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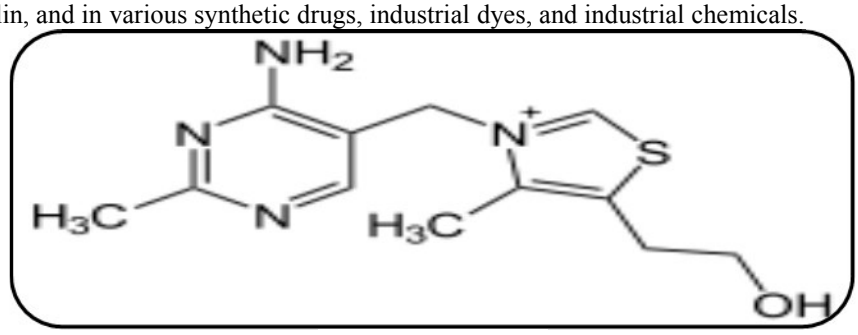
Synthesis with Investigation of New Bio- Chemical Compounds and Studying of (Bio-Chemical ,Chromatography ,Spectral , Analytical) - Behavior

Dr. Nagham Mahmood Aljamali*[1] Dr.Ahmed Mousa Essa[2] Dr.Eman Abdulwahab Alkuwaity[1]
Dr.Amal Saadoon Majeed[3] Rajaa Abed Alameer Gafel[1]
1.Chemistry Department ,Faculty of Education for women., Iraq
2.Jabir Ibn Hayyan – Medical University , Iraq
3.Sub-Department of Basic Science ,Faculty of Nursing ,Iraq

Abstract:
Series bio - compounds prepared in this work which bearing bio – nuclei like thiazole ring via cyclization by acid or azotation reaction in cold medium or condensation by refluxing process , some these compounds included two process at same compound like formazan compound [9 and 10].All formatted compounds [1-10] were identified by using (TLC) and techniques((FT.IR ,^{1}H.NMR ,^{13}C.NMR ,Chromatography Analysis)) ,then studying((biological activity studying , chromatography behavior , studying of physical characterization and other analytical studies like solubility in various solvents)) .
Keywords: formazan , thiazole , bio , active,azo, imine .

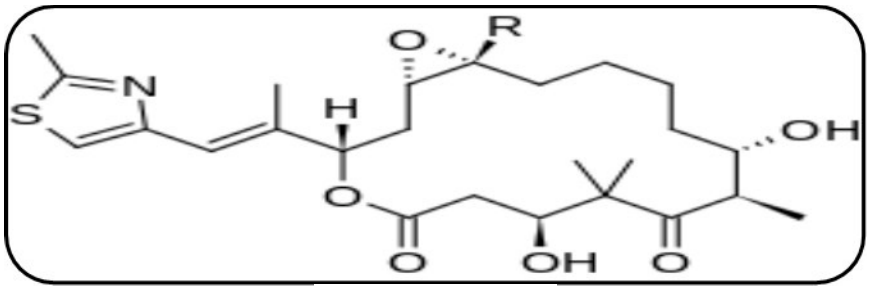
Introduction

Thiazole ring structure included in most of important biologically active natural products like thiamine (vitamin B_1) , the penicillin, and in various synthetic drugs, industrial dyes, and industrial chemicals.



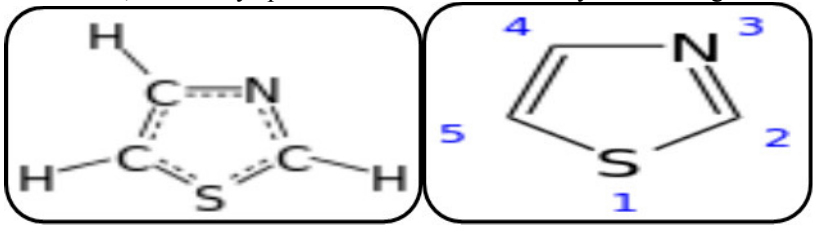
Thiamine - Thiazole

This structure of thiazole cycle leads to that its derivatives exhibit wide potential application in many fields like chemical , biological and material sciences , pharmaceutical in Epothilones which used in treatment types of cancers [(1-5)] .



Epothilons - Thiazole

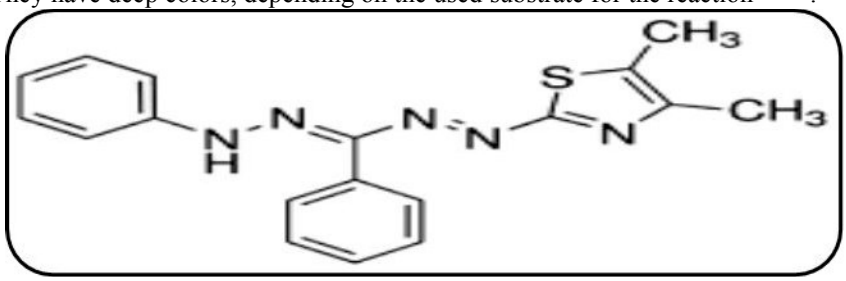
Other thiazole compounds include in some chemical compounds like rhodamine and the dye rhodamine red derived from it, and the dye primuline which used in analytical and organic chemistry[(6-10)] .



Thiazol Ring

Formazan are chromogenic products of the reduction of tetrazolium salts by dehydrogenases process

and reductases. They have deep colors, depending on the used substrate for the reaction[11-18] .



Formazan Structure - Example

Experiments & Instruments

Melting points were recorded on Gallenkamp melting point apparatus and were uncorrected . FT-IR spectra were recorded by using (FT-IR 8300 Shimadzu) in the range (400-4000) cm^{-1} as KBr discs .

^{1}H.NMR–spectra in DMSO– d6 solvent were carried out in Canada , ^{13}C.NMR–spectra in DMSO– d6 solvent were carried out in Canada , and chromatographic Analysis in Canada, physical and analytical studies in Baghdad university , biological studying in Kufa university .

