



Synthesis and Characterization of some 2,3-dihydroquinoxaline and evaluation of their biological activity

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Abstract

This work included the synthesis of azo dye (A_1) by the reaction of diazonium salt to benzidine with salicylaldehyde at (0-5) °C and synthesis of schiff base (A_2 - A_6) through reaction substituted aromatic amine with aldehyde group in azo compound (A_1) in ethanol compounds (A_2 - A_6) and 2,3-dihydroquinoxaline derivatives prepared by reaction schiff base with anthranilic acid in ethanol compounds (A_7 - A_{11}), and characterization by using spectroscopic techniques Uv/Vis, FT-IR, C.H.N. and H^1 -NMR of some the prepared compounds using DMSO- d^6 as a solvent, in addition to melting point and determination a purity of TLC, This work consisted a study of biological activity for some prepared compounds against four types of pathogenic bacteria to detect their resistance to anti biotic, drugs.

Introduction

Azo dyes are known as colored organic materials used in the coloring of other materials by binding to the materials to be dyed, and gain different colors not to be affected by light and washing [1]. There are two types of dyes: natural dyes and synthetic dyes [2]. Schiff base organic compounds containing the group of azomethene (-CH=N-), named after the world Schiff, which first attended in 1864 from a simple condensation reaction of aldehydes or ketones with primary amines [3]. Hydroquinazolin-4-one is a hexagonal ring containing two nitrogen atoms and a carbonyl group and containing a carbonyl group at site 4-called hydroquinazolin 4-one [4].

Experimental

Materials: All chemicals were used through this work purchased from Fluka, Aldrich, BDH Companies and were used without further purifications.

Devices used: Melting points were recorded using a measuring device melting point type: Automatic melting point \ SMP40. Thin layer chromatography (T.L.C.) was carried out using sheet polygram silica-gel as stationary phase, the spots were enhanced using UV rays. UV-Vis. spectra were recorded with spectrophotometer type: SHIMADZU UV spectrophotometer -1800 using Ethanol as a solvent. Infrared spectra were recorded using FT-IR-600

Fourier - Transform infrared (FT-IR) Spectrophotometer by KBr disc. H^1 -NMR spectra were recorded on Fourier Transform Varian spectrophotometer operating at 400 MHz with DMSO- d^6 . Quantitative analysis of the elements.

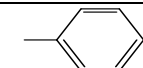
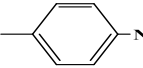
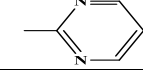
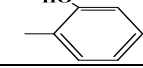
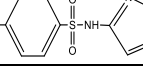
Synthesis of Azo dye (A_1) [5]: Azo dye was prepared in two main steps:

Step 1 / Preparation of diazonium salt: (0.02 mol, 3.684 gm) of benzidine dissolved in (30 ml) 37% HCl at a temperature of (0-5) °C with continuous stirring, then adding a solution of sodium nitrite.

Step 2 / Coupling reaction: (0.04 mol, 4.2 ml) of salicylaldehyde dissolved in (30 ml) of the Pyridine, and cooled to (0-5) °C in an ice bath. This solution was then slowly added to the cooled diazonium salt solution to yield azo compound. physical properties of azo colored dark red, M.P. (234-236) °C, yield 77% and Rf was 0.79.

Synthesis of Schiff Bases (A_2 - A_6) [6,7]: A solution of (0.01 mol, 4.5 gm) of azo dye (A_1) in 50 ml of absolute ethanol was added to (0.02 mol) of different aromatic amines in 20 ml absolute ethanol and 4 drops of glacial acetic acid was added then the mixture was refluxed for (3-5) hrs. The mixture was cooled to room temperature, filtered, dry and recrystallized in absolute ethanol. Physical properties, yield and Rf are given in Table (1).

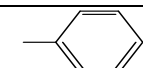
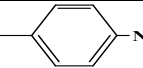
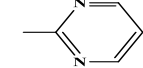
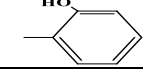
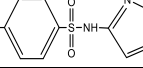
Table (1): physical properties, yield and Rf of schiff base (A₂-A₆).

Comp. No.	Ar	Molecular Formula/ M.Wt g/mol	Color	M.P (°C)	T. Ref. (hr.)	Yield (%)	Rf
A ₂		C ₃₈ H ₂₈ N ₆ O ₂ 600.68	Dark green	175-177	3	71	0.60
A ₃		C ₃₈ H ₂₆ N ₈ O ₆ 690.68	Light green	145-147	3	82	0.78
A ₄		C ₃₄ H ₂₄ N ₁₀ O ₂ 604.63	Brown	231-233	5	81	0.80
A ₅		C ₃₈ H ₂₈ N ₆ O ₄ 632.68	Red	259-260	4	57	0.84
A ₆		C ₄₆ H ₃₆ N ₁₀ O ₈ S ₂ 920.98	Brown	270-272	5	66	0.76

Synthesis of 2,3-Dihydroquinazolin 4-One derivatives (A₇-A₁₁) [8]: A mixture (0.002 mol) of Schiff's Bases (A₂-A₆) with (0.004 mol, 0.55 gm) of anthranilic acid in 30 ml of absolute ethanol and (3 ml) of tri ethylamine the mixture was refluxed for (5-

7) hrs. then the solvent evaporated with 10% sodium bicarbonate, formed precipitate and recrystallized from mixture (benzene-ether 1:1). Physical properties, yield, and Rf, are given in Table (2).

Table (2): physical properties, yield and Rf of 2,3-dihydroquinazolin 4-one (A₇-A₁₁).

