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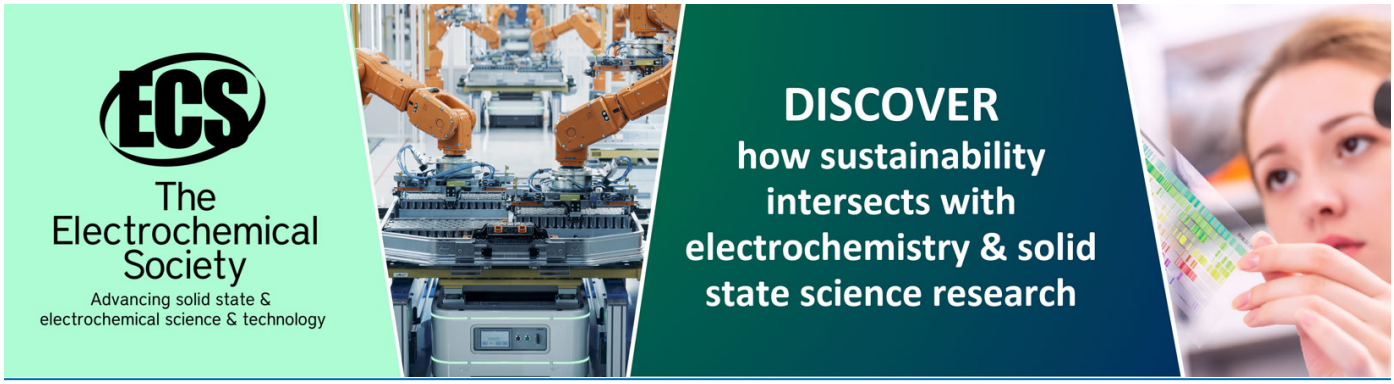

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Synthesis, Characterization, and Study of Antibacterial Activity of Some New Formazan Dyes Derivatives, Derived from 2-Mercapto Benzoxazole

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Abstract. This research describes the preparation of new 2-mercapto benzoxazole (1) pre-prepared was reacted with 2-Aminophenol and carbon disulfide in presence of ethanolic potassium hydroxide in a single step, Then hydrazine benzoxazole (2) was synthesized from the reaction of the compound (1) with hydrazine hydrate in presence of alcohol. And aromatic aldehydes react with compound (2) to give aryl substituted (1,3-benzoxazole-2-yl) hydrazone (3a-f). Then formazan derivatives were prepared by a reaction hydrazon and diazonium salt different compensation for aromatic amines (4a-c, 5d-f). All compounds were confirmed by their Physical data,^{1}H- NMR, ^{13}CNMR, and FTIR for some of them, the biological activities of these compounds have been assayed against two kinds of bacteria.
Keywords: 2-Aminophenol, 2-mercapto benzoxazole, 2- hydrazine benzoxazole, formazan, diazonium salt, Antimicrobial activity.

1. Introduction

In recent years Formazans compounds have found increasing applications in diverse fields [1-2]. In recent years Formazans compounds have found increasing applications in diverse fields. Formazan Skeleton is (-N=N–C=N–NH-) [2]. Formazans are polydentate ligands with donor atoms so that's why they have the ability to form complexes with metal atoms. Metal complex formazan is derived from non-metal complex formazan by treating it with metal salts such as FeSO4•7H2O, CrCl3•6H2O, and CuSO4•5H2O[3-6]. Formazans and their metal complex are ranging in color from red to orange as well as blue color [7-8]. Formazans and heterocyclic hydrazones are known for their spectrum of biological activities such as antiviral [9] antimicrobial [10], anti-inflammatory, antifungal [11], anticancer [12], anti-HIV [13], etc. Several formazans show promising antifertility [14] and antiparkinsonian activity [15] In the present study, we have synthesized formazan derivatives by coupling Schiff base prepared from 2-Hydrazinobenzoxazole (2) and various aldehydes with appropriate aniline derivatives in pyridine. (Scheme 1) The structures of these derivatives were assigned on the basis of IR, and ^{1}HNMR and ^{13}C-NMR spectral data, which are used widly to characterize materials and chemicals [16-19]. The synthesized compounds were screened for their antimicrobial activities.

2. Experimental

All the chemicals and solvents used were of (Fluka, Aldrich, BDH) products and were used without recrystallization, Melting Point Electro thermal 9300 melting point Apparatus. Infrared spectra were recorded Spectrophotometer model Shimadzu 8400, Type (KBr), and ^{1}H- NMR spectrometer for

proton (^{1} H-NMR) Bruker 400MHz, was to measure in Jordan in Ahl– Albate University, by a device Ultra shield 400 MHz.