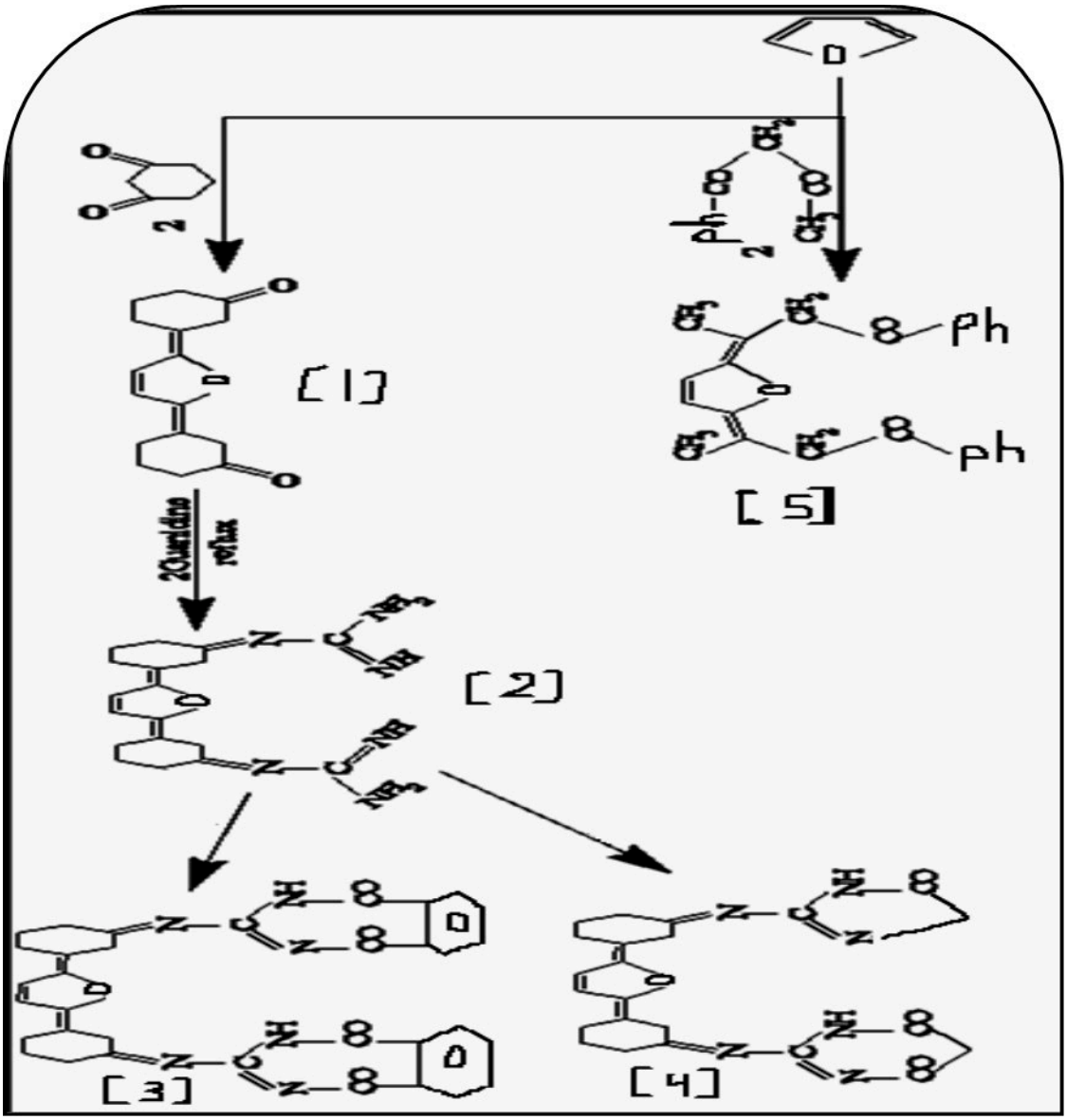
Methodology

Synthesis of Compounds [1 - 4]:

(0.01mole) Of furan and 1,3-dione cyclohexan (0.02mole) were reacted in presence of acetic acid to produce compound [1] ,which (0.01mole) refluxed with guanidine (0.02mole) in presence of absolute ethanol with drops of glacial acetic acid for (3hrs) , the precipitate was filtered and dried with re crystallized to yield compound [2] ,which (0.01mole) reacted with (0.02mole) of (di ethyl phthalate or chloro ethyl acetate) respectively in presence of absolute ethanol with refluxing for (7hrs) according to studies[12], the precipitates filtered, re crystallized from ethanol to produce compounds[3] and [4] respectively.

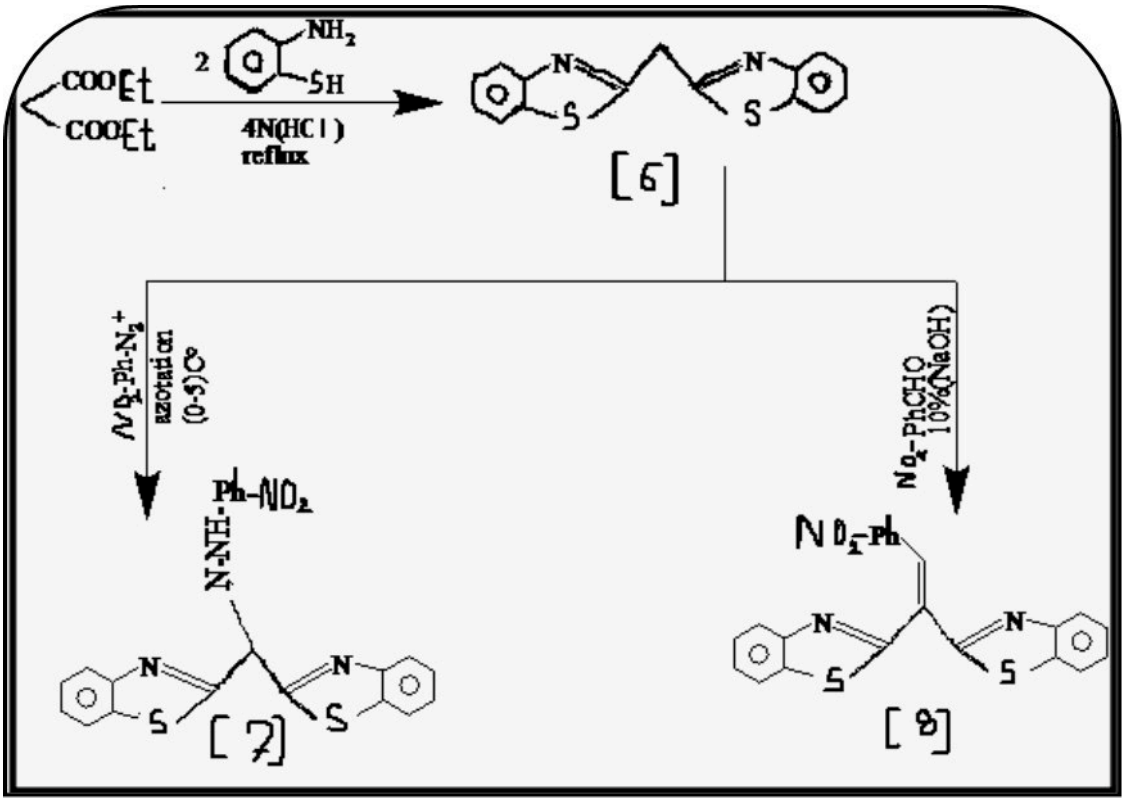
Synthesis of compound [5] :