Comp. No.	Ar	Molecular Formula/ M.Wt g/mol	Color	M.P (°C)	T. Ref. (hr.)	Yield (%)	Rf
A ₇		C ₅₂ H ₃₈ N ₈ O ₄ 838.93	Light orange	144-146	7	61	0.63
A ₈		C ₅₂ H ₃₆ N ₁₀ O ₈ 928.92	Violet	278-280	6	34	0.87
A ₉		C ₄₈ H ₃₄ N ₁₂ O ₄ 842.88	Brown	215-217	7	77	0.71
A ₁₀		C ₅₂ H ₃₈ N ₈ O ₆ 870.93	Orange	281-283	5	71	0.77
A ₁₁		C ₆₀ H ₄₆ N ₁₂ O ₁₀ S ₂ 1159.22	Dark red	318-320	5	85	0.46

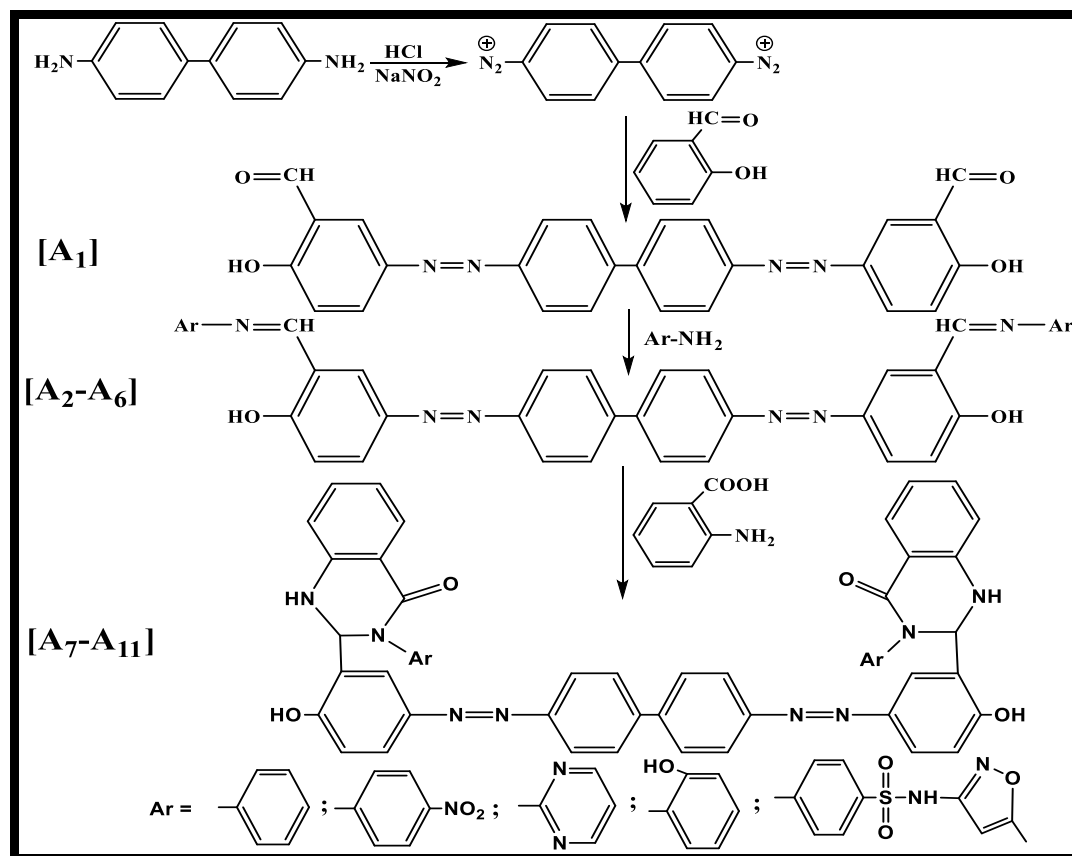
The Biological Activity [9]: The bacteria species used are listed in tables (5). All strains were obtained from College of Education for Women, Tikrit University. They were grown up to the stationary phase nutrient bath at 37 °C and a sample of 0.5 ml of each bacteria was spread over a surface of a nutrient agar plate.

Antibacterial assay [10]: Disc of filter paper (6 mm diameter) is sterilized at 140 °C for 1hr., and impregnated with the germs. DMSO was used as a solvent for compounds (A₁, A₄, A₅, A₆, A₇, A₉, A₁₁).

The same solvent was used for antibiotics (Amoxicillin, Ampicillin). Blank paper discs of DMSO was used as control. The inoculated plates are incubated at 37 °C for 24 hrs., the inhibition zone was measured. In all experiments the mean of each triplicate was measured.

Results and Discussion

In this work many compounds were synthesized which includes azo, schiff bases and 2,3-dihydroquinazolin as in the following Scheme:

**Characterization of Azo dye (A₁) [11, 12]:**

Azo dye has synthesized from the reaction of diazonium salt with salicylaldehyde. The FT-IR spectra of azo dye general showed disappearance of (NH₂) absorption of benzidine and appearances of (N=N) absorption band in 1482 cm⁻¹, besides bands in 1693 cm⁻¹ is due to (C=O) aldehyde and band at 3461 cm⁻¹ due to (OH) of salicylaldehyde,

Beside UV spectra the transitions $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ have confirmed the presence of the un-bonded pair electrons on nitrogen, oxygen atoms and aromatic system (double bond). UV and IR absorbance spectra were given in table (3) see fig (1) and (4).

¹H-NMR spectrum of compound (A₁) showed singlet signal at $\delta =$ (2.68) ppm due to DMSO-d₆ solvent, multiple signal (7.52 - 9.04) ppm due to aromatic rings, singlet signal (10.52) ppm due to CHO aldehyde and singlet signal (15.68) ppm due to H-O phenolic. ¹H-NMR spectrum is given in table (3) see fig (7).

