Contents lists available at https://digitalcommons.aaru.edu.jo/huj_nas/.

Hadhramout University Journal of Natural & Applied Science

Article

Digital Object Identifier:
Received 14 Augustus 2022,
Accepted 5 November 2022,
Available online 25 March 2023

Synthesis and Characterization of New Azo Dyes Derived from Acyclovir and Sulfamethoxazole and Study Their Biological Activity

Abdul Rahman Y. Wahoud[1*], Thanaa Shritch [2], Ali Yousef [2], Mohammad Mazen Kondakji [2]

[1]Department of Chemistry, College of Sciences, Hadhramout University, Yemen.

[2]Department of Chemistry, Faculty of Science, Al Baath University, Syria.

*Corresponding author: a.wahoud@hu.edu.ye

Abstract: In this research, two new azo dyes, 9-(2-Hydroxy-ethoxymethyl)-2-(2-hydroxy5nitrophenylazo)-1,9-dihydro-purin-6-one and 4-(2-Hydroxy-napHthalen-1-ylazo)-N-(5-methyl-isoxazol-3-yl)-benzenesulfonamide, derived from Acyclovir and Sulfamethoxazole was synthesized. The first dye synthesis was started by diazation of acyclovir, then combined with 4-nitrophenol activated with potassium hydroxide 10%, yielding 80%, and its purity was verified by thin layer chromatography (TLC). The second dye synthesis was started by diazation of Sulfamethoxazole then combined with β-naphthol activated with potassium hydroxide 10%, yielding 87%, and its purity was verified by thin layer chromatography (TLC). The identity of the resulting pigments was determined based on spectroscopic methods such as infrared spectroscopy, proton nuclear magnetic resonance spectrum and UV-VIS spectrum. The results of this study showed that they were in agreement with the blistered formula of the dyes. The biological activity of the resulting pigments was studied on two common and clinically pathogenic bacteria E.Coli and Steaphylococcus. It was found that the dye has a good inhibitory ability towards the growth of both bacteria, more than it is in the case of acyclovir and Sulfamethoxazole.

Keywords: Acyclovir, Azo; Biological activity; Inhibitory diameter Sulfamethoxazole.

1. Introduction:

Man's love for colors in general goes back to prehistoric times; however, this matter did not happen in reality until the modern era, when access to the full range of rainbow colors was available to the majority of humanity. Azo compounds occupy about 60-70% of the dyes, and the reason for their name is due to the presence of the azo group -N=N- with sp2 hybridization associated with the aromatic system. Titration such as methyl orange and methyl red.

Acyclovir is one of the most common antivirals that has been shown to be effective against human herpes viruses, including herpes simplex virus 1, which enters the body and hides in the cells of the nervous system and is associated with the outbreak of the herpes lesion of the face and mouth known as cold sores, while herpes virus type 2 is associated with systemic ulcers. In addition to a third type called the herpes zoster virus, the same virus that causes chickenpox.

The azo compounds derived from Sulfamethoxazole have also demonstrated great biological efficacy, as the sulfanilamide and Sulfamethoxazole compounds have great efficacy in treating many diseases, reducing the effect of toxins that cause respiratory problems, and preventing the transmission of bacterial infection.

On this research, we aim to preparation of azo dye in the laboratory, determine of the structure of the resulting pigment using the available spectroscopic methods and study the biological activity of the resulting dye against Gram-positive and Gram-negative bacteria.

2. Materials and Methods:

2.1. Equipment and tools:

Nuclear magnetic proton resonance device model 400MHz from Bruker/Swiss company, infrared spectrophotometer model IRAffinity-1S from Shimazu/Japan, visible and ultraviolet spectrophotometer model UV-VIS-1800 Shimazu/Japan, electro thermal melting point devise from Kruss/Germany, sensitive balance type Sartorius BL-210S, heater equipped with a magnetic drive type P-Selecta 243 and UV lamp with two lamps (254nm, 366nm) from DESAGA company / Germany were used.

2.2. Chemicals:

Acyclovir, potassium hydroxide 10%, hydrochloric acid, sodium nitrite, concentrated sulfuric acid, ethanol, para-

nitrophenol, dimethylformamide (DMF), ethyl acetate, hexane, sodium bicarbonate, acetonitrile, dimethyl sulfoxide (DMSO), sodium chloride, Sulfamethoxazole ,β-Naphthol, sulfuric acid, silica gel 60F254 size /20X20/ from Merck company/ Germany with analytical grade were used.

2.3. Preparation of azo dye derived from acyclovir:

First, mix (0.01mol) acyclovir with (10ml) concentrated hydrochloric acid and (10ml) distilled water in a single-hole swab fitted with a magnetic stirrer. Then, the mixture was placed in an ice bath, in order to maintain a temperature (0oC), and sodium nitrite solution was added to the dialysis solution. A pale-yellow transparent solution was observed, indicating the formation of highly soluble diazonium salt and the stirring was continued at the temperature (0oC). An aqueous solution of (0.01mol) of para-nitrophenol in an appropriate amount of 10% potassium hydroxide was slowly added dropwise at the same reduced temperature (0oC) to start the coupling reaction, forming a yellow solution and the pH of the resulting solution (pH = 5-6) is adjusted using a saturated solution of potassium carbonate and the mixture was continued to stir for 3-4 hours. The yellow precipitate is filtered off and washed with distilled water, then with sodium chloride solution and again with distilled water. The resulted compound is purified by recrystallization by ethanol, a fine yellow precipitate with a melting point of (217 oC) was obtained, with a yield of 80%, the purity was verified using thin layer chromatography (Fig. 1).

