Synthesis of new 1,3- oxazepine - 4,7- dione derivatives containing azo group and 1,3,4- thiadiazole moiety and preliminary evaluation of their antibacterial activity

تحضير مشتقات 3,1- أوكسازبين -7,4- دايون جديدة حاوية على مجموعة الأزو و وحدة 4,3,1- ثياديازول وتقييم أولي لفعاليتها ضد البكتريا

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Abstract

In this work new 1,3-oxazepine-4,7-dione derivatives containing azo group and 1,3,4-thiadiazole moiety have been synthesized. At first, the primary aromatic amine 2-Amino-5-mercapto-1,3,4-thiadiazole was converted to the corresponding azoaldehyde derivative containing 1,3,4-thiadiazole moiety 1 via coupling reaction between its diazonium salt and alkaline solution of 2-hydroxybenzaldehyde as coupling reagent. Next , the resulting azoaldehyde derivative 1 was respectively introduced in acid-catalyzed condensation reactions with the primary aromatic amines (3-nitroaniline, 4-methoxyaniline, 2,4- dimethylaniline, 4-hydroxyaniline) in absolute ethanol to give four new azoimine derivatives containing1,3,4-thiadiazole moiety 2a-d respectively . Later , treatment of the resulting azoimine derivatives 2a-d with each maleic and Phthalic anhydrides under the same cycloaddition reaction conditions afforded eight new 1,2-disubstituted-1,3-oxazepine -4,7-dione derivatives 3a-d and 4a-d respectively.

The structures of all synthesized oxazepine derivatives 3a-d and 4a-d were confirmed by (C.H.N.S.) elementary micro analysis and the spectral methods including FT-IR and ^{1}H NMR for most derivatives (3a, 3b, 3d, 4a, 4c and 4d) . The synthesized oxazepine compounds (3a, 3b, 3c and 4b) were tested for their antibacterial activity against two pathogenic strains of bacteria, *Staphylococcus aureous* (Gram - positive) and *Escherichia coli* (Gram - negative). The results revealed that the tested oxazepine derivatives showed inhibition activity against Gram - positive bacteria is relatively higher than that of Gram - negative.

Keywords : 1,3- oxazepine, 1,3,4-thiadiazole, antibacterial activity , أوكسازبين , 1, 3, 4- ثياديازول
فعالية ضد البكتريا

الخلاصة

تم من خلال هذا العمل تحضير مشتقات 3,1- أوكسازبين - 7,4- دايون جديدة حاوية على مجموعة الأزو و وحدة 4,3,1- ثياديازول . في البداية تم تحويل الأمين الاولي الأروماتي 2- أمينو-5- مركبتو- 4,3,1 - ثياديازول الى مشتق الأزوالديهايد المقابل الحاوي على وحدة 4,3,1- ثياديازول 1 عن طريق تفاعل أزدواج ما بين ملح الدايازونيوم لهذا الأمين ومحلول قاعدي لمركب 2- هيدروكسي بنزالديهايد ككاشف إزدواج . تم لاحقا إدخال مشتق الأزوالديهايد الناتج 1 في تفاعلات تكثيف محفزة بحامض مع الامينات الأولية الأروماتية (3- نايتروأنيلين, 4- ميثوكسي أنيلين, 4,2- ثنائي مثيل أنيلين, 4- هيدروكسي أنيلين) على التوالي في الأيثانول المطلق فتم الحصول على أربع مشتقات أزوإيمين جديدة حاوية على وحدة 4,3,1- ثياديازول 2a-d على التوالي . ان معاملة مشتقات الأزوإيمين الناتجة 2a-d مع كل من أنهيدريد الماليك و أنهيدريد الفثالك كمكونات خماسية الذرات وعلى التوالي و تحت نفس شروط الإضافة الحلقية أعطت ثمان من مشتقات 2,1- ثنائي التعويض -3,1- أوكسازبين -7,4- دايون 3a-d و 4a-d على التوالي .

شخصت تراكيب كافة مشتقات الأوكسازبين المحضرة 3a-d و 4a-d بوساطة التحليل الكمي الدقيق للعناصر (C.H.N.S.) والطرائق الطيفية المتضمنة مطيافية الأشعة تحت الحمراء والرنين النووي المغناطسي للبروتون لغالبية المركبات المحضرة وهي (3a, 3b, 3d, 4a, 4c, 4d). تم أختبار الفعالية البايولوجية لمشتقات الأوكسازبين المحضرة (3a, 3b, 3c, 4b) ضد سلالتين من البكتريا هما *Staphylococcus aureous*) الموجبة لصبغة كرام و *Escherichia coli*) (السالبة لصبغة كرام وقد دلت النتائج المستحصلة بأن المشتقات المدروسة أظهرت فعالية تثبيطيه تجاه البكتريا الموجبة لصبغة كرام أعلى نسبيا مما أبدته تجاه البكتريا السالبة لصبغة كرام .

1.Introduction

Oxazepine derivatives showed various biological activities, such as antibacterial[1], Antimicrobial and anticonvulsant activity[2], as telomerase inhibitors[3], as Potent agonists of the human TRPA1 receptor[4], inhibitors for some enzymes action[5], Some of oxazepine derivatives are used in another applied fields[6].

The classical methods for preparing 1,3-oxazepine ring are limited[7]. Recently, cycloaddition reaction type (2+5→7) is used for the synthesis of various 1,3-oxazepine ring[8-11]. In this type of cycloaddition, two atoms of imine group as two-membered component was added to five-membered component such as maleic or phthalic anhydrides to give a seven-membered hetrocycle[12,13].

Several five-membered aromatic systems having three hetero atoms at symmetrical position have been studied because of their interesting physiological properties , thiadiazole is a five-membered ring system containing two nitrogen and one sulphur atom , They occur in nature in four isomeric forms viz. 1,2,3-thiadiazole ; 1,2,5-thiadiazole ; 1,2,4-thiadiazole and 1,3,4-thiadiazole[14] . The thiadiazoles have occupied an important place in drug industry[15], antimicrobial[16], antituberculosis[17], anti-inflammatory[18], anticonvulsants[19], antihypertensive[20], anticancer[21] and antifungal[22].

Azo dyes constitute one of the largest and most varied groups of synthetic organic dyes in use today [23]. Azo compounds are highly important, wellknown and widely used substances in the textile, paper, coloring agents for foods and cosmetics industries. Other applications include emerging technologies like liquid crystals, organic photoconductors and non-linear optics [24-25], azo compounds were reported to show a variety of biological activities including antibacterial[26], antifungal [27], antimicrobial [28], antiviral [29]and anti-inflammatory [30] activities.

2. Experimental

2.1. General

The chemicals used in this work were obtained from Fluka, sigma aldrich, GCC, Merck and S.D.Fine and were used without another purification. Silica TLC plates were used with an aluminum backing (0.2 mm, 60 F_{254}). The reactions were monitored by TLC and visualized by development of the TLC plates with an alkaline potassium permanganate dip or with Iodine vapor. Melting points were determined on an Electro thermal Stuart SMP 30 capillary melting point apparatus. Infrared spectra were recorded on SHIMADZU FTIR-8400S Infrared Spectrophotometer as potassium bromide discs. ^{1}H NMR spectrum of oxazepine derivatives (3a, 3b, 3d, 4a, 4c and 4d) were collected on NMR spectrometer, Bruker 2009 spectrometer at400MHz in DMSO-d_6 as solvent and TMS as an internal standard at Kashan University, Iran. Elemental Analysis (C. H. N.S.) was carried out with Micro analytical unit, Euro vector S.P.A. E.A 3000- CHNS Elemental analyzer at Kufa University. Autoclave was used to sterilize agar media, supplied from Prestige Medical-England. Incubator was used to maintain different temperature required for the growth of organism, supplied from Binder - Germany. Azoaldehyde derivative 1 was prepared following the method described by Acton [31].

2.2. Synthesis

2.2.1. General procedure for the Synthesis of azoimine derivatives (2a-d)

Azoaldehyde derivative **1** (0.798 g, 0.003 mol) was dissolved in (15 mL) of absolute ethanol containing two drops of glacial acetic acid, then (3-nitroaniline, 4-methoxyaniline, 2,4-dimethylaniline and 4-hydroxyaniline) respectively (0.003 mol) was dissolved in (15 mL) of absolute ethanol and added drop wise . The reaction mixture was refluxed with stirring on a water bath at 70 ^{o}C for 12-16 h. and monitored by TLC . The mixture was then allowed to cool down to room temperature , the colored precipitate was filtered and recrystallized from ethanol. The physical properties and other characteristics for the synthesized azoimine derivatives **2a-d** were shown in table (1).

2.2.2. General procedure for the Synthesis of 1,3- oxazepine - 4,7- dione derivatives (3a-d)

A mixture of azoimine derivatives **2a-d** (0.002 mol) and maleic anhydride (0.196 g , 0.002 mol) in dry benzene (20 mL**)** was refluxed on a water bath at 70 ºC for 20-24 h. and monitored by TLC. The mixture was then allowed to cool down to room temperature . dried upon filter paper then in oven and recrystallized from ethanol. The physical properties and other characteristics for the synthesized oxazepine derivatives **3a-d** were shown in table (2). The (C.H.N.S.) elementary analysis of 1,3-oxazepine derivatives **3a-d** was listed in table (4) .

2.2.3. General procedure for the Synthesis of 1,3- oxazepine - 4,7- dione derivatives (4a-d)

A mixture of azoimine derivatives **2a-d**(0.002 mol) and phthalic anhydride(0.296 g ,0.002 mol) in (20 mL**)** of dry benzene , was refluxed on a water bath at 70 ºC for 20-24 h. and monitored by TLC. The mixture was then allowed to cool down to room temperature . dried upon filter paper then in oven and recrystallized from ethanol . The physical properties and other characteristics for the synthesized oxazepine derivatives **4a-d** were shown in table (3). The (C.H.N.S.) elementary analysis of 1,3-oxazepine derivatives **4a-d** was listed in table (4) .

Table (1): physical properties and other characteristics for the synthesized Azoaldehyde derivative (1) and azoimine derivatives (2a-d)

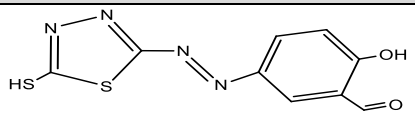
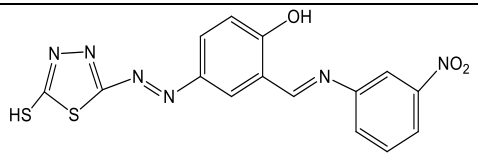
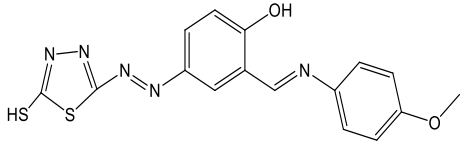
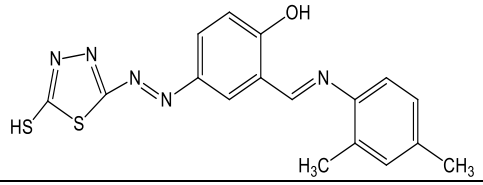
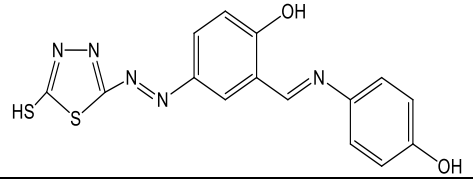
Com. no.	Structure	Molecular formula	M.Wt. g/mol	RN. time (h.)	Color	Yield %	m.p. ºC	R_f
1		$C_9H_6N_4O_2S_2$	266.30	-	orange	58	189-191	0.46 *n*-hexane : Et_2O 1 : 4
2a		$C_{15}H_{10}N_6O_3S_2$	386.40	16	light orange	83	137-139	0.55 *n*-hexane : Et_2O 2 : 1
2b		$C_{16}H_{13}N_5O_2S_2$	371.43	13	dark yellow	65	166-168	0.45 *n*-hexane : Et_2O 1 : 3
2c		$C_{17}H_{15}N_5OS_2$	369.46	14	brown	60	121-123	0.76 *n*-hexane : Et_2O 1 : 3
2d		$C_{15}H_{11}N_5O_2S_2$	357.41	12	dark brown	55	148-150	0.71 *n*-hexane : Et_2O 1 : 3

Table (2): physical properties and other characteristics for the synthesized 1,3-oxazepine derivatives (3a-d)

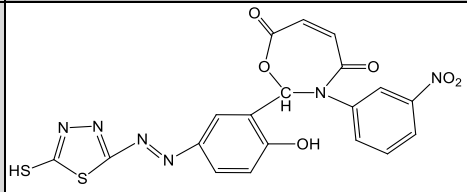
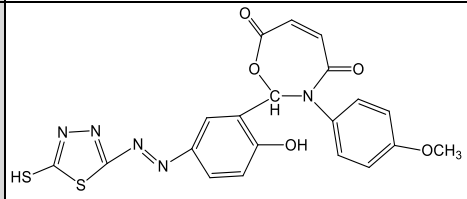
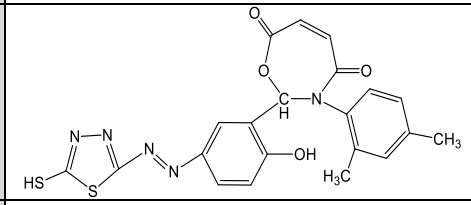
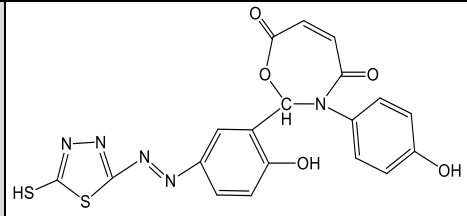
Com. no.	Structure	Molecular formula	M.Wt. g/mol	RN. time (h.)	Color	Yield %	m.p. ºC	R_f
3a		$C_{19}H_{12}N_6O_6S_2$	484.47	24	orange	69	208-210	0.50 *n*-hexane : Et_2O 2 : 1
3b		$C_{20}H_{15}N_5O_5S_2$	469.50	20	brown	64	211-213	0.28 *n*-hexane : Et_2O 1 : 3
3c		$C_{21}H_{17}N_5O_4S_2$	467.52	23	dark brown	73	216-218	0.54 *n*-hexane : Et_2O 1 : 3
3d		$C_{19}H_{13}N_5O_5S_2$	455.47	21	dark brown	57	205-207	0.56 *n*-hexane : Et_2O 1 : 3

Table (3): physical properties and other characteristics for the synthesized 1,3-oxazepine derivatives (4a-d)