Characterization of Schiff Bases (A₂-A₆) [13, 14]:

Schiff Bases derivatives have synthesized from the reaction of compound (A₁) with deferentes aromatic amines. The FT-IR spectra of Schiff Bases derivatives in general showed disappearance of (C=O) absorption of azo dye and appearances of (C=N) absorption band in (1622-1672) cm⁻¹, beside UV spectra, the transions $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ have

confirmed the presence of the un-bonded pair electrons on nitrogen, oxygen atoms and aromatic system (double bond). UV and IR absorbance spectra were given in table (3) see fig (2) and (5).

Characterization of 2,3-Dihydroquinazolin 4-One derivatives (A₇-A₁₁) [14]:

2,3-Dihydroquinazolin 4-One derivatives (A₇-A₁₁) have synthesized from the reaction of compound (A₂-A₆) with anthranilic acid.

The FT-IR spectra of 2,3-dihydroquinazolin 4-One derivatives in general showed disappearance of (C=N) absorption band in (1622-1672) cm⁻¹ of schiff bases derivatives and appearances of (C-N) absorption band in (1244-1340) cm⁻¹ and appearances of (C=O) absorption band in (1652-1690) cm⁻¹, beside UV spectra, the transions $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ have confirmed the presences of the un-bonded pair electrons on nitrogen, oxygen atoms and aromatic system (double bond). UV and IR absorbance spectra were given in table (3) see fig (3) and (6).

¹H-NMR spectrum of compound (A₉) showed singlet signal at $\delta =$ (6.31) ppm due to C-H dihydroquinazolin ring, singlet signal at $\delta =$ (6.60) ppm due to N-H dihydroquinazolin ring, multiple signal (6.98 - 9.07) ppm due to aromatic rings, and singlet signal (10.20) ppm due to H-O phenolic. ¹H-NMR spectrum is given in table (3) see fig (8).

Table (3): FT-IR, UV/Vis. and ¹H-NMR data of the prepared compounds.

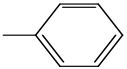
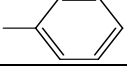
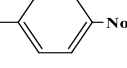
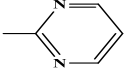
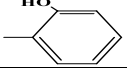
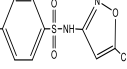
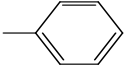
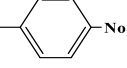
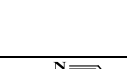
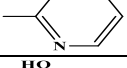
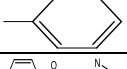
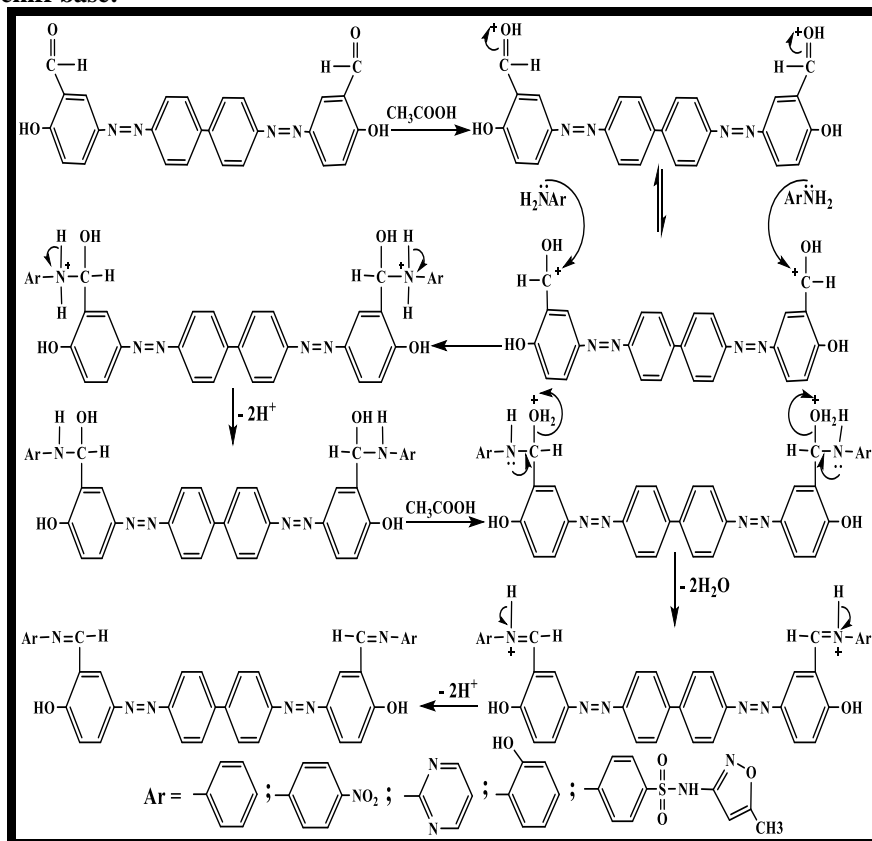
Comp. No.	Ar	λ_1 max 2λ max	IR (KBr) cm^{-1}						
			ν (C=N) or ν (C=O)	ν (C-H) Arom. Aliph.	ν (N-H)	ν (C=C)	ν (N=N)	ν (O-H)	Others
A ₁		270 337	1693	3080 2918	1568 1609	1482	3461	ν (N-N) 1192
A ₂		261 393	1622	3041 2912	1506 1559	1435	3373
A ₃		248 398	1650	3049 2900	1485 1594	1438	3407	ν (NO ₂). asy.(1565) sym.(1367)
A ₄		217 342	1672	3035 2944	1490 1578	1440	3320	ν (C-N) 1370
A ₅		239 305	1659	3091 2956	1555 1591	1485	3446
A ₆		218 384	1646	3093 2958	3172	1492 1595	1448	3299	ν (SO ₂). asy.(1360) sym.(1172)
A ₇		240 388	ν (C=O) 1670	3037 2945	3185	1520 1583	1490	3459	ν (C-N) 1340
A ₈		229 368	ν (C=O) 1687	3071 2928	3140	1529 1580	1464	2234	ν (C-N) 1244 ν (NO ₂). asy.(1510) sym.(1389)
A ₉		209 249	ν (C=O) 1652	3055 2925	3103	1504 1596	1452	3415	ν (C-N) 1278
A ₁₀		237 367	ν (C=O) 1690	3066 2971	3177	1530 1581	1467	3404	ν (C-N) 1315
A ₁₁		246 392	ν (C=O) 1662	3076 2943	3182	1530 1590	1477	3449	ν (C-N)1281 ν (SO ₂). asy.(1358) sym.(1142)
A ₁	2.68 (DMSO-d ⁶ solvent), 7.52-9.04 (multiple, 14H, 14×H aromatic rings), 10.52 (S, 2H, 2×CHO aldehyde), 15.68 (S, 2H, 2×H-O phenolic).								
A ₉	6.31 (S, 2H, 2×C-H dihydroquinazolin), 6.60 (S, 2H, 2×N-H dihydroquinazolin), 6.98-9.07 (multiple, 14H, 14×H aromatic rings), 10.20 (S, 2H, 2×H-O phenolic).								