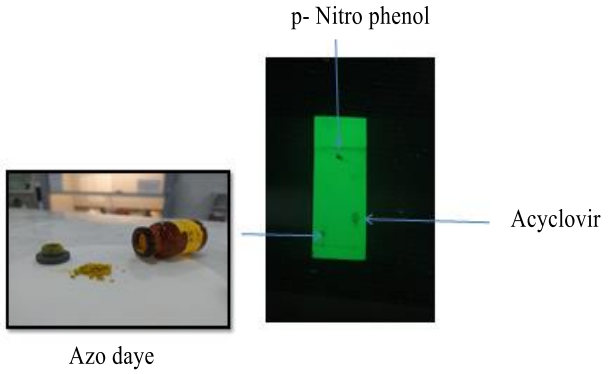
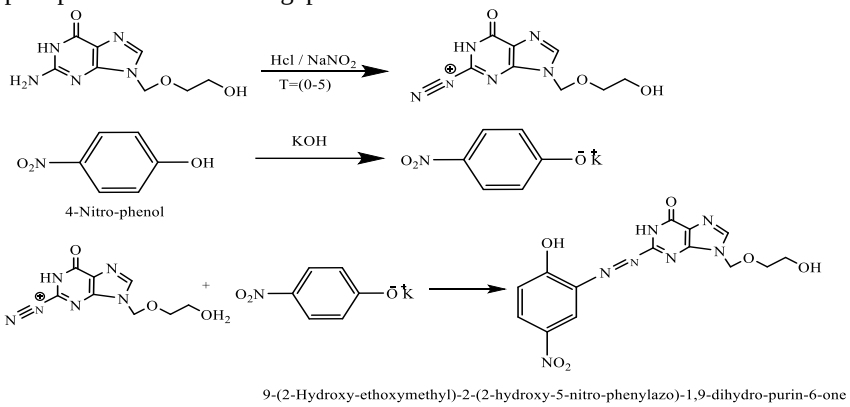


Figure 1: 4-nitrophenol azocyclovir dye

The azo dye was prepared from the diazotization reaction of the drug compound acyclovir in order to obtain the diazonium chloride salt, and then the coupling reaction with para-nitrophenol was carried out according to the following scheme (1).



Scheme 1: Synthesis of azo dye from acyclovir

2.4 Preparation of azo dye derived from Sulfamethoxazole:

First, put in a single-hole flask fitted with a magnetic stirrer (0.001mol 0.5gr,) of Sulfamethoxazole, add 5ml of concentrated hydrochloric acid and 10ml of distilled water, and stir until complete dissolution process with cooling in an ice bath to 0°C temperature. The prepared sodium nitrite solution (0.19gr in 2ml of concentrated sulfuric acid and 5ml of distilled water) was added dropwise, while continuing the process of stirring and cooling and maintaining the zero-degree temperature to ensure the completion of the dialysis process. Then diazium salt was slowly added dropwise at the same reduced temperature (0°C) to start the mating reaction and after that activated solution (0.288gr beta-naphthol in 20ml of potassium hydroxide 10%) was added very slowly to the dialysis solution, where an orange-coloured solution is formed, the pH of the resulting azo dye solution is adjusted to pH = 8 by a saturated solution of potassium bicarbonate, and the mixture is left to stir for five hours. Then the orange-colored precipitate is filtered and washed with cold distilled water, sodium chloride solution and distilled water respectively. The precipitate was dried after washing for 24 hours at laboratory temperature, and purified by recrystallization with ethanol, so an orange-coloured dye., melting point 225-226°C, was obtained with a yield of 87%. The product was confirmed by TLC thin layer chromatography using (ethyl acetate: hexane) with a ratio of 1:1 mobile phase and 70% alcohol as a solvent (Fig. 2).

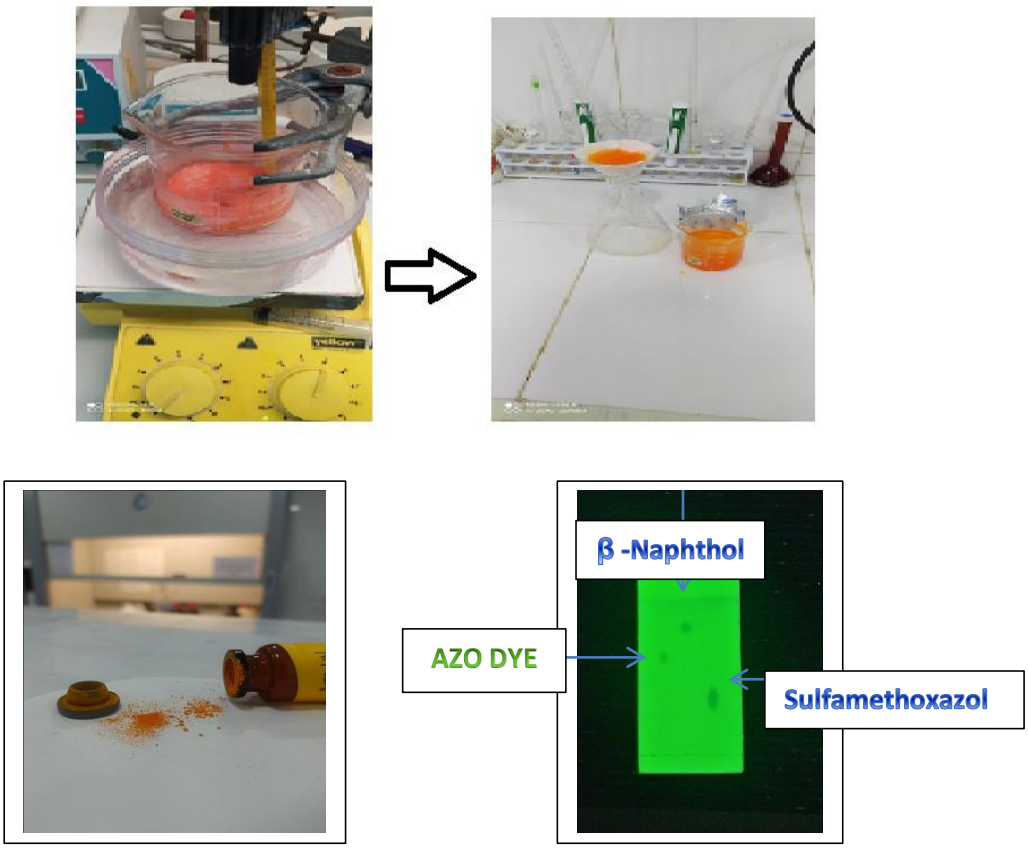
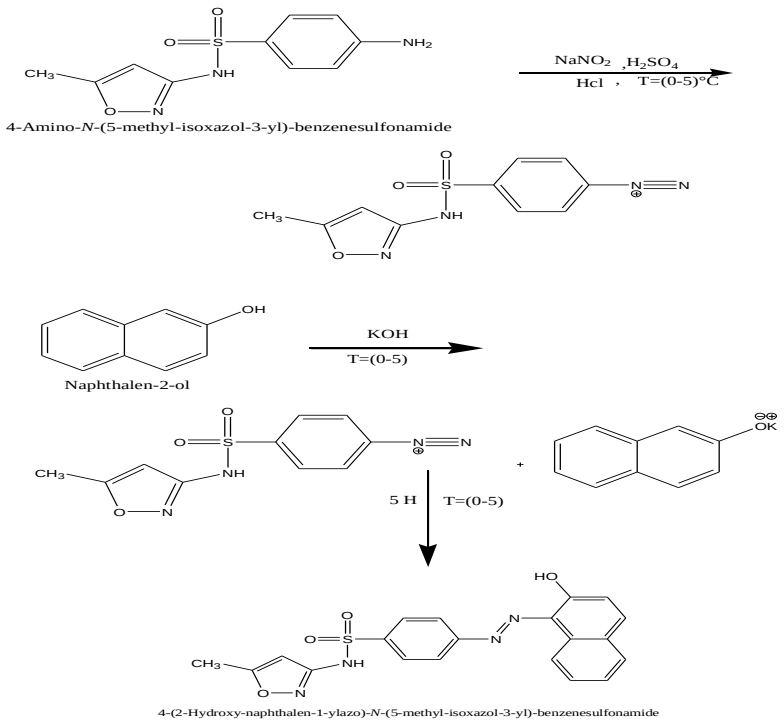