According to studies[12] ,(0.01mole) of furan refluxed with (0.02mole) of acetyl acetophenone in presence of acetic acid for (4hrs) , the precipitates filtered , dried to give compounds [5] .



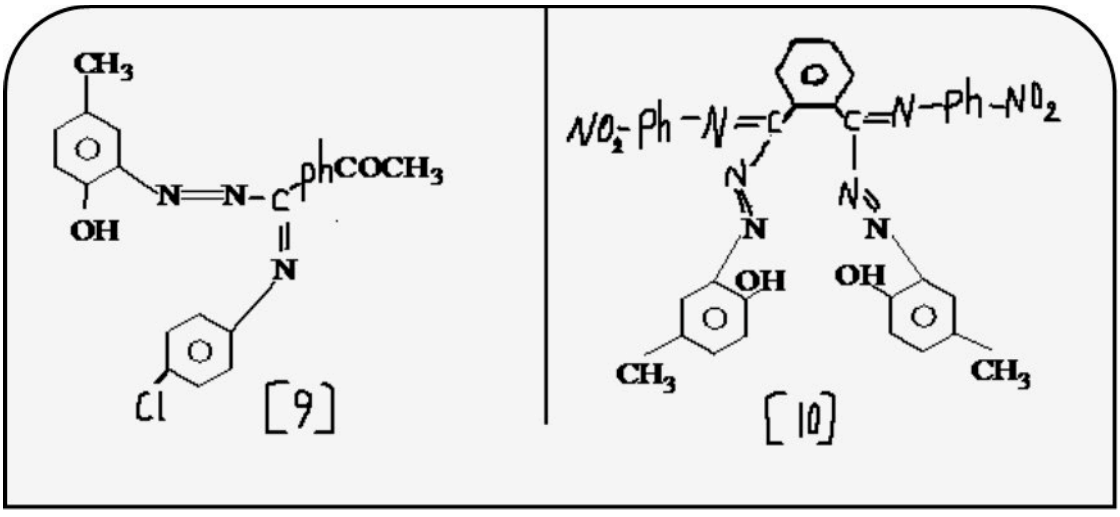
Synthesis of Compounds [6 - 8]:

Di ethyl malonate (0.01mole) refluxed with o-thiol aniline (0.02mole) in presence of (4N) of concentrated hydrochloric acid for (7hrs) according to studies[12] , then , the precipitation filtered , dried to give compound [6] , which reacted in base medium with [4-nitro phenyl azo at (0-5)C° or with 4-nitro benzaldehyde] respectively to give compounds [7] and [8] respectively .



Synthesis of Compounds [9 ,10]:

(0.01mole) Of p-chloro aniline was refluxed with (0.01mole) of 4-aceto benzaldehyde for (4hrs) in presence of (glacial acetic acid), to produce precipitation which filtered and dried then re crystallized to yield imine compound , which reacted in ethanol in beaker , while 2-amino-4-methyl- phenol dissolved in (3ml) of concentrated hydrochloric acid ,then solution of sodium nitrite was added ,after that reaction with mixture solution according to papers[15 ,16] , after (48 hrs) gave precipitation which filtered and dried then re crystallized to yield Compound [9] , while (0.01 mole) of 0-diformal benzene refluxed with (0.02mole) of p-nitro aniline in presence of ethanol for (4 hrs) to yield di imine compound which reacted with azo compound (4-methyl hydroxy benzene azo) salt to produce compound [10].



Results and Discussion :

In the present work of our work, we synthesized Monomers [1- 10] and will identified them by spectral methods like (FT.IR , H.NMR , ^{13}C.NMR)and studying of various analytical measurements with bio chemical behavior like

(biological activity , Solubility in series solvents , chromatography behavior) :

Spectral Studying :

The FT.IR-spectrum showed an absorption bands at (1705 -1716)cm^{-1} in compounds [1 ,5, 9, 10] due to the carbonyl of ketone group (-CO-)., while other bands are appeared at {(3340- 3190)cm^{-1} for (NH) amide and amine groups in compounds [2 ,3,4,7] respectively .While compounds [9 and 10]] appeared bands at (1467 , 1484)cm^{-1} for azo groups (-N=N-) ., and other bands in Table (1) , and some Figures (1 -4)

Table (1): FT.IR- data (cm^{-1}) of Compoundss [1 - 10] .

