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Syntheses of some new Formazan derivatives , derived frome isoniazid and study their biological activity

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ABSTRACT

This research includes the preparation of a new series of formazan derivatives . In the beginning was prepared hydrazon derivatives through condensation izonzaid with compensators benzaldehyd different , then formazan derivatives were prepared by condensation hydrazon and diazonium salt different compensation for aromatic amines. All the reaction completion were routinely monitored by TLC.The structures of the compounds have been confirmed by ,(C.H.N) analysis of the elements for some compounds , 1H NMR, IR spectral data and melting points. The antibacterial activity of the compounds has also been screened.

Keywords: *formazans, diazonium salt , hydrazone ,Antimicrobial activity.*

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تحضير مشتقات فورمازان جديدة ، مشتقة من الايزونزايد ودراسة فعاليتها البايولوجية

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الملخص

يتضمن هذا البحث تحضير سلسله جديدة من مشتقات الفورمازان . في البداية تم تحضير مشتقات الهيدرازون من خلال تكاثف ألايزونزايد مع معوضات البنزالديهايد المختلفة ،ثم بعد ذلك تم تحضير مشتقات الفورمازان بواسطة تكاثف الهيدرازون وملح الدايازونيوم لامينات اروماتية مختلفة التعويض . وتمت متابعة التفاعلات باستخدام تقنية كروموتوغرافيا الطبقة الرقيقة وشخصت المركبات المحضرة بواسطة أطياف الرنين النووي المغناطيسي للبروتون والأشعة تحت الحمراء وطيف التحليل الدقيق للعناصر،وقيست درجة الانصهار لجميع المركبات المحضرة . كما تم فحص الفعالية المضادة للبكتريا لبعض المركبات .

الكلمات الدالة : الفورمازان ، ملح الدايازونيوم ، الهيدرازون ، الفعالية المضادة للبكتريا .

١. INTRODUCTION

Isoniazid (isonicotinic acid hydrazide, INH) has been a front_line anti_tubercular agent for decades, but only recently has an understanding of its action against Mycobacterium tuberculosis emerged [1] . It was shown that superoxide stimulates INH activity in mycobacteria. Activation of INH may also affect DNA , proteins,and other macromolecules

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through formation of (ROS, reactive oxygen species) [2] .Thus, oxidative activation of INH can both have a specific effect on mycolic acid synthesis and be generally toxic for proteins and nucleic acids. The limited number of effective anti_tuberculosis drugs available and the problems associated with drug resistance and potential adverse reactions such as hepatotoxicity became a prerequisite for synthesis of more effective analogs of INH. We have synthesized isonicotinoylhydrazones**,** analogs of INH with different substitutes in the benzene ring at the N′ position [3] .The present study was undertaken to determine the superoxide scavenging activity (SSA, superoxide scavenging activity) of newly synthesized isonicotinoilhydrazone analogs of isoniazid with respect to their tuberculostatic activity and acute toxicity.Further, we hypothesized that antioxidant action of the isonicotinoylhydrazones may be responsible for the bene ficial effects of these compounds .

Formazans are compounds which contain the characteristic chain of atoms N=N–C=N–NH [4] , Formazans have been found to possess important medical applications due to their various activities [5]. such as antimicrobial [6] , analgesic, antifungal [7] , anticancer, anti-HIV [8] etc. Several formazans showed promising anti-fertility, anti-parkinsonian and anticonvulsant activities. Our idea was to combine Azomethine group (−CH=N−) and azo group (−N=N−) in one single molecule to get formazan derivative [9] .

2. Experimental

Melting points were determined in open capillaries on Electrothermal melting point apparatus(Electrothermal Engineering LTD S-N 10853) and are uncorrected. (^{1}HNMR) &(^{13}C-NMR) spectra were recorded on a Bruker AM 300 (300 MHz) instrument using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in parts per million (ppm). Splitting patterns are designated as follows: s- singlet, d- doublet, t-triplet , q- quartet and m- multiplet. The reactions were followed on pre-coated TLC plates (Silica gel 60 F254, Merck), visualizing the spots in ultraviolet light. The IR spectra were recorded on Shimadzu FTIR 8400S by using KBr disc method scan(400-4000)cm-1.