Figure 2: Sulfamethoxazole azo dye

The azo dye was prepared from the diazotization reaction of the drug compound Sulfamethoxazole in order to obtain the diazonium chloride salt, and then the coupling reaction with β-naphthol was carried out according to the following scheme (2).



Scheme 2: Synthesis of azo dye from Sulfamethoxazole

2.5. Antibacterial assay:

Two samples of the resulting dye were prepared at concentrations (50-100 µg/ml), the pharmaceutical substance (acyclovir-Sulfamethoxazole) at concentrations (50-100 µg/ml) and the reference substance (gentamicin) at concentration (100 µg/ml) using DMSO solvent were prepared. The samples were placed inside dishes by stainless steel cylinders with inner diameter (6mm) and outer (8mm) containing Gram-positive (Staphylococcus Aureus) and Gram-negative (Pseudomonas aeruginosa) bacteria with 100000 CFU. The dishes with a cellular medium were incubated for 24 hours at temperature of 36.5-37oC.

3. Results & Discussion:

3.1. Chemical synthesis and characterization of Acyclovir Azo dyes:

3.1.1. FT-IR Spectrum:

The FT-IR spectra of the resulting dye and the raw materials (acyclovir and para-nitrophenol) used to prepare the product were recorded in Figure (3) and (4) respectively. It was observed that the absorption due to the amine group (NH2) in acyclovir had disappeared, which appears at the wave number (3341,3284 cm-1). The emergence of a new absorption band at the site (1508 cm-1) belonging to the azo group (-N=N-) in the product. Other absorption bands are also noted in the table (1).