Compounds	I.R$_{(KBr)}$ ((Only Important Groups))
[1]	(C-O-C) ether of furan : 1180 ., (C=0) ketone :1705
[2]	(C=N) :1619 , (NH_2) amine : 3340 ,3310
[3]	(NH): 3320, (C=N) endo cycle :1605, (C=N) imine: 1630 , (CO-NH)carbonyl of amide :1688.
[4]	(NH): 3330, (C=N) endo cycle :1610, (C=N) imine: 1627 ,(CO-NH)carbonyl of amide :1692.
[5]	(C-O-C) ether of furan : 1194 ., (C=0) ketone :1712
[6]	(C-S) thiazol: 690, (C=N) endo cycle: 1608, (CH)aliph: 2950
[7]	(C-S) thiazol: 690, (C=N) endo cycle: 1608 , (NH) :3190 ,(NO_2): 1390
[8]	((C-S) thiazol: 696, (C=N) endo cycle: 1614 , (=CH)alkene: 3097 ,(NO_2): 1376
[9]	(OH): 3400, (C=N):1620 , (N=N) Azo: 1484 , (CO)ketone: 1716 , (C-Cl): 734 ,.
[10]	(OH): 3455, (C=N):1615 , (N=N) Azo: 1467 , (CO)ketone: 1714 ,(NO_2): 1364.

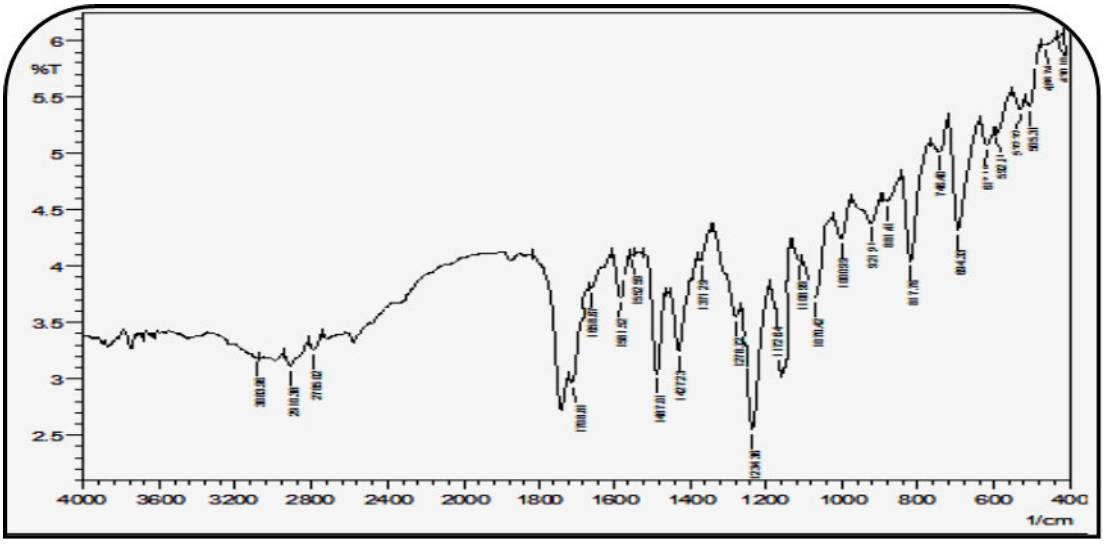


Fig (1) :FT.IR of Compound[1]

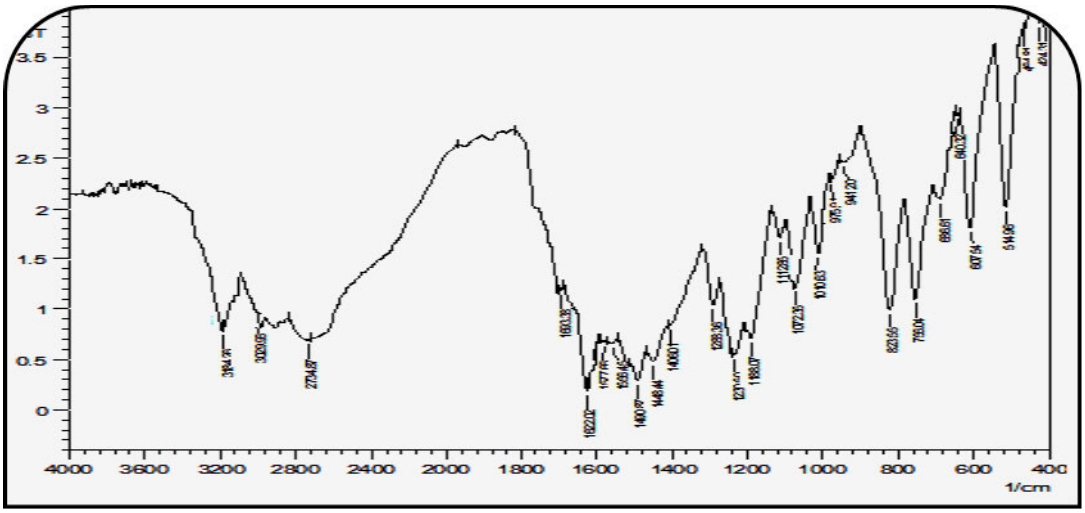


Fig (2) :FT.IR of Compound[3]

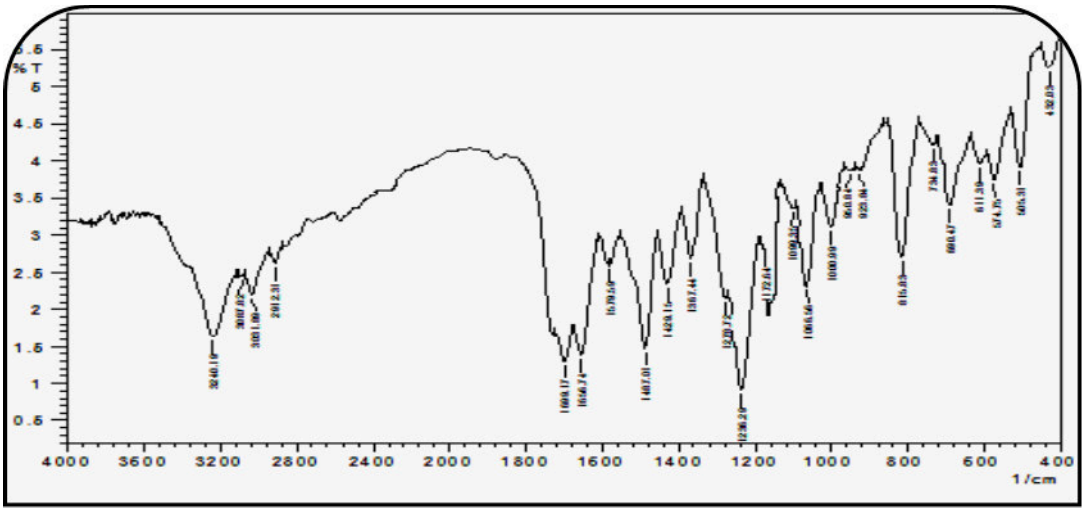


Fig (3) :FT.IR of Compound[4]