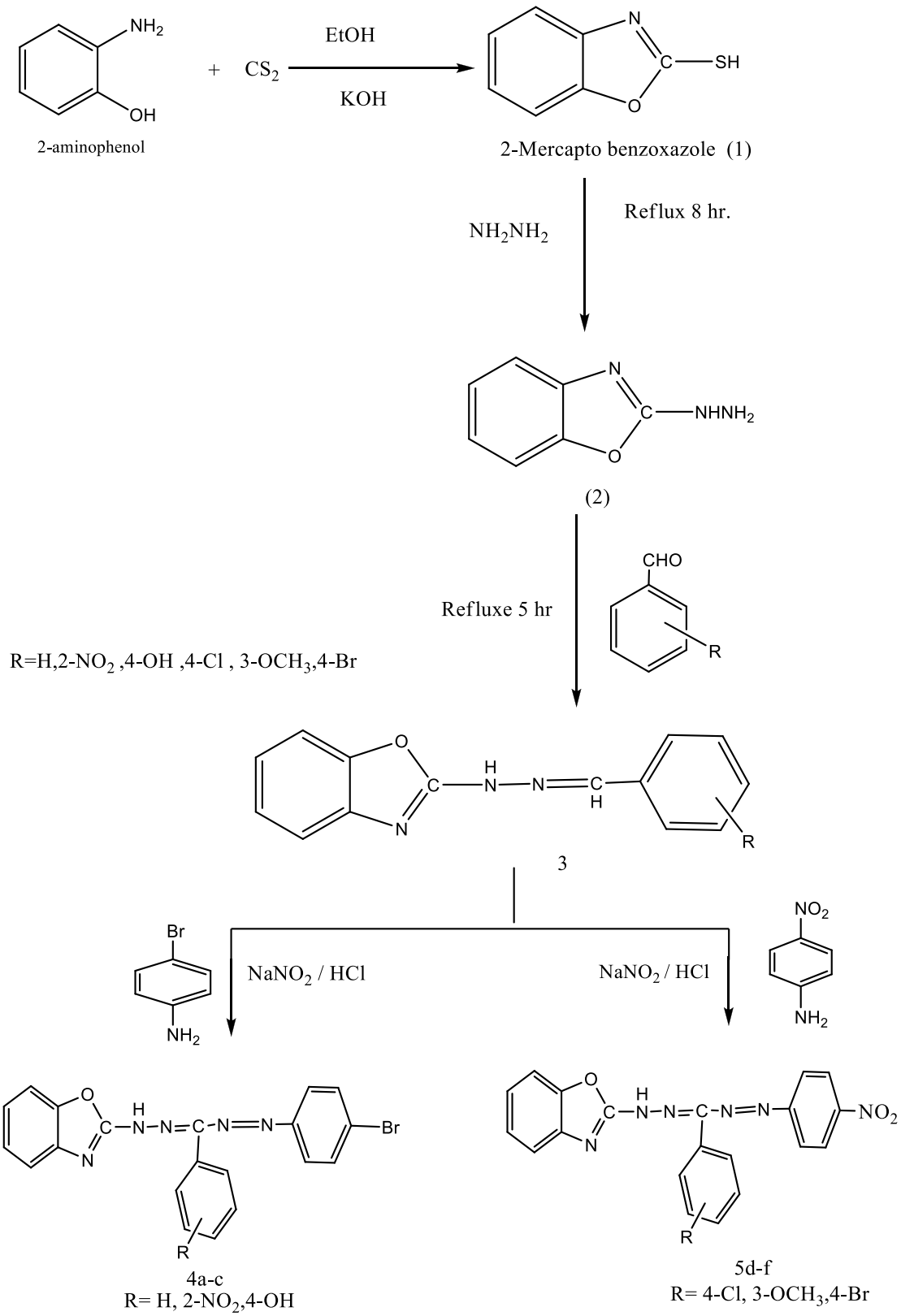
2.1 Synthesis of 2-Mercapto benzoxazole (1)
O - amino phenol 10.91gm (0.1mole) of was mixed with, 5.65 gm. (0.1mole) of potassium hydroxide and 7.67 gm. (0.1mole, 6.19ml) of carbon disulfide, 100ml of 95% ethanol and 15 ml of water in a 500ml round bottom flask heated under reflux for (3) hours. Then added 1-1.5 gm. of charcoal cautiously and the mixture is heated at for 10 minutes, the charcoal is removed by filtration. The filtrate is heated to 60-70^{o}C, 100ml of warm water is added, and then acidified with dilute acetic acid with stirring. The product separated as glistening white crystals and the mixture is placed in a refrigerator for (3) hours to complete the crystallization. The product is collected on a Buckner funnel and dried overnight at 40^{o}C. The dried product is recrystallized with ethanol [24]. The melting point is 183-185 ^{o}C, yield 85 Mol.wt. is 151.02 gm./mole. IR = Data: Ar CH = 3034, Ar C=C =1570, C=N= 1624, - C=S, 670.