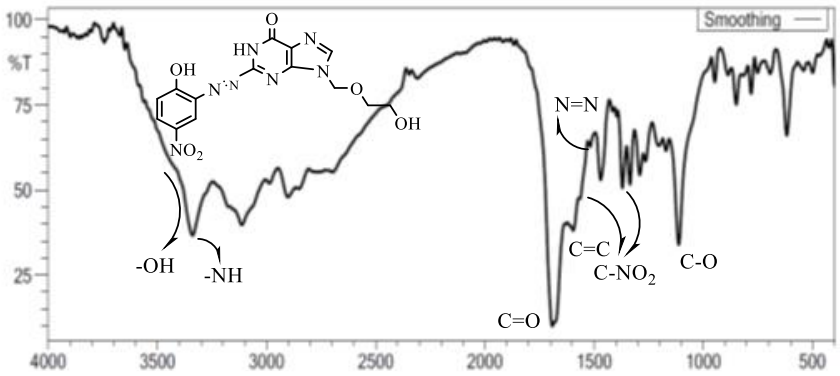


Figure 3: Infrared spectrum of azo dye

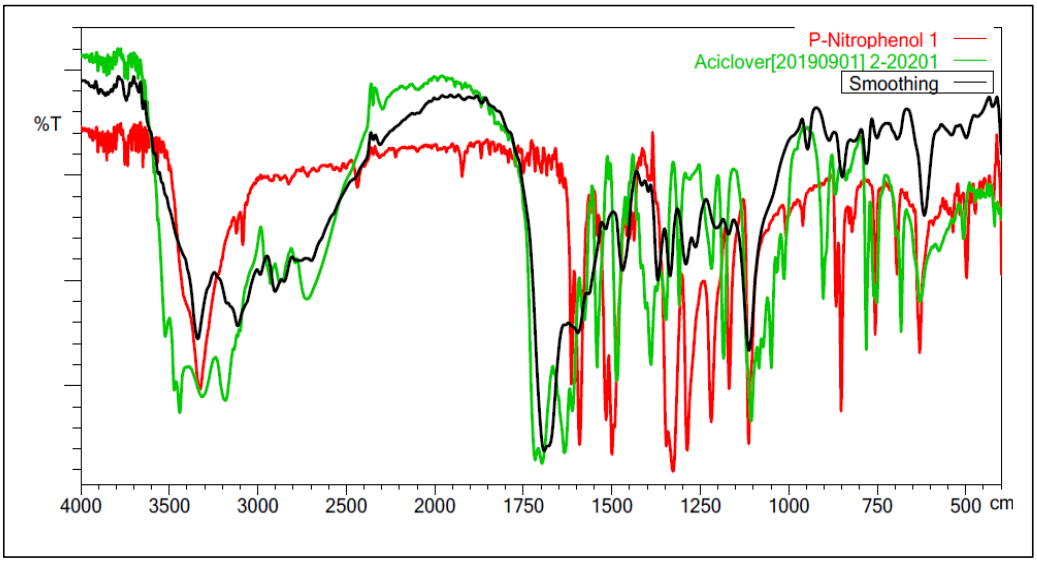
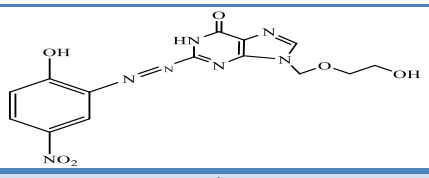


Figure 4: Comparison of IR spectra of pigment and starting materials

Table 1: Absorption bands of functional groups characteristic in IR spectroscopy of Azo Dye and reactants



(Cm^{-1}) ῡ										
O-H Stretch	**NH_2 Stretch**	**N-H Stretch**	**C-H(Sp^2)**	**C=O**	**C=C**	**N=N**	**C- NO2**	**C-O**	**C-N**	**Comp**
3522	3341-3284 1633(Bend)	3471	3095	1720	1485-1610	-	-	1107	1217 1575(C=N)	**Acyclovir**
3327	-	-	3084	-	1591-1498	-	1515-1346		1256	**P-nitro phenol**
3523	-	3336	3115	1692	1543-1489	1508	1521-1336		1290 1593(C=N)	**AZO**

3.1.2. 1H-NMR Spectrum:

The 1H-NMR spectrum of 1H-NMR was recorded using chloroform moderator dye solvent, where two triple signals of Hb and Hc appear at the shift (1.50 and 1.11 ppm) respectively, and five single signals at 4.75ppm) belong to proton Hd, and at 8.12 ppm for He, 3.70 ppm for Ha, 10.11 ppm for Hg, 12.04 ppm for Hf, in addition to other shifts shown in Table (2).

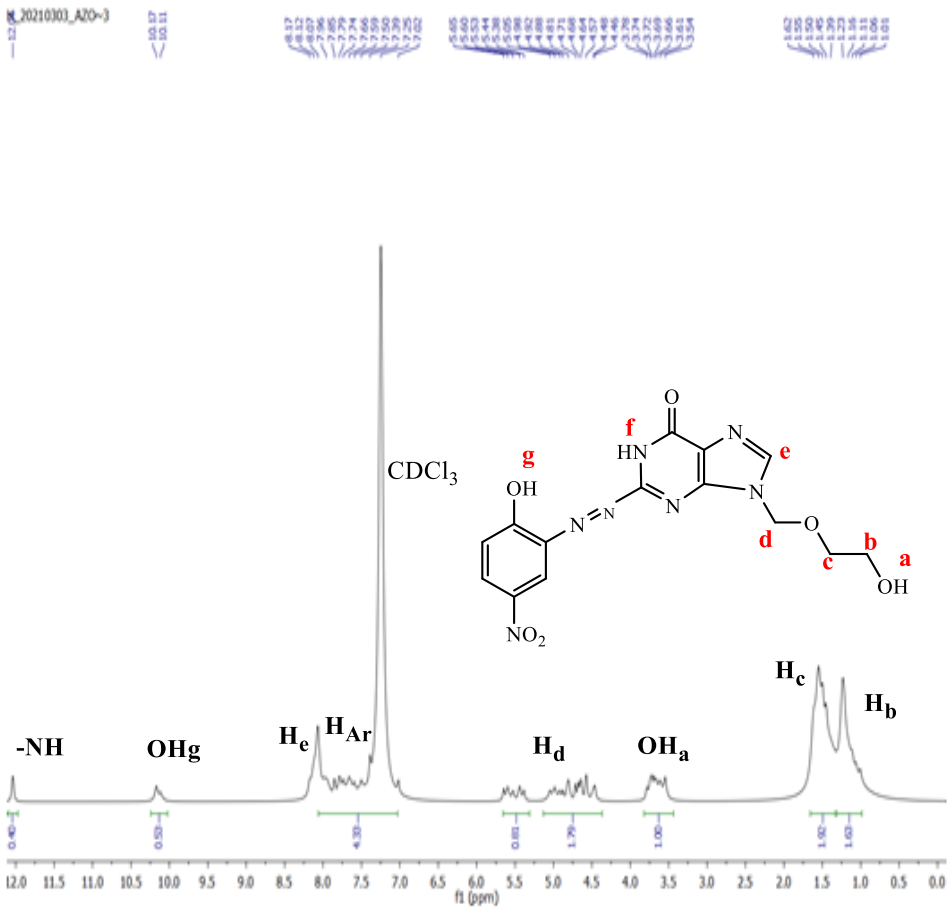
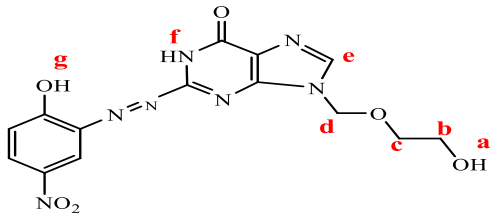