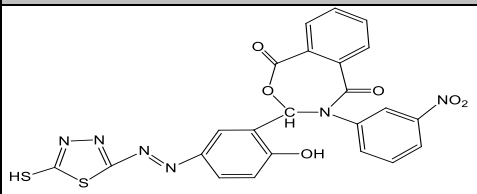
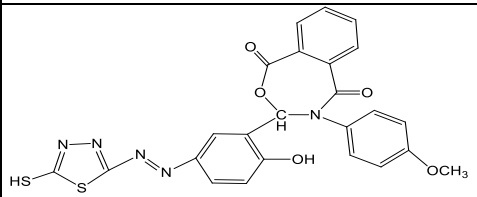
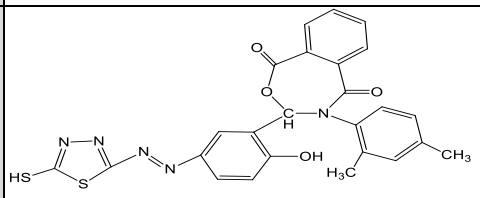
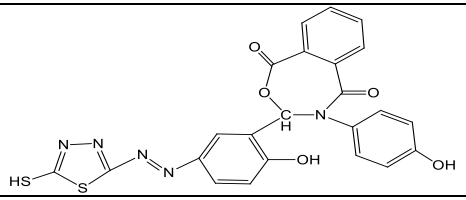
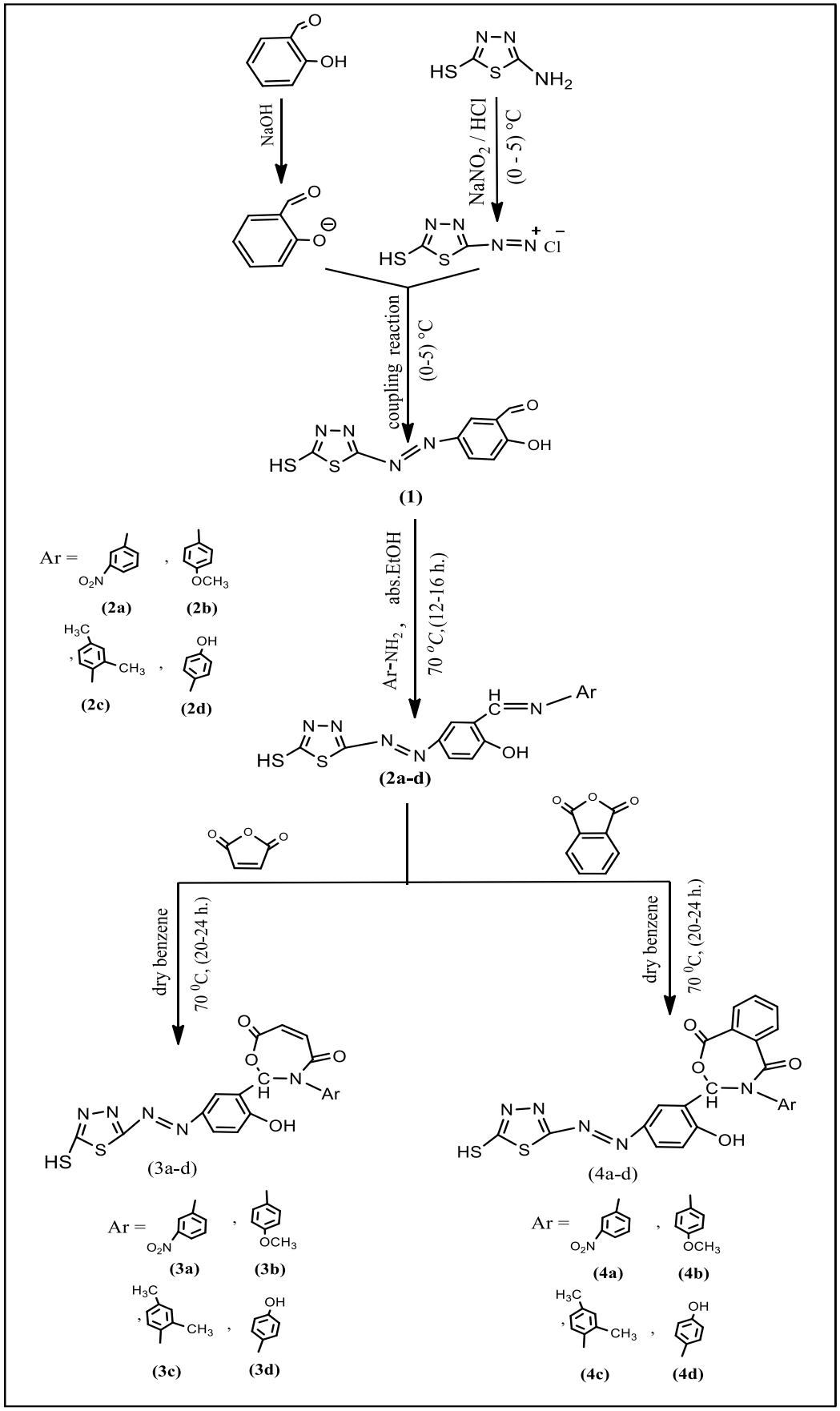
Com. no.	Structure	Molecular formula	M.Wt. g/mol	RN. time (h.)	Color	Yield %	m.p. ºC	R_f
4a		$C_{23}H_{14}N_6O_6S_2$	534.53	24	light orange	63	189-191	0.56 *n*-hexane : Et_2O 2 : 1
4b		$C_{24}H_{17}N_5O_5S_2$	519.56	21	dark brown	59	176-178	0.49 *n*-hexane : Et_2O 1 : 3
4c		$C_{25}H_{19}N_5O_4S_2$	517.58	22	dark brown	62	197-199	0.62 *n*-hexane : Et_2O 1 : 3
4d		$C_{23}H_{15}N_5O_5S_2$	505.53	20	dark brown	70	200-202	0.66 *n*-hexane : Et_2O 1 : 3

Table (4): (C.H.N.S.) elementary micro analysis of the synthesized 1,3-oxazepine derivatives 3a-d and 4a-d

Comp. no.	C%		H%		N%		S%	
	Calculated	Found	Calculated	Found	Calculated	Found	Calculated	Found
3a	47.10	47.18	2.50	2.69	17.35	17.93	13.24	13.59
3b	51.16	51.06	3.22	3.11	14.92	14.70	13.66	13.31
3c	53.95	53.06	3.67	3.81	14.98	14.70	13.72	13.07
3d	50.10	50.44	2.88	2.54	15.38	15.79	14.08	14.74
4a	51.68	51.94	2.64	2.48	15.72	15.46	12.00	12.59
4b	55.48	55.58	3.30	3.56	13.48	13.26	12.34	12.19
4c	58.01	58.54	3.70	3.34	13.53	13.06	12.39	12.10
4d	54.65	54.06	2.99	2.34	13.85	13.45	12.68	12.52

3. Results and Discussion

The target of the present work is to synthesize new 1,3- oxazepine derivatives containing biologically active azo group and 1,3,4- thiadiazole moiety . Scheme (1) shows the full synthetic plan of this research .


NaOH
NaNO2 / HCl
(0 - 5) °C
coupling reaction
(0-5) °C
(1)
Ar =
(2a)
(2b)
(2c)
(2d)
Ar-NH2 , abs.EtOH
70 °C,(12-16 h.)
(2a-d)
dry benzene
70 °C, (20-24 h.)
(3a-d)
(4a-d)
(3a)
(3b)
(3c)
(3d)
(4a)
(4b)
(4c)
(4d)

Scheme (1)

A coupling reaction between the diazonium salt of the primary aromatic amine 2-amino-5-mercapto-1,3,4-thiadiazole and phenoxide salt of 2-hydroxybenzaldehyde at (0-5) ^{o}C afforded azoaldehyde derivative **1** .

The FT-IR spectrum of azoaldehyde derivative **1**, fig. (1) is devoid the sharp bands at 3394 cm^{-1} and 3277 cm^{-1} attributed to the asymmetric and symmetric stretching vibrations of (-NH_2) group respectively and appearance of strong band at 1635 cm^{-1} attributed to the stretching vibration of (C=O) group. The intramolecular hydrogen bonding between carbonyl group oxygen atom and *O*-hydroxy group causes shifting the stretching vibration of carbonyl group toward lower frequency. Other characteristic bands shown in table (5).

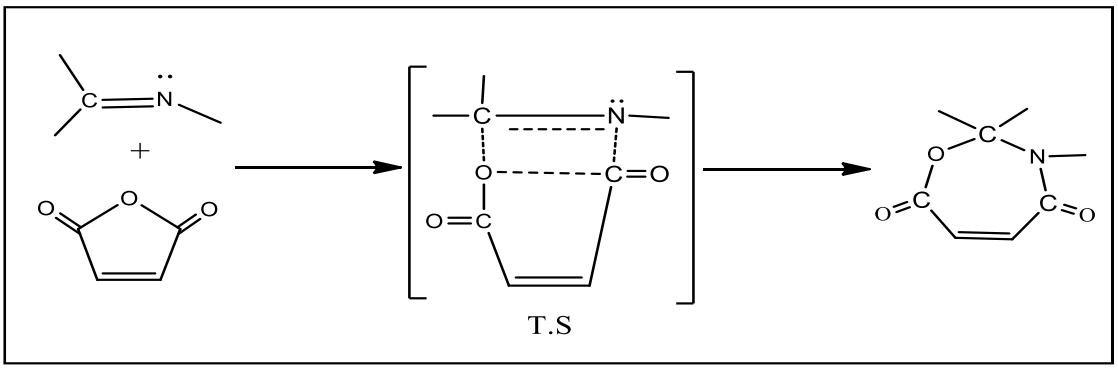
Azoimine derivatives **2a-d** were prepared through condensation reaction between the prepared azoaldehyde derivative **1** and some of aromatic primary amines (3-nitroaniline, 4-methoxyaniline, 2,4-dimethylaniline and 4-hydroxyaniline) respectively in the presence of glacial acetic acid as catalyst in absolute ethanol.

FT-IR speatra of azoimine derivatives **2a-d**, figs. (2-5) illustrate good evidence that the condensation reactions happened successfully by disappearing the medium band at 1635 cm^{-1} which attributed to the υ (C=O) in azoaldehyde derivative **1** and appearing weak-medium band at frequency less than that of (C=O) group at the range (1604-1618) cm^{-1} assigned to the stretching vibration of exocyclic imine group (C=N) , also the spectra showed disappearance the two sharp band at the range (3250-3400) cm^{-1} for asymmetric and symmetric stretching vibrations of (-NH_2) group. Other characteristic bands shown in table (5).

Table (5): FT-IR data of the prepared azoaldehyde (1) and azoimine derivatives (2a-d) in cm^{-1}

Com. no.	FT-TR bands
1	3284 (υ $_{O-H, phenolic}$), 3099$_{br}$ (υ $_{N-H, thioketone form}$ and υ $_{C-H, benzene ring, vib. coupling}$), 2613 (υ $_{S-H, thioenol form}$), 1635 (υ $_{C=O}$), 1550, 1539 and 1502 (υ $_{C=C, benzene}$ and υ $_{C=N, thiadiazole ring}$), 1425 (υ $_{N=N}$), 1392 (υ $_{C=S, thioketone form}$), 835, 752 and 700 (δ $_{o.o.p.C-H, benzene rings}$).
2a	3255 (υ $_{O-H}$), 3128$_{br}$ (υ $_{N-H, thioketone form}$ and υ $_{C-H, benzene rings, vib. coupling}$), 2791 (υ $_{C-H, imine}$), 1610 (υ $_{C=N, exocyclic}$), 1554 and 1491(υ $_{C=C}$ and υ $_{C=N, aromatic rings}$), 1525 (υ as.NO_2), 1410 (υ $_{N=N}$), 1373 and 1271 (υ $_{C-N}$), 1348 (υ s.NO_2), 1051 (υ $_{C=S, thioketone form}$), 881, 800, 756, 736 and 719 (δ $_{o.o.p.C-H, benzene rings}$) .
2b	3282 (υ $_{O-H}$), 3103$_{br}$ (υ $_{N-H, thioketone form}$ and υ $_{C-H, benzene rings, vib. coupling}$), 2953 (υ as.C-H, CH_3), 2903 (υ s.C-H, CH_3), 2830 (υ $_{C-H, imine}$), 1612 (υ $_{C=N, exocyclic}$), 1539 and 1502 (υ $_{C=C}$ and υ $_{C=N, aromatic rings}$), 1390 (δ s.C-H, CH_3), 1310 and 1247 (υ $_{C-N}$), 1035 (υ $_{C=S, thioketone form}$), 825, 725 and 672 (δ $_{o.o.p.C-H, benzene rings}$) .
2c	3265 (υ $_{O-H}$), 3090 (υ $_{N-H, thioketone form}$), 3016 (υ $_{C-H, benzene rings}$), 1604 (υ $_{C=N, exocyclic}$), 1550 and 1502 (υ $_{C=C}$ and υ $_{C=N, aromatic rings}$), 1448 (δ as. C-H, CH_3), 1390 (δ s.C-H, CH_3), 1317 and 1265 (υ $_{C-N}$), 1051 and 1037 (υ $_{C=S, thioketone form}$), 889, 813, 756 and 713 (δ $_{o.o.p.C-H, benzene rings}$) .
2d	3279 (υ $_{O-H}$), 3090 (υ $_{N-H, thioketone form}$ and υ $_{C-H, benzene rings, vib. coupling}$), 1618 (υ $_{C=N, exocyclic}$), 1560 and 1500 (υ $_{C=C}$ and υ $_{C=N, aromatic rings}$), 1388 and 1249 (υ $_{C-N}$), 1047 (υ $_{C=S, thioketone form}$), 825 (δ $_{o.o.p.C-H, benzene rings}$) .

1,3-oxazepine derivatives **3a-d** and **4a-d** were prepared via introducing of the synthesized azoimine derivatives **2a-d** in (2+5→7) cycloadation reaction with maleic and Phthalic anhydrides, respectively, in dry benzene. The reaction proceeds via formation of four-membered cyclic transition state. The participating orbitals in this concerted process should be in the same plane as indicated in scheme (2) .



scheme (2)

The (C.H.N.S.) elementary micro analysis of the synthesized 1,3-oxazepine derivatives **3a-d** and **4a-d**, table (4) showed good agreement between the calculated and found values.

The FT-IR data of the synthesized 1,3-oxazepine derivatives **3a-d**, figs. (6-9) provide good evidence that the cycloaddition reactions proceeded successfully and produced the desired products by disappearing the weak-medium band at the range (1604-1618) cm^{-1} which attributed to the stretching vibration of imine group (C=N) and appearing band at the range (1687-1710) cm^{-1} attributed to the stretching vibration of (C=O) group inside oxazepine ring, moreover the spectra of derivatives **3a-d** appeared weak-medium band at the range (1620, 1612, 1606 and 1614) cm^{-1} due to υ (C=C) inside oxazepine ring. Other band were summarized in table (6).

Table (6): FT-IR data of the prepared 1,3- oxazepine derivatives (3a-d) in cm^{-1}

Com.no.	FT-TR bands
3a	3272 (υ $_{O-H}$), 3187(υ $_{N-H,\ thioketone\ form}$), 3097 (υ $_{C-H,\ benzene\ rings}$), 2899 (υ $_{C-H,\ oxazepine}$), 1697 (υ $_{C=O,\ O=C-O\ and\ O=C-N,\ vib.\ coupling,\ oxazepine}$), 1614(υ $_{C=C,\ oxazepine}$), 1531 (υ $_{C=C,}$ υ $_{C=N,\ aromatic\ rings\ and}$ υ as.NO_2 , $_{vib.\ coupling}$), 1348 (υ s.NO_2), 1301 and 1257 (υ $_{C-N}$), 1058 (υ $_{C=S,\ thioketone\ form}$), 868, 804, 736 and 711(δ $_{o.o.p\cdot C-H,\ benzene\ rings}$) .
3b	3404 and 3304 (υ $_{O-H}$), 3059 (υ $_{N-H,\ thioketone\ form}$), 3016 (υ $_{C-H,\ benzene\ rings}$), 2953 (υ as.C-H, CH_3), 2868 (υ s.C-H, CH_3), 2837 (υ $_{C-H,\ oxazepine}$), 1710 (υ $_{C=O,\ O=C-O\ and\ O=C-N,\ vib.\ coupling,\ oxazepine}$), 1606 (υ $_{C=C,\ oxazepine}$), 1560 and 1510 (υ $_{C=C\ and}$ υ $_{C=N,\ aromatic\ rings}$), 1458 (δ as.C-H, CH_3), 1377 (δ s.C-H, CH_3), 1287 and 1247 (υ $_{C-N}$), 1031 (υ $_{C=S,\ thioketone\ form}$), 827, 765, 744 and 696 (δ $_{o.o.p.C-H,\ benzene\ rings}$) .
3c	3279 (υ $_{O-H}$), 3095$_{br}$ (υ $_{N-H,\ thioketone\ form\ and}$ υ $_{C-H,\ benzene\ rings,\ vib.\ coupling}$), 2955 (υ as.C-H, CH_3), 1705 (υ $_{C=O,\ O=C-O\ and\ O=C-N,\ vib.\ coupling,\ oxazepine}$), 1612 (υ $_{C=C,\ oxazepine}$), 1573 and 1502 (υ $_{C=C\ and}$ υ $_{C=N,\ aromatic\ rings}$), 1454 (δ as.C-H, CH_3), 1388 (δ s.C-H, CH_3), 1317 and 1259 (υ $_{C-N}$), 1045 (υ $_{C=S,\ thioketone\ form}$), 856, 817 and 677 (δ $_{o.o.p.C-H,\ benzene\ rings}$) .
3d	3286 (υ $_{O-H}$), 3093$_{br}$ (υ $_{N-H,\ thioketone\ form\ and}$ υ $_{C-H,\ benzene\ rings,\ vib.\ coupling}$), 1687 (υ $_{C=O,\ O=C-O\ and\ O=C-N,\ vib.\ coupling,\ oxazepine}$), 1620 (υ$_{C=C,\ oxazepine}$), 1562 and 1500 (υ $_{C=C\ and}$ υ $_{C=N,\ aromatic\ rings}$), 1273 (υ $_{C-N}$), 1045 (υ $_{C=S\ ,\ thioketone\ form}$), 896, 831, 751 and 692 (δ $_{o.o.p.C-H,\ benzene\ rings}$).

The FT-IR data of the synthesized 1,3-oxazepine derivatives **3a-d** ,figs. (10-13) provide good evidence that the cycloaddition reactions proceeded successfully and produced the desired products by disappearing the weak-medium band at the range (1604-1618) cm^{-1} which belong to the stretching vibration of imine group (C=N) and appearing band or two band at the range (1624-1708) cm^{-1} attributed to the stretching vibration of (C=O) group inside oxazepine ring. Other band were summarized in table (7) .

