Synthesis, Characterization and Biological Activity Study of New Compounds Tetrazole Derivatives Azo-Schiff Base

Hiba Ibrahim Abdulla AL-Joubory1 and
Khalid Mohamad Motny Al-janaby2
1 Department of chemistry, college of Education for pure science, University Tikrit, Tikrit, Iraq.
2 College of Petroleum and Minerals Engineering, University of Tikrit, Tikrit, Iraq.

Abstract:

This work included synthesis of azo dye (H1) by the reaction of diazonium salt to sulfacetamide with 4-hydroxy benzaldehyde at (0-5) °C and synthesis of schiff base (H2-H6) through reaction substituted aromatic amine (aniline, 4-nitro aniline, 4-chloro aniline, 4-amino benzoic acid and phenyl hydrazine) with aldehyde group in azo compound (H1) in ethanol compounds (H2-H6) and tetrazole derivatives prepared by reaction schiff base with sodium azide in ethanol compounds (H7-H11) 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 a solvent, in addition melting point and determination a purity of TLC, and this work consists a study of biological activity for the some prepared compounds against four types of pathogenic bacteria and know to be resistant to anti biotic.

Key words: Azo, Schiff's base, Tetrazole and Biological activity.

تحضير وتشخيص ودراسة الفعالية البايولوجية لمركبات التترازول الجديدة المشتقة من قواعد شيف - أزو

هبة إبراهيم عبد الله الجبوري[١]

وخالد محمد مطني الجنابي[٢]

[١] قسم الكيمياء، كلية التربية للعلوم الصرفة، جامعة تكريت. تكريت – العراق.

[٢] كلية هندسة النفط والمعادن، جامعة تكريت. تكريت – العراق.

الخلاصة:

تضمن هذا البحث تحضير صبغة الأزو(H1) عن طريق تكوين ملح الدايزونيوم من تفاعل سلفاسيتاميد مع ٤–هيدروكسي بنزالديهايد بدرجة حرارة (٠–٥)C^0، ومن ثم تحضير قواعد شيف (H2–H6) من تفاعل معوضات الأمينات الأروماتية مع مجموعة الألديهايد الموجودة في صبغة الأزو المحضرة ثم تحضير حلقة خماسية لمشتقات التترازول (H6–H11) من مفاعلة قواعد شيف المحضرة مع أزيد الصوديوم في الايثانول، ثم شخصت المركبات المحضرة بالطرائق الطيفية مثل طيف الأشعة فوق البنفسجية [UV] وطيف الأشعة تحت الحمراء [IR] وأطياف الرنين النووي المغناطيسي [^{1}H– NMR] والتحليل الكمي للعناصر (C.H.N.) وتعيين درجات الانصهار، وتقييم الفعالية البايولوجية لبعض المركبات المحضرة على نمو نوعين من العزلات البكتيرية وهي ***الزائفة الزنجارية والمكورات العنقودية الذهبية.***

الكلمات المفتاحية: أزو، قواعد شيف، تترازول، الفعالية البايولوجية.

Introduction:

Azo dyes are a class of compounds containing a N=N double bond and due to their ability to absorb visible light [1]. For many years, the azo compounds have been the main class of dyes used in various application such as textile fibers dyeing, coloring of different materials and advanced organic synthesis [2]. The synthesis and dyeing properties of azo compounds are assigned in many papers [3,4]. Azo compounds are widely used as dyes and pigments. Another application is analytical chemistry. On the other hand, azo compounds shown biological activities containing antibacterial [5]. Schiff bases and azo compounds are important structures in the medicinal and pharmaceutical fields [6]. The compounds carrying azomethine functional group (-C=N-) which are known as Schiff bases gain importance in medicinal [7,8] and pharmaceutical field due to the most versatile organic synthetic intermediates and also showing a broad range of biological activities [9] such as antituberculosis, anticancer, analgesic, anti-inflammatory [10], anticonvulsant, antibacterial and antifungal activities [11]. On the other hand, cyclic imides represent an important class of bioactive molecules that shows a wide range of pharmacological activities such as androgen receptor antagonistic [12]. Tetrazoles are a representative class of poly-aza-heterocyclic compounds, which consisting of a 5-membered ring of four nitrogen and one carbon atoms [13]. The first tetrazole was prepared by the Swedish chemist Bladin [14] in 1885. Katritsky et al. synthesized 1,5-disubstituted tetrazoles in high yields from imidoylbenzotriazoles includes short reaction times and mild reaction conditions [15]. Tetrazoles are unknown in the nature the ring systems of tetrazoles are very resistant to reduction [16]. Tetrazoles are a class of heterocycles with a wide range of applications including nanomaterials5 and specialty explosives [17]. The tetrazoles are representative of active pharmacophores for several therapeutic active molecules such as antiallergic [18], anti-inflammatory, antibiotic, antihypertensive and antitubercular agents [19]. For example, the β-lactam antibiotics A of the cephalosporin class is an example of drugs containing a 1,5-disubstituted tetrazole moiety. Losartan B is sartan derivatives that was the first nonpeptide angiotensin receptor antagonist to appear on the market followed by Valsartan C which include the regulation of blood pressure and volume homeostasis [20].

Experimental:

Material: All chemicals were used through this work purchased from Alfa Aesar, Chem-Lab, HIMDIA, Oxford, Aldrich, Companies and were used without further purifications.

Devices used: Melting points were recorded using a measuring device melting point type: Automatic melting point\SMP40 and were uncorrected. 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 spectrometer -1800 using Ethanol as a solvent. Infrared spectra were recorded using FT-IR-600 Fourier- Transform infrared (FT-IR) Spectrophotometer by KBr disc. ^{1}H-NMR spectra were recorded on Fourier Transform Varian spectrophotometer operating at 400 MHz with DMSO-d^{6}(Ibn –Al –Hatham college).

Methods of preparation:

Synthesis of Azo dye (H1) [21]:

Azo dye was prepared in two main steps:

Step 1 / Preparation of diazonium salt: (0.04 mol, 9.44 gm) of sulfacetamide dissolved in (50 ml) 37% HCl at a temperature of

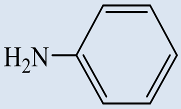
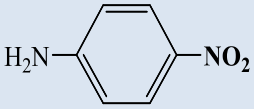
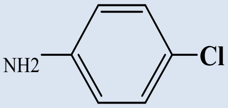
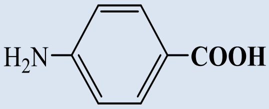
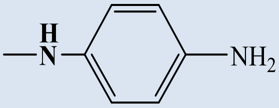
(0-5) ^{0}C with continuous stirring, then add a solution of sodium nitrite.

Step 2 / Coupling reaction: (0.04 mol, 4.88gm) of 4-hydroxy benzaldehyde dissolved in (50 ml) of the Pyridine, and cooled to (0–5) ^{0}C in an ice bath. This solution is then slowly added to the cooled diazonium salt solution to yield azo compound. physical properties of azo is color red, M.P. (260-261) ^{0}C, yield 81% and R.f. 0.65.

Synthesis of Schiff Bases (H2-H6) [22, 23]:

A series of Schiff bases were prepared from the reaction of azo prepared (H1) (3.69 gm 0.01 mole) with (0.01 mole) from different aromatic amine (aniline, 4-nitro aniline, 4-chloro aniline, 4-amino benzoic acid and phenyl hydrazine) in (30 ml) ethanol absolute and few drops of glacial acetic acid. This mixture was refluxed for (4-9) hr. The mixture was cooled to room temperature, filtered, dry and rec-rystallized in absolute ethanol, physical properties, yield and R.f. are given in Table (1).