٢.1. Preperation of N-Benzyldin isonicotino hydrizide [1a-b] .

A solution of isoniazid (0.0072 mol , 1gm) in absolute ethanol (15 ml) was slowly added to a solution of aromatic aldehydes (0.0072 mol) in absolute ethanol (15 ml) with addition (3-4)drop Glatial acetic acid. The stirred reaction mixture was refluxed for 4 h. After cooling, a

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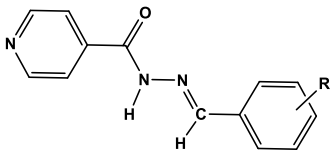
precipitate was formed which was collected by filtration, then washed with cold ethanol, and recrystallized from appropriate solvent [10]. The results are shown in Table (1).

2.2. Preparation of (E)-5-Isonicotinoyl-1,3-dipheny formazan[2a-d , 3a-e].

A cold stirred solution of amine derivatives (0.001 mol) previously dissolved in aqueous HCl (10 mL) was diazotized over crushed ice by drop wise addition of cold aqueous solution of $NaNO_2$(0.0069 g) with stirring till a clear solution of diazonium salt of respective amine was obtained[11].

The temperature was at 0-5 ^oC. This mixture was then poured into a cold solution of hydrazone (0.3gm , 0.001 mol) dissolved in dry pyridine (7 mL). The reaction mixture was further stirred for 2 h maintaining temperature 0-5 ^oC. The mixture was then poured into water with continuous stirring. then filtered and washed with water till the exceess of pyridine was removed , dried and recrystallized from appropriatd solvent[12]. The results are shown in Table (2).

Table (1): Physical properties of the derivative hydrazon (1a-b) record



Comp. No.	R	Molecular Formula M.Wt. g/mol	Colour	M.P ^0C	Yield%	RF	Rec.Solv.
1a	**$-N(CH_3)_2$**	$C_{15}H_{16}N_4O$ 268	Yellow	190-192	52	0.91	EtOH
1b	**3,4,5- $-OCH_3$**	$C_{16}H_{17}N_3O_4$ 315	White	217-219	89	0.92	EtOH

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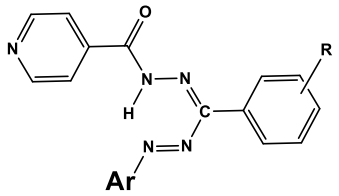
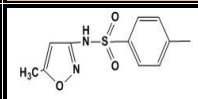
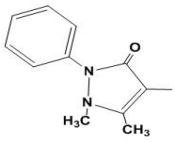
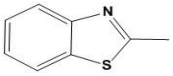
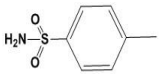
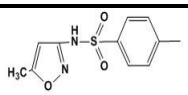
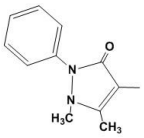
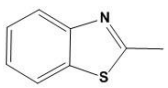
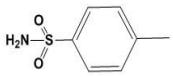
Table (2): Physical properties of the derivative Formazan (2a-d,3a-e) record



Comp No.	R	Ar	Molecular Formula M.Wt. g/mol	Colour	M.P ^{0}C	Yield %	RF	Rec.Solv.
2a	**-N(CH3)$_2$**		$C_{25}H_{24}N_8O_4S$ 532	light green	170-172	60	0.62	Diethyl ether
2b	**-N(CH3)$_2$**		$C_{26}H_{26}N_8O_2$ 469	light green	130-132	51	0.53	Acetone
2c	**-N(CH3)$_2$**		$C_{22}H_{20}N_7OS$ 430	light green	154-156	80	0.70	EtOH
2d	**-N(CH3)$_2$**		$C_{20}H_{21}N_7O_3$ 407	Yellow Shiny	205-207	58	0.50	EtOH
3a	**3,4,5-OCH$_3$**		$C_{23}H_{20}N_6O_4S$ 476	Yellow	190-192	54	0.75	Diethyl ether
3b	**3,4,5-OCH$_3$**		$C_{27}H_{27}N_7O_5$ 529	Milky	195-197	60	0.91	Acetone
3c	**3,4,5-OCH$_3$**		$C_{22}H_{21}N_6O_6S$ 497	Yellow	116-118	62	0.88	EtOH
3d	**3,4,5-OCH$_3$**		$C_{22}H_{21}N_6O_6S$ 497	Dark yellow	250*	59	0.50	Diethylether Chloroforme +
3e	**3,4,5-OCH$_3$**		$C_{22}H_{20}N_5O_4Br$ 498	Yellow	١٤٧-١٤٥	٦١	0.62	EtOH