Figure 5: 1H-NMR spectrum of Azo Dye

Table 2: Chemical Shifts in the 1H- NMR Spectrum of the Azo Dye



^{1}H-NMR (δ-ppm)	Sign symbol
3.7 (1H,s)	**a**
1.11 (2H,t, J=20Hz)	**b**
1.50 (2H,t, J=20Hz)	**c**
4.75 (2H , m)	**d**
8.12 (1H,s)	**e**
12.04 (1H,s)	**f**
10.11 (1H,s)	**g**
7.02-7.85 (3H,m)	**H-Aromatic**

3.1.3. UV-VIS Spectrum:

Figure (6) shows the absorption spectrum of the azo dye prepared by UV-VIS spectroscopy using dimethylformamide solvent. We notice from the spectrum the appearance of a maximum absorption band λmax at 275 nm due to the π→π* transition, and the appearance of a peak with a maximum absorption at 450 nm due to the n→π* transition, which is a sterically permissible transition.

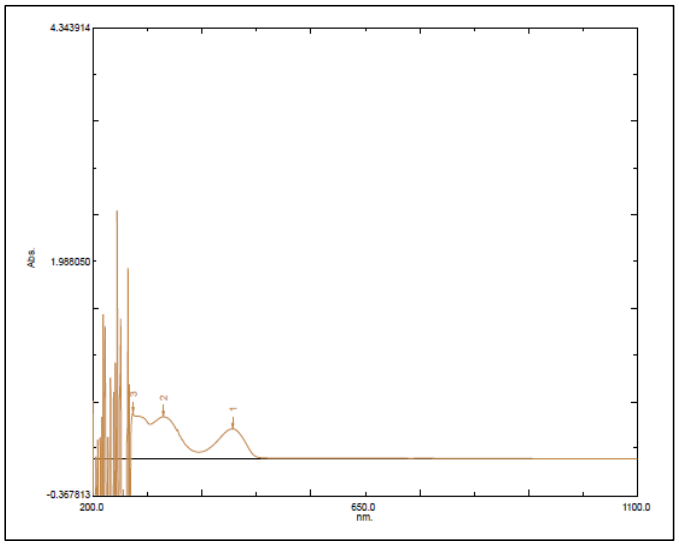


Figure 6: UV-VIS spectrum of Azo Dye

3.2. Chemical synthesis and characterization of Sulfamethoxazole Azo dye:

3.2.1. FT-IR Spectrum:

The FT-IR spectrum of the Sulfamethoxazole azo dye was recorded in Figure (7), and its data were compared with the data of the FT-IR spectrum of the raw materials (Sulfamethoxazole, beta-naphthol) used to prepare the dye (Fig.8), the disappearance of the absorption due to the amine group (NH2)) in Sulfamethoxazole, which appears at the wave number (3341-3284 cm-1), and the appearance of a new absorption band in the site (1506 cm-1) dating back to (-N=N-) in pigment , other absorption bands shown in Table (3) were also observed.

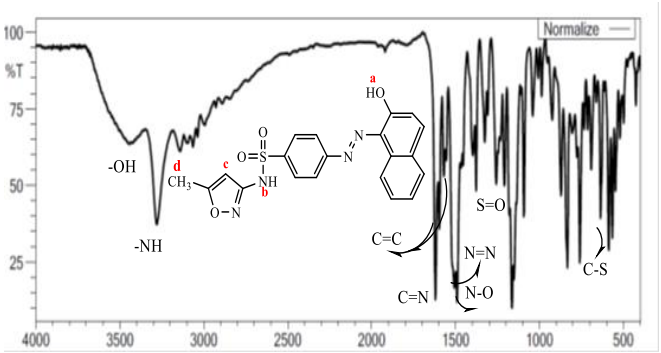


Figure 7: Infrared spectrum of azo dye

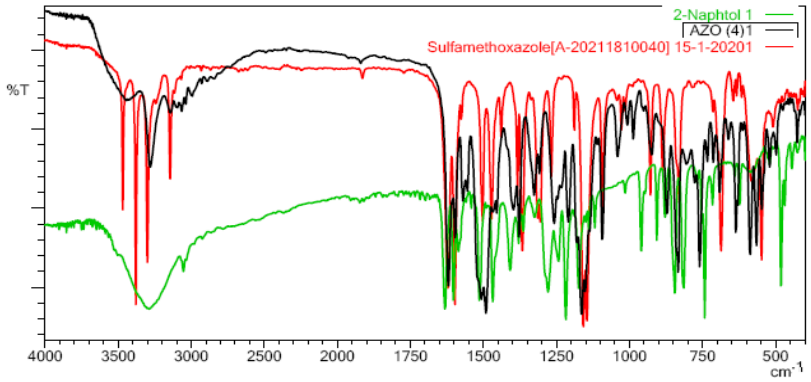
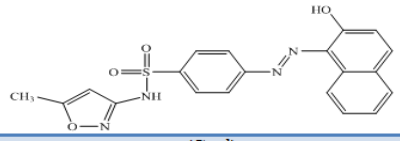


Figure 8: Comparison of IR spectra of pigment and starting materials

Table 3: Absorption bands of functional groups characteristic in IR spectroscopy of Azo Dye and reactants



$\bar{\upsilon}$ (Cm^{-1})										
C_{SP^2}-H	S=O	C=C	C-N	C-O	NH_2 Stretch	N-H Stretch	O-H Stretch	N-O	N=N-	Comp.
3095	1320-1152 668(C-S)	1485-1610	1217 1575(C=N)	1107	3341-3284 1633(Bend)	3471	3522	1552-1365	-	Sulfamethoxazol
3080	-	1602-1468	-	1174	-	-	3300	-	-	β-naphthol
3115	1327-1163 628(C-S)	1597-1490	1258 1620(C=N)	1093	-	3281	3490	1554-1377	1506	AZO

3.2.2. 1H-NMR Spectrum:

The proton NMR spectrum (Fig.9) of the Sulfamethoxazole azo dye was recorded in the modulator chloroform, where the spectrum shows the appearance of four single signals at 9.38ppm (for the proton Ha, and at 11.17 ppm) for Hb, and 6 .40 ppm for Hc, 2.36 ppm for Hd, 12.04 ppm for Hf, and the aromatic protons are shown within the range (7.40-8.01 ppm according to Table (4).