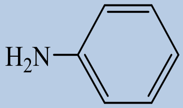
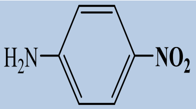
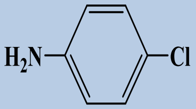
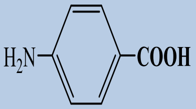
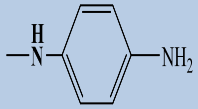
Table (1): physical properties, yield and R.f. of schiff base (H2-H6).

Comp. No.	Ar	Molecular Formula/ M.Wt g/mol	Color	M.P (^{0}C)	T. Ref. (hr.)	Yield (%)	R.f.
H2		$C_{21}H_{16}N_4O_4SNa$ 444.44	Dark Brown	125- 127	5	92	0.87
H3		$C_{21}H_{16}N_5O_6SNa$ 489.44	Orang	159- 161	9	73	0.50
H4		$C_{21}H_{16}N_4O_4SClNa$ 478.88	Brown	147- 150	6	86	0.69
H5		$C_{22}H_{17}N_4O_6SNa$ 488.45	Yellow	136- 138	7	79	0.43
H6		$C_{21}H_{17}N_5O_4SNa$ 456.44	Light Brow	-١٧٠ ١٧٢	٩	٩١	0.75

Synthesis of Tetrazole derivatives (H7-H11) [24]:

Compound (H2-H6) (0.02 mole) was added to solution of (0.02 mole, 1.3 gm) of sodium azide in (25ml) of ethanol. The reaction mixture was refluxed for (5-9) hrs. The mixture was cooled to room temperature, filtered, dry and rec-rystallized in absolute ethanol, physical properties, yield and R.f. are given in Table (2).

Table (2): physical properties, yield and R.f of 1,3-oxazepine derivatives (H6-H٩).

Comp. No.	Ar	Molecular Formula/ M.Wt g/mol	Color	M.P (^{0}C)	T. Ref. (hr.)	Yield (%)	R.f.
H 7		$C_{21}H_{18}N_7O_4SNa$ 487.47	Light Yellow	232-234	8	89	0.60
H 8		$C_{21}H_{17}N_8O_6SNa$ 532.47	Dark Brown	263-265	7	67	0.82
H 9		$C_{21}H_{17}N_7O_4SClNa$ 521.91	Orang	167-169	6	74	0.93
H 10		$C_{22}H_{18}N_7O_6SNa$ 531.48	Yellow	248-250	5	73	0.66
H11		$C_{21}H_{17}N_5O_4SNa$ 456.44	Brown	-216 218	5	70	0.85

The biological activity [25]:

The bacteria species used are listed in tables (7). All strains were obtained from College of Science department of Biology, Tikrit University. They were grown up to the stationary phase nutrient bath at 37 ^{0}C and a sample of 0.5 ml of each bacteria was spread over a surface of a nutrient agar plate.

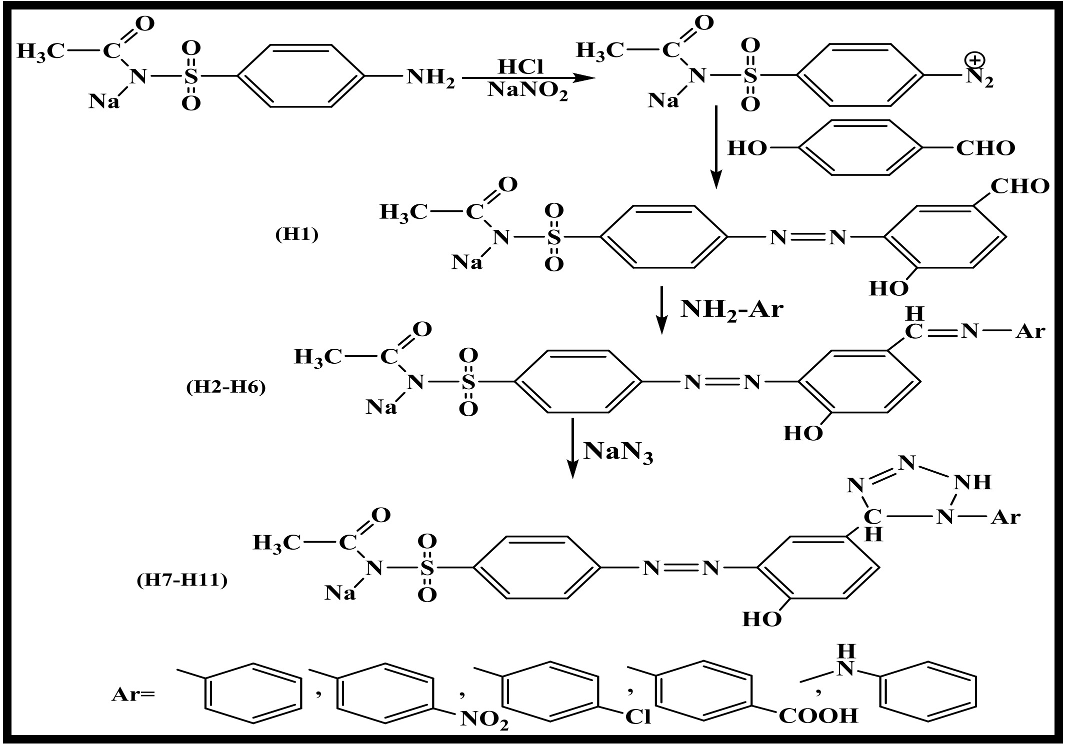
Antibacterial assay [26]:

DMSO was used as a solvent for compounds (H1, H2, H4, H6, H9, H11). The same solvent was used for antibiotics

(Amoxicillin, Ampicillin, Ciprofloxacine). Blank discs of DMSO was used as control. The inoculated plates are incubated at 37 ^{0}C for 24 hrs., and the inhibition zone (mm) were measured. In all experiments the mean of each triplicate was measured.

Results and Discussion:

In this work many compounds were synthesized azo, schiff bases derivatives and tetrazole derivatives and as in the following Scheme:



Scheme (1): synthesis of compounds (H1-H11).

Characterization of Azo dye (H1) [27, 28]:

Azo dye has synthesized from the reaction of diazonium salt with 4-hydroxy benzaldehyde.

UV spectra show the transions n-π* (255 nm) and π-π* (371 nm) which have confirmed the presences of the un-bonded pair electrons on nitrogen, oxygen atoms and aromatic system (double bond).

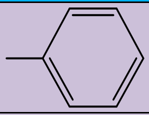
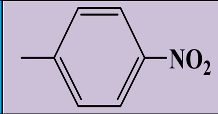
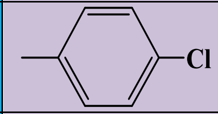
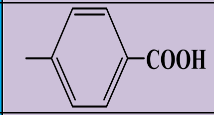
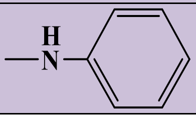
The FT-IR spectra of azo dye general showed disappearance of (NH_2) absorption of sulfacetamide and appearances of (N=N) absorption band in 1468 cm^{-1}, besides bands in 1658 cm^{-1} is due to (C=O) aldehyde and band at 3435 cm^{-1} due to (OH) of salsaldehyde. IR spectra is given in fig (1).

^{1}H-NMR spectrum of compound (H2) showed singlet signal at δ= (2.84) ppm due to (CH_3), multiple signal (6.65-7.99) ppm due to aromatic rings, singlet signal at δ= (9.09) ppm due to (CH) and singlet signal (9.61) ppm due to (OH). ^{1}H-NMR spectrum of compound (H2) is given in fig (11).