***Decomposation**

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3. Results and discussion

The physical properties of hydrazon and novel formazans derivatives are Presented in Table (1) .The compounds are quite stable in dry air and they are soluble in most organic solvent. Synthetic routes leading to target compounds are summarized in Scheme1.The structure of these compounds were proven on the basis of melting points and spectral data.

3.1. IR spectra

The IR spectra of all compounds in this study are recorded in the solid state using KBr disk technique. Selected bands of diagnostic importance are listed in Table (3,4). The formation of hydrazone (1a-b) was indicated by their IR spectra from the appearance of azomethine (CH=N) stretching band at 1674,1612 cm^{-1} combined with the disappearance of IR absorption band in region 3378 cm^{-1} and 1710 cm^{-1} corresponding to NH_2 group and C=O group of 2-amino benzothiazole (1) and 3,4,5-tri methoxy benzaldehyde respectively. While formazans derivatives (2a-d ,3a-e) confirmed by the appearance of IR absorption band in the region1437-1455 cm^{-1} due to -N=N- group[13] .See Figures (1,2) .

3.2 . (^{1}H-NMR) spectra

^{1}H-NMR spectra of formazans derivatives ,shows the disappearance of signal at 8.2 ppm due to (CH=N), and the appearance of the aromatic ring protons in the range (7.00 – 8.50 ppm) [14]. ^{1}H- NMR(DMSO- d^6) δ (ppm) Spectra showed the following data: (2c) ^{1}HNMR 3.35 (s,6H,$(CH_3)2$) , 6.96-7.60 (m,13H,Phenyl group-H) , 2.47 (s,(^{6}d-DMSO) , 3.81(d,H_2O) , 10.37 (s,1H,NH) . (3a)^{1}H-NMR 6.57-8.00 (m,11H,Phenyl group-H) , 9.78 (s,1H,NH) , 2.35 (s,6H,$(CH_3)_2$) , 3.72-3.83 (s,9H,$(CH_3)_3$) 2.50 (s,d^6-DMSO) . (3c) ^{1}H-NMR , 11.89 (s,1H,NH), 6.75-8.75 (m,10H,Phenyl group-H), 3.37 (s,9H,$(OCH_3)_3$) , 2.49 (s,(^{6}d-DMSO) , 2.92-2.97(d,H_2O) .(3e) 1HNMR , 8.90 (s,1H,NH) , 3.57 (s,9H,$(OCH_3)_3$) , 6.35-7.43 (m,10H,phenyl group-H) , 2.67 (s,(^{6}d-DMSO).See Figures (3,4) .

3.3. (C.H.N) analysis of the elements for some compounds [14]

The results of element analysis C.H.N of synthesized formazan derivative(2c,3c) is shown in Table (6).

3.4. Antibacterial activity

The effect of some of the prepared compounds in this research on the growth of bacteria , namely **: 1- *Eschershia coli* , 2- *Klebislla* , 3- *Staphylococcus aureus***:

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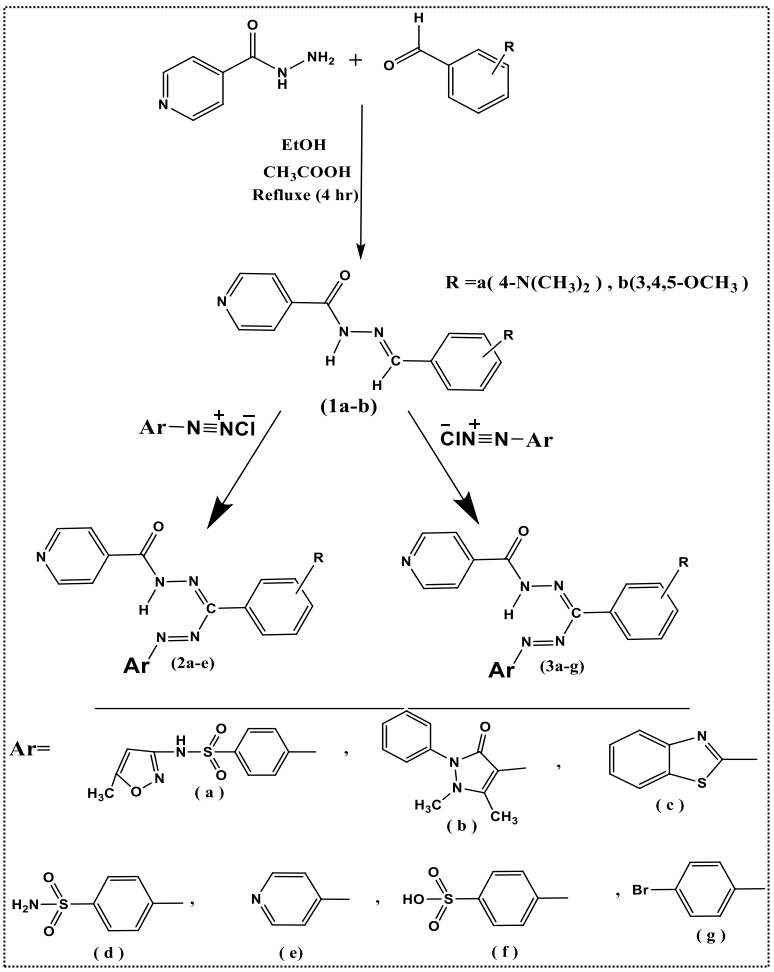
Antibacterial activity of the prepared compounds are studied and the results showed that some of the prepared compounds possess good antibacterial activity[15]. The results are shown in Table (5) , See Fig. (5) .



Scheme (1): Reactions pathways for prepared compounds.

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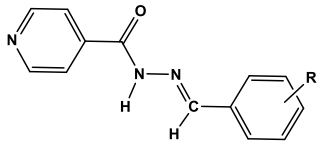
Table (3) : IR absorption bands (cm^{-1}) of Synthesized hydrazon compound(1a-b)



Comp. NO.	R	IR(KBr),cm^{-1}					
		=C-H ν Ar	C=N ν C-N ν	C=C ν Ar	N-H ν C=O ν	ν C-H Out of plane	Others
1a	4-$N(CH_3)_2$	3088	1602 1228	1525 1589	3199 1666	709 817	ν (CH)aliph asy. (2972) ν ν sy.(2819)
1b	3,4,5-OCH_3	3003	1602 1236	1458 1577	3189 1681	748 840	ν (CH)aliph ν asy.(2945) ν Sy. (2838) ν C-O-C asy. (1230) ν ν Sy.(1184)

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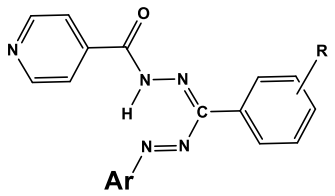
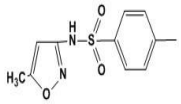
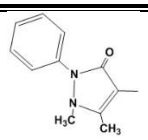
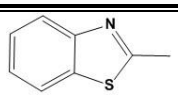
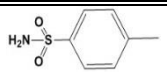
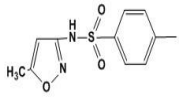
Table (4) : IR absorption bands (cm^{-1}) of Synthesized Formazan Compound (2a-d.)