Table (4): Elemental analysis of some of the prepared compounds.

Comp. No.	Molecular Formula	Found				Calculated			
		C%	H%	N%	O%	C%	H%	N%	O%
A ₁	C ₂₆ H ₁₈ N ₄ O ₄	69.02	4.01	12.18	14.04	69.33	4.03	12.44	14.21
A ₂	C ₃₈ H ₂₈ N ₆ O ₂	75.85	4.66	13.81	5.18	75.98	4.70	13.99	5.33
A ₅	C ₃₈ H ₂₈ N ₆ O ₄	72.05	4.29	13.06	10.00	72.14	4.46	13.28	10.12
A ₁₀	C ₄₈ H ₃₄ N ₁₂ O ₄	68.19	4.00	19.80	7.41	68.40	4.07	19.94	7.59

Mechanism schiff base:

**Biological activity [9]:**

The antimicrobial activity of the synthesized compounds [A₁, A₄, A₅, A₆, A₇, A₉, A₁₁] were examined by the agar diffusion method using four different bacterial species *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Staphylococcus Epidermidis*. The results indicated

that some of the assayed compounds showed a microbial activity against the used bacteria. Antibacterial activity of compounds [A₁, A₅, A₆] were given in fig (9) and (10). Evaluation of inhibitory activity of compounds prepared is given in fig (11 - 14).

Table (5): Antibacterial activity of some of the prepared compounds.

Comp. No.	Conc. mg/ml	<i>E. Coil</i>	<i>K. Pneumonia</i>	<i>S. Aureus</i>	<i>S. Epidermidis</i>	Inhibition Distance
A ₁	25	-	-	-	-	0
	50	+	-	+	+	1-2
	100	++	-	++	+	1-4
A ₄	25	+	-	+	+	1-2
	50	++	+	++	+++	1-5
	100	+++	+++	+++	+++	4-5
A ₅	25	+	+	++	+	1-4
	50	+++	++	++	+++	2-5
	100	+++	+++	+++	+++	4-5
A ₆	25	+	+	+	++	1-3
	50	+++	++	+++	+++	1-5
	100	+++	+++	+++	+++	4-5
A ₇	25	-	-	-	-	0
	50	+	+	+	+	1-2
	100	++	++	++	++	3-4
A ₉	25	-	-	-	-	0
	50	+	+	++	++	1-4
	100	++	+	++	+++	1-5
A ₁₁	25	+	-	+	+	1-2
	50	++	+	+	++	1-4
	100	+++	+++	+++	+++	4-5

(-) = No inhibition

(++) = Inhibition zone (2-4) cm

(+) = Inhibition zone (1-2) cm

(+++)= Inhibition zone (4-5) cm

Table (6): Antibacterial efficacy of control treatments (antibiotics) in the growth of a number of negative and positive bacteria (diameter of the inhibition circuit measured by cm).

Comp. No.	Name	<i>E. Coil</i>	<i>K. pneumonia</i>	<i>S. Aureus</i>	<i>S. Epidermidis</i>
1	Amoxicillin	2.8	2.7	3.0	2.5
2	Ampicillin	3.7	2.5	2.5	2
3	Blank disk	0	0	0	0

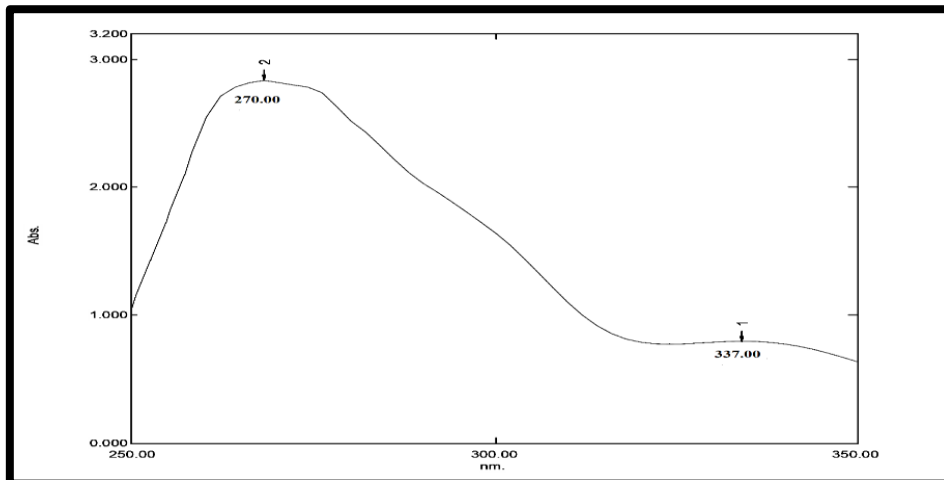


Fig (1): UV/Vis spectrum of compound [A₁].