Characterization of Schiff Bases (H2-H6)]:

Schiff Bases derivatives have synthesized from the reaction of azo prepared (H1) with different aromatic amine (aniline, 4-nitro aniline, 4-chloro aniline, 4-amino benzoic acid and phenyl hydrazine). Beside UV spectra show the transions n-π* and π-π* which have confirmed the presences of the un-bonded pair electrons on nitrogen, oxygen atoms and aromatic system (double bond). UV absorbance spectra is given in table (3). The FT-IR spectra of Schiff Bases derivatives in general showed disappearance of (C=O) absorption of azo prepared (H1) and appearances of (C=N) absorption band in (1647-1684) cm^{-1}. IR spectra is given in table (3) see fig. (2) and fig. (3). ^{1}H-NMR spectrum of compound (H_2) showed singlet signal at δ= (2.50) ppm due to DMSO-d^6 solvent, multiple signal (8.20 - 9.02) ppm due to aromatic rings, singlet signal at δ= (10.01) ppm due to N-H amic acid, and singlet signal (13.03) ppm due to O-H carboxylic acid. ^{1}H-NMR spectrum of compound (H_{11}) is given in fig (12).

Table (3): FT-IR and UV/Vis. data of Schiff Bases (H2-H6).

Comp. No.	Ar	λ_1 max λ٢ max	IR (KBr) cm^{-1}						
			ν (O-H)	ν (C-H) Arom.	ν (C=N)	ν (C=C)	ν (N=N)	ν (S=O)	Others
H2		219 310	3469	3053	1684	١٤٩٥ ١٥٩٩	1439	1135	-----------
H3		269 332	3479	3059	1658	1475 1581	1441	1155	ν (NO_2). *asy*.1581 *sym*.1377
H4		261 308	3469	3057	1658	1496 1599	1441	1155	ν (C-Cl) 1092
H5		226 359	3377	3072	1647	1473 1576	1437	1169	ν (C=O) 1695
H6		248 340	3442	3061	1674	1512 1614	1454	1161	ν (N-H) 3265

Characterization of tetrazole derivatives (H7-H11):

tetrazole derivatives (H7-H11) have synthesized from the reaction of compound (H2-H6) with sodium azide. UV spectra show the transions n-π* and π-π* which have confirmed the presences of the un-bonded pair electrons on nitrogen, oxygen atoms and aromatic system (double bond). UV absorbance spectra is given in table (4). The FT-IR spectra of tetrazole derivatives in general showed disappearance of (C=N) absorption band in (1647-1684) cm^{-1} of schiff bases derivatives and appearances of (N-H) absorption band in (3221-3276) cm^{-1}, appearances of (C-N) absorption band of tetrazole in (1232-1290) cm^{-1} and appearances of (N-N) absorption band of tetrazole in (1111-1157) cm^{-1}. IR spectra is given in table (4) see fig (4), (5) and (6).

^{1}H-NMR spectrum of compound (H11) showed singlet signal at δ= (2.89) ppm due to (CH_3), singlet signal at δ= (4.71) ppm due to (CH), singlet signal at δ= (5.02) ppm due to (NH), multiple signal (6.84-8.42) ppm due to aromatic rings, singlet signal at δ= (8.67) ppm due to (NH) and singlet signal (9.72) ppm due to (OH). ^{1}H-NMR spectrum of compound (H11) is given in fig (12).

Table (4): FT-IR and UV/Vis. Characterization of tetrazole derivatives (H7-H11).

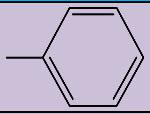
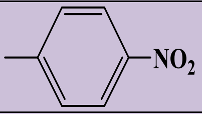
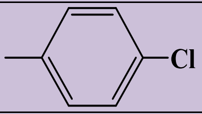
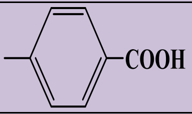
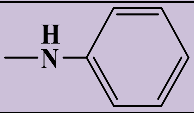
omp. N	Ar	λ_1 max ٢λ max	IR (KBr) cm^{-1}							
			ν (O-H)	ν (N-H)	ν (C-H) Arom.	ν (C=C)	ν (N=N)	ν (C-N)	ν (N-N)	Others
H7		258 377	3408	3255	3034	1493 1587	1438	1269	1123	-----------
H8		245 380	3477	3221	3050	1500 1633	1442	1232	1111	ν (NO_2). *asy*.1597 *sym*.1396
H9		221 339	3473	3240	3060	1485 1599	1441	1290	1157	ν (C-Cl) 1090
H10		208 383	3388	3226	3066	1506 1595	1421	1252	1153	ν (C=O) 1660
H11		230 367	3386	3276	3068	1508 1585	1441	1284	1144	----------

Table (5): Elemental analysis of some of the prepared compounds.

Comp. No.	Molecular Formula	Found				Calculated			
		C%	H%	N%	O%	C%	H%	N%	O%
H1	$C_{15}H_{12}N_3O_5SNa$	48.67	3.20	11.40	21.64	48.78	3.28	11.38	21.66
H2	$C_{21}H_{17}N_4O_4SNa$	56.81	3.82	12.70	14.43	56.75	3.86	12.61	14.40
H5	$C_{22}H_{17}N_4O_6SNa$	54.11	3.59	11.34	19.67	54.10	3.51	11.47	19.65
H7	$C_{21}H_{17}N_8O_6SNa$	47.42	3.17	20.95	18.08	47.37	3.22	21.04	18.03
H8	$C_{21}H_{17}N_7O_4SClNa$	48.26	3.27	18.83	12.30	48.33	3.28	18.79	12.26
H11	$C_{21}H_{19}N_8O_4SNa$	50.10	3.74	22.39	4.55	50.20	3.81	22.30	4.58

Biological activity [32]:

The antimicrobial activity of the synthesized compounds (H1-H11) were examined by the agar diffusion method using two different bacterial species ***Staphylococcus aurous*** and ***Pseudomonas aeruginosa***. The results indicated that some of the assayed compounds showed antimicrobial activity against the used bacterial. Antibacterial activity of compounds (H1and H9) is given in fig (13) and (14).

Table (6): Antibacterial activity of some of the prepared compounds.

Comp. No.	Conc. mg/ml	*Pseudomonas aeruginosa*	*Staphylococcus aurous*
H1	0.0001	-	-
	0.001	+	-
	0.01	++	-
H2	0.0001	+	-
	0.001	++	+
	0.01	+++	++
H3	0.0001	+	+
	0.001	+++	++
	0.01	++	++
H4	0.0001	+	+
	0.001	+++	++
	0.01	+++	++
H5	0.0001	-	-
	0.001	+	+
	0.01	++	+
H6	0.0001	-	-
	0.001	+	+
	0.01	++	+
H7	0.0001	+	-
	0.001	++	+
	0.01	+++	++
H8	0.0001	-	-
	0.001	-	+
	0.01	-	+++
H9	0.0001	+	-
	0.001	++	-
	0.01	++	-
H10	0.0001	+	-
	0.001	++	+
	0.01	++	+++
H11	0.0001	+	-
	0.001	++	+
	0.01	+++	+++

(-) = No inhibition **(+) = Inhibition zone (10-15) mm**
(++) = Inhibition zone (15-25) mm **(+++) = Inhibition zone (25-30) mm**

Table (7): Antibacterial efficacy of control treatments (antibiotics) in the growth of a number of negative and positive bacteria (mm).

No.	***Name***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
١	Amoxicillin	17	٢٠
٢	Ampicillin	18	15
٣	Ciprofloxacine	12	16
4	Blank disk	٠	٠

Fig (1): FT-IR spectrum of compound [H1].