Table (7): FT-IR data of the prepared 1,3- oxazepine derivatives (4a-d) in cm^{-1}

Com.no.	FT-TR bands
4a	3290 (υ_{O-H}), 3095$_{br}$ ($\upsilon_{N-H,\ thioketone\ form\ and}$ $\upsilon_{C-H,\ benzene\ rings,\ vib.\ coupling}$), 1707 ($\upsilon_{C=O,\ O=C-O,\ oxazepine}$), 1633 ($\upsilon_{C=O,\ O=C-N,\ oxazepine}$), 1535 and 1502 ($\upsilon_{C=C,}$ $\upsilon_{C=N,\ aromatic\ rings\ and}$ υ as.NO_2, vib. coupling), 1433 ($\upsilon_{N=N}$), 1394 and 1269 (υ_{C-N}), 1348 (υ s.NO_2), 1035 ($\upsilon_{C=S,\ thioketone\ form}$), 898, 831, 763, 738 and 678 ($\delta_{o.o.p\cdot C-H,\ benzene\ rings}$).
4b	3261 (υ_{O-H}), 3134 ($\upsilon_{N-H,\ thioketone\ form}$), 3070 ($\upsilon_{C-H,\ benzene\ rings}$), 2926 ($\upsilon$ as.C-H, CH_3), 2847 (υ s.C-H, CH_3), 1707 ($\upsilon_{C=O,\ O=C-O\ and\ O=C-N,\ vib.\ coupling,\ oxazepine}$), 1556 and 1508 ($\upsilon_{C=C\ and}$ $\upsilon_{C=N,\ aromatic\ rings}$), 1462 ($\delta$ as.C-H, CH_3), 1375(δ s.C-H, CH_3), 1296 and 1249 (υ_{C-N}), 1053 ($\upsilon_{C=S,\ thioketone\ form}$), 891, 823, 744 and 686 ($\delta_{o.o.p.C-H,\ benzene\ rings}$) .
4c	3284 (υ_{O-H}), 3093$_{br}$ ($\upsilon_{N-H,\ thioketone\ form\ and}$ $\upsilon_{C-H,\ benzene\ rings,\ vib.\ coupling}$), 2953($\upsilon$ as.C-H, CH_3), 2802 (υ s.C-H, CH_3), 1708 ($\upsilon_{C=O,\ O=C-O,\ oxazepine}$), 1624 ($\upsilon_{C=O,\ O=C-N,\ oxazepine}$), 1496 ($\upsilon_{C=C\ and}$ $\upsilon_{C=N,\ aromatic\ rings}$), 1384 ($\upsilon$ s.C-H, CH_3), 1309 (υ_{C-N}), 1045 ($\upsilon_{C=S,\ thioketone\ form}$), 896, 802 and 678 ($\delta_{o.o.p.C-H,\ benzene\ rings}$) .
4d	3281 (υ_{O-H}), 3095 ($\upsilon_{N-H,\ thioketone\ form\ and}$ $\upsilon_{C-H,\ benzene\ rings,\ vib.\ coupling}$) 1703 ($\upsilon_{C=O,\ O=C-O,\ oxazepine}$), 1624 ($\upsilon_{C=O,\ O=C-N,\ oxazepine}$), 1548 and 1500 ($\upsilon_{C=C\ and}$ $\upsilon_{C=N,\ aromatic\ rings}$), 1386 and 1271 ($\upsilon_{C-N}$), 1043 ($\upsilon_{C=S,\ thioketone\ form}$), 893, 827 and 677 ($\delta_{o.o.p.C-H,\ benzene\ rings}$).

^{1}H NMR spectra of 1,3-oxazepine derivatives

^{1}H NMR spectrum, fig. (14), (400 MHz, DMSO) of compound (**3a**) appeared the following signals at δ (ppm): 2.509 - 2.513 (DMSO solvent), 3.359 -3.412 (H_2O in DMSO), 6.217 (s,1H , 1×olefinic =C-H proton bonded with O-C=O inside oxazepine ring), 6.535 - 6.540 (d, 1H, 1×olefinic =C-H proton bonded with O=C–N inside oxazepine ring), 6.634-7.956 (Ar-H and C-H proton of oxazepine ring), 8.458 - 8.471 (d, 1H, 1× H-O phenolic proton).

Appearance of olefinic protons signals in addition of identity of integration of protons in the down field (11 protons) (olefinic, aromatic, C-H proton of oxazepine and O-H phenolic proton) with that in the proposed structure of product illustrate good evidence that the cycloaddition reaction happened successfully and formed oxazepine derivative (**3a**).

The expanded spectrum of compound (**3a**), fig.(14a) showed appearance eight signals at the range (6.634-7.956) ppm attributed to seven types of nonequivalent aromatic protons in addition of (C-H) proton of oxazepine, these signals could be interpreted according to their theoretical chemical shifts, fig. (14b) as follows :

6.634 (s, 1H, 1×Ha), 6.705 - 6.743 (d, 1H, 1×Hb), 6.840 (s, 1H, 1×Hc), 7.112 - 7.195 (m, 1H, 1× Hd, oxazepine ring), 7.509 - 7.533 (d ,1H , 1×He), 7.657 - 7.684 (t , 1H, 1×Hf), 7.845 - 7.889 (m, 1H, 1×Hg), 7.956 (s, 1H, 1×Hh).

^{1}H NMR spectrum, fig. (15), (400 MHz, DMSO) of compound (**3b**) appeared the following signals at δ (ppm): 2.509 (DMSO solvent), 3.354 - 3.396 (H_2O in DMSO), 3.738 (s, 3H, 1× CH_3-O), 6.294 - 6.324 (d, 1H , 1×olefinic =C-H proton bonded with O–C=O inside oxazepine ring), 6.450 - 6.481 (d, 1H , 1×olefinic =C-H proton bonded with O=C–N inside oxazepine ring), 6.909-6.932 and 7.538-7.560 (Ar-H and C-H proton of oxazepine ring), 8.375 (s, 1H, 1×O-H phenolic proton).

Appearance of only two signals for aromatic protons may be due to presence of two types of nonequivalent protons in two benzene rings.

Appearance of olefinic protons signals is considered good evidence that the cycloaddition reaction took place successfully and formed oxazepine ring, this conclusion is assisted by the identity between integration of protons in the down field (11 protons) and number of these protons in the proposed structure of compound (**3b**).

^{1}H NMR spectrum, fig. (16), (400 MHz, DMSO) of compound (**3d**) appeared the following signals at δ (ppm): 2.504-2.513 (DMSO solvent), 6.179 - 6.185 (d, 1H, 1×olefinic =C-H proton bonded with O–C=O inside oxazepine ring), 6.636 (s, 1H , 1×olefinic =C-H proton bonded with O=C–N inside oxazepine ring), 7.137 and 7.753 (Ar-H and C-H proton of oxazepine ring), 13.177 (s, 2H, 2×O-H phenolic proton).

Appearance of only two signals for aromatic protons may be due to presence of two types of nonequivalent protons in two benzene rings, moreover appearance of olefinic protons signals indicates that the cycloaddition reaction proceeded successfully and formed the desired oxazepine ring.

^{1}H NMR spectrum, fig. (17), (400 MHz, DMSO) of compound (**4a**) appeared the following signals at δ (ppm): 2.509 (DMSO solvent), 3.435 - 3.452 (H_2O in DMSO), 7.100 - 8.754 (Ar-H and C-H proton of oxazepine ring), 13.171-13.174 (s, 1H, 1×O-H phenolic proton).

The integration of aromatic protons and proton of oxazepine ring (12 protons) is agreed with number of these protons in the proposed structure of compound (**4a**).

The expanded spectrum of compound (**4a**), fig.(17a) showed appearance ten signals at the range (7.100-8.754) ppm due to ten types of nonequivalent aromatic protons in addition of (C-H) proton of oxazepine ring, these signals could be interpreted according to their theoretical chemical shifts, fig. (17b) as follows :

7.100 (s, 1H, 1×Ha), 7.371 (s, 1H, 1×Hb), 7.592 - 7.623 (s, 1H, 1×Hc + t, 1H, 1× Hd), 7.644 (s, 1H, 1× He , oxazepine), 7.665 (s ,1H , 1×Hf), 7.683 - 7.702 (d , 1H, 1×Hg), 7.756 - 7.791 (d, 1H, 1×Hh), 7.919 - 7.941 (d, 1H, 1×Hi), 7.975 - 7.998 (d, 2H, 2×Hj), 8.754 (s, 1H, 1×Hk), The signal of C-H proton of oxazepine ring may be interacted with signals of aromatic protons.

^{1}H NMR spectrum, fig. (18), (400 MHz, DMSO) of compound (**4c**) appeared the following signals at δ (ppm): 1.271 (s, 6H, 2× CH_3), 2.515 (DMSO solvent), 3.451 (H_2O in DMSO), 4.27 (br, 1H, 1×N-H, thioketone form), 7.182-8.488 (Ar-H and C-H proton of oxazepine ring), 13.172 (s, 1H, 1×O-H phenolic proton).

The expanded spectrum of compound (**4c**), fig.(18a) showed appearance eleven signals at the range (7.082-8.488) ppm due to ten types of nonequivalent aromatic protons in addition of (C-H) proton of oxazepine, these signals could be interpreted in association with their theoretical chemical shifts, fig. (18b) as follows :

7.082 - 7.116 (d, 1H, 1×Ha), 7.182 - 7.213 (d, 1H, 1×Hb), 7.515 - 7.537 (m, 1H, 1×Hc may be due to long-rang coupling with methyl groups protons), 7.691 (d, 1H, 1× Hd), 7.818 (s ,1H , 1×He), 7.976 (d, 1H, 1×Hf), 8.034 - 8.062 (d, 1H, 1×Hg), 8.132 - 8.137 (d, 1H, 1×Hh), 8.164 (s, 1H, 1×Hi), 8.416 - 8.430 (d, 1H, 1×Hj), 8.476 - 8.488 (d, 1H, 1×Hk).

^{1}H NMR spectrum, fig. (19), (400 MHz, DMSO) of compound (**4d**) appeared the following signals at δ (ppm): 2.090 (s, 1H, 1×S-H), 2.508 (DMSO solvent), 3.437 - 3.454 (H_2O in DMSO), 7.099-7.789 (Ar-H and C-H proton of oxazepine ring), 13.174 (s, 2H, 2×O-H phenolic protons).

The expanded spectrum of compound (**4d**), fig.(19a) showed appearance seven signals at the range (7.099-7.789) ppm due to six types of nonequivalent aromatic protons in addition of (C-H) proton of oxazepine, these signals could be interpreted according to their theoretical chemical shifts, fig. (19b) as follows :

7.099 (s, 1H, 1×Ha), 7.577-7.600 (dd, 2H, 2×Hb), 7.638 (s, 1H, 1×Hc), 7.669-7.683 (s, 1H, 1× Hd + d, 1H, 1× He), 7.754 (m ,5H , 5×Hf), 7.789 (s, 1H, 1×Hg) .

Antibacterial activity

In this research the antibacterial test was carried out according to the disc diffusion method. Four synthesized oxazepine derivatives (**3a**, **3b**, **3c** and **4b**) were screened for their antibacterial activity in **vitro** against two types of bacteria ***staphylococcus aureous*** (Gram-positive) and ***Escherichia coli*** (Gram-negative). Prepared agar and petridishes were sterilized by autoclaving for 15 min. at 121 ^{o}C. The agar plates were surface inoculated uniformly from the both culture of the tested microorganism. In the solidified medium suitably spaced apart holes were made all 6mm in diameter. These holes were filled with 40 μL of the prepared compounds (5mg of the compound dissolved in 1mL of DMSO solvent). These plates were incubated at 37 ^{o}C for 24 h. for both bacteria. The zones of microbial growth inhibition around the discs were measured in (mm). The test results presented in table (8).

Table (8): The antibacterial activity for some of synthesized 1,3-oxazepine derivatives

Comp. no.	Diameter of inhibition zone in (mm)	
	*Staphylococcus aureou*s	*Escherichia coli*
3a	13	5
3b	9	0
3c	15	8
4b	12	7

Key of symbols : Highly active = +++ (inhibition zone > 15 mm)
Moderately active = ++ (inhibition zone 11-15 mm)
Slightly active = + (inhibition zone 5-10 mm)
Inactive = - (inhibition zone < 5 mm)

The results revealed that all tested oxazepine derivatives showed relatively higher activity against Gram - positive bacteria (*Staphylococcus aureou*s) than that of Gram-negative bacteria (*Escherichia coli*). Oxazepine derivatives (3a, 3c and 4b) appeared moderate activity against Gram - positive bacteria while oxazepine derivative (3b) appeared slight activity against the same bacteria.

Conclusions

1. All cycloaddition reactions for the synthesis of oxazepine derivatives required relatively long time for completion, the reason may be due to the relative stability of the synthesized azoimine derivatives due to the extending conjugation with azo group which leads to decrease the characteristic of π-bond in (C=N) group.
2. In general, the rates of cycloaddition reactions are relatively increased in presence of electron-donating groups substituted in benzene ring and relatively decreased in presence of electron with-drawing groups substituted in the same ring. The reason may be attributed to increase stability of transition state for the cycloaddition.
3. All tested oxazepine derivatives showed relatively higher activity against Gram - positive bacteria (***Staphylococcus aureou*s**) than that of Gram - negative bacteria (***Escherichia coli***).
4. Oxazepine derivatives (**3a**, **3c** and **4b**) appeared moderate activity against Gram - positive bacteria while oxazepine derivative (**3b**) appeared slight activity against the same bacteria.

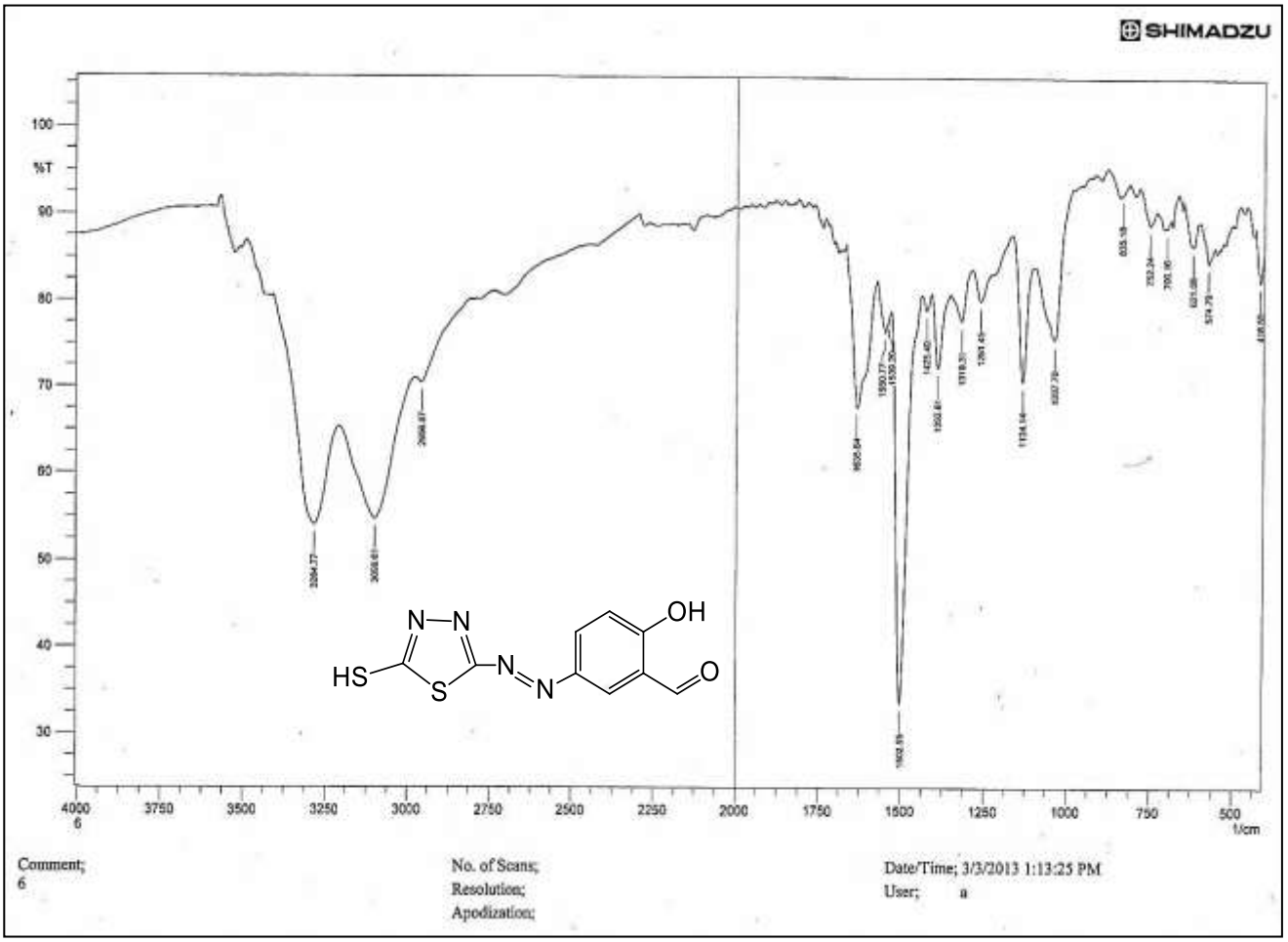


Fig. (1): FT-IR spectrum of compound (1)