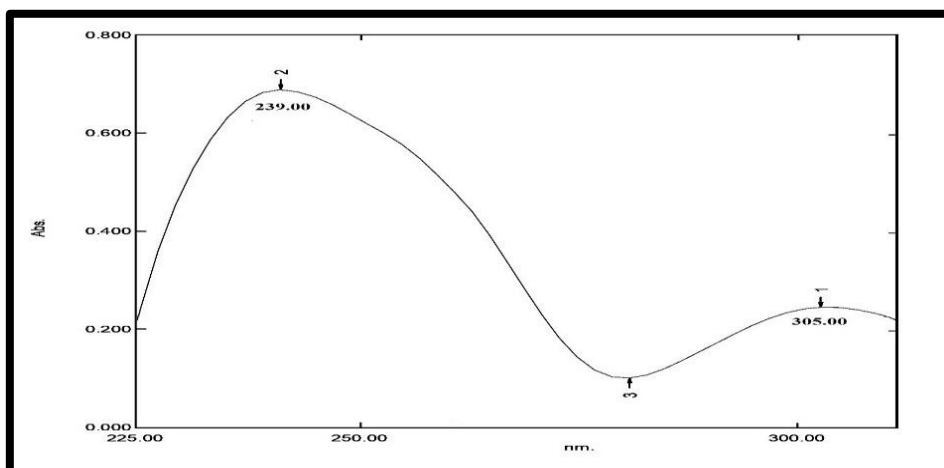


Fig (2): UV/Vis spectrum of compound [A₅].

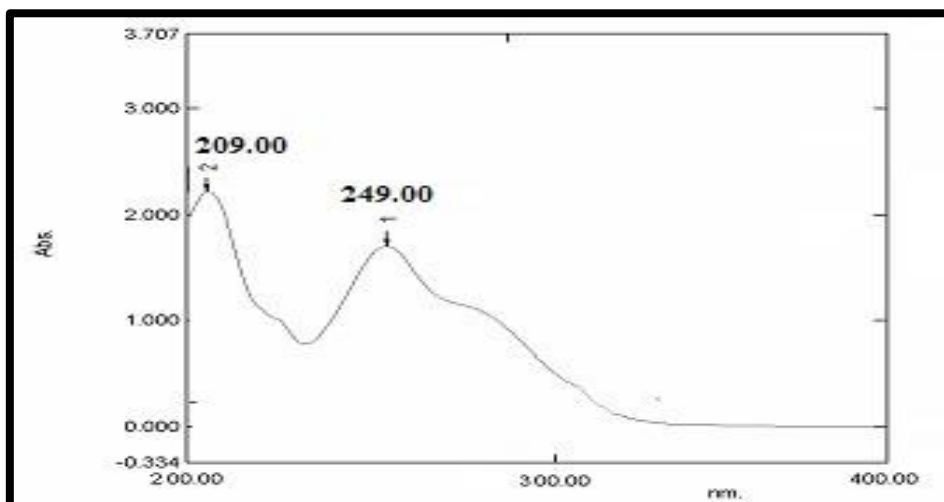


Fig (3): UV/Vis spectrum of compound [A₉].

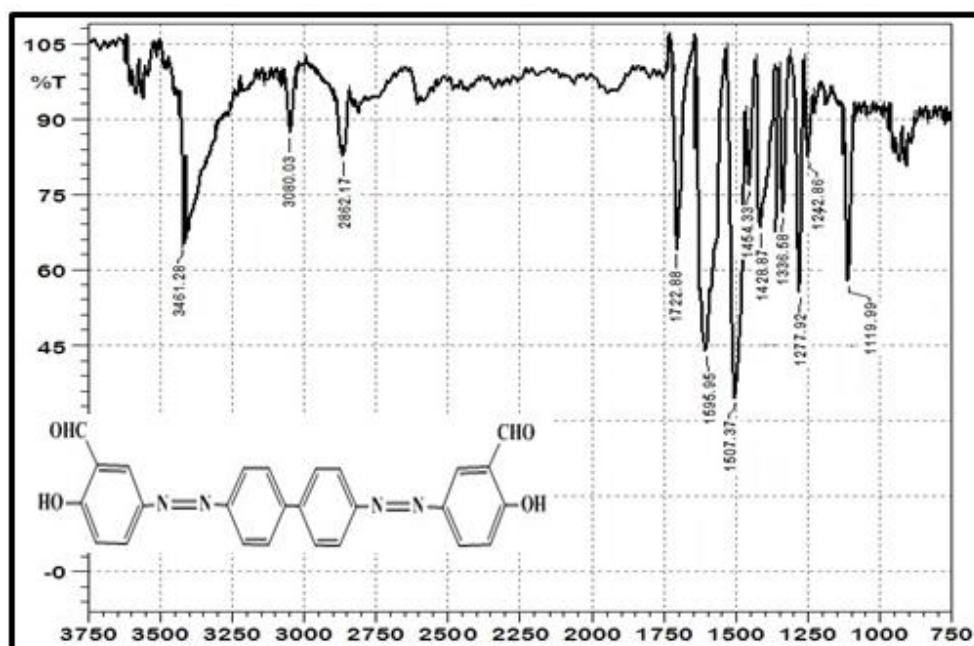


Fig (4): FT-IR spectrum of compound [A₁].