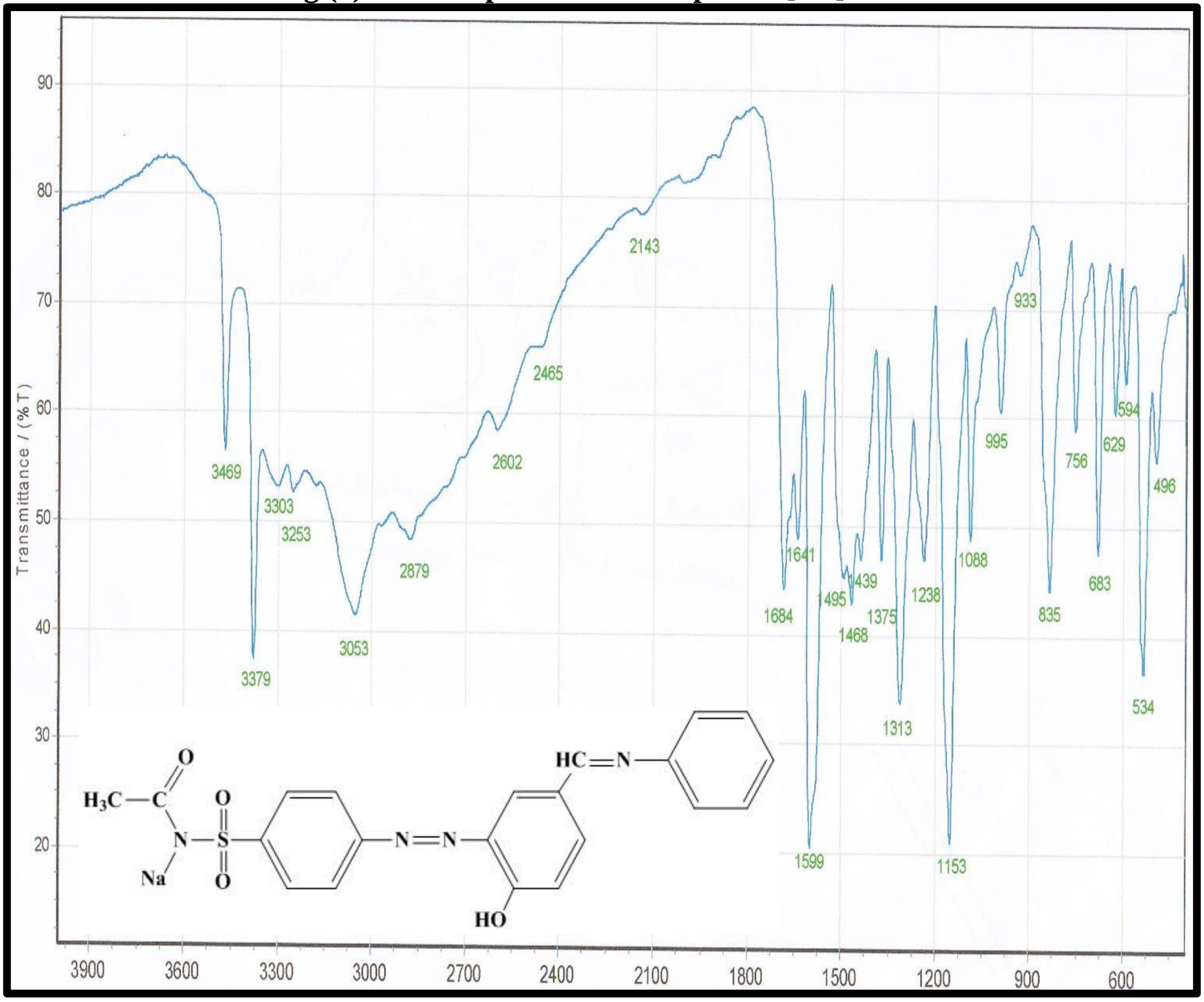


Fig (2): FT-IR spectrum of compound [H2].

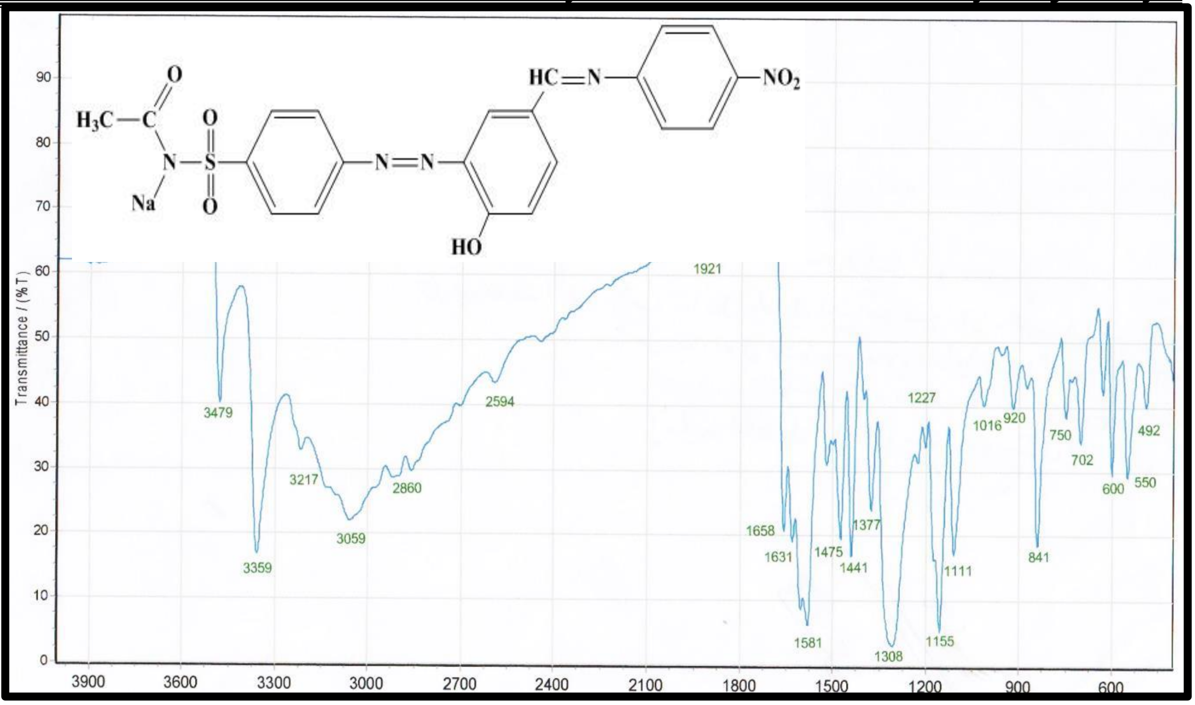


Fig (3): FT-IR spectrum of compound [H3].