Comp. NO.	R	Ar	IR(KBr),cm^{-1}					
			V =C-H Ar	*V*C=N *V*C-N	*V*C=C Ar	*V*N-H *V*C=O	*V*N=N	Others
2a	**4-N(CH₃)₂**		3048	1676 1256	1487 1572	3312 1665	1455	(C-H)aliph Vas(2920) Vsy(2855)
2b	**4-N(CH₃)₂**		3002	1620 1254	1591 1525	3402 1651	1477	(C-H)aliph Vasy.(2930) Vsy.(2843)
2c	**4-N(CH₃)₂**		3055	1625 1232	1506 1527	3394 1643	1446	(C-H)aliph Vasy.(2850) Vsy.(2750)
2d	**4-N(CH₃)₂**		3332	1670 1274	1489 1595	3297 1653	1455	(C-H)aliph Vasy.(2930) Vsy.(2780)
3a	**3,4,5-OCH₃**		3084	1620 1270	1459 1482	3188 1599	1457	(C-H)aliph Vas.(2943) Vsy.(2825) VC-O-C(1236)

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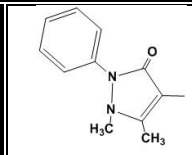
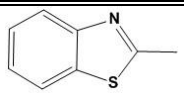
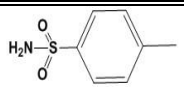
3b	**3,4,5-OCH_3**		3001	1602 1286	1504 1577	3178 1613	1458	(C-H)aliph Vas.(2943) Vsy.(2825) VC-O- C(1236)
3c	**3,4,5-OCH_3**		3150	1604 1230	1525	3394 1591	1444	(C-H)aliph Vas.(2981) Vsy.(2843) VC-O- C(1230)
3d	**3,4,5-OCH_3**		3035	1684 1254	1430 1573	3295 1540	1447	(C-H)aliph Vas.(2899) Vsy.(2812) VC-O- C(1199)
3e	**3,4,5-OCH_3**		3190	1612 1294	1500 1606	3200 1588	1450	(C-H)aliph Vas.(2900) Vsy.(2831) VC-O- C(1238)

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Table (5): In vitro antibacterial activity substituted formazans [2a-d ,3a-e] .

Comp. NO.	Conc.	*E.coli*	*Klebisella pneumonia*	*Staphylococcs aureus*
2b	50	++	–	–
	100	++	–	–
	150	+++	–	–
2c	50	–	–	–
	100	–	++	–
	150	–	+++	–
3a	50	++	–	–
	100	++	–	–
	150	+++	–	–
3b	50	–	–	–
	100	–	–	–
	150	–	–	–
3e	50	–	–	–
	100	–	–	–
	١٥٠	–	–	–

Key to symbols :, Highly active = +++ , Moderately active = ++ , Slightly active = + , Inactive = -

Tabl (6): (C.H.N) analysis of the elements for some compounds [2c , 3c]

Comp NO	Molecular Formula	found					Calculated				
		C%	H%	N%	S%	O%	C%	H%	N%	S%	O%
2c	$C_{22}H_{19}N_7OS$	61.82	3.98	22.9	7.49	3.81	61.85	3.91	23.4	7.52	3.32
3c	$C_{23}H_{20}N_6O_4S$	57.7	4.20	17.70	6.72	13.68	57.90	4.25	17.91	6.74	13.2

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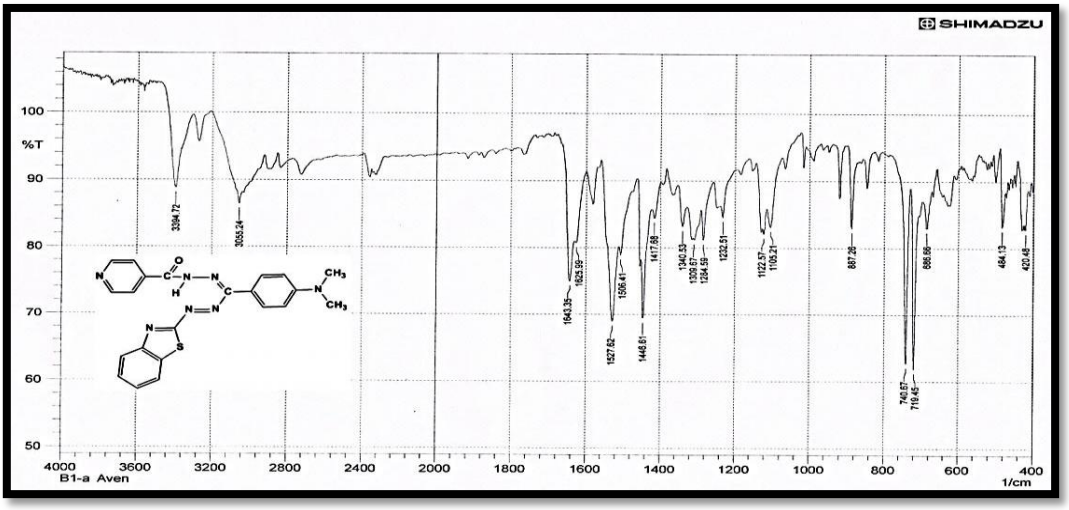