2.2 Synthesis of 2-hydrazino benzoxazole (2)
2-Mercapto benzoxazole (1) (0.01 mole.) was mixed with (0.04 mole) and Hydrazine hydrate (25) ml (0.05 mole) are mixed well and heated on a water bath for 10 min. then dissolved in (50) ml methanol, the reaction mixture is heated with the reflux condenser for (8) hours, cooled to room temperature, the product filtered to give crystals colorless, then recrystallized with ethanol [25]. %, m.p.166-168°C (lit 171 °C) 10.Anal Calculate for C7H7N3S (M.wt. 218), IR, Cm-1: 3392, 3276, (-NH2), 3355(-NH), 3020,1597, 1035(arylring), 1650 (C=N), 1192, 1078, 669(C-S-C).;(

2.3 Synthesis of (2-benzylidene) hydrazine benzoxazole 3(a-f)
2-Hydrazinobenzoxazole (2) (0.001mol) was mixed with an aromatic aldehyde (0.001mol) in ethanol (50 ml) then added (3-4) drop of glacial acetic acid were refluxed on a water bath for (5) hours. After cooling to room temperature. The crystalline solid, which separated, was filtered and recrystallized from a suitable solvent [22]. The structure of synthesis compounds (3a-e) were confirmed by melting - point, the physical properties, and I-R spectral characterization data are given in below tables (1,3)**.**

2.4 Synthesis of 1-(benzoxazole -2-yl)-5-(4-sub.phenyl)-3-arylformazan (4a-c,) (5 d-f)
Prepared by aniline derivatives (0.01mol) in glacial acetic acid (2ml) and HCl (1.5ml) was diazotized with NaNO2 (0.2g in 2ml water) at (0-5ºC). The resultant phenyl diazonium chloride solution was added with stirring to compound (3) (0.01mol) in pyridine (7ml) was added in an ice bath. The reaction mixture was left at room temperature for two days. Then filtered and washed repeatedly with distilled water and recrystallized from a suitable solvent [23].then were confirmed by physical properties and I-R spectral data are given in below tables (2,4)**.**

Antimicrobial Activity: All the newly synthesized compounds were screened for their antimicrobial activity determined by the ager diffusion method. Against *Escherichia Coli (G-)* and *Streptococcus (G+)*. Using EtOH as solvent at 50 and (100µg/ml) concentration, the plate was incubated at the appropriate temperature at (37 ^{0}C) by using the cup-plate method. After (24) hours the zone of inhibition was measured [24]. Results in Table (5).



Scheme 1. Path ways for synthesized compounds

3. Results and Discussion

2-mercaptobenzoxazole (1) was prepared from the reaction of O - aminophenol with CS_2 in ethanolic potassium hydroxide, yield was 85%, The melting point is183-185 ^{0}C, and the data infrared = Ar- CH = 3034, Ar- C=C =1570, C=N= 1624, - C=S, 670, as described in preparation method [1], 2-Hydrazinobenzoxazole (2) was prepared by reaction of the compound (1) with hydrazine hydrate, The was melting point of (166-168°C), the infrared was band at (3737 cm-^{1}) due to (NH_2), While (N-H) at (3296 cm-^{1}), band at (1650 cm-^{1}) for (C=N), (3064cm-^{1}) (Ar=CH), (1552-1600 cm-^{1}) (ArC=C) ,and at (1197 cm-^{1}) for (C-O-C) group, ^{13}CNMR: 133.29 (N=C-N ring), 169.13 (C-O-C ring), (122.82-131.23) (C=C). as shown in Figure (1,6) .prepared 2-benzylidene) hydrazine benzoxazole (3a- f) of reaction the substituted aldehydes with compound (2) in ethanol. IR spectrum of the compound 2-[2-(4-chlorobenzylidene) hydrazine ebenzoxazole (2), showed clear absorption bands at (3194-3409 cm-^{1}) (N-H), (2825-2955 cm-^{1}) (=C-H), (3030-3070 cm-^{1}) (Ar=CH), (1610-1652 cm-^{1}) (C=N), and (1430-1581 cm-^{1})(C=C), On the other hand of ^{1}H-NMR, showed, at δ=(2.50) ppm (C – H Aliph),at δ =(3.67)ppm (OCH_3),at δ =(7.13-7.96) ppm (C=C) of aromatic ring , at δ =(8.47)ppm(CH=N), and δ(12.46)ppm (NH). the Compound (3e) ,As shown in the table (3) and Figure (2,4). prepared 1-(benzoxazole -2-yl) -5-(4-sub.phenyl)-3-arylformazan(4a-c) (5d-f) of reaction, the compound (3a-f) with aniline derivatives and $NaNO_2$, added to pyridine, showed clear absorption bands at (3170-3420 cm-^{1}) (N-H), (3023-3080 cm-^{1}) (Ar=CH), (1606-1645 cm-^{1}) (C=N), and (1500-1594 cm-^{1})(C=C),(1407-1510 cm-^{1}) (N=N).On the other hand of ^{1}H-NMR , showed, at δ =(6.33-7.78)ppm (C=C) of the aromatic ring, and δ=(9.75) ppm (NH), the Compound (4b).^{13}CNMR (133.44-133.67) (N=C ring) ,(171.12) (C-O-C ring), (118.49-131.63) (C=C).(165.50) (N=C-N) , the Compound (5d), As shown in the table (4) and Figure (3,5,7) [25].

Biological activity: All the newly synthesized compounds (3a, 3c, 4b, 5f) showed biological activities against gram-positive and gram-negative bacteria including *Streptococcus Pyogene* and *Escherichia coli*. The test results showed that the compounds (3a, 5f) showed highly active against *Streptococcus Pyogene* and *Escherichia Coli.* The compound (4b) showed no activity against of bacteria used. Results in Table (5).