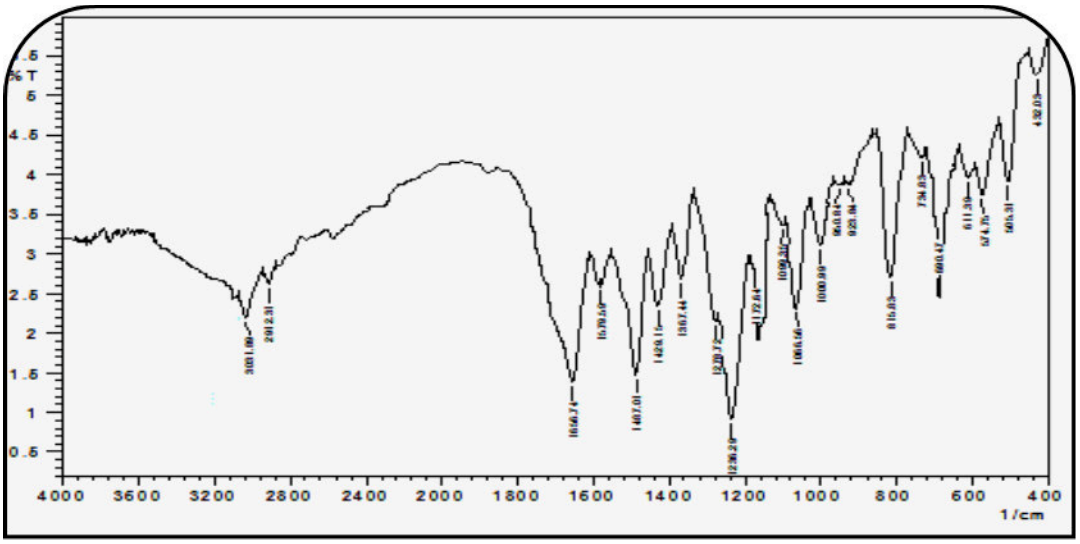


Fig (4) :FT.IR of Compound[6]

The ^{1}H.NMR- Spectra: showed signals at Ɓ {Ɓ (0.66 - 1.55) for proton of cyclo hexane in compounds [1- 4] respectively .

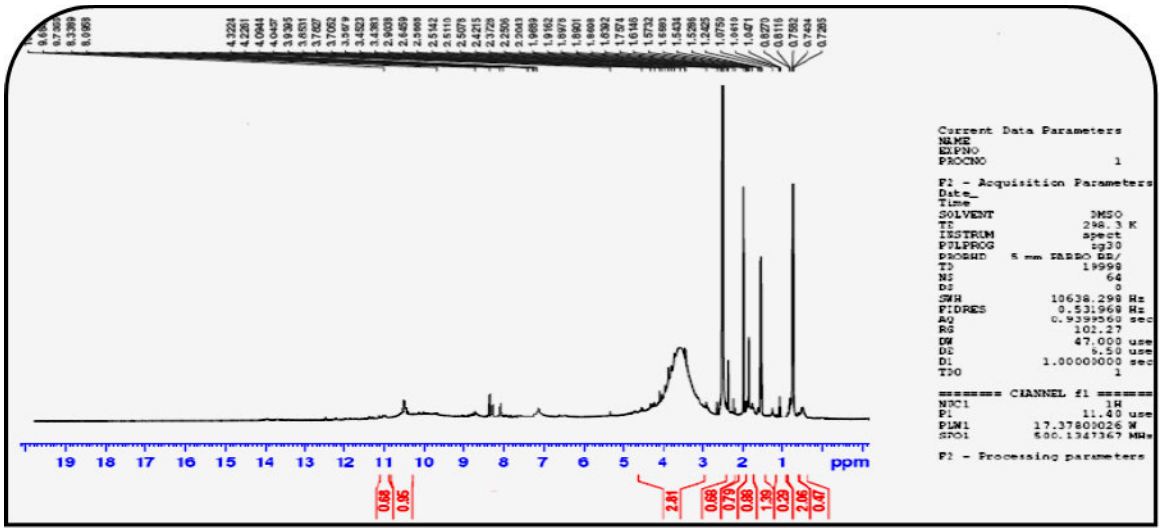
While compounds [2 ,7] showed signal for protons of (NH) amine at Ɓ (5.10 -5.56) respectively .

Compounds [9 , 10] showed signals at Ɓ(11.14 – 11.23) for proton of (OH) phenol groups, respectively .

Compound [3 ,4] showed signals at Ɓ(10.09 , 10.12) proton of amide (NH-CO-)., and other signals in table (2) , and Figures (5-8) .

Table (2): H.NMR- data (Ɓ - ppm) of Compounds [1-10]

Compounds	H.NMR ((Important Peaks))
[1]	protons of cyclo hexane : (0.78 – 1.55).
[2]	(NH_2) proton of amine group: 5.56 , protons of cyclo hexane :(0.66 – 1.52).
[3]	protons of cyclo hexane : (0.72 – 1.43) ,(NH-CO)amide: 10.09, (Ph-)protons of phenyl group :(6.97-7.65).
[4]	protons of cyclo hexane : (0.86 – 1.31) ,(NH-CO)amide: 10.12, (N-CH_2-CO) : 3.35 .
[5]	(CH_3) :0.69 ,(CH_2-CO) ketone: 2.78 ,(Ph-)protons of phenyl groups :(6.88-7.74). .
[6]	(Ph-)protons of phenyl groups :(6.86-7.49) ,(-CH-) :1.07 .
[7]	(Ph-)protons of phenyl groups :(6.90-7.54) ,(-CH_2-) :0.95 , (NH):5.10.
[8]	(Ph-)protons of phenyl groups :(6.89-7.75) ,(C=CH-) :6.08 .
[9]	(OH): 11.23 , (Ph-)protons of phenyl groups :(6.86-7.63) , (CH_3):0.91 ,(CH_3-CO): 2.98 .
[10]	(OH): 11.14 , (Ph-)protons of phenyl groups :(6.73-7.82) , (CH_3):1.19