Fig.(1): FT- IR of Compound [2c]

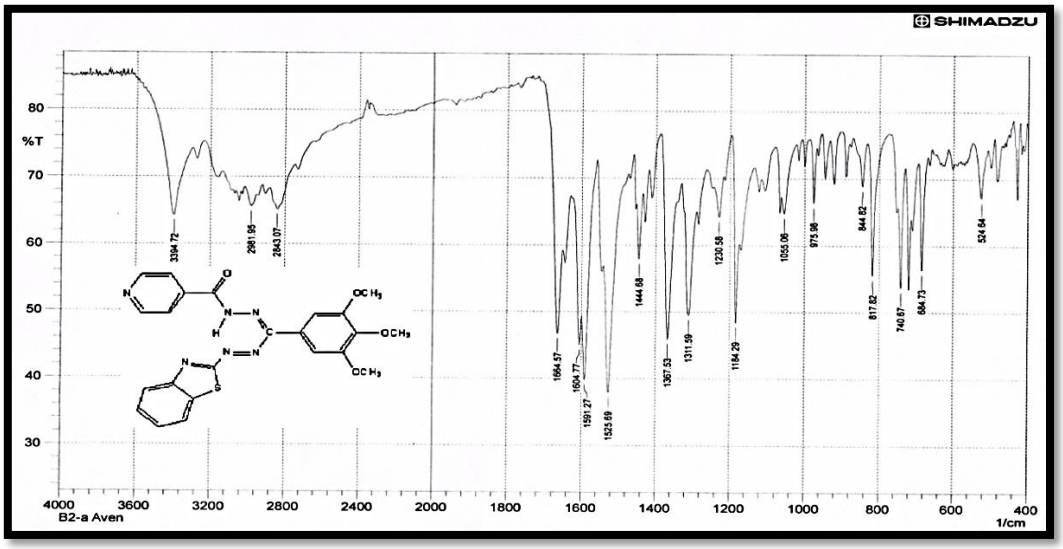

Fig.(2:) FT- IR of Compound [3c]

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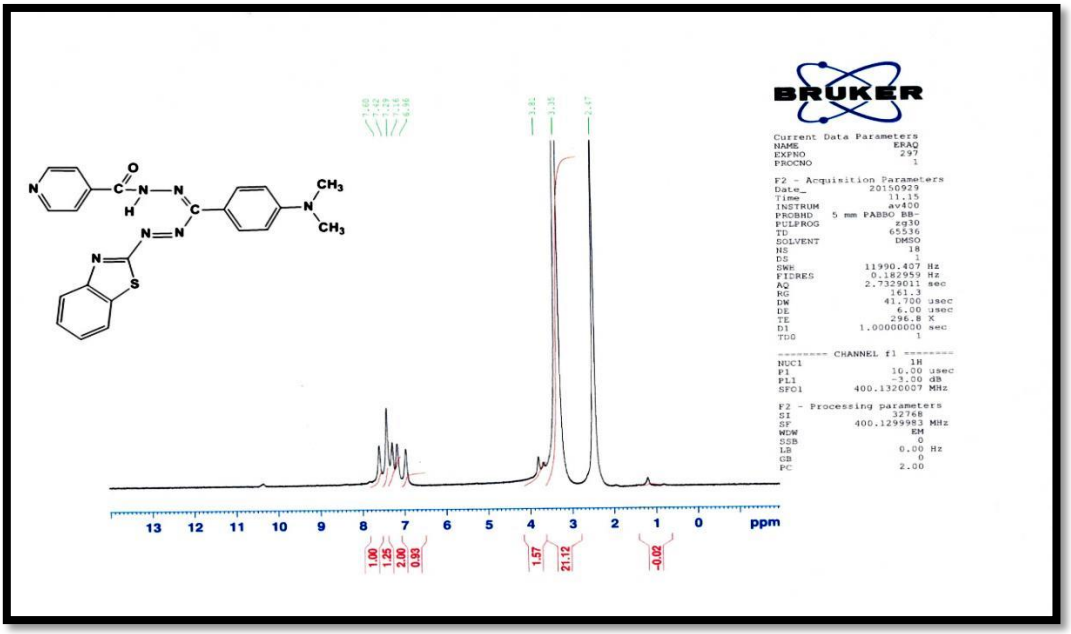