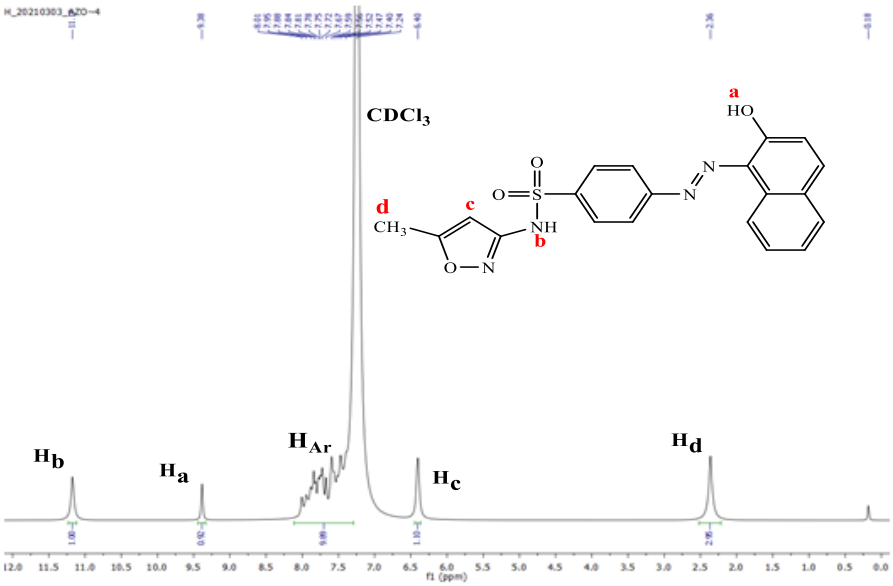
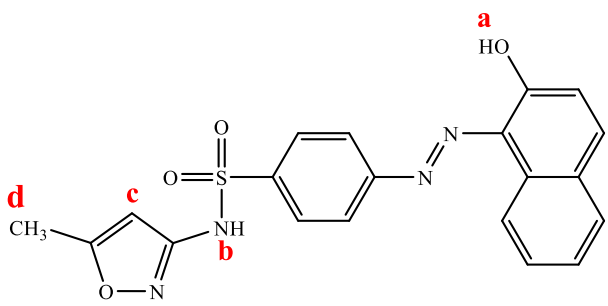


Figure 9: 1H-NMR spectrum of Azo Dye

Table 4: Chemical Shifts in the 1H- NMR Spectrum of the Azo Dye



H-NMR (δ-ppm)[1]	**Sign symbol**
9.38 (1H,s)	**a**
11.17 (1H,s)	**b**
6.40 (1H,s)	**c**
2.36 (3H,s)	**d**
7.40-8.01 (10H,m)	**H-Aromatic**

3.2.3. UV-VIS Spectrum:

Figure (10) shows the absorption spectrum of the azo dye prepared by UV-VIS spectroscopy using dimethylformamide solvent. We notice from the spectrum the appearance of a maximum absorption band λmax at 295 nm due to the π→π* transition, and the appearance of a peak with a maximum absorption at 435 nm due to the n→π* transition, which is a sterically permissible transition.

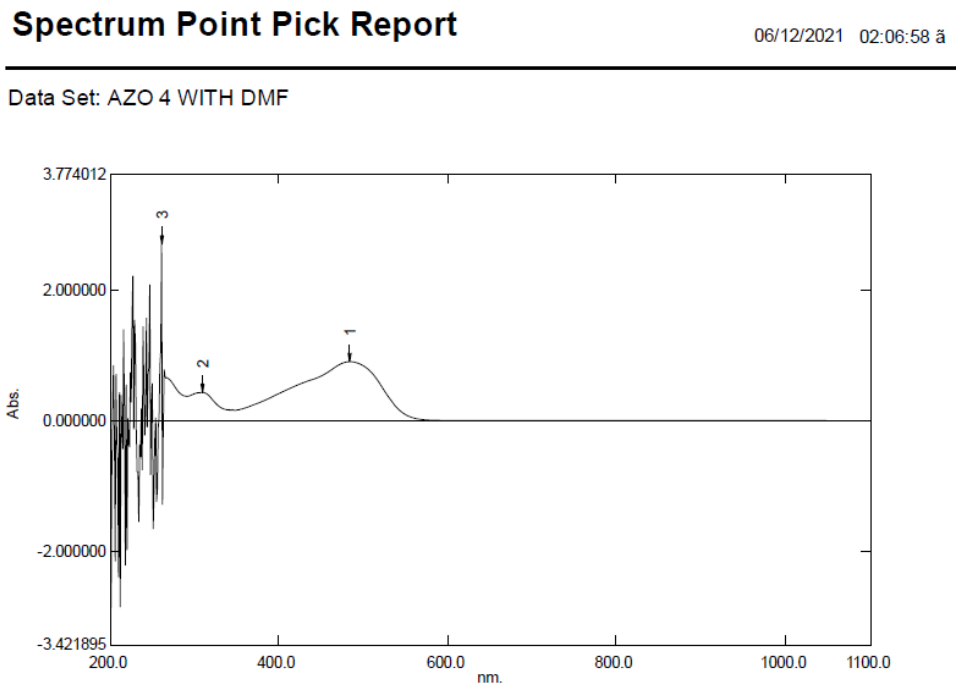
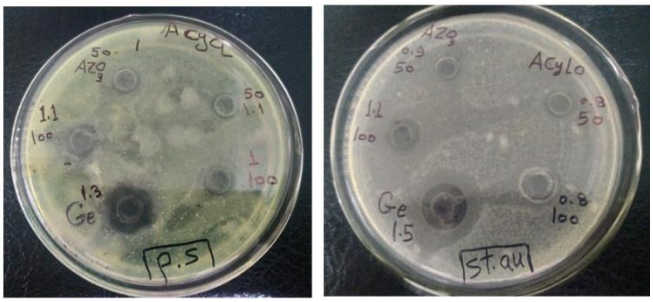