Fig(5): H.NMR of Compound [4]

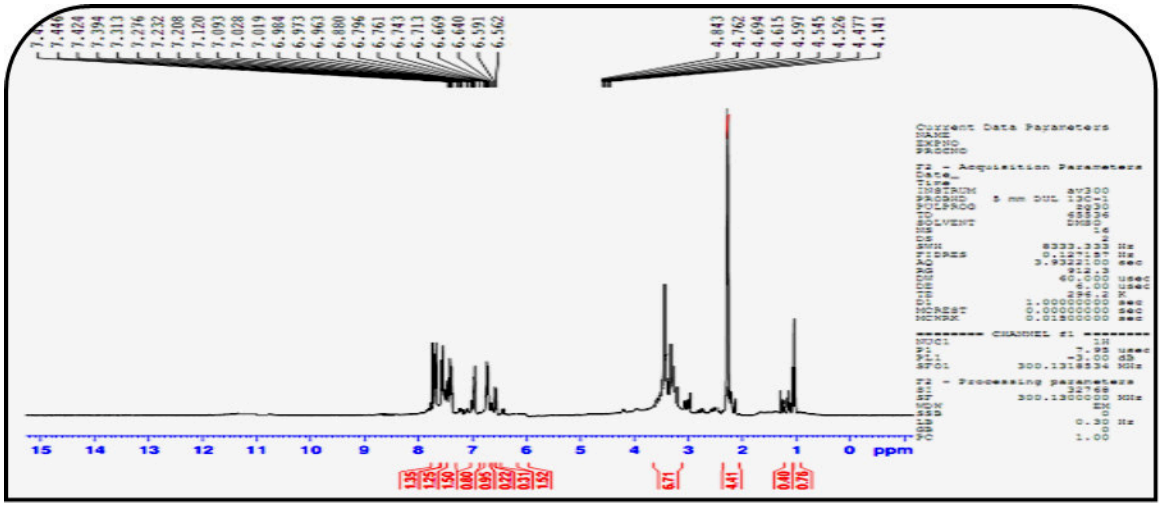


Fig (6): H.NMR of Compound [5]

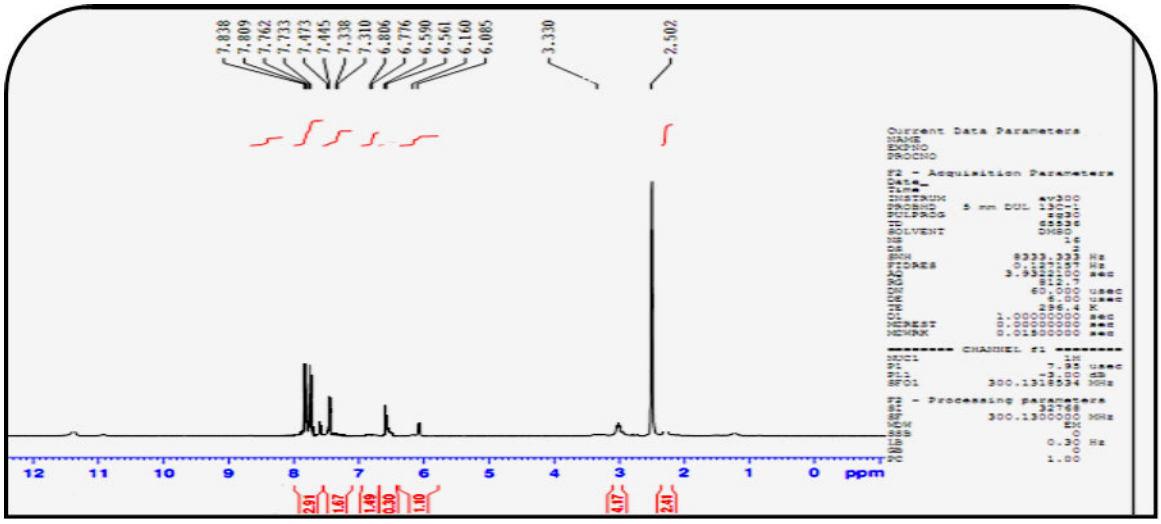


Fig (7): H.NMR of Compound [8]

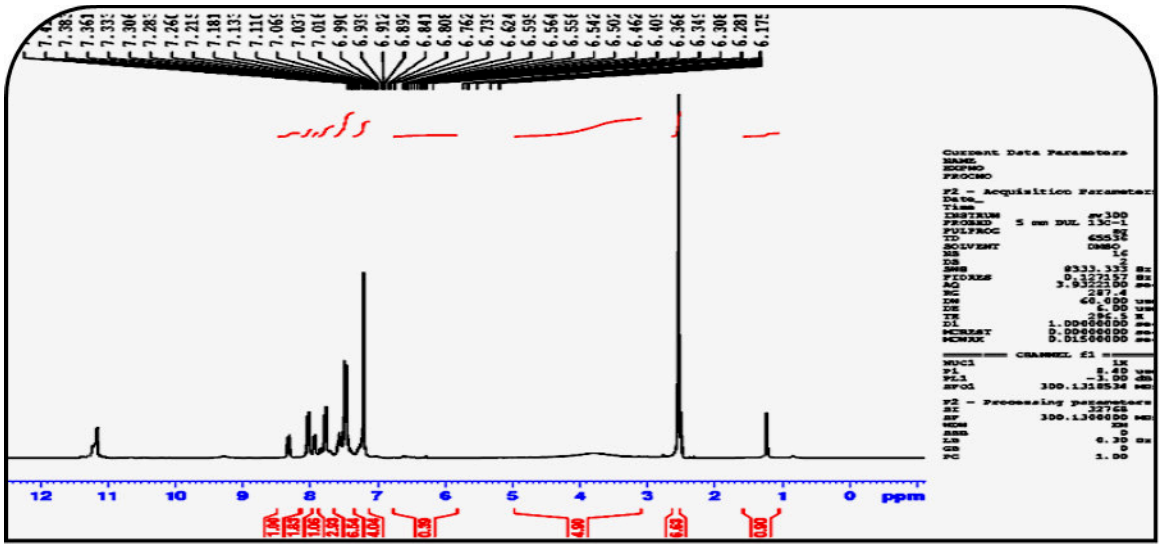


Fig (8): H.NMR of Compound [10]

The ^{13}C.NMR spectral data of some compounds showed peaks indicated to important active groups[15-18] in these compounds , table (3) and figures (9-11) .