Table 1. Physical data and molecular formulae of the prepared compounds (3 (a- e).

Comp. No.	R	Molecular formula	Color	M.P(^{0}C)	Yield (%)	Recryst. Solvent
3a	H	C14H11N3O	Yellow	200-202	70	EtOH
3b	2-NO_2	C14H10N4O$_3$	Orang	222-224	92	MeOH
3c	4-OH	C14H11N3O$_2$	Dark Yellow	218-220	65	MeOH
3d	4-Cl	C14H10N3OCl	Pale Brown	210-212	84	EtOH
3e	3-OCH_3	C15H13N3O$_2$	Pale Yellow	230-232	80	MeOH
3f	4-Br	C14H10N3OBr	Yellow	240-242	80	EtOH

Table 2. Physical data and molecular formulae of the prepared compounds(4a-c,) (5 d-f).

Comp. No.	R	Molecular formula	Color	M.P(^{0}C)	Yield (%)	Recryst. Solvent
4a	H	$C_{20}H_{14}BrN_5O$	Light Brown	150-152	70	Diethyl ether
4b	2-NO_2	$C_{20}H_{13}BrN_6O_3$	Red Brown	Gum	88	Chloroform

4c	4-OH	$C_{20}H_{14}BrN_5O_2$	Brown	114-116	50	EtOH
5d	4-Cl	$C_{20}H_{13}ClN_6O_3$	Brown	234-236	65	EtOH
5e	3-OCH_3	$C_{21}H_{16}N_6O_4$	Dark Brown	Gum	73	Acetone
5f	4-Br	$C_{20}H_{13}BrN_6O_3$	Milky	Gum	75	EtOH

Table 3. IR spectroscopy for synthesized compounds (3a-f).

Comp. No.	R	IR, (KBr), cm^{-1}							
		Fixed bands in structure							Changed bands in structure
		ν N-H	ν(=CH)Ar	νC – H Aliph	ν C=N.	νC=CAr	ν C – N	N-N	
3a	H	3370	3035	2833-2857	1620	1432,1580	1227	1030	
3b	2-NO_2	3402	3070	2850,2900	1614	1460,1543	1312	1056	ν(NO_2) 1350
3c	4-OH	3295	3055	2866,2940	1610	1485,1565	1265	1025	ν(OH) 3342
3d	4-Cl	3367	3030	2825-2900	1645	1500-1596	1260	1012	ν (C – Cl) 955
3e	3-OCH_3	3194	3068	2888-2905	1652	1430-1575	1376	1070	ν (C–O–C) 1170
3f	4-Br	3409	3064	2955--2933	1620	1581-1502	1240	1122	ν (C – Br) 748

Table 4. FTIR spectroscopy for synthesized compounds (4a,4b,4c,5d,5e,5f).

Comp. No.	R	IR, (KBr), cm^{-1}							
		Fixed bands in structure							Changed bands in structure
		ν N-H	ν(=CH)Ar	ν C=N.	νC=CAr	ν C – N	N-N	νN=N	
4a	H	3413	3033	1606	1512	1230	1099	1407	
4b	2-NO_2	3320	3034	1617	1576	1290	1022	1470	ν(NO_2) 1390
4c	4-OH	3170	3054	1622	1500	1398	1100	1482	ν(OH) 3378
5d	4-Cl	3245	3080	1635	1594	1277	1047	1504	ν (C – Cl) 960
5e	3-OCH_3	3390	3023	1620	1570	1290	1083	1510	ν (C–O–C) 1173
5f	4-Br	3420	3034	1645	1534	1300	1018	1490	ν (C – Br) 782

Table 5. Antibacterial activity of some prepared compounds [3a,3c,4b,5f] .

Comp. No.	Conc.	*G-Escherichia coli (G-)*	*Streptococcus Pyogene(G+)*
3a	50	++	+ +++