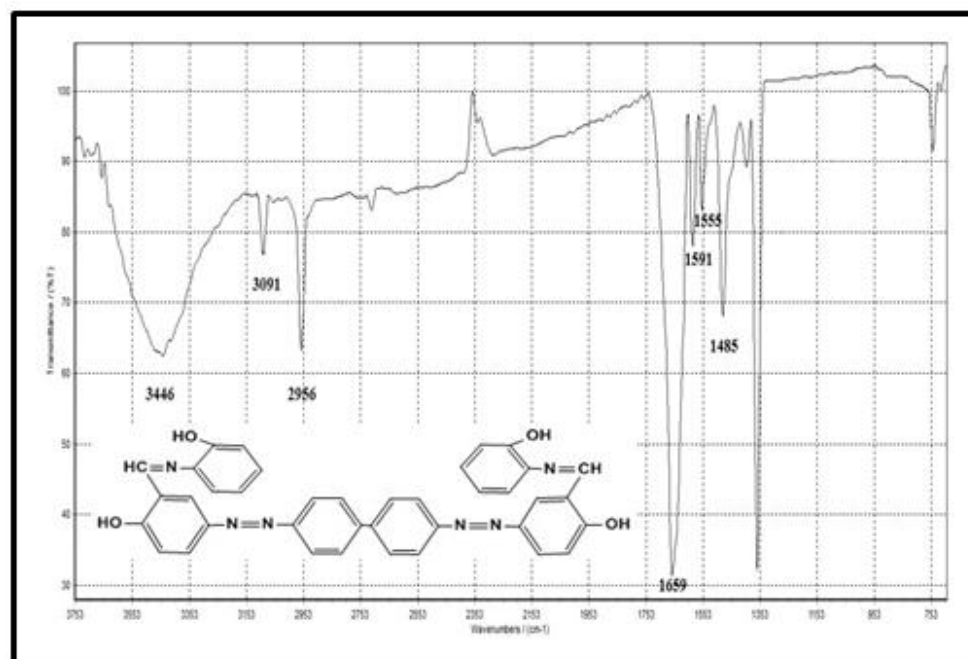


Fig (5): FT-IR spectrum of compound [A₅].

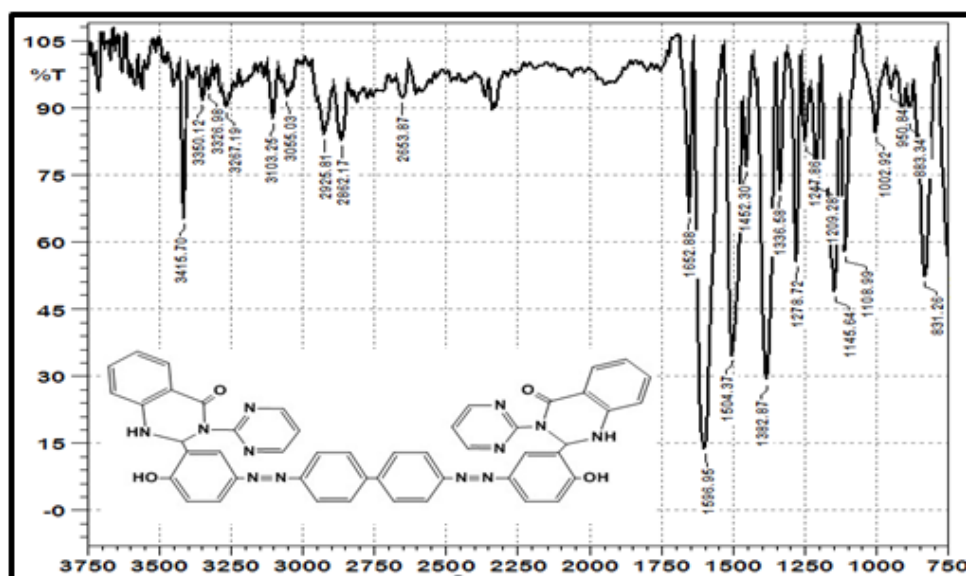


Fig (6): FT-IR spectrum of compound [A₉].

