12}	-	-
A_{13}	-	+++
A_{14}	-	++
A_{15}	-	-
A_{16}	-	-
A_{17}	-	-

Key to interpretation: Less than 10 mm=inactive (-), 10-15 mm=slightly active (+), 15-20 mm= moderately active (++), more than 20 mm=highly active (+++)

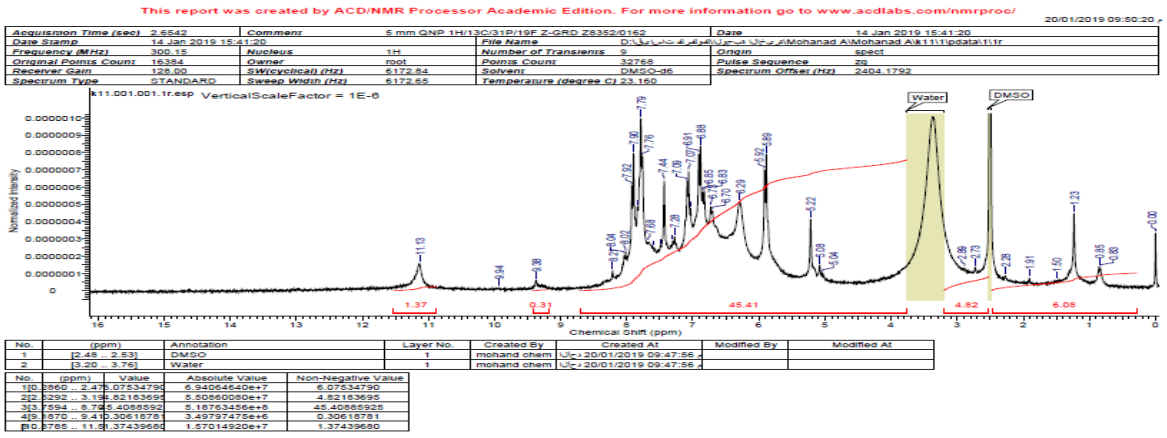


Figure -1- nmr spectrum for compound A4

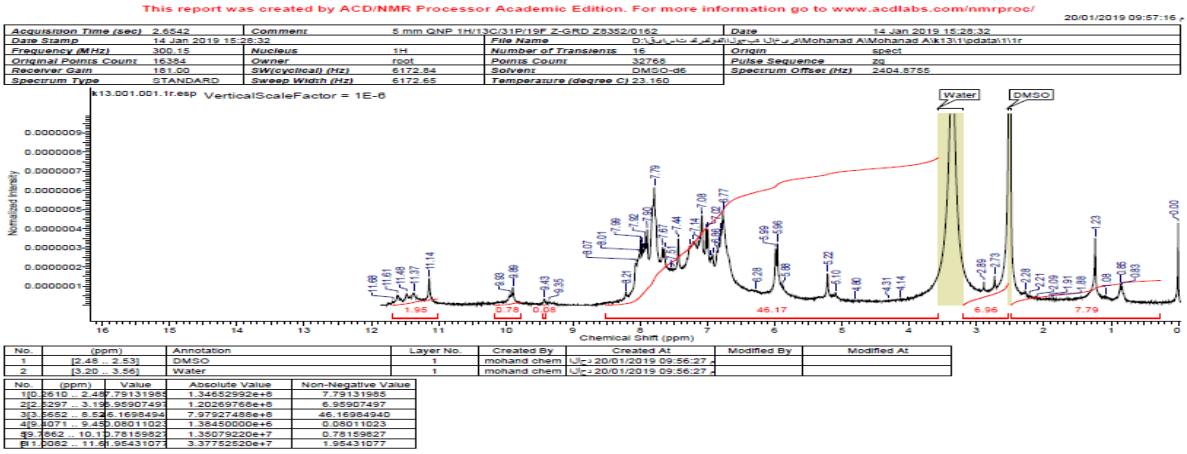


Figure -2- nme spectrum for compound A6

References

1-Xu,H.and Zeng,X.(2010) *Bioorg.Med.Chem.Lett.,20,pp.4193-4195*.

2-Tonelli,M., Vazzana, I., Tasso, B., Boido, V., Sparatore, F., Fermeglia,M., Paneni, M.S., Posocco, P., Pricl, S., Colla, P., Ibba, C., Secci, B., Collu, G., and Loddo, R., (2009) *Bioorg.Med.Chem.Lett.,17,pp. 4425-4440.*

3-Jarrahpour, A. and Zarei, M.(2010) *Tetrahedron, 66,pp. 5017-5023.*

4- Jarrahpour, A. and Zarei, M., (2009) *Tetrahedron,50, pp.1568-1570.*

5- Zarei, M., Mohamadzadeh, M., (2011) *Tetrahedron,67,pp. 5832-5840.*

6-Zhao, x., Li, C., Zeng , S. and Hu, W., (2011) *Eur.J. Med.Chem. 46 ,pp. 52-57.*

7-Sathe B.S., Jaychandran E., Jagtap V.A. and Sreenivasa G.M. ,Intern. (2011) J.of pharmacetical research and develepment , vol.3, no.3 , pp.164-169.

8-Sondhi S.M. , Singh N., kumar A. ,Lozach O. And Meijer L. , (2006),Bioorganic Medicinal Chemistry, vol. 14 ,no. 11 ,pp. 3758-3765.

9-Miri R., Razzaghi-asl and Mohammad M.K., (2013), J. Of Molecular modeling , vol. 19, no. 2 , pp. 727-735.

10- Mounika k. , Anupama B., Pragathi J. And Gyanakumari C.,(2010), J. of scientific research , vol.2 , no. 3, pp. 513-524.

11- Venkatesh P. ,(2011) , J. of pharmaceutical and Health Science ,vol. 1 , no. 1 , pp. 8-11.

13- Chung K. T., (2016) Azo dyes and human health: a review. Journal of Environmental Science and Health, Part C, 34(4), 233-261.

14- Taha N. I., Tapabashi N. O. and El-Subeyhi (2018), , Intern. J. of Org. Chem., 8, pp. 309-318.

15- Taha N. I. and Eehab. M. Rasheed E. M., (2018), Kirkuk University Journal/Scientific Studies (KUJSS), vol. 13, no.(3), pp.109-125.

14- Walksman S. A., (1947) "Microbial Antagonism and Antibiotic Substances", 72, Commonwealth Fund, N. Y., 2nd Ed., p. 72