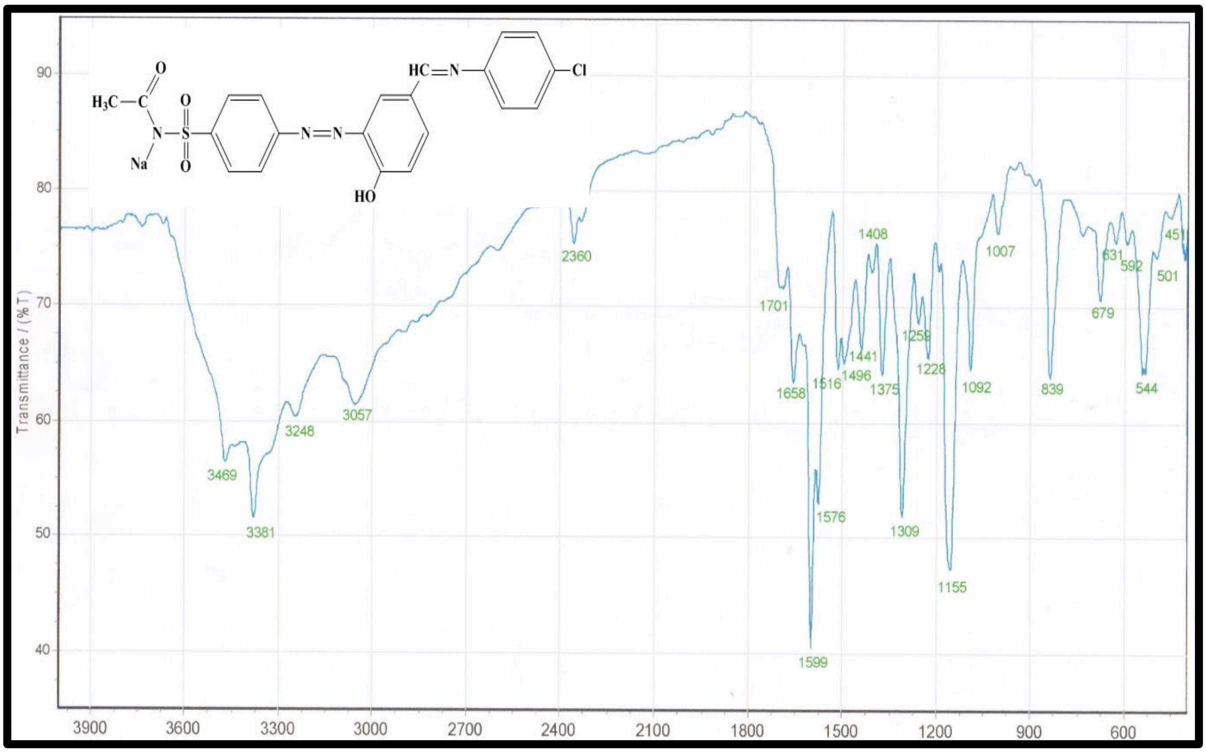


Fig (4): FT-IR spectrum of compound [H4].

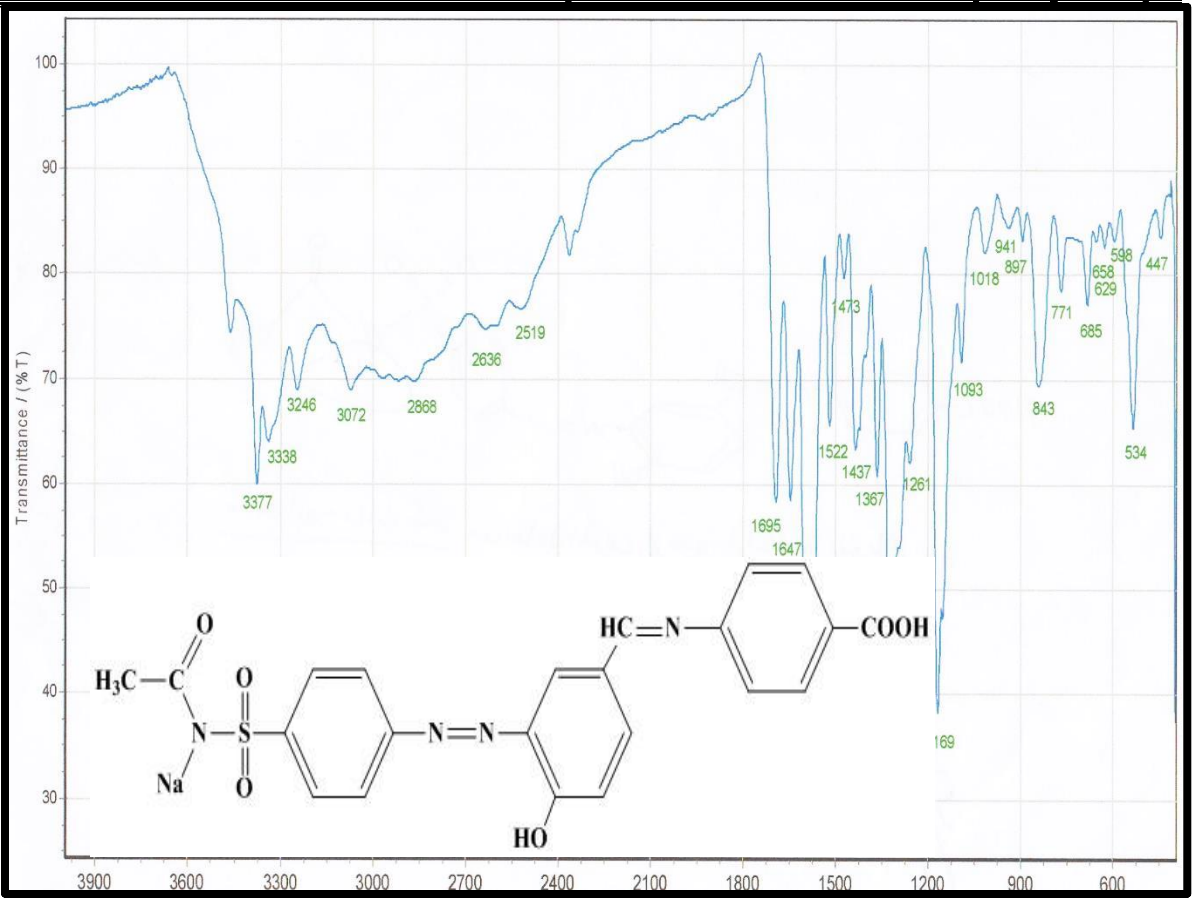


Fig (5): FT-IR spectrum of compound [H5].

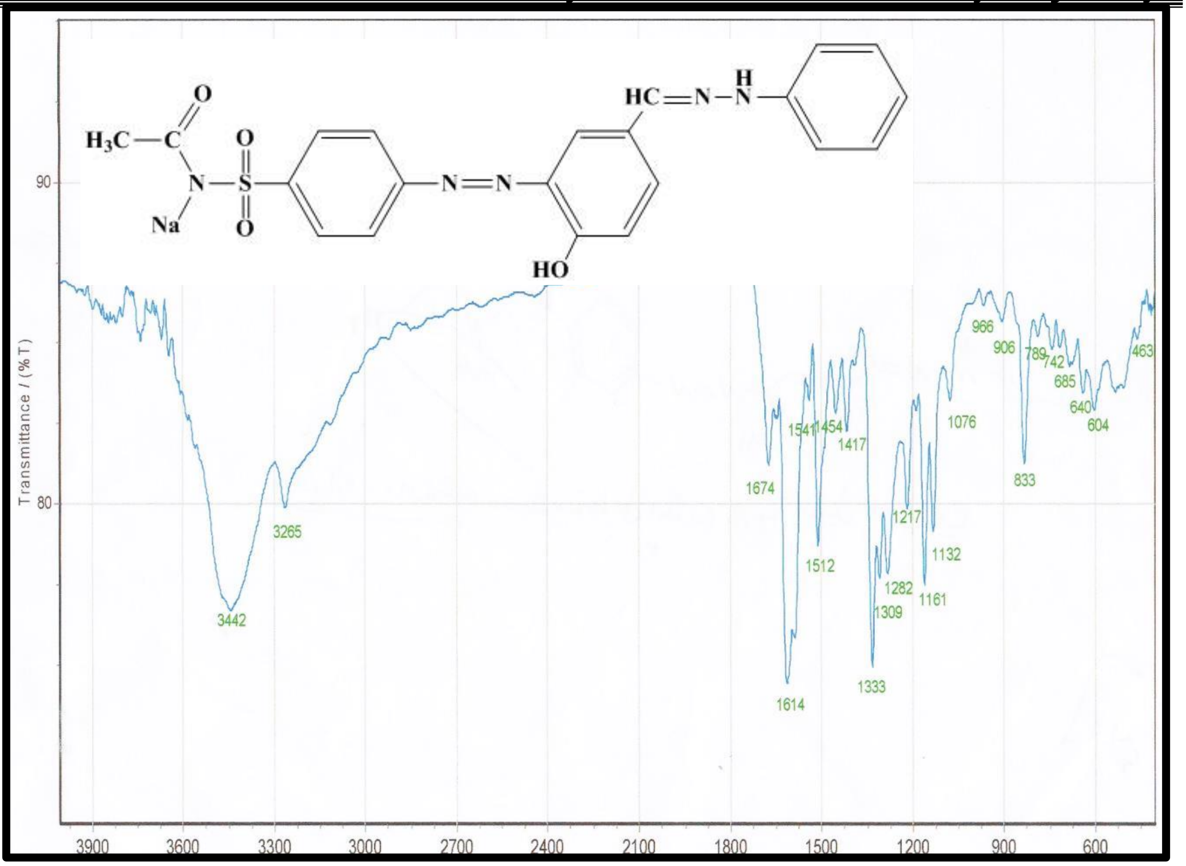


Fig (6): FT-IR spectrum of compound [H6].