	100	+	+
3c	50	+++	++
	100	-	++
4b	50	--	
	100	-	-
5f	50	+	++
	100	+++	++

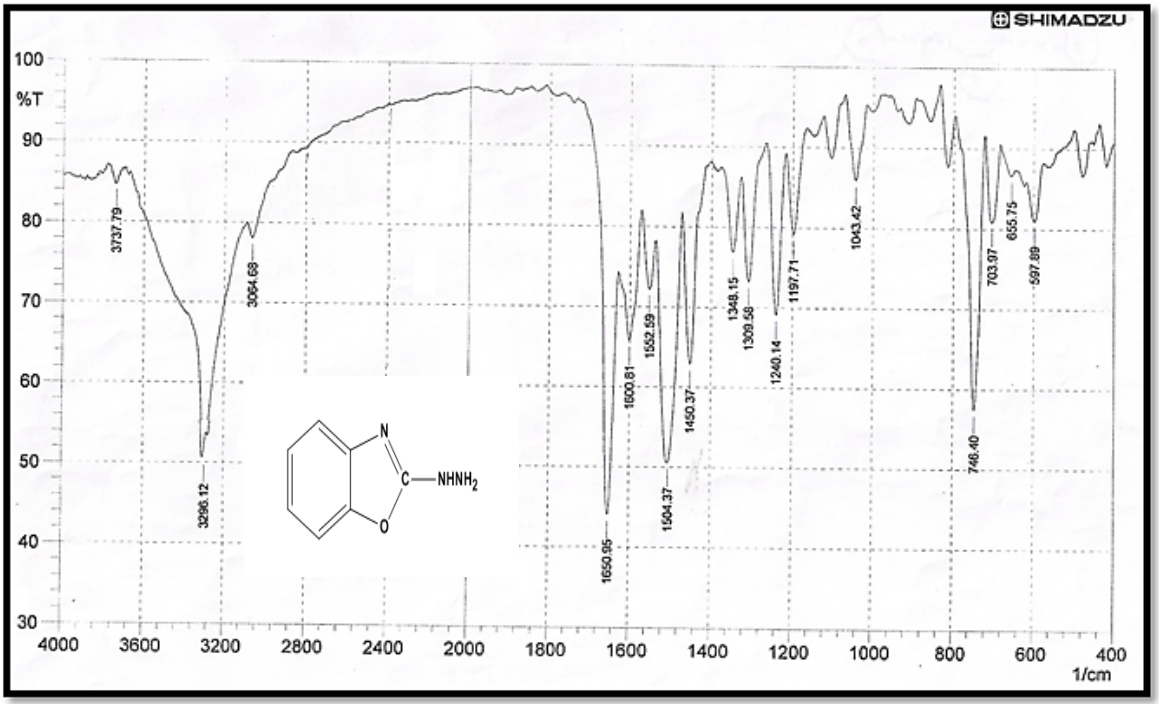


Figure. **1.** FT-IR spectrum for compound(2).

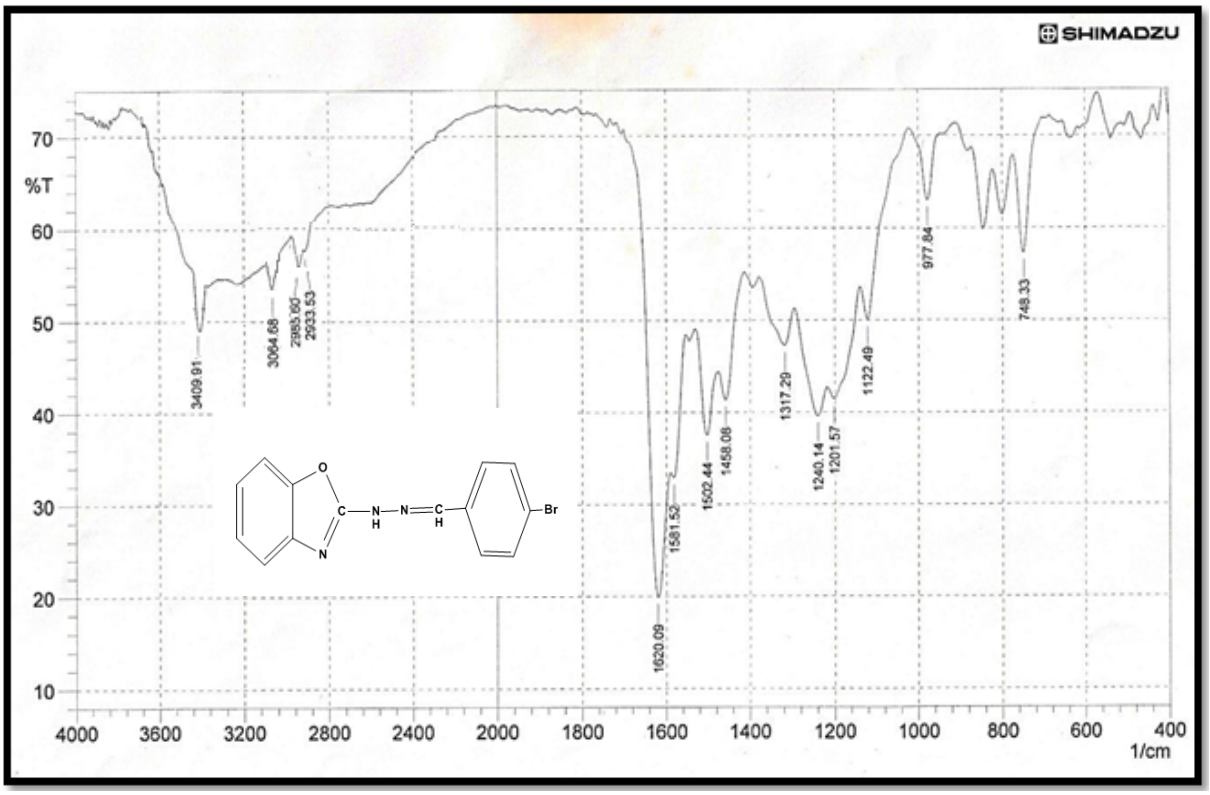


Figure 2. FT-IR spectrum for compound(3f).