Table (3): ^{13}C.NMR- data of Compounds

Comp. No.	^{13}C.NMR-data ((only Important Peaks))
[1]	(136-145):(C, furan)., (11.1-20.3): (C, aliphatic carbon of cyclo hexane), (100 ,105): (C=C)alkene , (192): (CO)ketone.
[2]	(132-150):(C, furan)., (10.8-28.7): (C, aliphatic carbon), (101 ,103): (C=C)alkene , (114,112):(C=N) ,(117, 119):(C=NH): (98.6)
[3]	(138-155):(C, furan)., (15 -22): (C, aliphatic carbon), (100 ,105): (C=C)alkene , (110,114):(C=N)exo ,(118, 120):(C=N)endo , (161):(CO-NH)amide , (123-127): (C , phenyl groups).
[4]	(144-153):(C, furan)., (9 -18): (C, aliphatic carbon), (104 ,108): (C=C)alkene , (113,115):(C=N)exo ,(118, 121):(C=N)endo , (161):(CO-NH)amide .
[6]	(26.3):(CH_2)., (140-135):(thiazole rings)., (117-129):(C, aromatic groups).
[8]	(105, 109):(CH=C) alkene, (148-140):(thiazole rings)., (116-135):(C, aromatic groups).
[9]	2(CH_3): (15 , 22) ,(197) :(CO-)ketone , (109): (C=N)imine , (118-140):(C, aromatic groups).

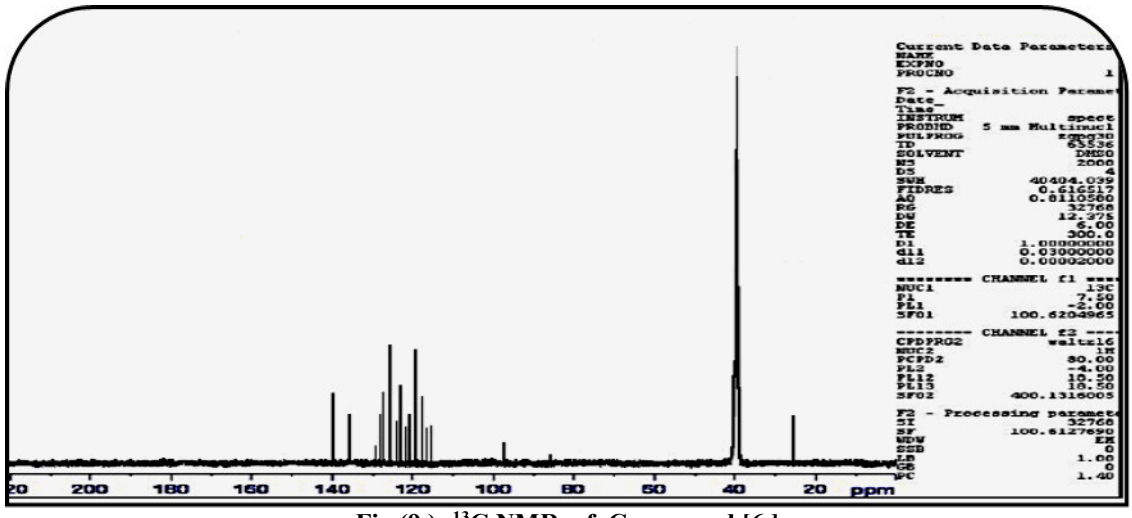


Fig (9): ^{13}C.NMR of Compound [6]

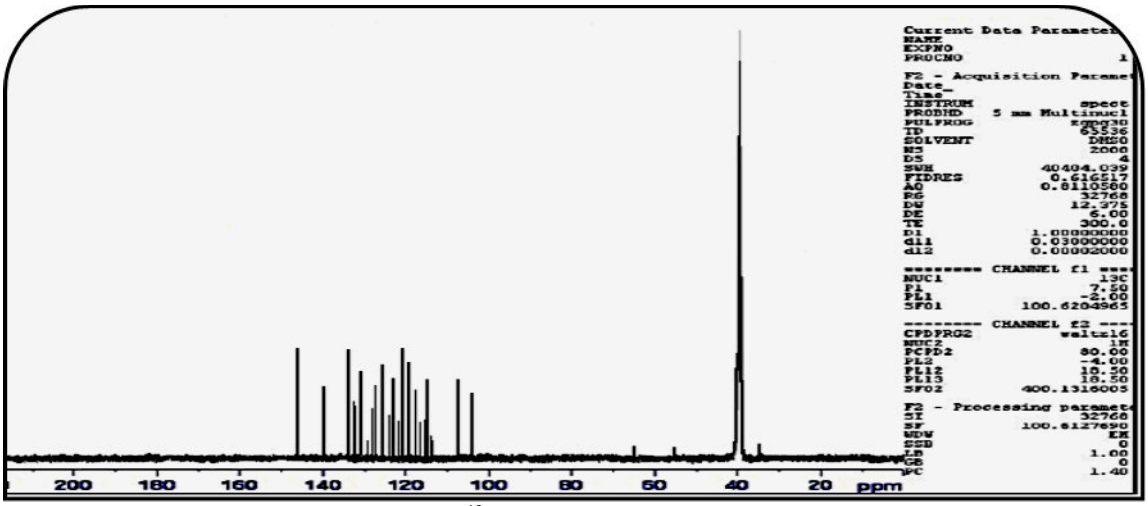


Fig (10): ^{13}C.NMR of Compound [8]