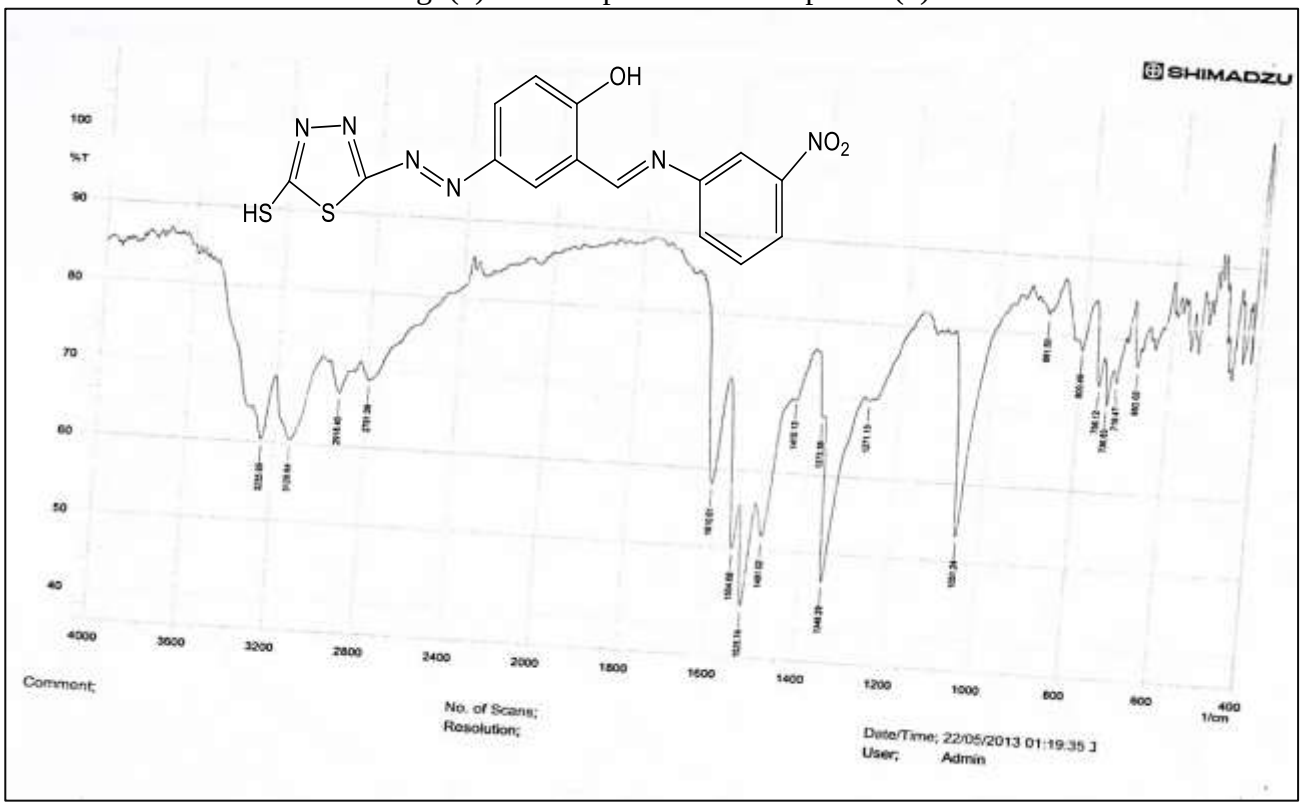


Fig. (2): FT-IR spectrum of compound (2a)

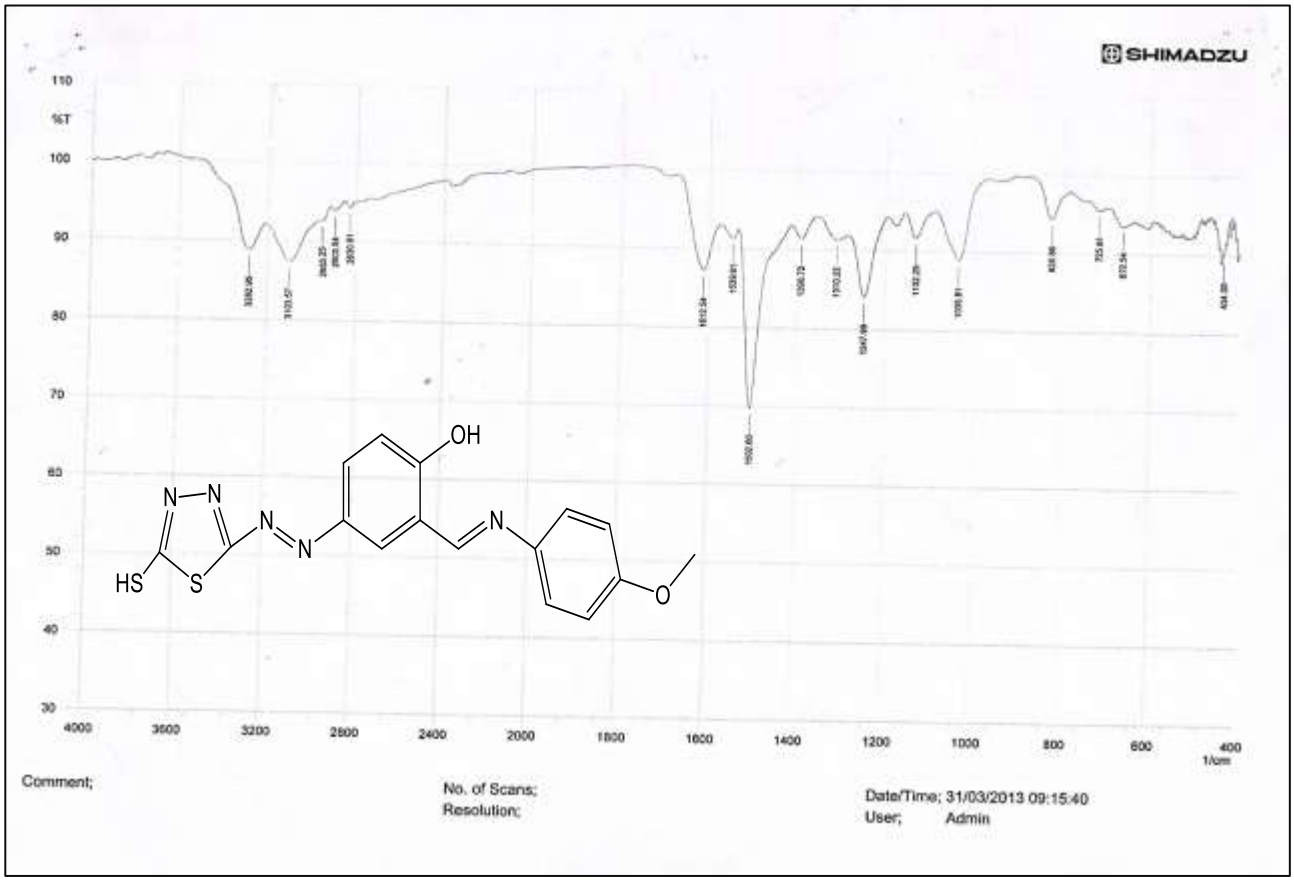


Fig.(3): FT-IR spectrum of compound (2b)

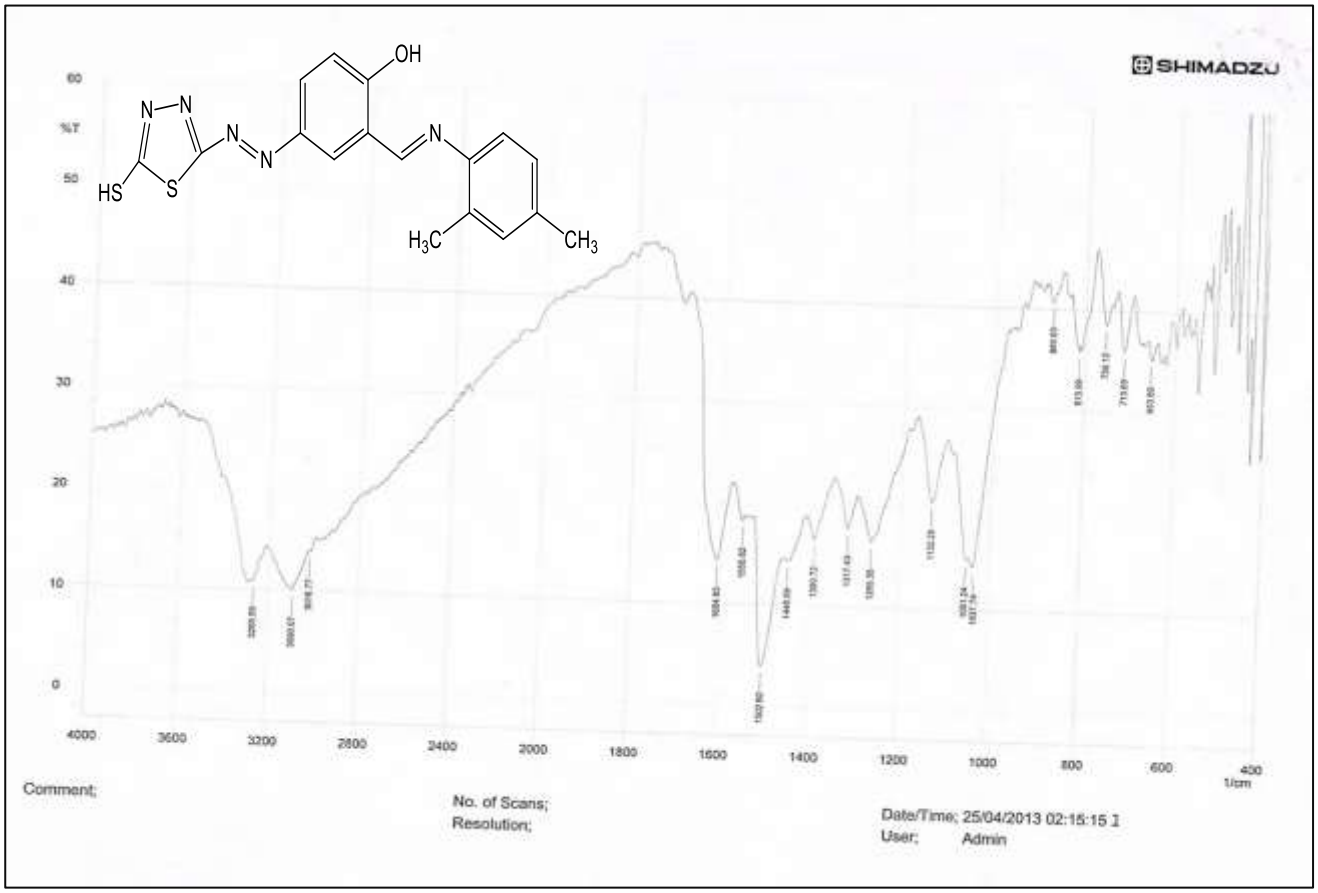


Fig.(4): FT-IR spectrum of compound (2c)

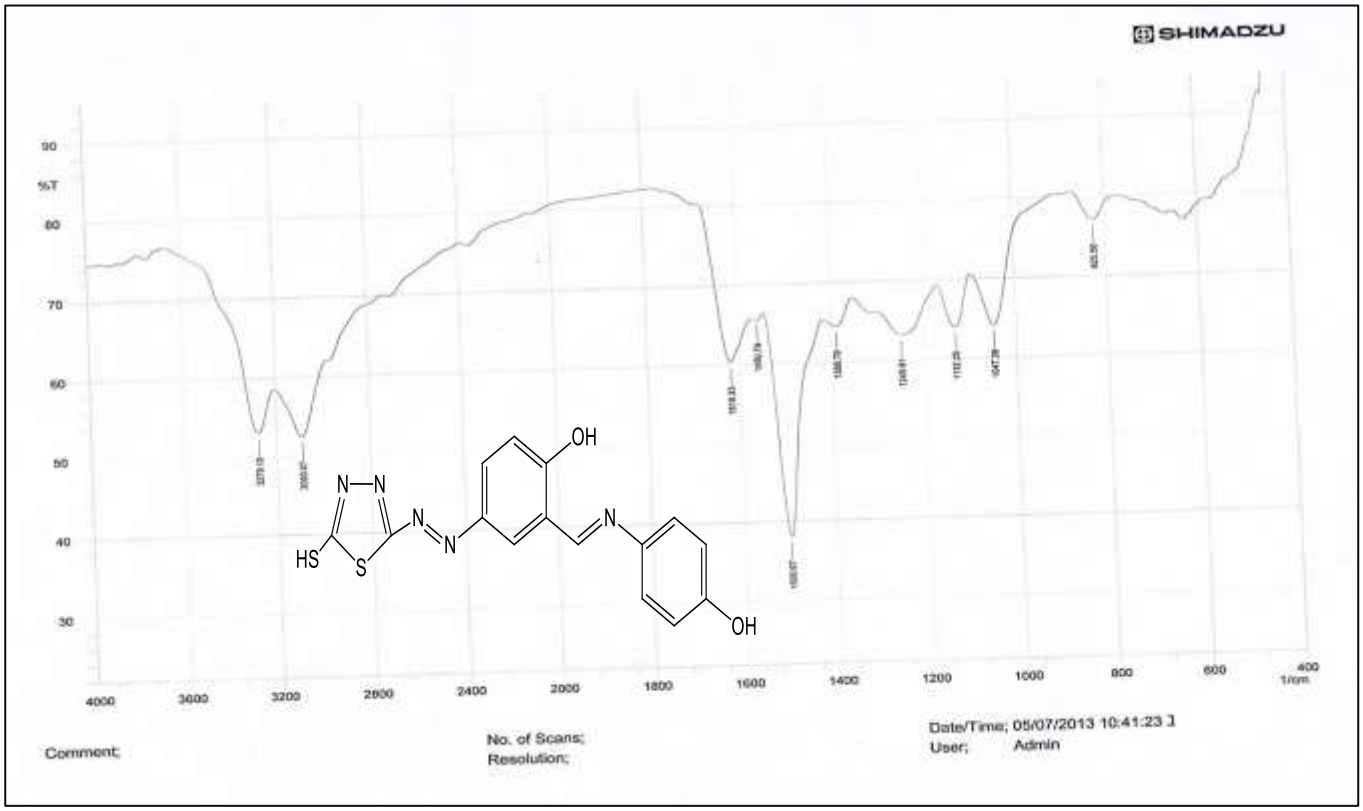


Fig.(5): FT-IR spectrum of compound (2d)

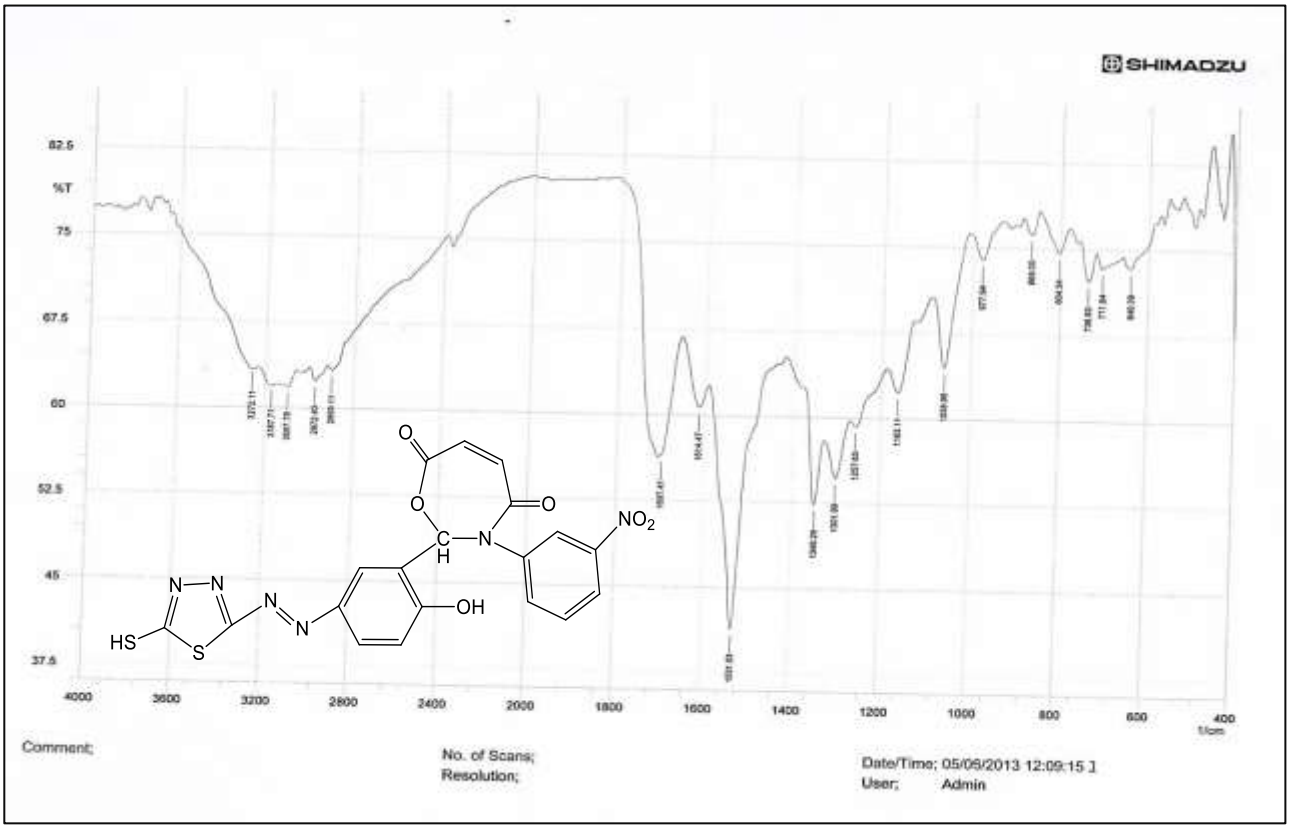


Fig.(6): FT-IR spectrum of compound (3a)