Fig.(3): ^{1}H-NMR of Compound [2c]

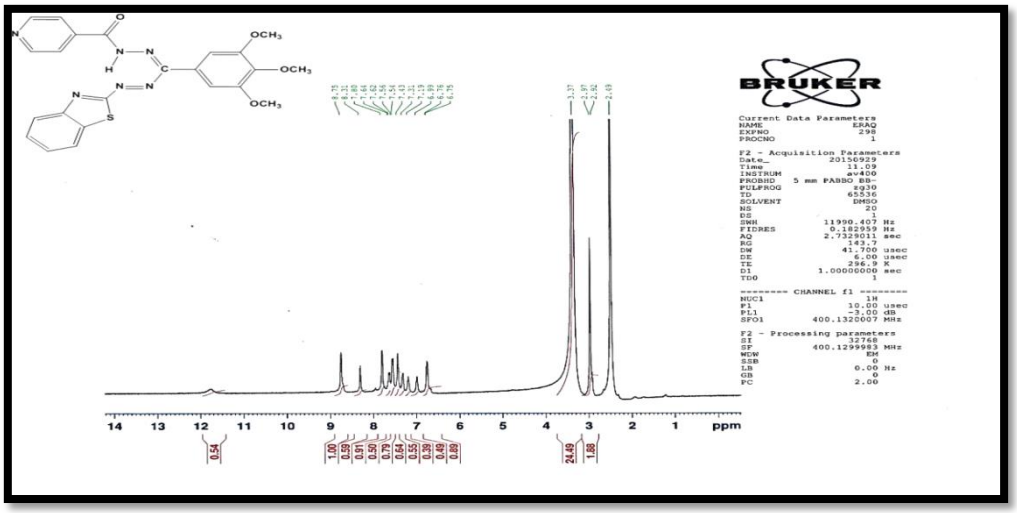

Fig.(4): ^{1}H-NMR of Compound [3a]

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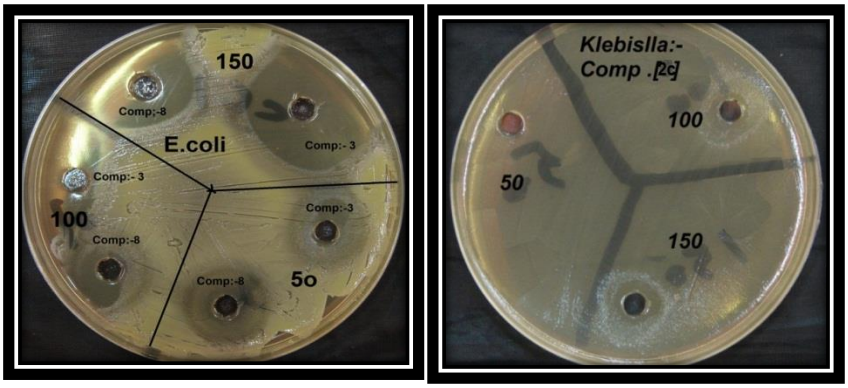

Fig.(5): Inhibition zones of the compounds [2c, 2b , 3a]

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