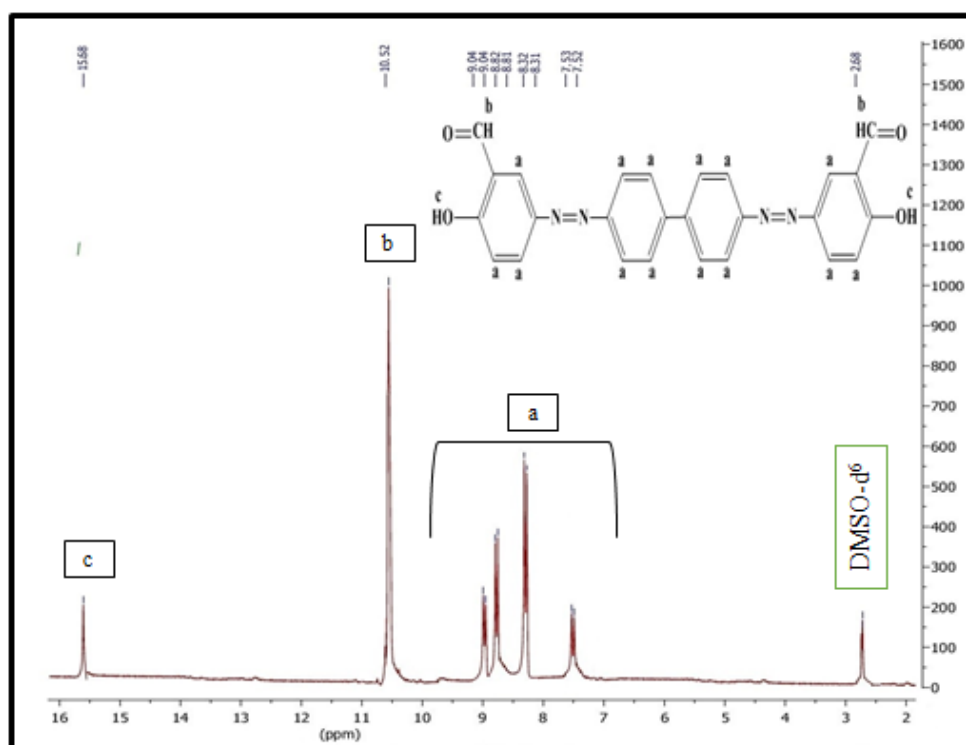


Fig (7): ¹H-NMR spectrum of compound [A₁].

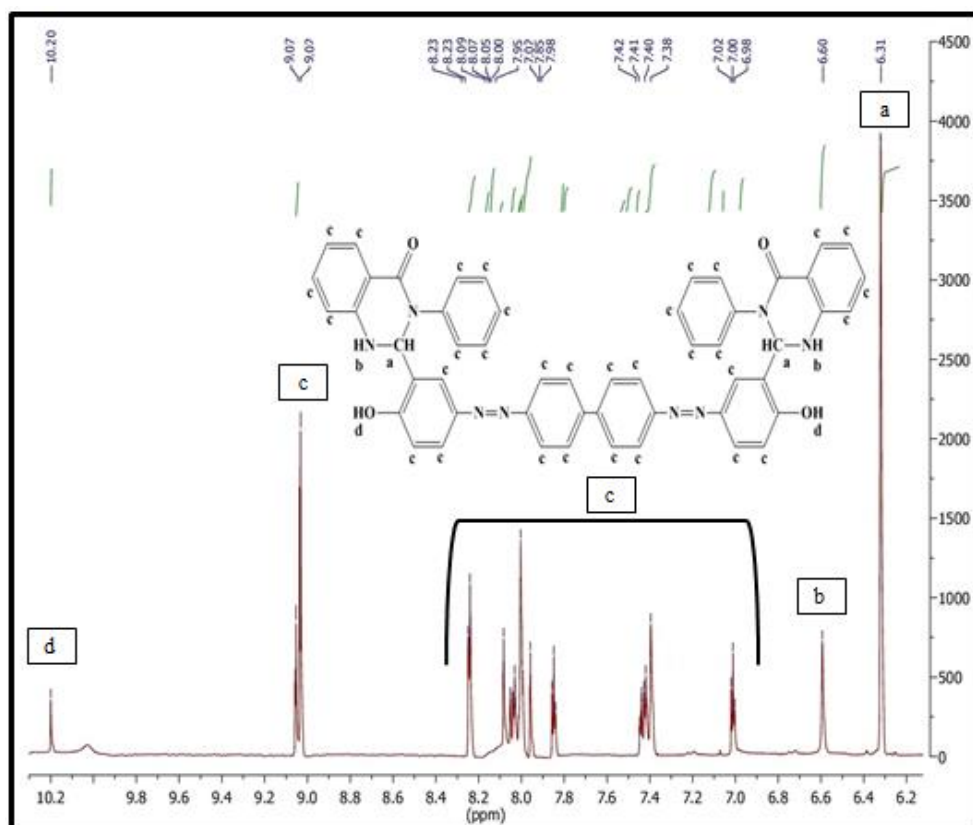


Fig (8): ¹H-NMR spectrum of compound [A₉].



Fig (9): Antibacterial activity of compounds [A₁] against *Klebsiella pneumoniae*.



Fig (10): Antibacterial activity of compounds [A₅, A₆] against *Escherichia coli*.

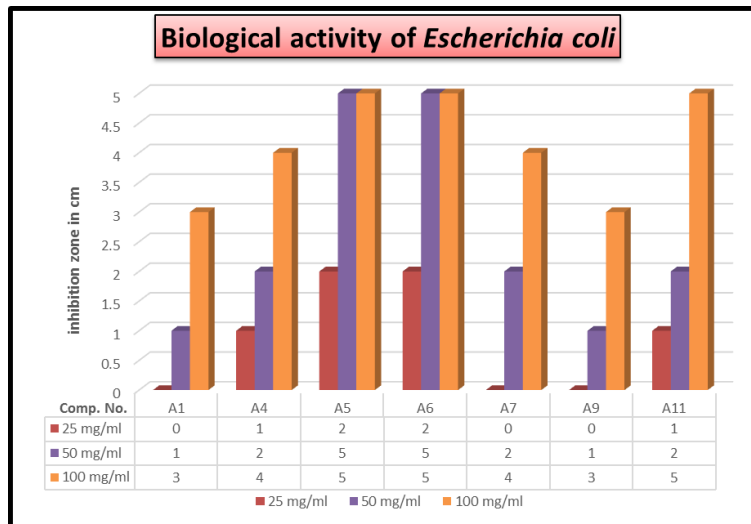


Fig. (11): Evaluation of inhibitory activity of compounds prepared for *Escherichia coli*.