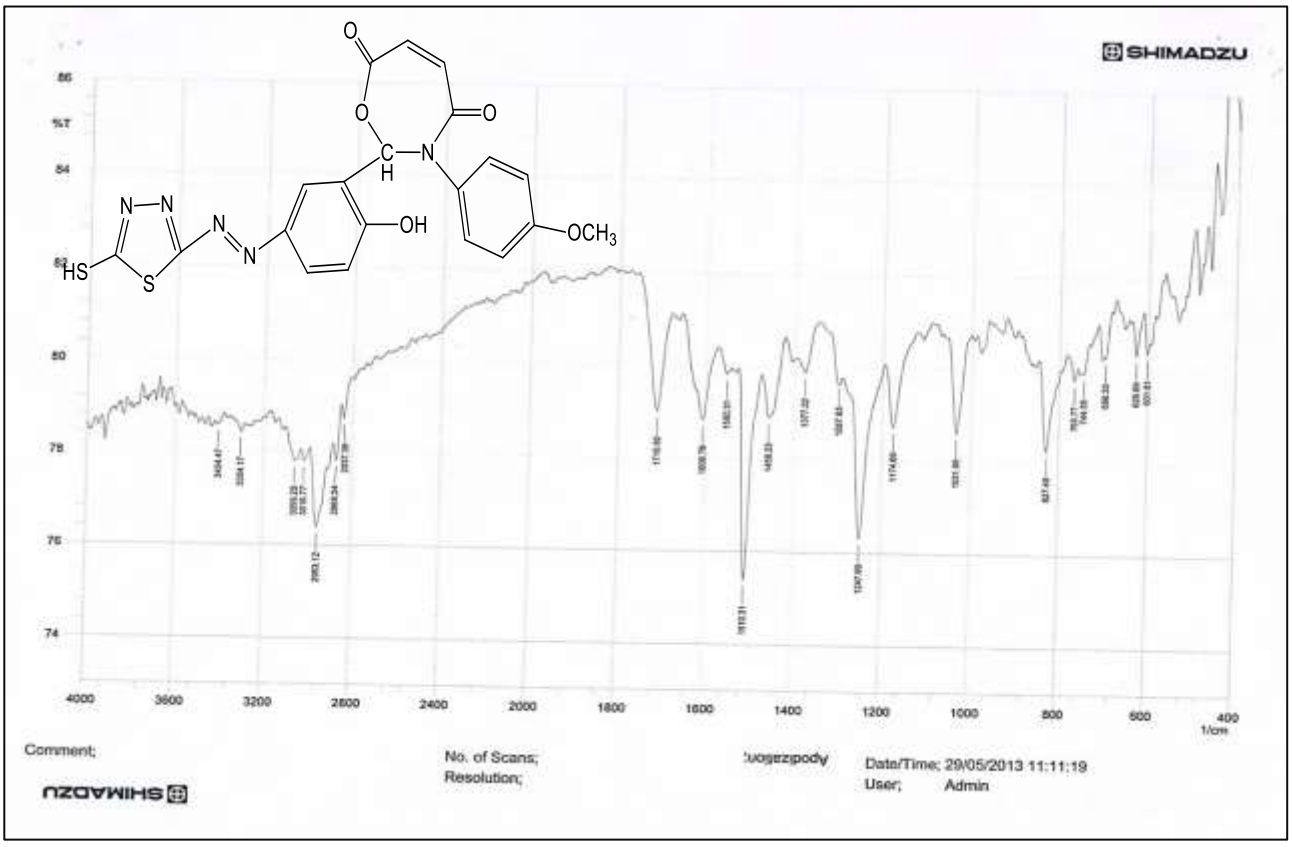


Fig.(7): FT-IR spectrum of compound (3b)

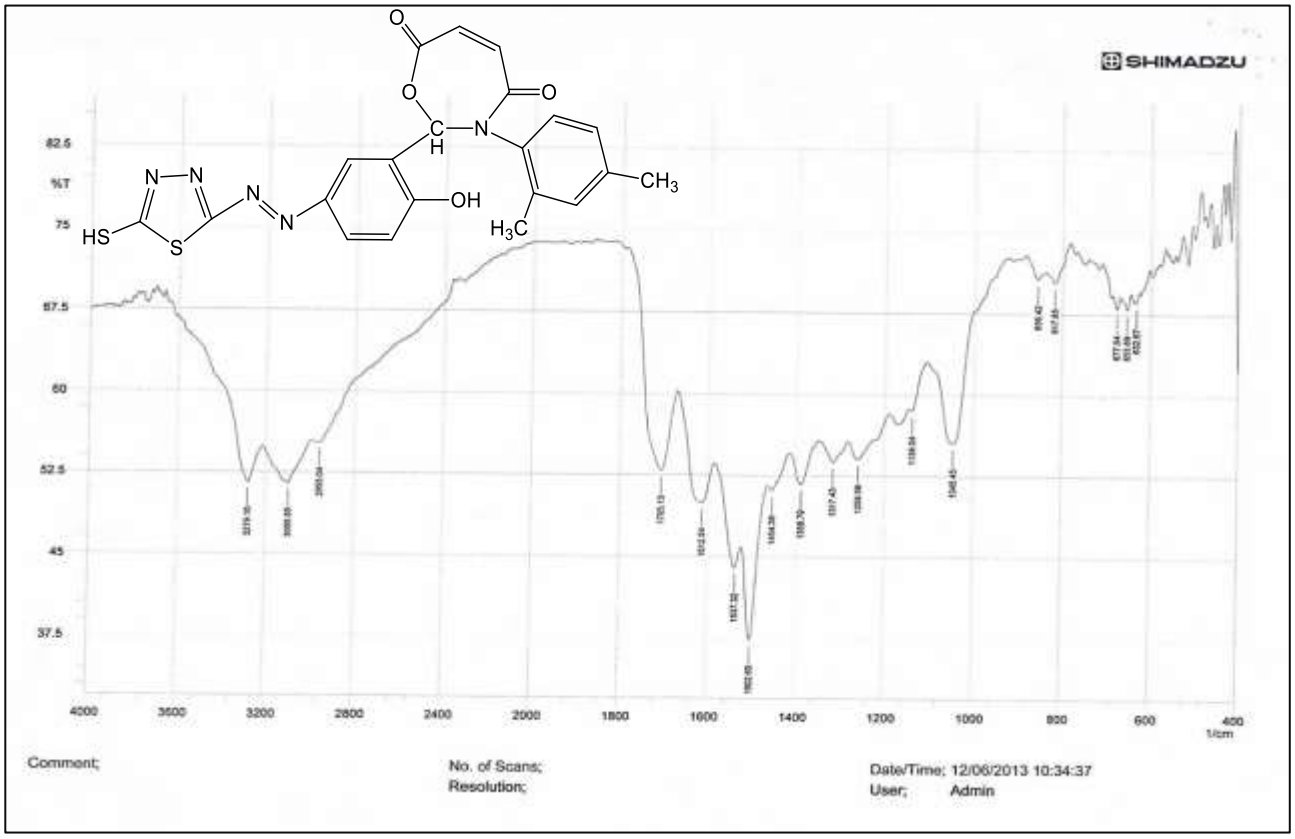


Fig.(8): FT-IR spectrum of compound (3c)

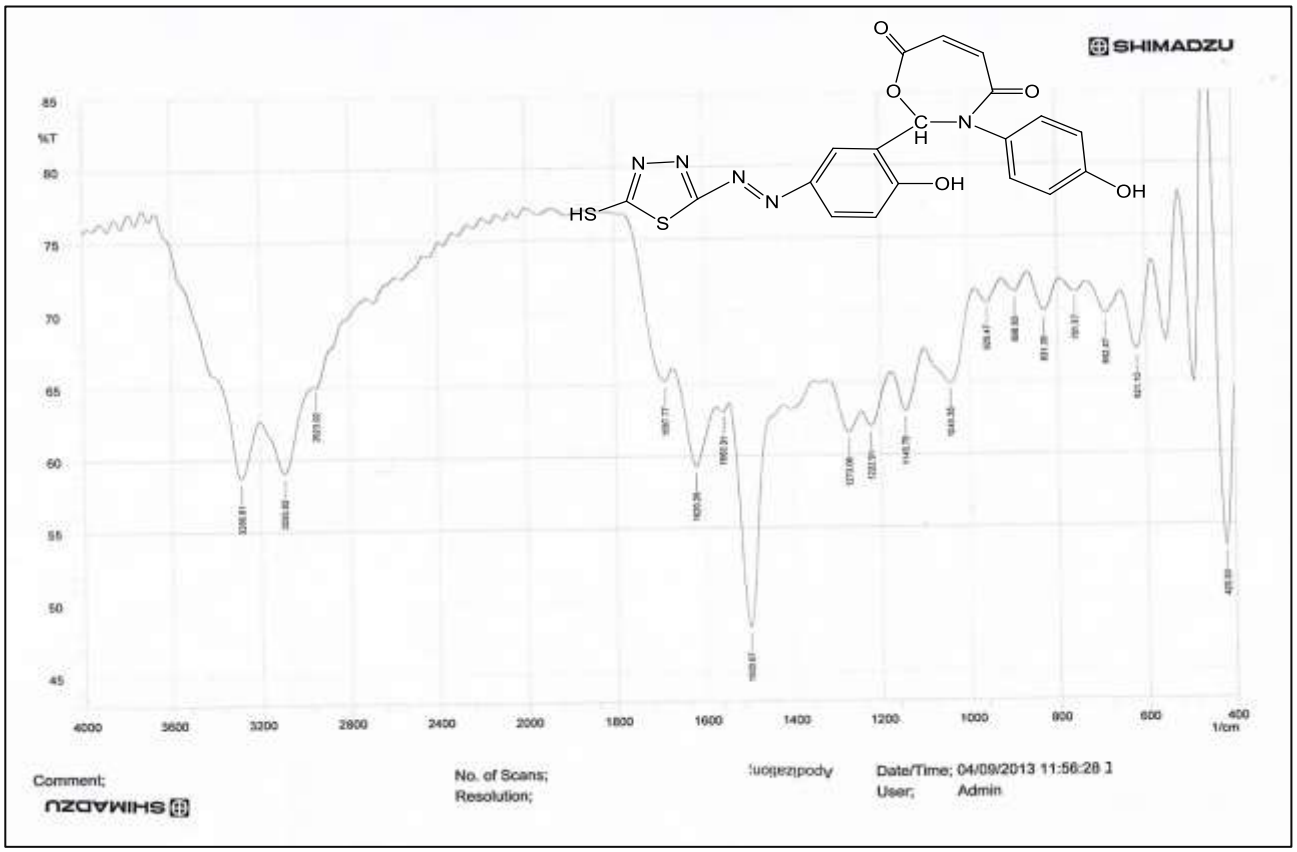


Fig.(9): FT-IR spectrum of compound (3d)

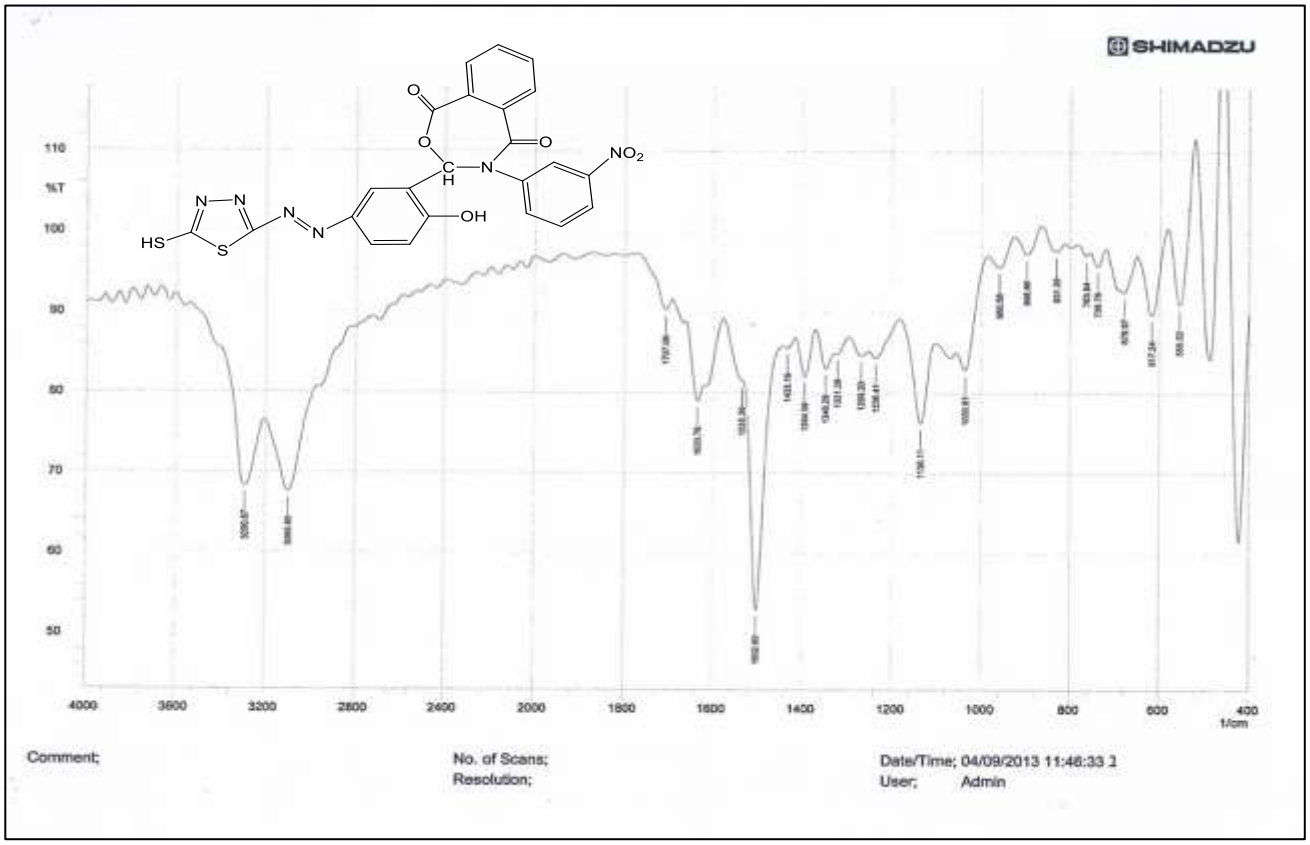


Fig.(10): FT-IR spectrum of compound (4a)

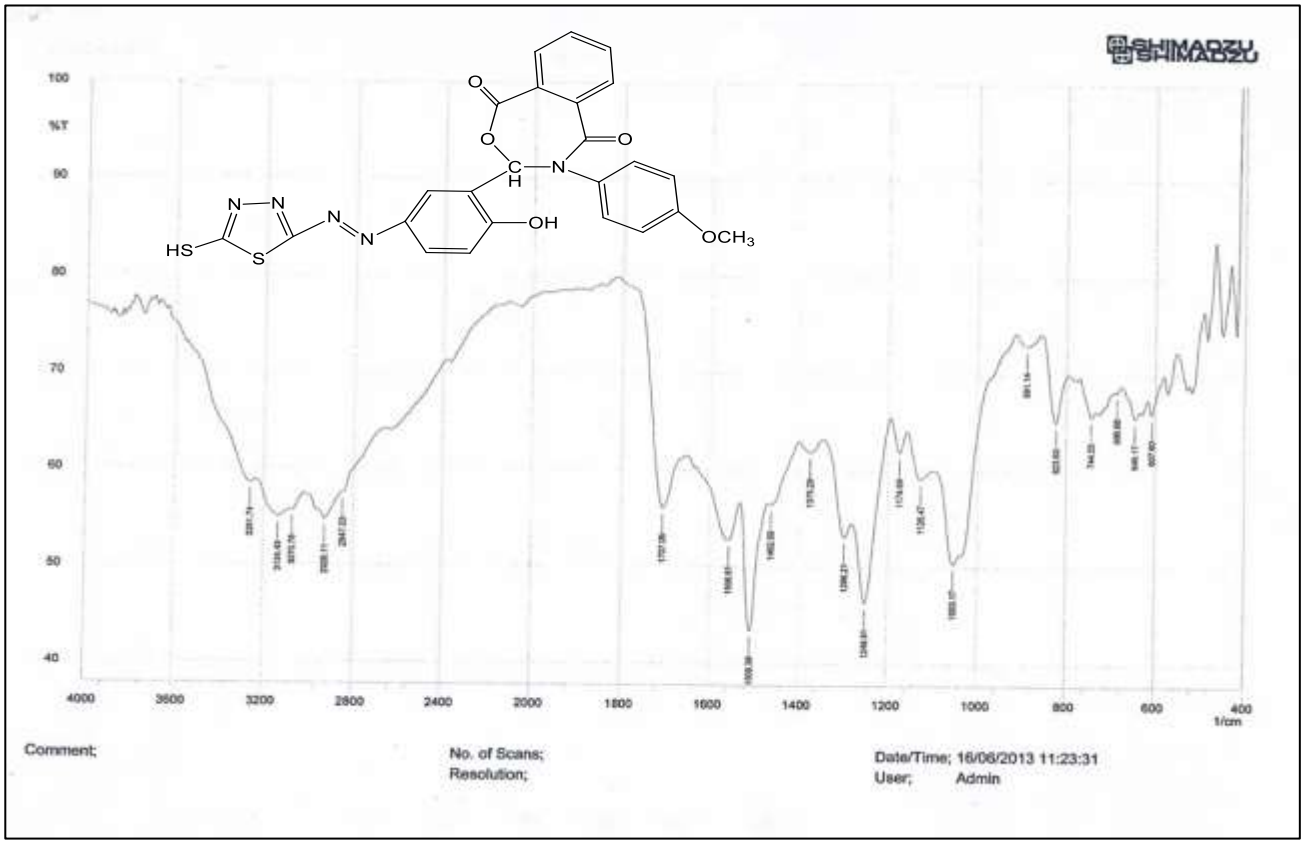


Fig.(11): FT-IR spectrum of compound (4b)