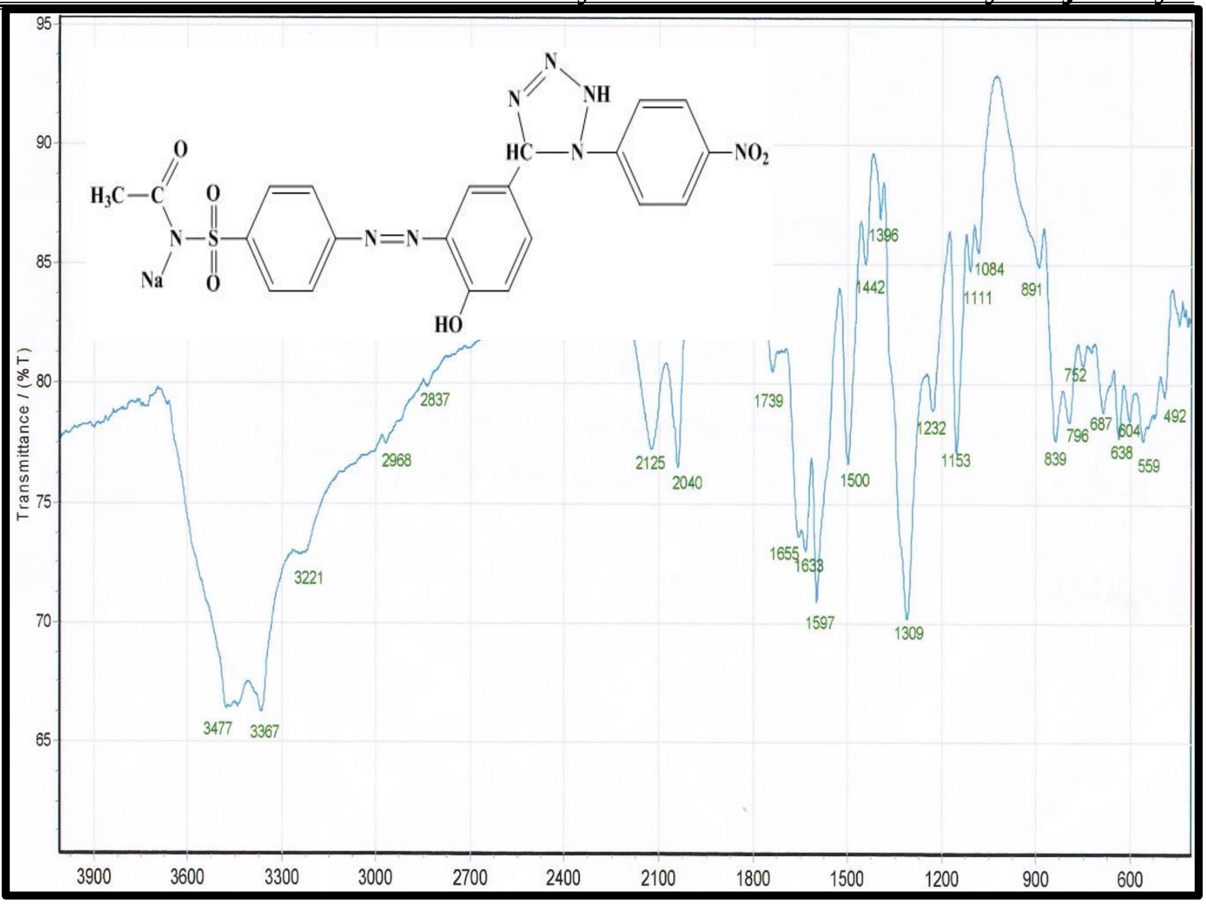


Fig (7): FT-IR spectrum of compound [H8].

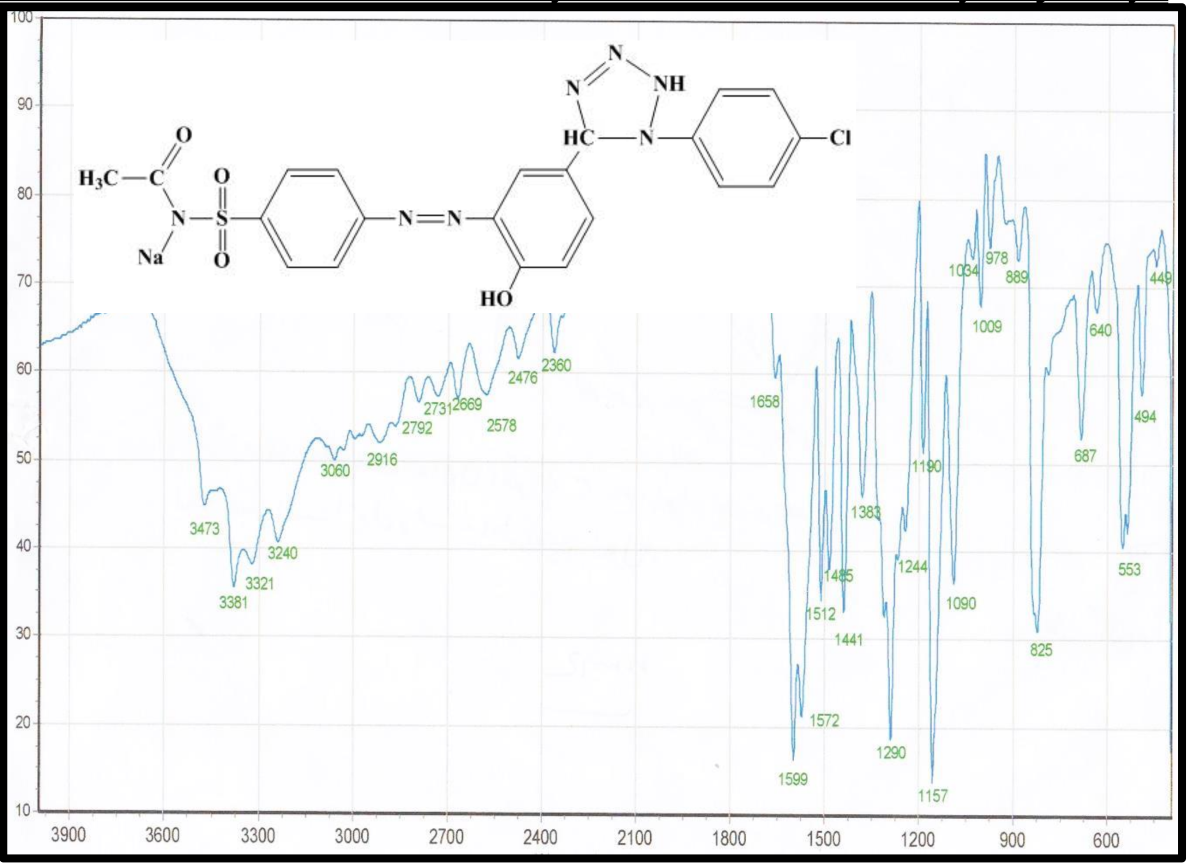


Fig (8): FT-IR spectrum of compound [H9].