Figure 10: UV-VIS spectrum of Azo Dye

3.2. Antibacterial assay:

After 24 hours of incubating the dishes in the microbiological incubator we found that: In the dish containing the gram-positive bacteria (Staphylococcus Aureus), areas of inhibition (a halo of no growth) were observed for the resulting pigment compared with the area of inhibition for the drug (acyclovir-Sulfamethoxazole) and the reference substance (gentamicin). In the dish containing the gram-negative bacteria (Pseudomonas aeruginosa)), the inhibition zones (the halo of no growth) compared with the inhibition zone for the drug (acyclovir-Sulfamethoxazole) and gentamicin. By measuring the diameter of the inhibition zones for the samples, as shown in Table (5) and Table (6), we conclude that the dye has activity and is able to stop the growth of Gram-negative and Gram-positive bacteria according to Concentrations used.



Pseudomonas aeruginosa Staphylococcus Aureus

Figure 11: Regions of inhibition of Azo dye, acyclovir and gentamicin towards both bacteria

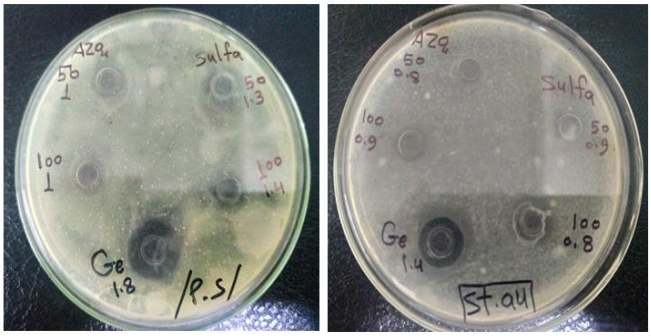


Figure 12: Regions of inhibition of Azo dye, Sulfamethoxazole and gentamicin towards both bacteria

Table 5. Antimicrobial activity of the compound's solutions against the tested bacterial strains.

Inhibitory diameter. (mm)	Sample concentration (µg/ml)	Sample	Type of germ
9	50	AZO	**Staphylococcus Aureus(S.T)**
8	50	acyclovir	
8	100		
11	100	AZO	
15	100	gentamicin	
10	50	AZO	**Pseudomonas Aeruginosa(P.S)**
11	50	acyclovir	
10	100		
11	100	AZO	
13	100	gentamicin	

Table 6. Antimicrobial activity of the compound's solutions against the tested bacterial strains

Inhibitory diameter. (mm)	Sample concentration(µg/ml)	Sample	Type of germ
8	50	AZO	**Staphylococcus Aureus(S.T)**
9	50	Sulfamethoxazole	
8	100		
9	100	AZO	
14	100	gentamicin	
10	50	AZO	**Pseudomonas Aeruginosa(P.S)**
13	50	Sulfamethoxazole	
14	100		
10	100	AZO	
18	100	gentamicin	

4. Conclusion:

1) New azo dyes were synthesized based on a pharmacologically active substance (Acyclovir)and (Sulfamethoxazole) with excellent selectivity and a good yield.

2) The biological activity of the dyes was studied, and the results showed that it possesses effectiveness and a good ability to stop the growth of Gram-negative bacteria and Gram-positive bacteria according to the concentrations used.

5. Acknowledgments:

The authors are grateful to Hadramout University and Al-Baath university- college of science for supporting this research.

6. **References:**

[1] B. W. Gung and R. T. Taylor, "Parallel Combinatorial Synthesis of Azo Dyes: A Combinatorial Experiment Suitable for Undergraduate Laboratories," Journal of Chemical Education, vol. 81, no. 11, p. 1630, Nov. 2004, doi: 10.1021/ed081p1630.

[2] Corinne. Decelles, "The story of dyes and dyeing.," Journal of Chemical Education, vol. 26, no. 11, p. 583, Nov. 1949, doi: 10.1021/ed026p583.

[3] B. Myek, O. B. Adesina, and M. L. Batari, "Synthesis of new azo dye and its iron complex derived from 3-aminophenol," International Journal of Modern Chemistry, vol. 7, no. 1, pp. 54–59, 2015, [Online]. Available: ttps://modernscientificpress.com/Journals/IJMChem.aspx

[4] S. Hojaghani, M. Hosaini Sadr, and A. Morsali, "Sonochemical synthesis of two new copper(II) complexes with azo ligands derived from anthranilic acid and β-naphtol," Ultrasonics Sonochemistry, vol. 26, pp. 305–311, Sep. 2015, doi: 10.1016/j.ultsonch.2015.02.009.

[5] J. Z. Chandanshive et al., "1,3-Dipolar cycloaddition of nitrile imines with α,β-unsaturated lactones, thiolactones and lactams: synthesis of ring-fused pyrazoles," Tetrahedron, vol. 68, no. 16, pp. 3319–3328, Apr. 2012, doi: 10.1016/j.tet.2012.02.068.

[6] S. Roscales and J. Plumet, "Metal-catalyzed 1,3-dipolar cycloaddition reactions of nitrile oxides," Organic & Biomolecular Chemistry, vol. 16, no. 44, pp. 8446–8461, 2018, doi: 10.1039/c8ob02072h.