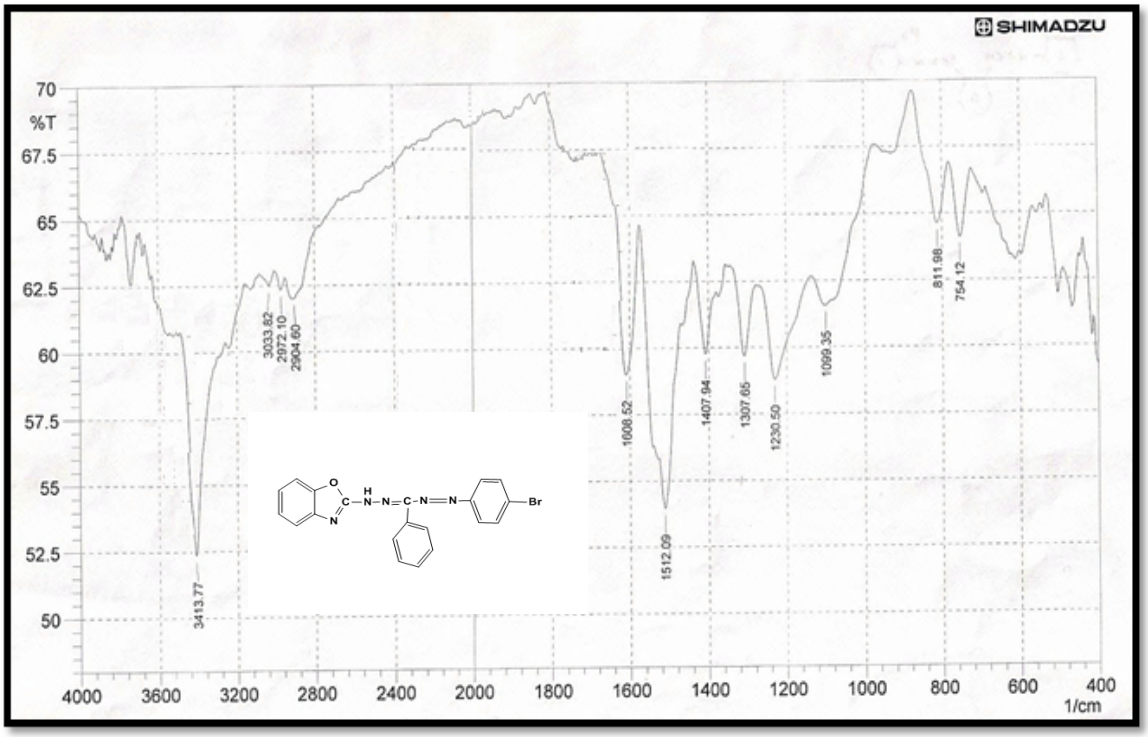


Figure 3. FT-IR spectrum for compound (4a).

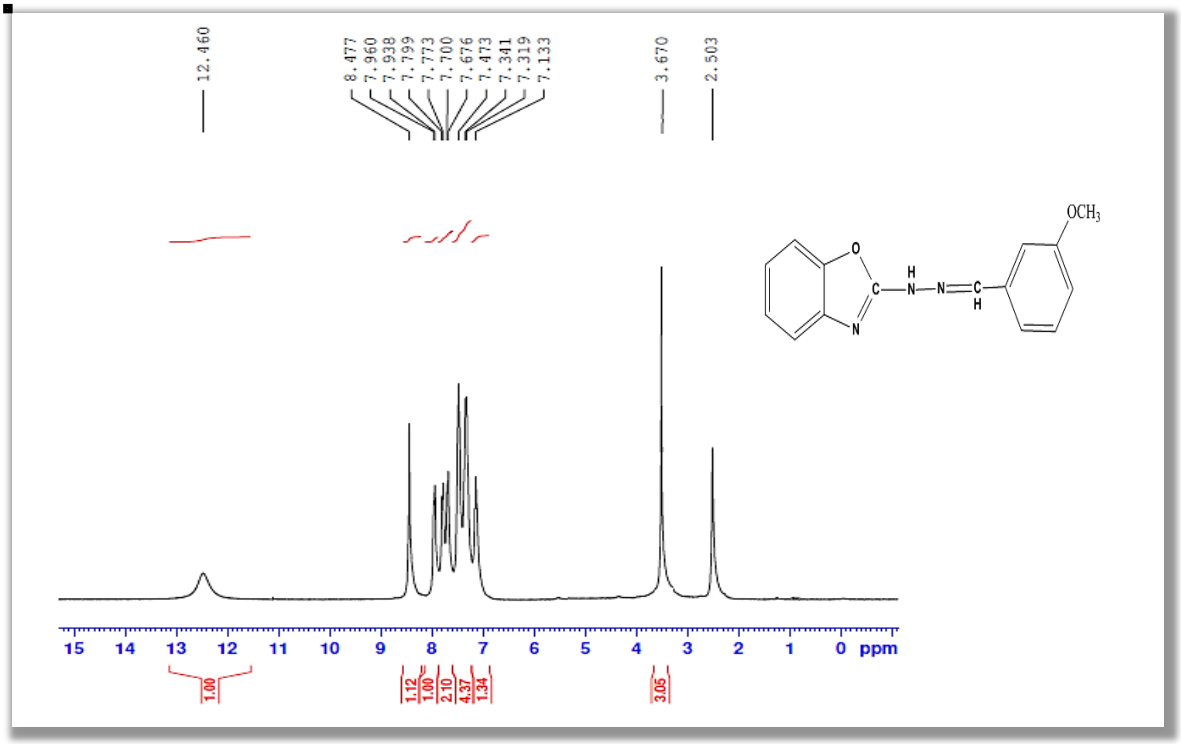


Figure 4. [1] H-NMR spectrum for compound(3e)