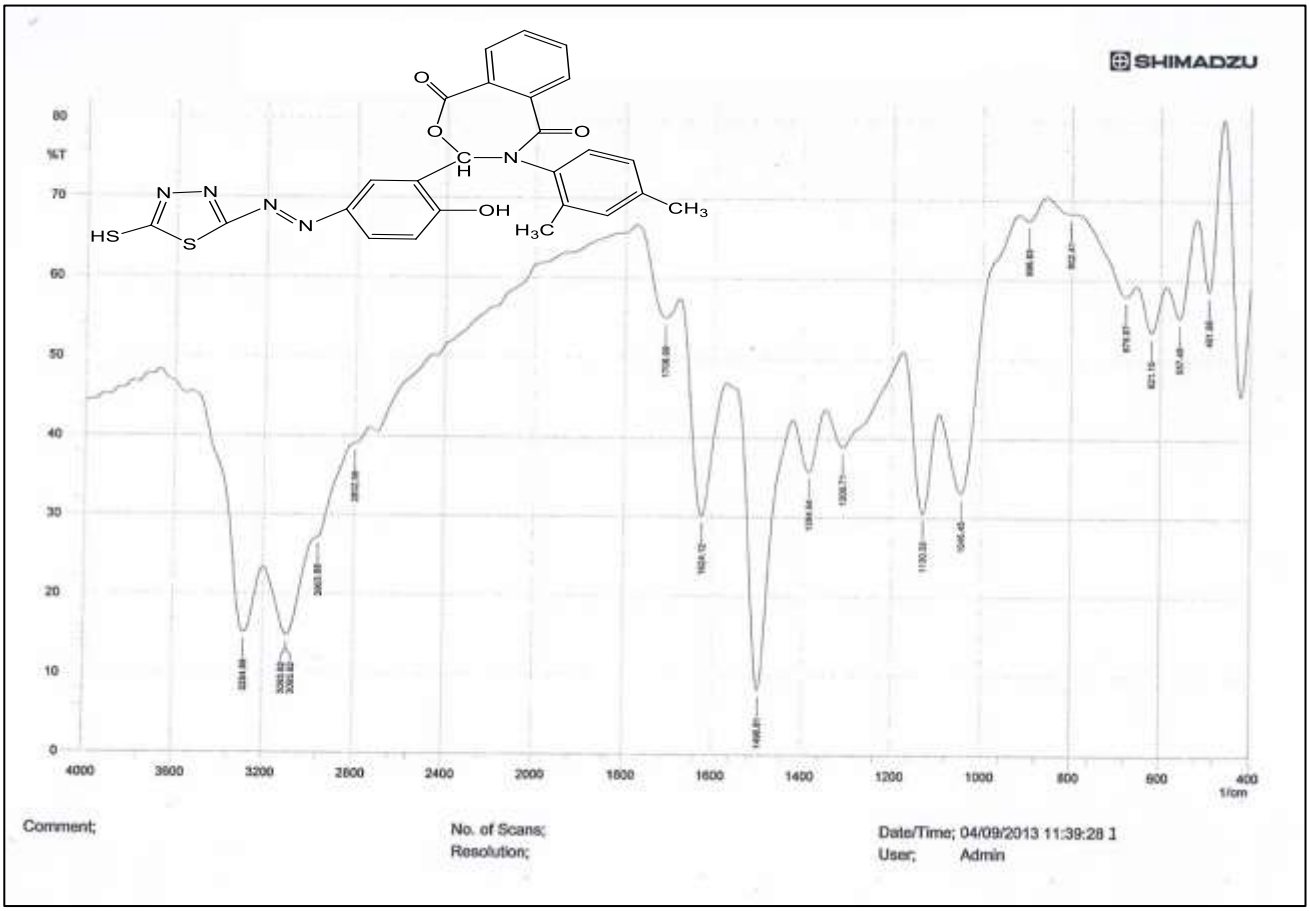


Fig.(12): FT-IR spectrum of compound (4c)

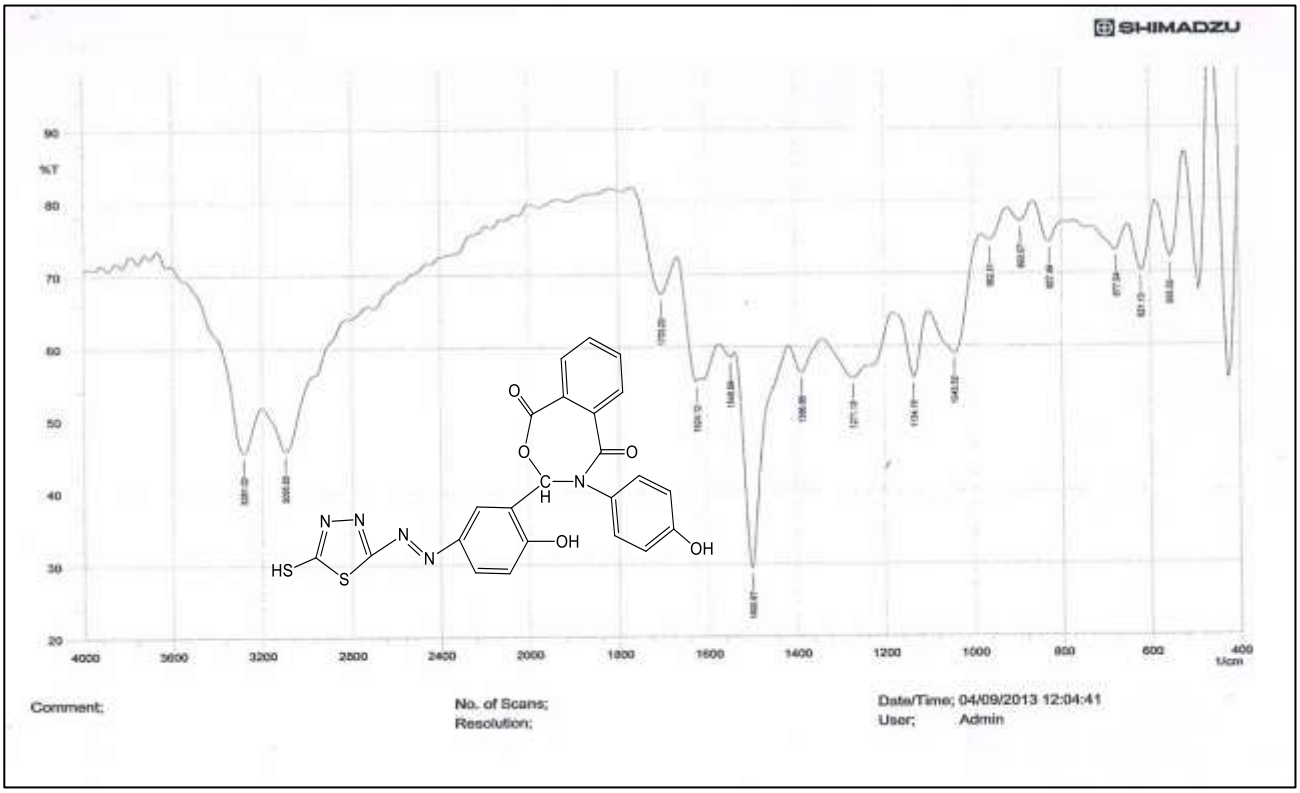


Fig.(13): FT-IR spectrum of compound (4d)

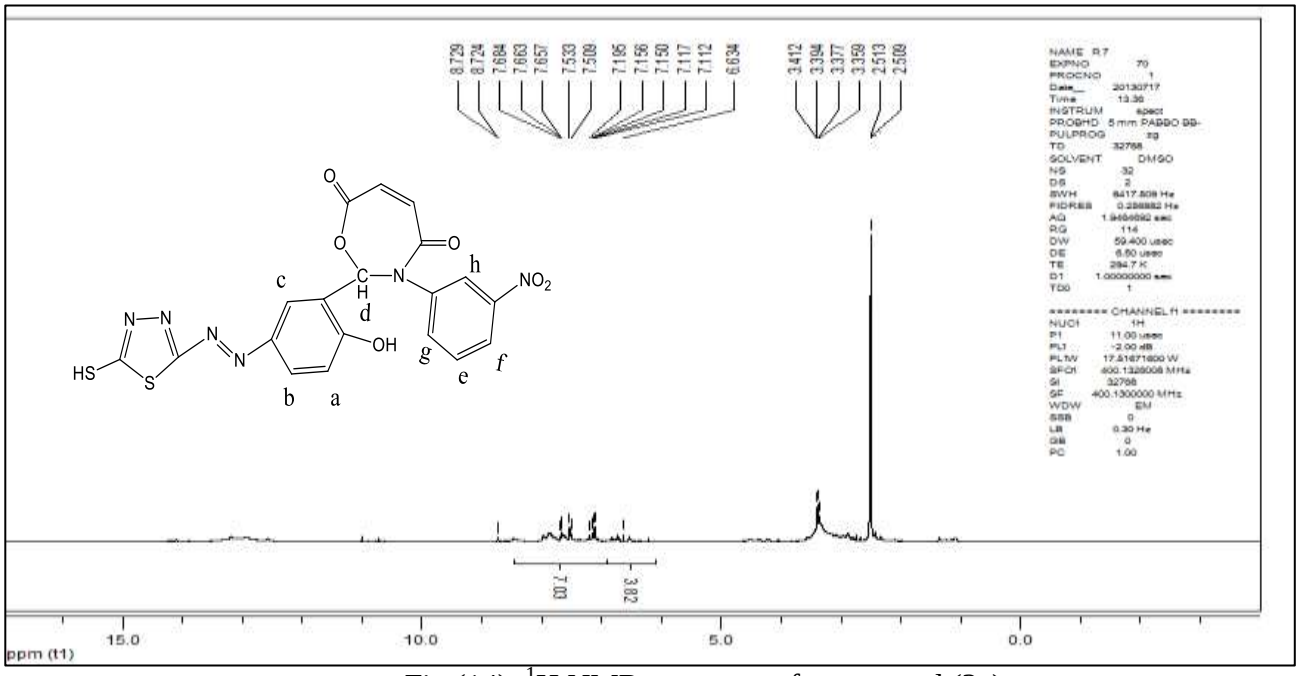


Fig.(14): ^{1}H NMR spectrum of compound (3a)

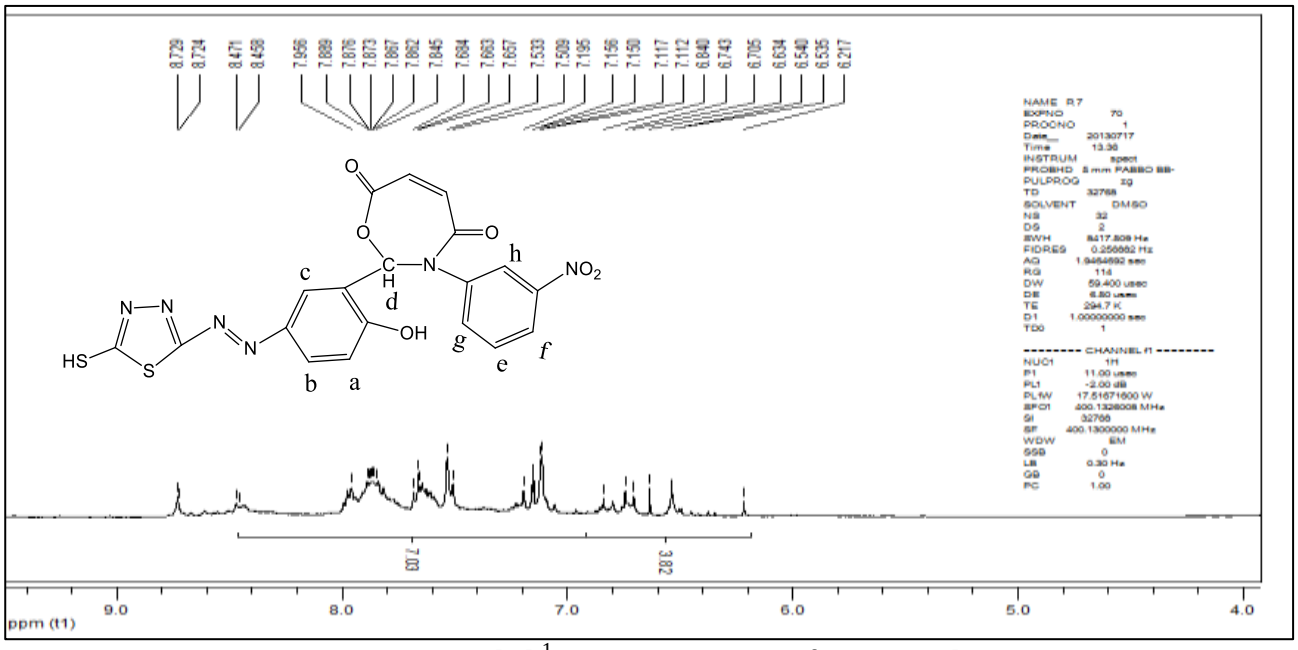


Fig.(14a): Expanded ^{1}H NMR spectrum of compound (3a)

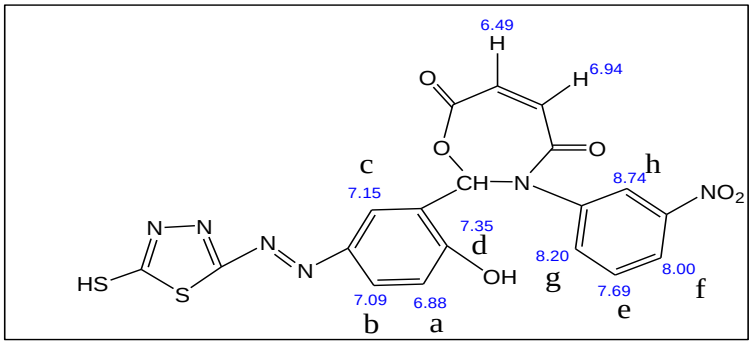


Fig.(14b): Theoretical δ (ppm) values of compound (3a)

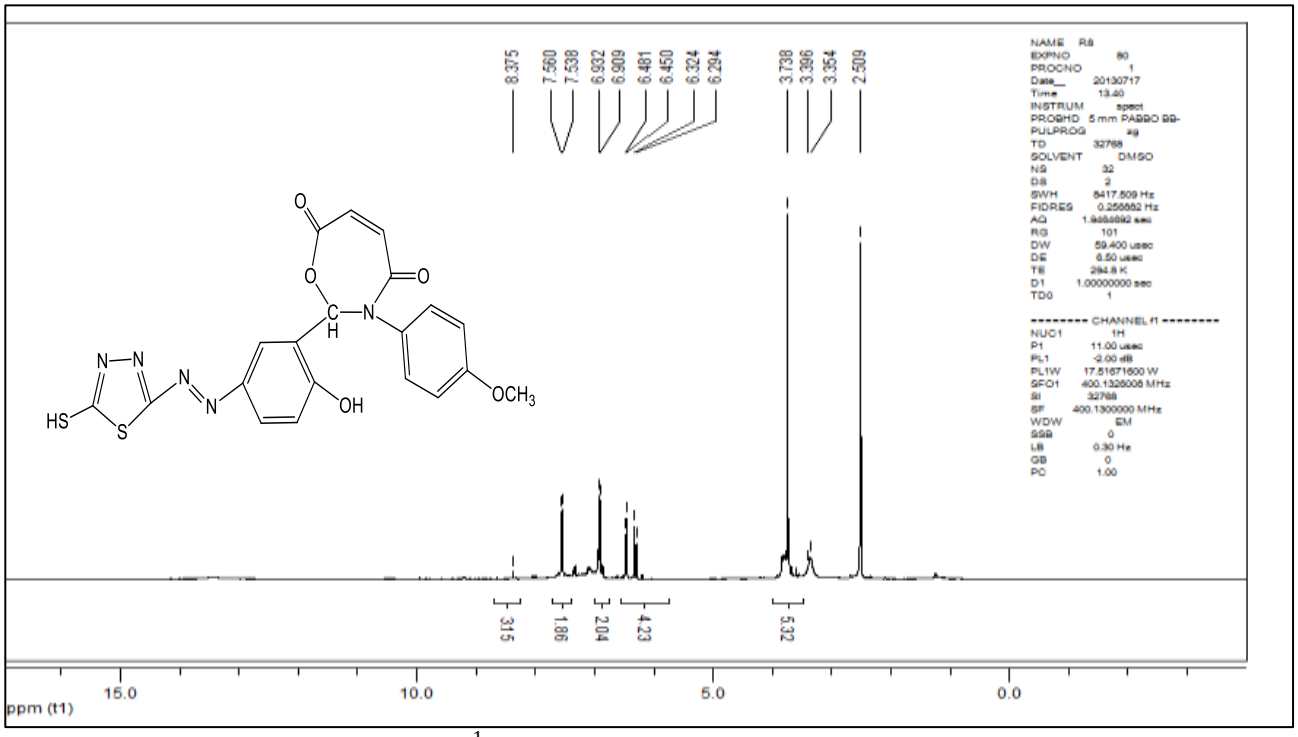


Fig.(15): ^{1}H NMR spectrum of compound (3b)