[7] H. Chen, "Recent Advances in Azo Dye Degrading Enzyme Research," Current Protein & Peptide Science, vol. 7, no. 2, pp. 101–111, Apr. 2006, doi: 10.2174/138920306776359786.

[8] B. Çatıkkaş, E. Aktan, and E. Yalçın, "Vibrational and electronic investigations, NLO, FMO analysis on a hetarylazoindole disperse dye by density functional theory," Journal of Molecular Structure, vol. 1117, pp. 218–226, Aug. 2016, doi: 10.1016/j.molstruc.2016.03.074.

[9]- N. Siddiqui, M. Alam, A.A. Siddiqui, Asian J. Chem. 16 (2004) 1005-1008.

[10] H. B. Hassib and S. A. Abdel-Latif, "Potentiometric, spectrometric, thermal and conductimetric studies on some 3-phenyl-4-(arylazo)-5-pyrazolones and their complexes with divalent cobalt metal ion," Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 59, no. 11, pp. 2425–2434, Sep. 2003, doi: 10.1016/s1386-1425(03)00010-6

[11] M. Akbar Ali, A. H. Mirza, R. J. Butcher, M. T. H. Tarafder, T. B. Keat, and A. M. Ali, "Biological activity of palladium(II) and platinum(II) complexes of the acetone Schiff bases of S-methyl- and S-benzyldithiocarbazate and the X-ray crystal structure of the [Pd(asme)2] (asme=anionic form of the acetone Schiff base of S-methyldithiocarbazate) complex," Journal of Inorganic Biochemistry, vol. 92, no. 3–4, pp. 141–148, Nov. 2002, doi: 10.1016/s0162-0134(02)00559-7.

[12] N. M. Mallikarjuna, J. Keshavayya, M. R. Maliyappa, R. A. Shoukat Ali, and T. Venkatesh, "Synthesis, characterization, thermal and biological evaluation of Cu (II), Co (II) and Ni (II) complexes of azo dye ligand containing sulfamethaxazole moiety," Journal of Molecular Structure, vol. 1165, pp. 28–36, Aug. 2018, doi: 10.1016/j.molstruc.2018.03.094.

[13] Ansary, A. L. E., Abd El-Fattah, H. M., Abdel-Kader, N. S., & Farghaly, A. "Synthesis and characterization of some sulfadrugs azodyes, potentiometric studies of the synthesized dyes and their Fe (III) complexes," Communications in Inorganic Synthesis, Jan. 01, 2013. http://dx.doi.org/10.21060/cis.2013.114

[14]- N. M. Mallikarjuna, J. Keshavayya, M. R. Maliyappa, R. A. Shoukat Ali, and T. Venkatesh, "Synthesis, characterization, thermal and biological evaluation of Cu (II), Co (II) and Ni (II) complexes of azo dye ligand containing sulfamethaxazole moiety," Journal of Molecular Structure, vol. 1165, pp. 28–36, Aug. 2018, doi: 10.1016/j.molstruc.2018.03.094.

[15] K. Nejati, Z. Rezvani, and M. Seyedahmadian, "The synthesis, characterization, thermal and optical properties of copper, nickel, and vanadyl complexes derived from azo dyes," Dyes and Pigments, vol. 83, no. 3, pp. 304–311, Dec. 2009, doi: 10.1016/j.dyepig.2009.05.007.

[16] N. M. Mallikarjuna and J. Keshavayya, "Synthesis, spectroscopic characterization and pharmacological studies on novel sulfamethaxazole based azo dyes," Journal of King Saud University - Science, vol. 32, no. 1, pp. 251–259, Jan. 2020, doi: 10.1016/j.jksus.2018.04.033.

[17] H. Henschel, A. T. Andersson, W. Jespers, M. Mehdi Ghahremanpour, and D. van der Spoel, "Theoretical Infrared Spectra: Quantitative Similarity Measures and Force Fields," Journal of Chemical Theory and Computation, vol. 16, no. 5, pp. 3307–3315, Apr. 2020, doi: 10.1021/acs.jctc.0c00126.

[18] "Infrared Spectroscopy". ColourLex. 12 March 2021. Retrieved 19 January 2022.

[19] "NASA Ames PAH IR Spectroscopic Database". The Astrophysics & Astrochemistry Laboratory. 12 September 2019. Retrieved 19 January 2022.

اصطناع وتوصيف أصبغة آزو جديدة مشتقة من الأسيكلوفير والسلفاميثوكسازول ودراسة الفعالية البيولوجية لها

عبد الرحمن وحود[1]، * , ثناء شريتح[2] , علي يوسف[3] , محمد مازن قندقجي[3]

الملخص: تم اصطناع صبغتي آزو جديدتين مشتقة من الأسيكلوفير والسلفاميثوكسازول. اصطنعت الصبغة الأولى باتحاد الأسيكلوفير مع نتروفينول المنشط بهيدروكسيد البوتاسيوم 10% وبمردود 80% , أما الصبغة الثانية فقد اصطنعت من اتحاد السلفاميثوكسازول مع بيتانفتول المنشط بهيدروكسيد البوتاسيوم 10% وبمردود 87% وحددت نقاوة كلا من الصبغتين بكروماتوغرافيا الطبقة الرقيقة .(TLC) وصفت الصبغتين الناتجتين بالأجهزة والطرق المطيافية IR, UV-Vis, NMR. درس النشاط المضاد للبكتيريا لكلا الصبغتين الناتجتين باستخدام نوعين من البكتيريا الشائعين E.Coli و Steaphylococcus ووجد بان الصبغتين المحضرتين لها مقدرة جيدة لمقاومة نمو نوعي البكتريا المذكورتين اكبر بكثير من مقاومة الأسيكلوفير والسلفاميثوكسازول لهما .

الكلمات المفتاحية: أسيكلوفير , أزو , النشاط البكتيري, قطر الكبح, سلفاميثوكسازول.