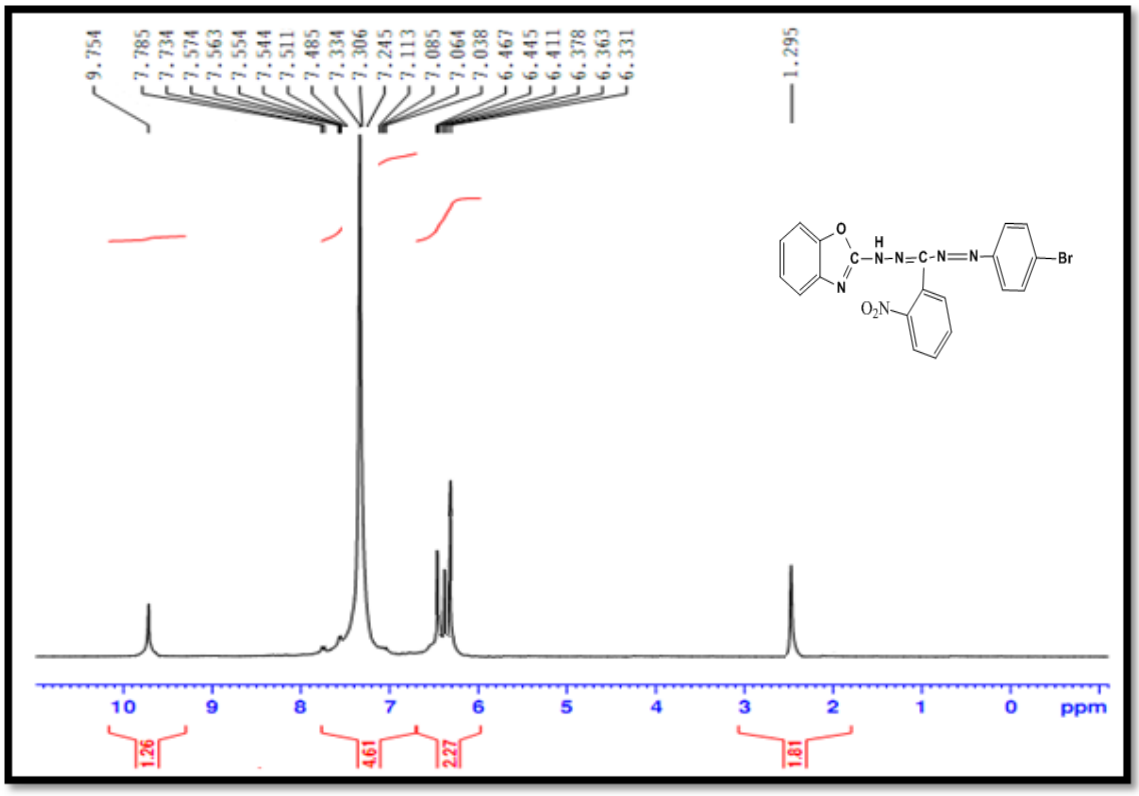


Figure 5. [1] H-NMR spectrum for compound(4b)