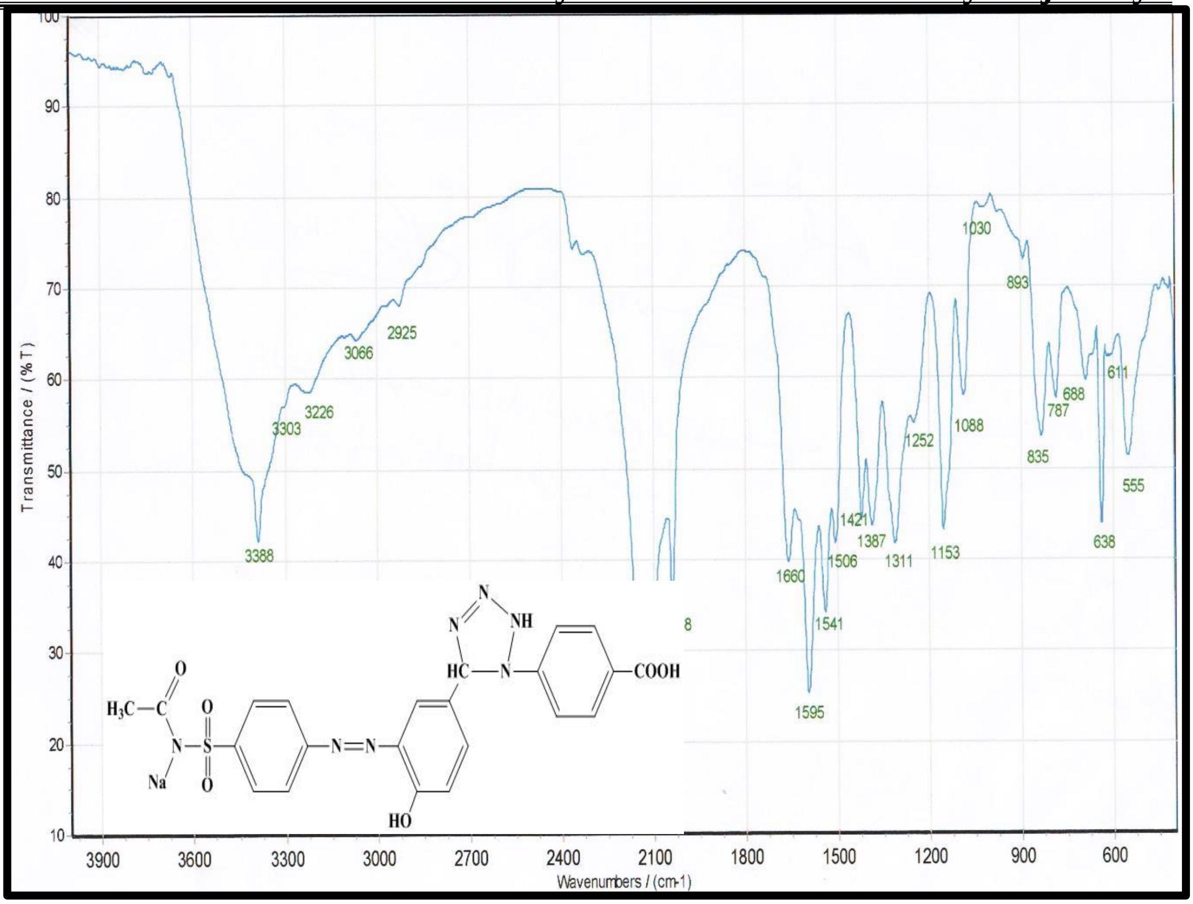


Fig (9): FT-IR spectrum of compound [H10].

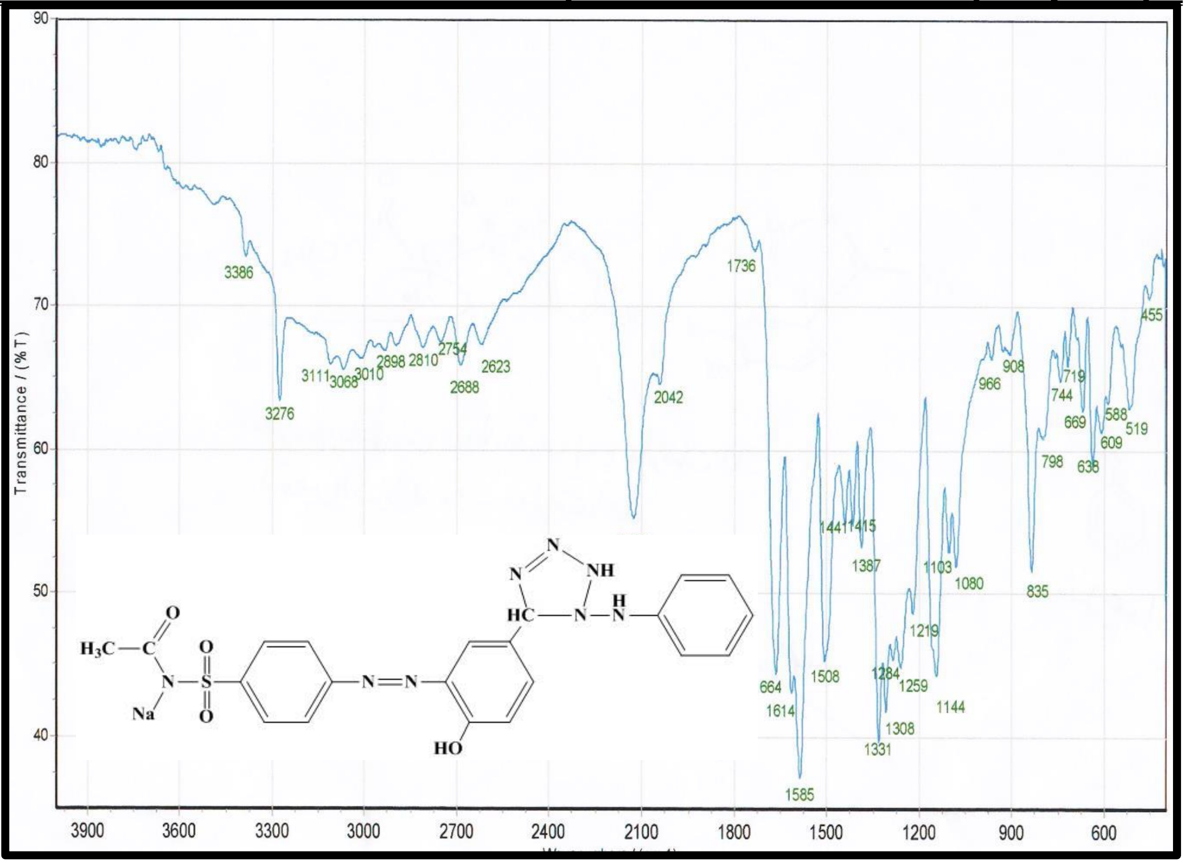


Fig (10): FT-IR spectrum of compound [H11].