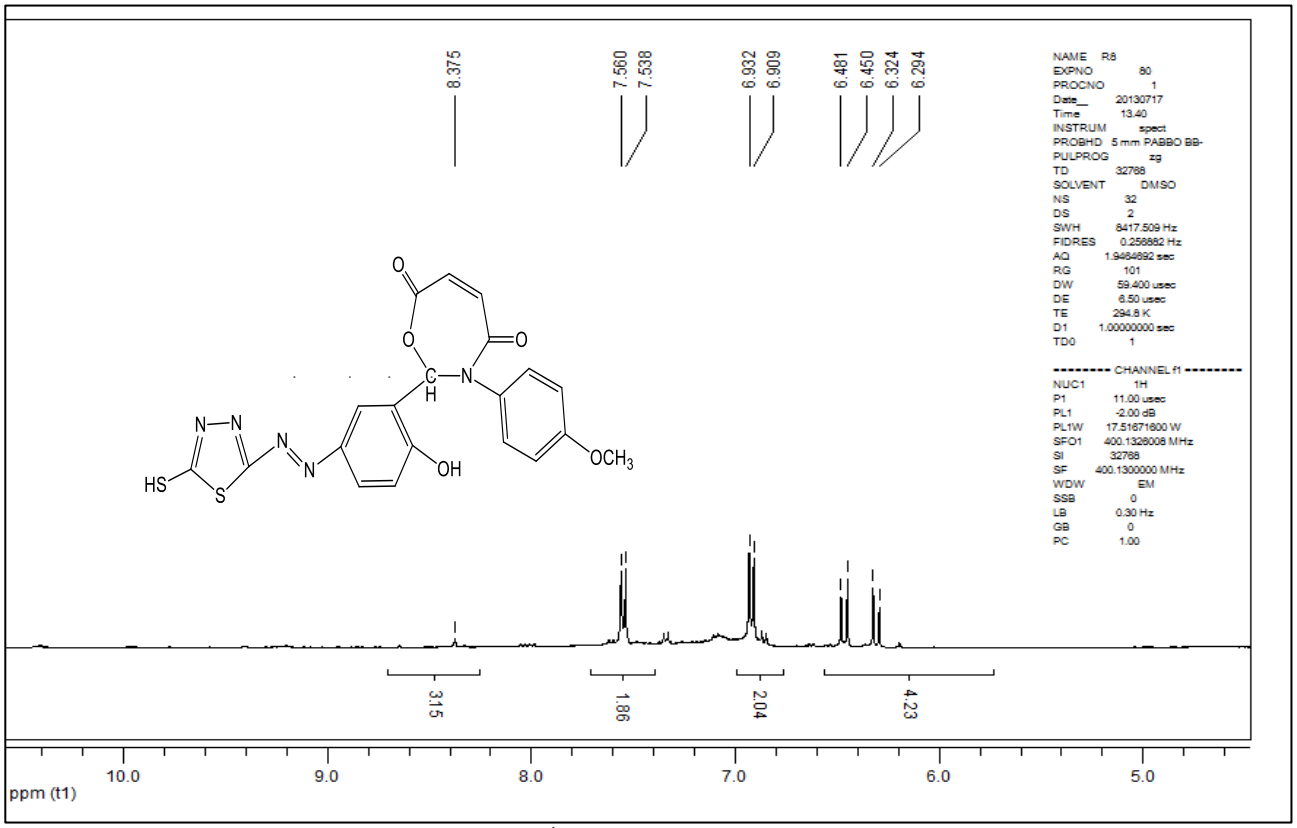


Fig.(15a): Expanded ^{1}H NMR spectrum of compound (3b)

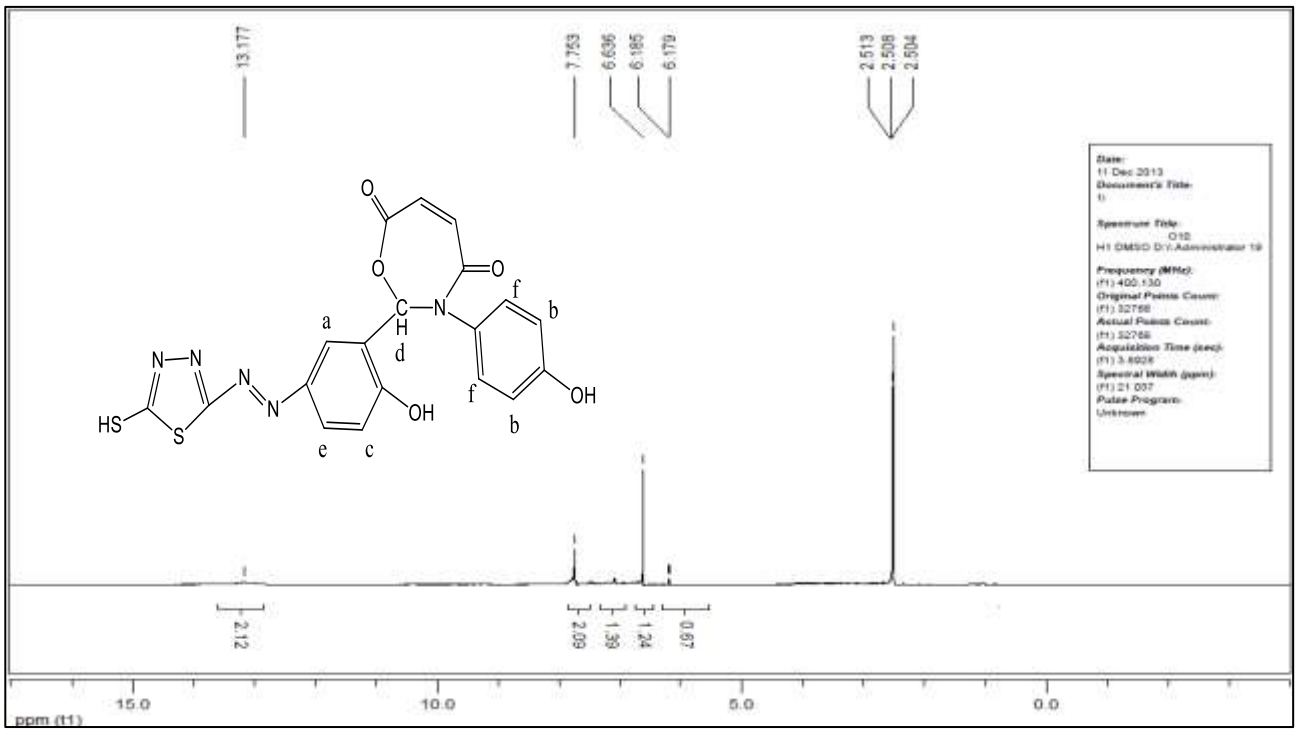


Fig.(16): ^{1}H NMR spectrum of compound (3d)

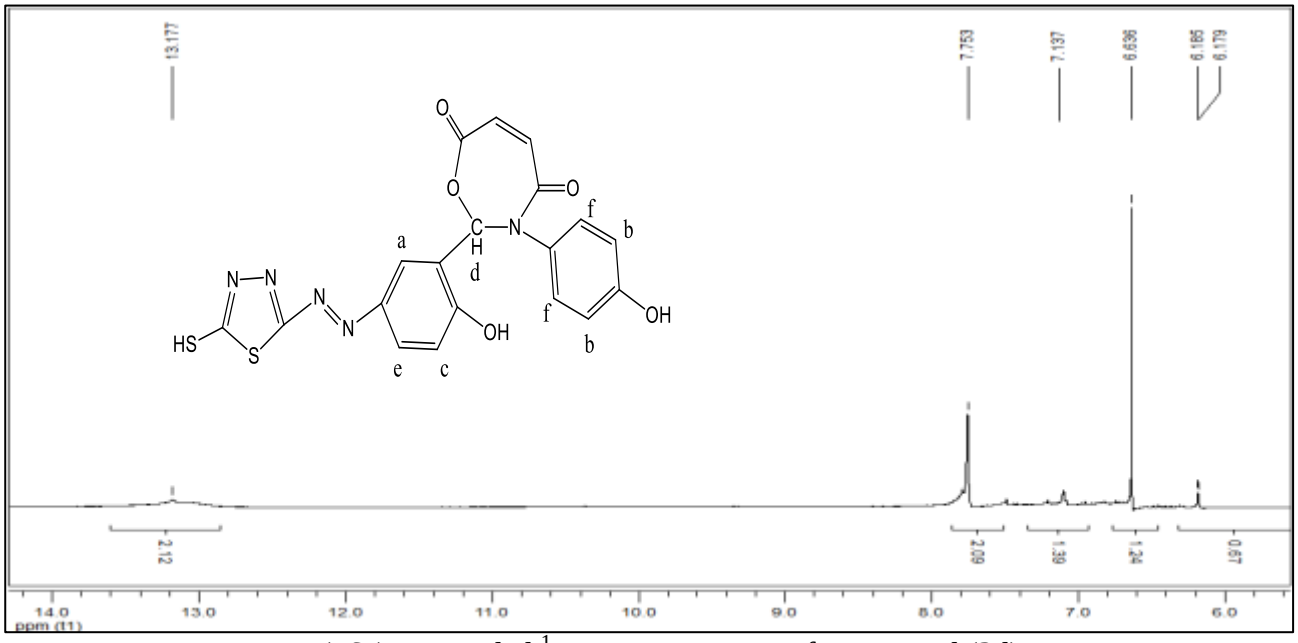


Fig.(16a): Expanded ^{1}H NMR spectrum of compound (3d)

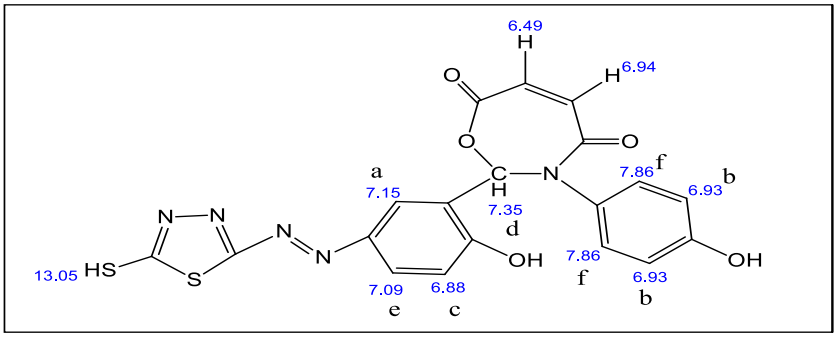


Fig.(16b): Theoretical δ (ppm) values of compound (3d)

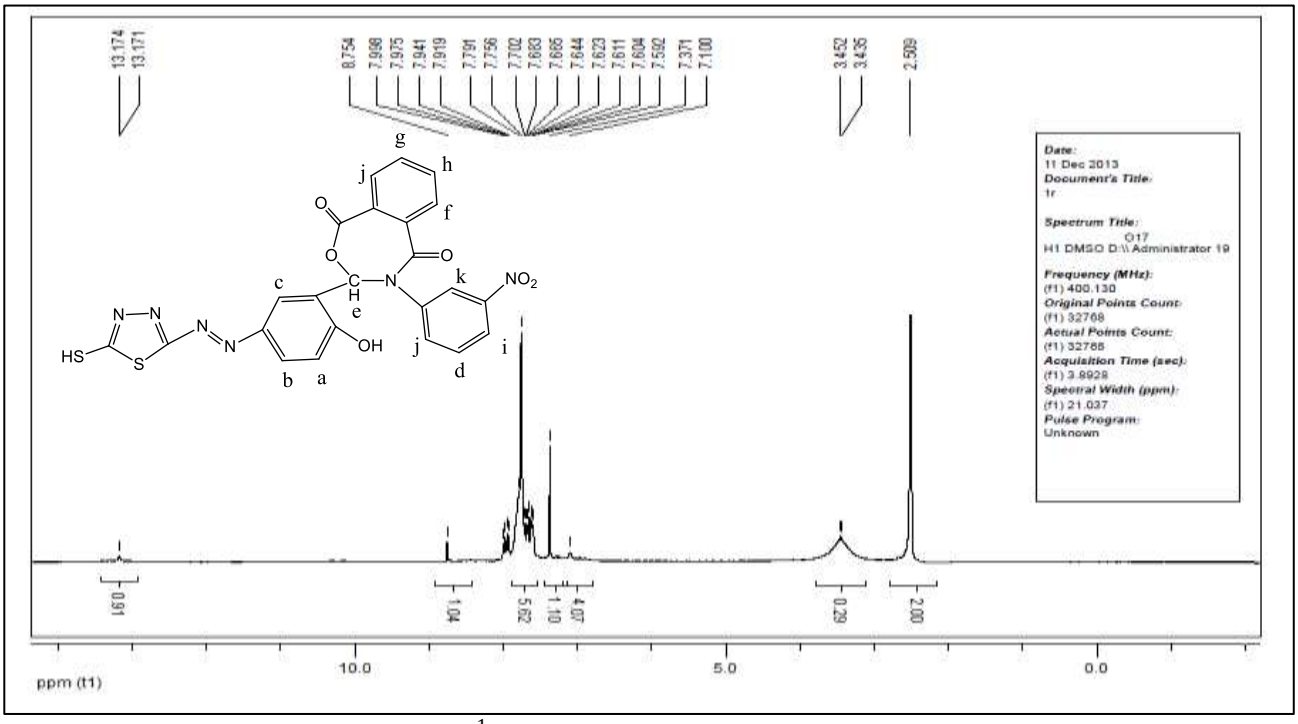


Fig.(17): ^{1}H NMR spectrum of compound (4a)

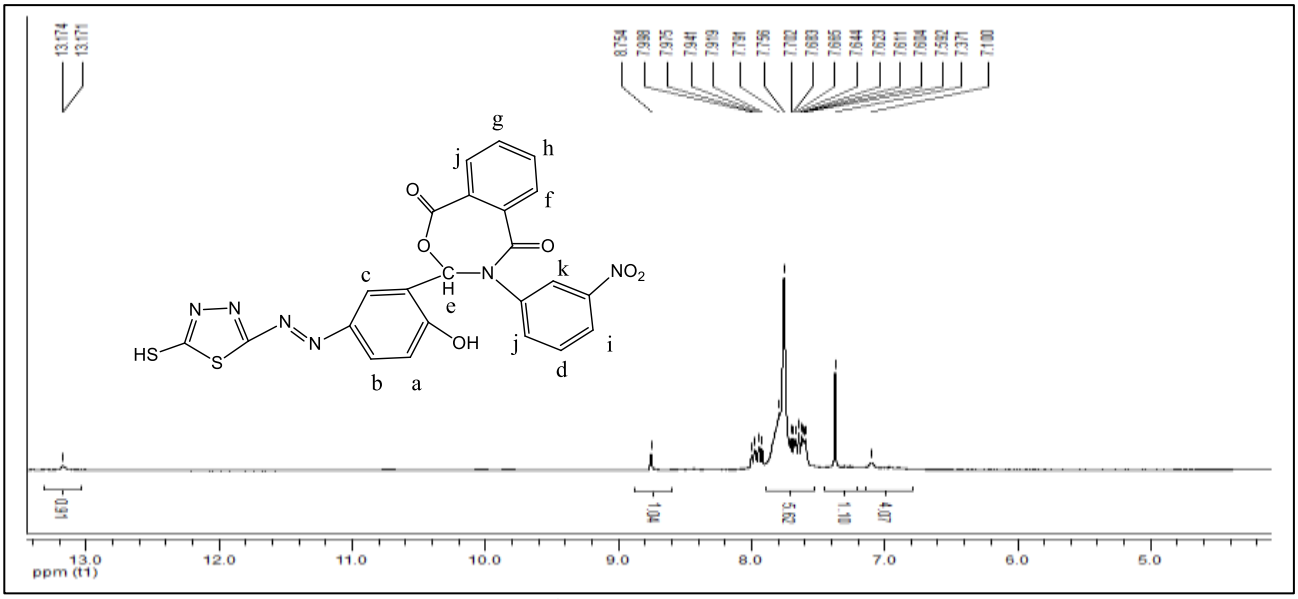


Fig.(17a): Expanded ^{1}H NMR spectrum of compound (4a)

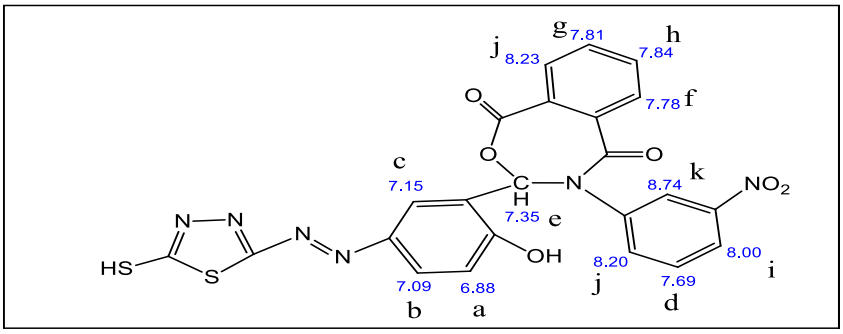


Fig.(17b): Theoretical δ (ppm) values of compound (4a)