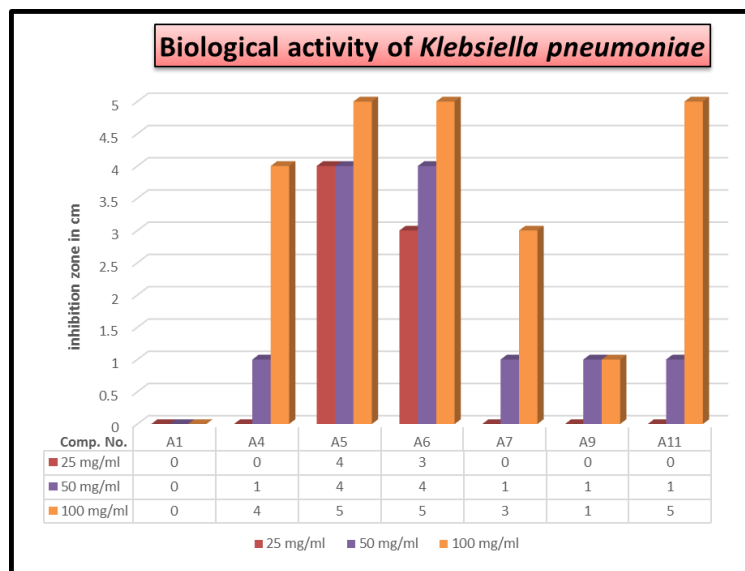


Fig. (12): Evaluation of inhibitory activity of compounds prepared for *Klebsiella pneumoniae*.

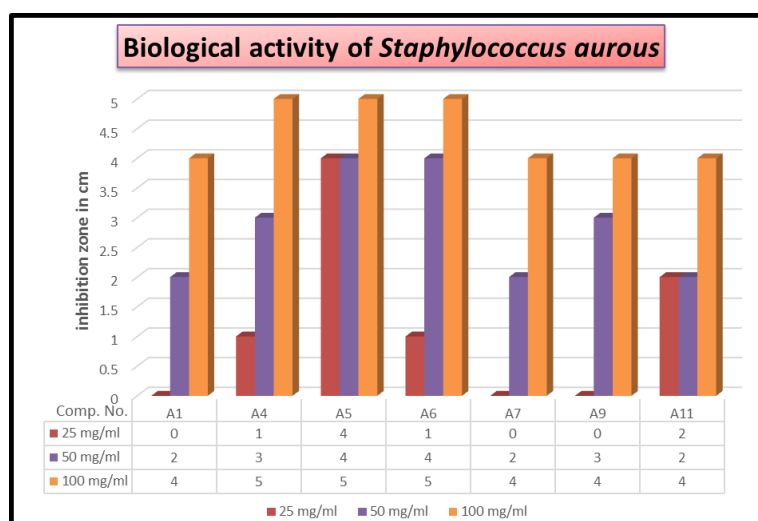


Fig. (13): Evaluation of inhibitory activity of compounds prepared for *Staphylococcus aureus*.

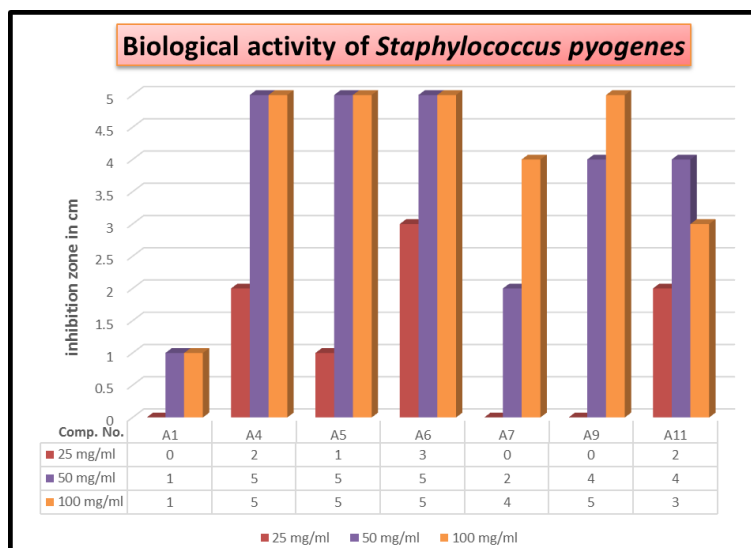


Fig. (14): Evaluation of inhibitory activity of compounds prepared for *Staphylococcus epidermidis*.

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تحضير وتشخيص بعض مشتقات 3,2- ثنائي هايدرو كوينازولين وتقييم الفعالية البيولوجية

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الملخص

تم تحضير صبغة الأزو [A₁] من تفاعل البنزيدين مع السلسلديهايد عن طريق ملح الديازانيوم، ومن ثم تحضير قواعد شيف [A₂-A₆] من تفاعل معوضات الأمينات الأروماتية مع مجموعة الألددهايد الموجودة في صبغة الأزو المحضرة ثم تحضير حلقة سداسية لمشتقات 3,2- ثنائي هايدرو كوينازولين [A₇-A₁₁] من مفاعلة قواعد شيف المحضرة مع حامض الانثرانيلك في الايثانول المطلق، ثم شخصت المركبات المحضرة بالطرائق الطيفية مثل طيف الأشعة فوق البنفسجية [UV] وطيف الأشعة تحت الحمراء [IR] وأطياف الرنين النووي المغناطيسي [¹H- NMR] والتحليل الكمي للعناصر (C.H.N.) وتعيين درجات الانصهار، ومتابعة سير التفاعلات بكميات صغيرة بالطريقة TLC، وتقييم الفعالية البيولوجية لبعض المركبات المحضرة على نمو أربعة أنواع من العزلات البكتيرية وهي أشريشيا القولون وكليسيلا الرئوية والمكورات العنقودية الذهبية والعنقودية البشروية.