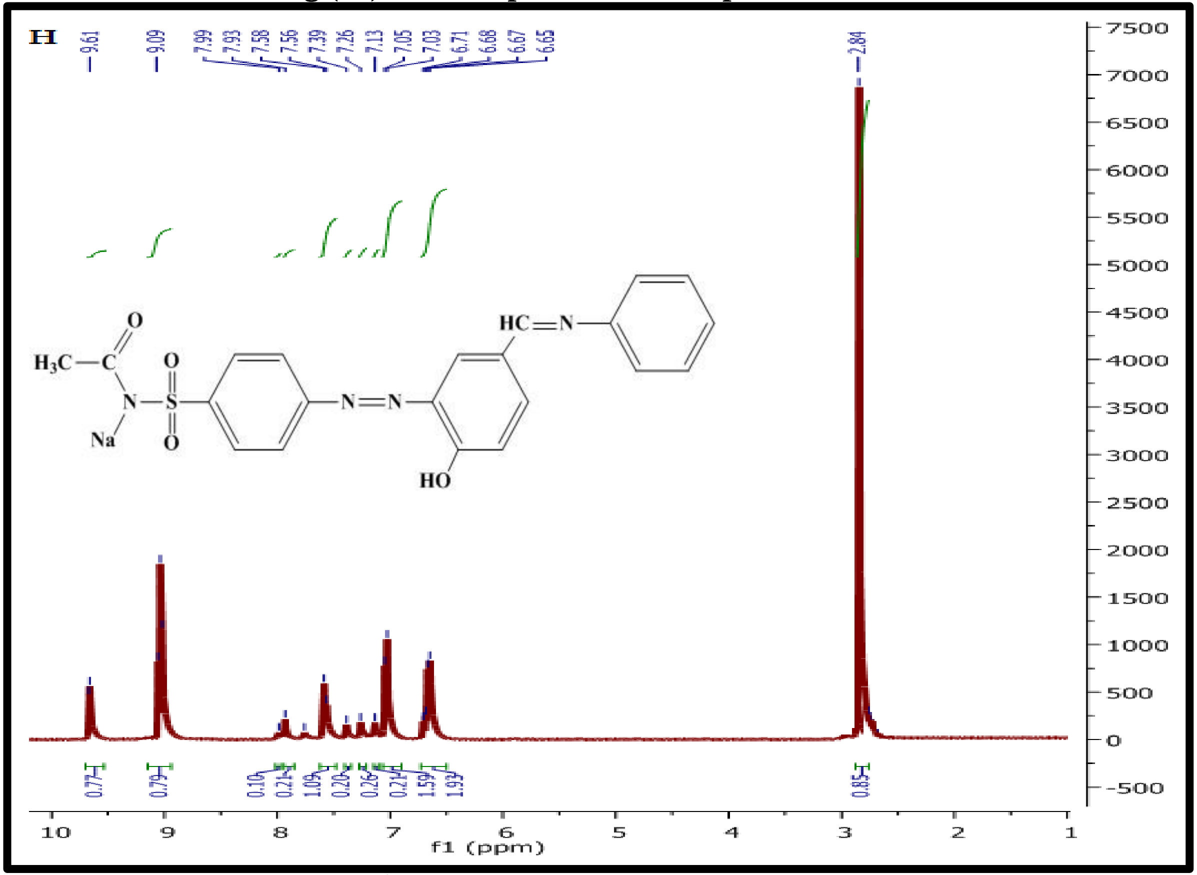


Fig (11): ^{1}H-NMR spectrum of compound [H2].

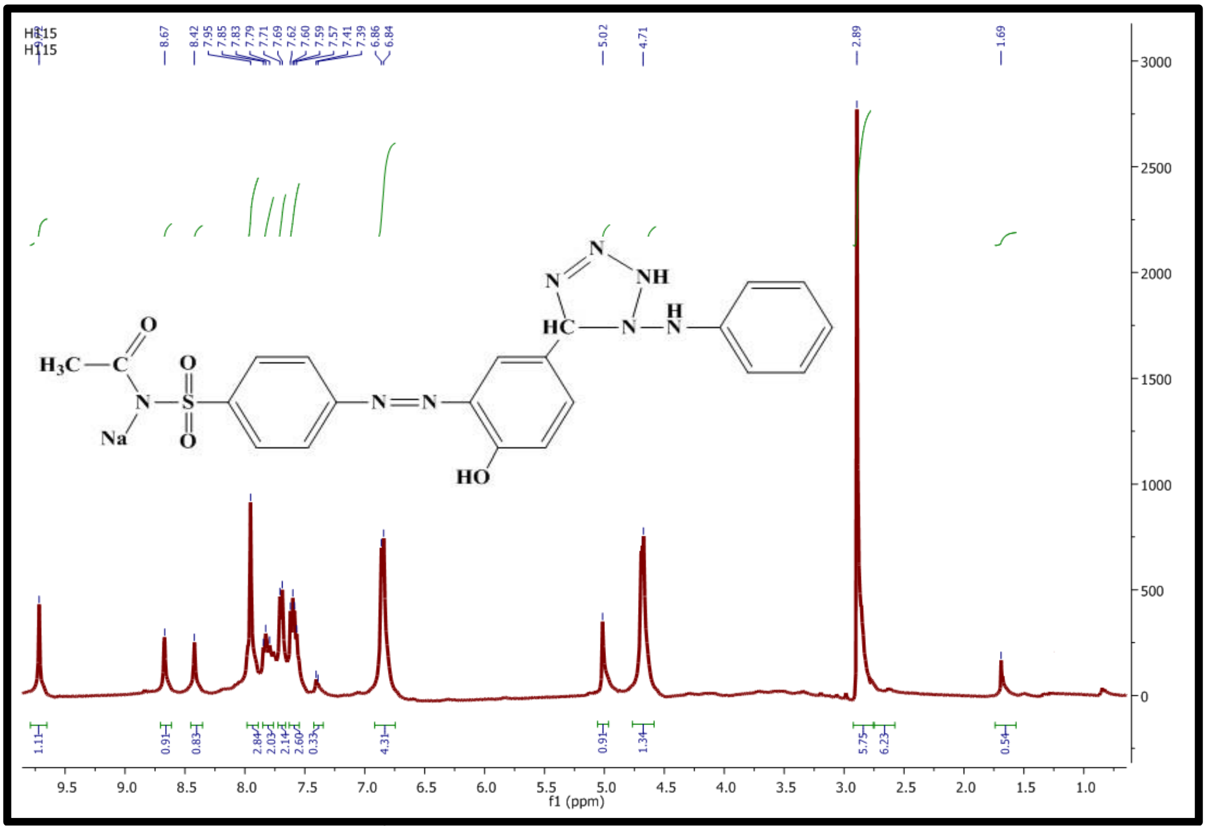


Fig (12): ^{1}H-NMR spectrum of compound [H11].

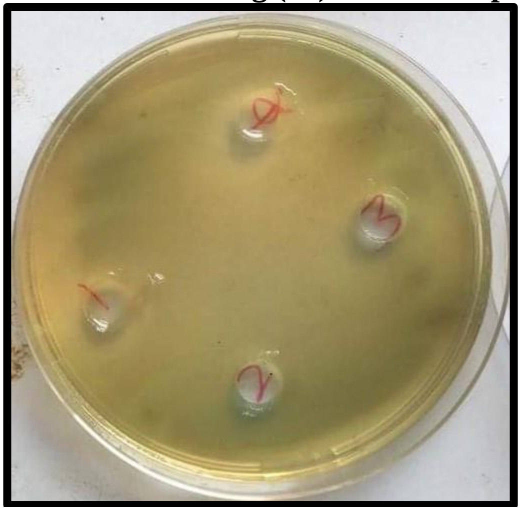


Fig (13): Antibacterial activity of compounds [H1] against *Pseudomonas aeruginosa*

Fig (14): Antibacterial activity of compounds [H9] against *Staphylococcus aurous*.

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