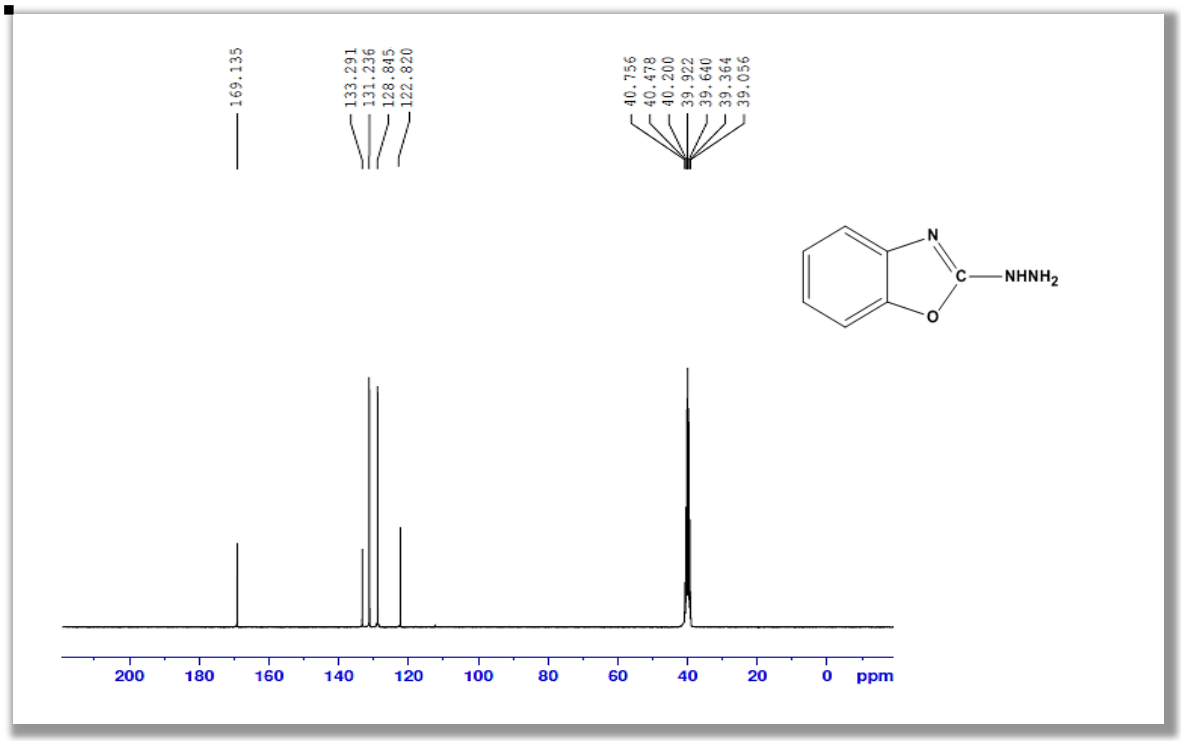


Figure 6. [13]C-NMR spectrum for compound(2).

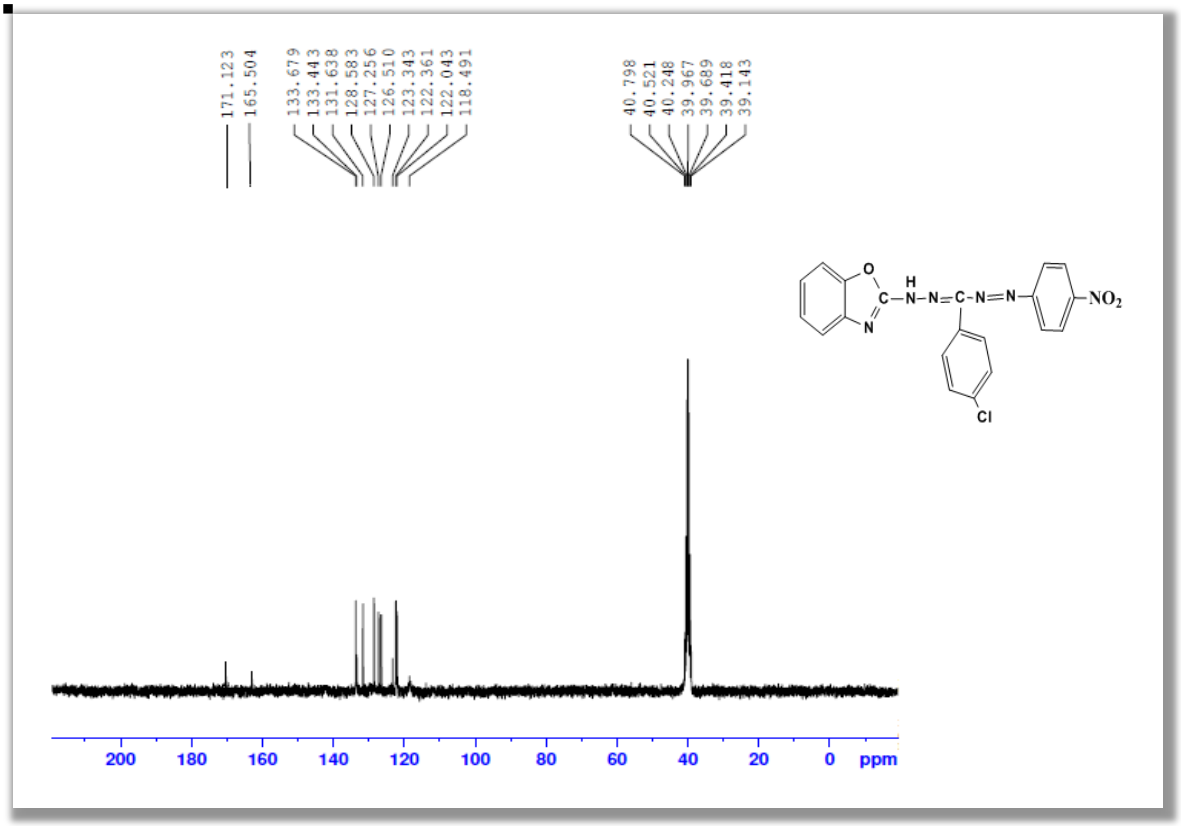


Figure 7. ^{13}C-NMR spectrum for compound(5d)

4. Conclusion

It could be concluded that all the compounds (3a,3c,4b,5f) showed biological activities against gram-positive and gram-negative bacteria including *Streptococcus Pyogene* and *Escherichia coli*. However, 3a, and 5f showed highly active against *Streptococcus Pyogene* and *Escherichia Coli.*

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