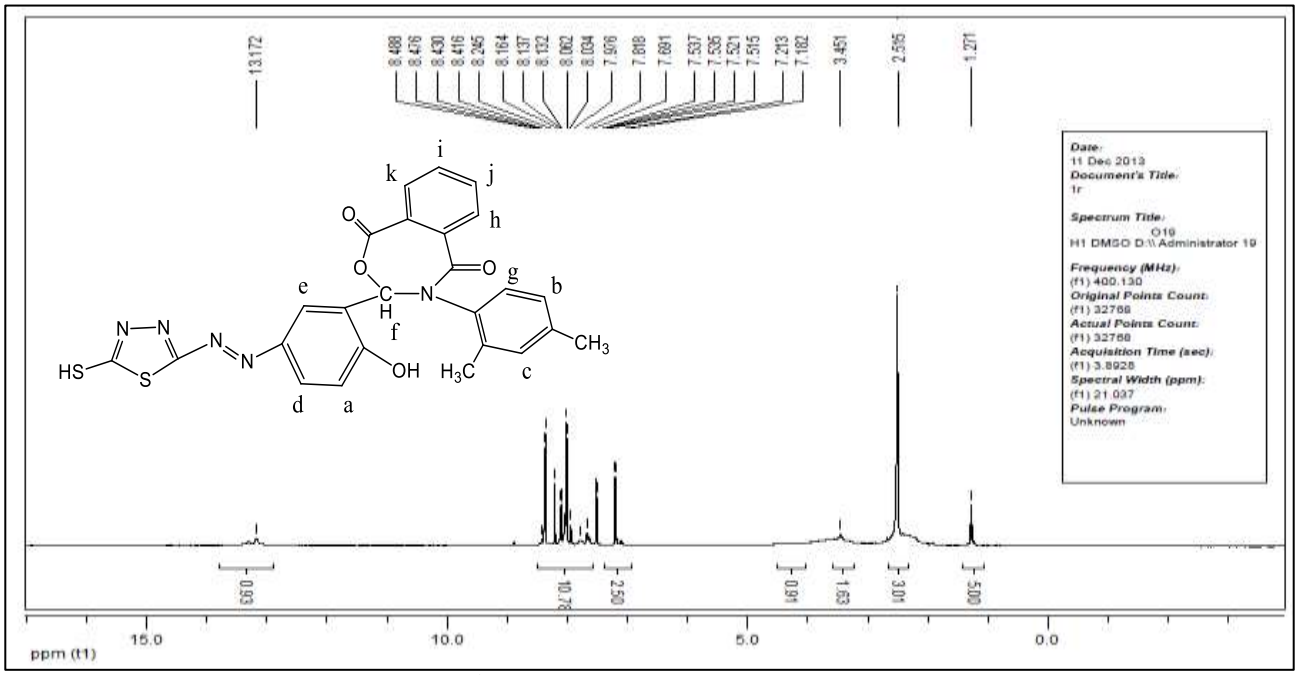


Fig.(18): ^{1}H NMR spectrum of compound (4c)

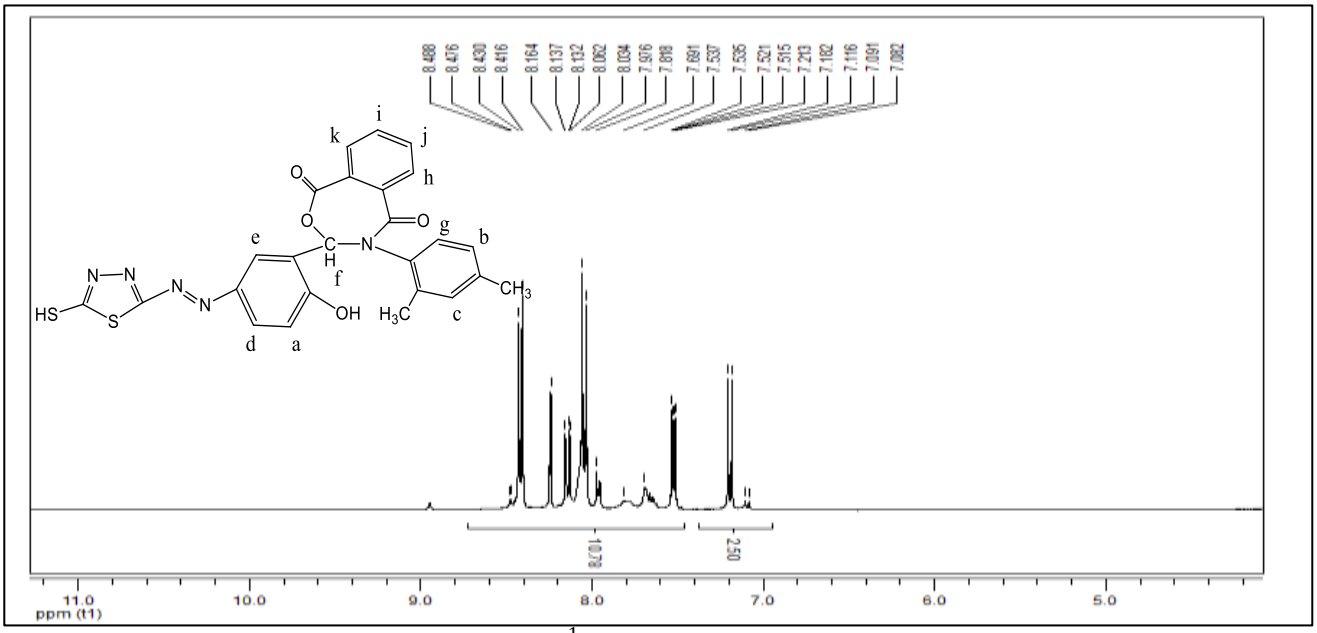


Fig.(18a): Expanded ^{1}H NMR spectrum of compound (4c)

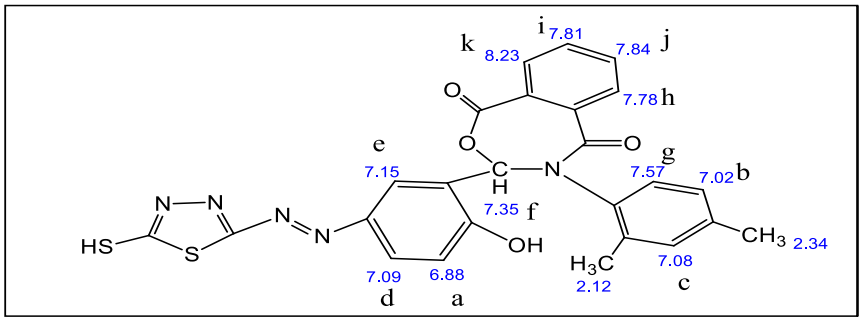


Fig.(18b): Theoretical δ (ppm) values of compound (4c)

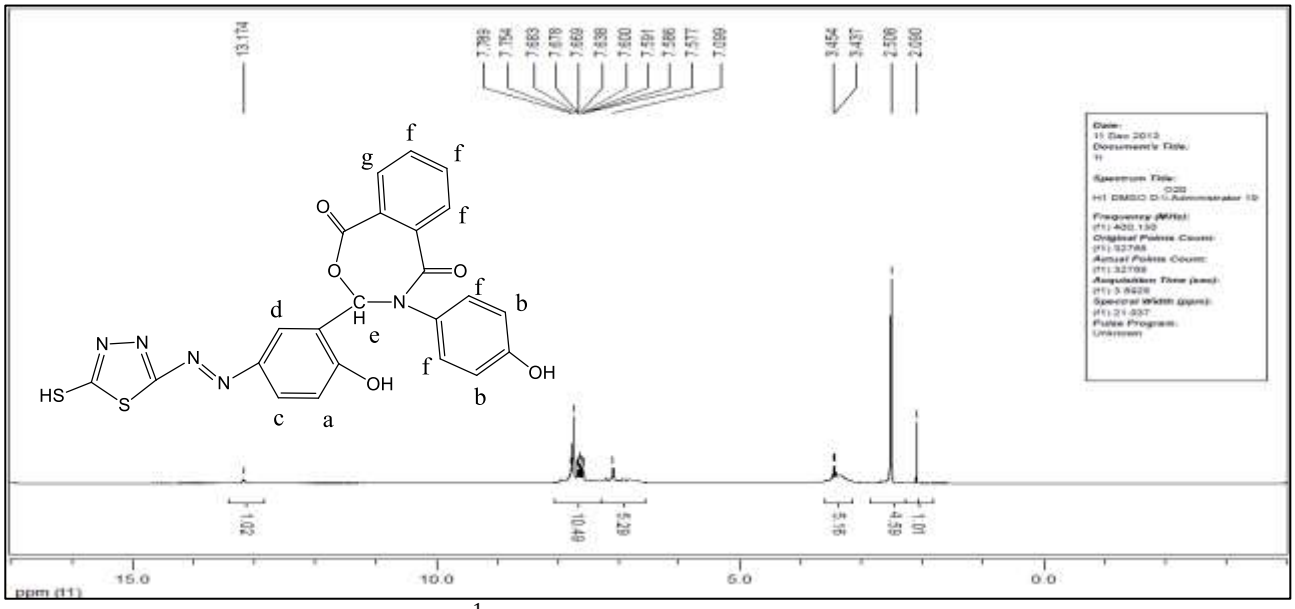


Fig.(19): ^{1}H NMR spectrum of compound (4d)

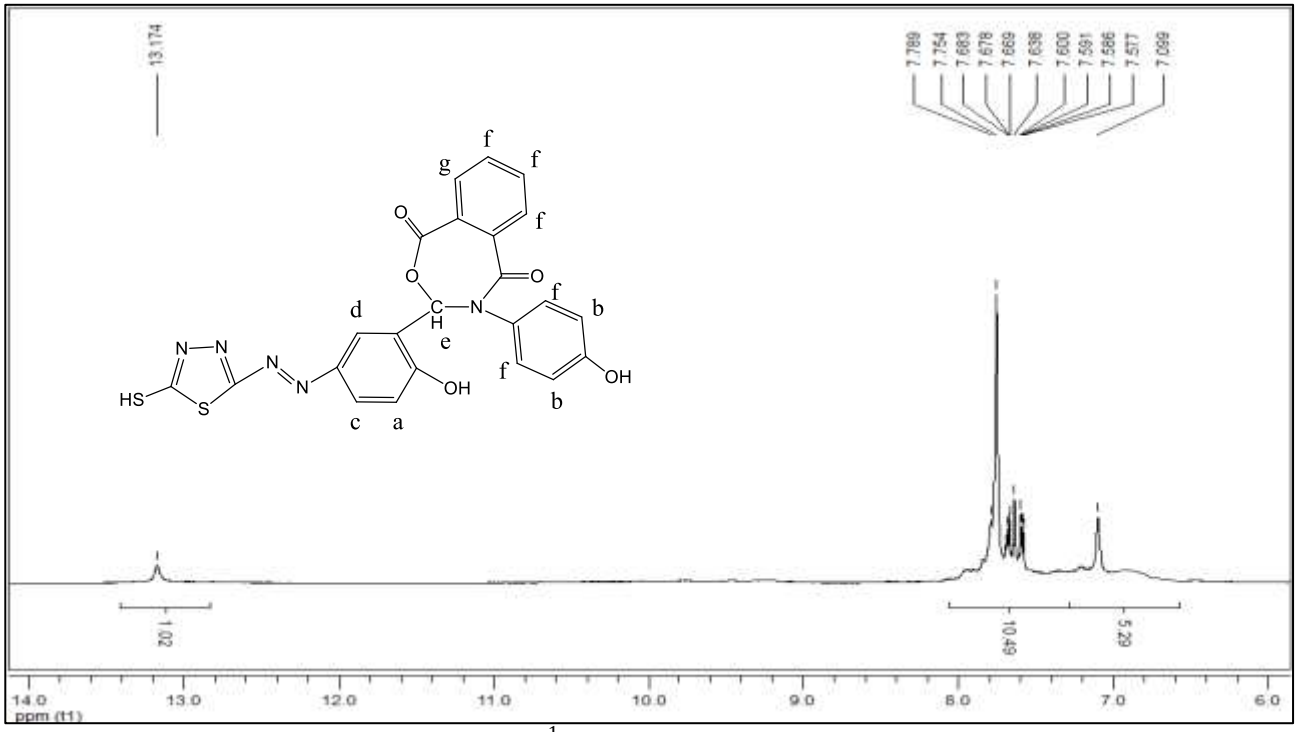


Fig.(19a): Expanded ^{1}H NMR spectrum of compound (4d)

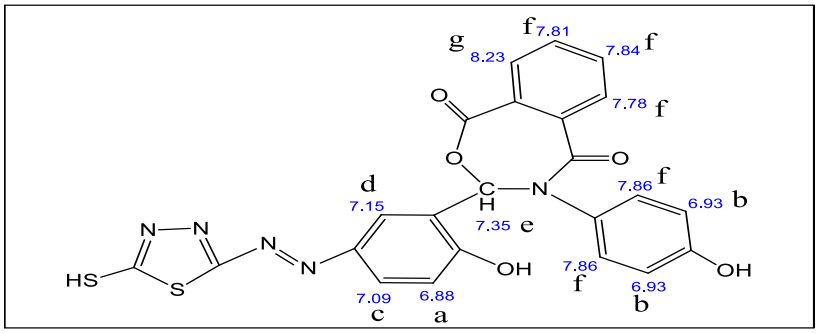


Fig.(19b): Theoretical δ (ppm) values of compound (4d)

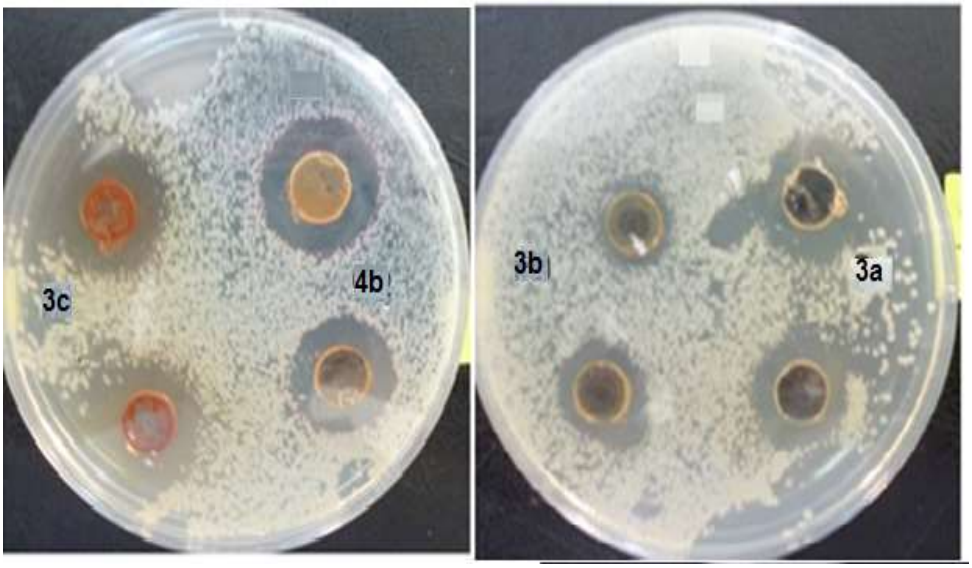


Fig. (20): Antibacterial activity of compounds (3a, 3b, 3c and 4b) against staphylococcus aureous

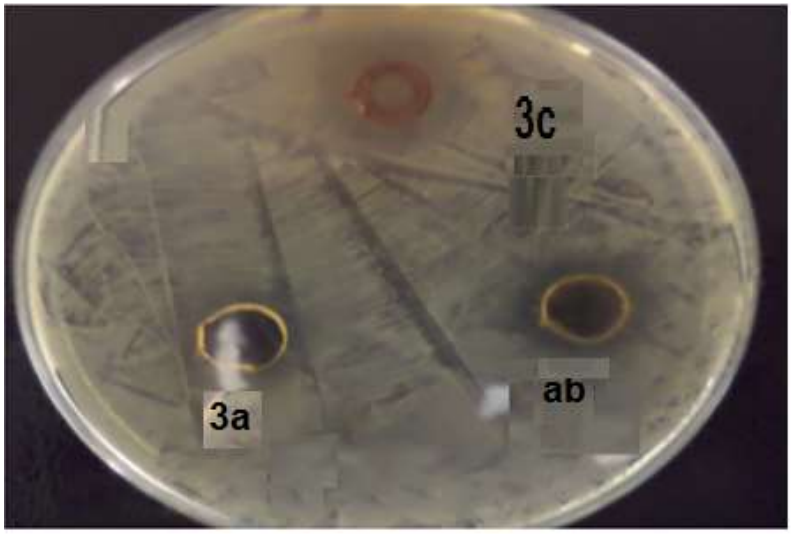


Fig. (21): Antibacterial activity of compounds (**3a**, **3c** and **4b**) against **Escherichia coli**

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