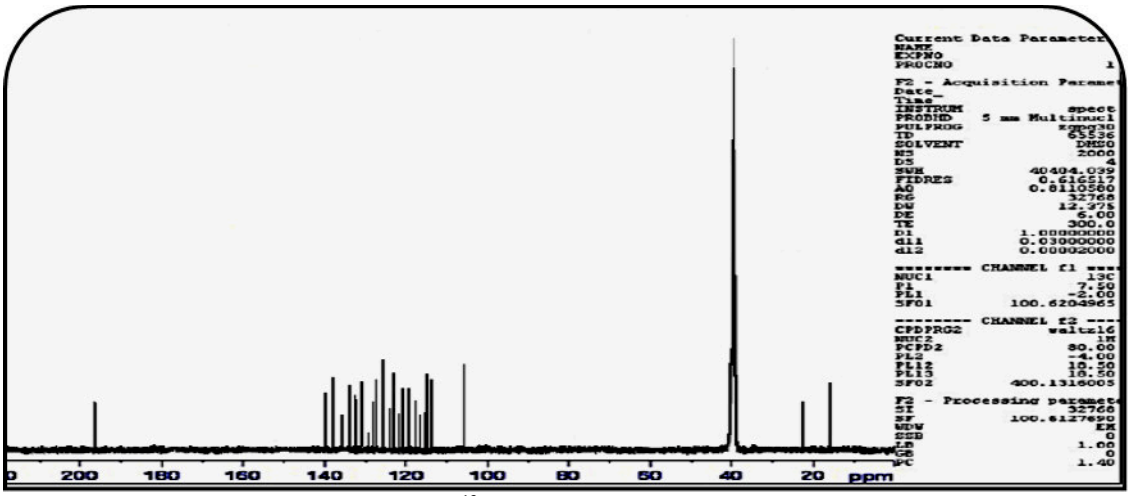
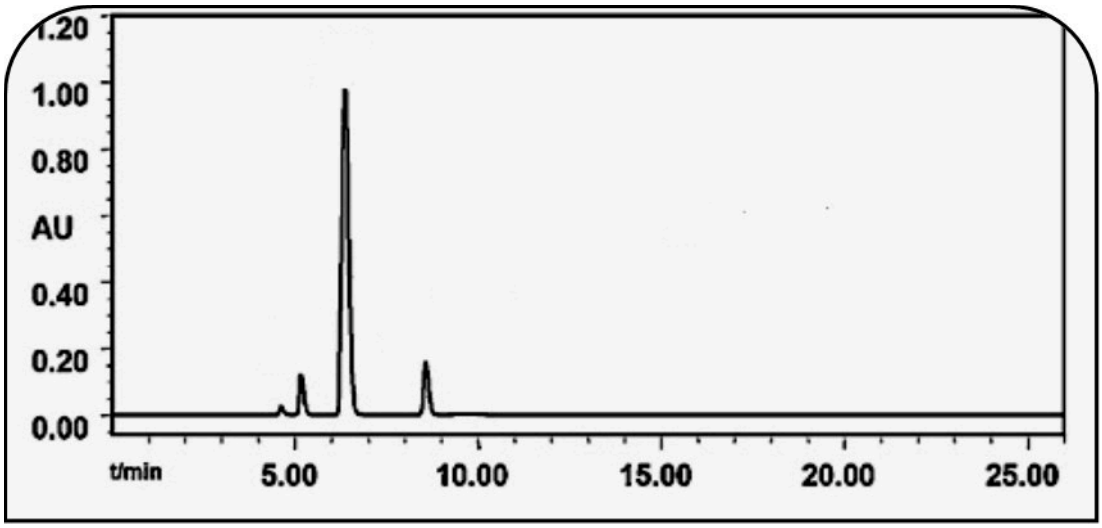


Fig (11): ^{13}C.NMR of Compound [9]

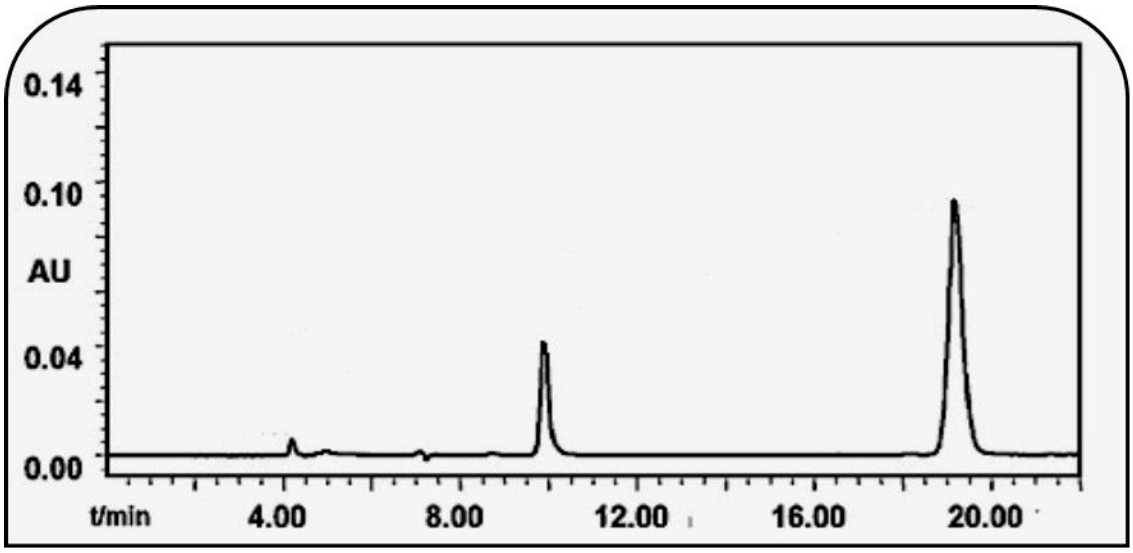
Studying of Chromatography Technique:

Analysis of some Compounds Through Chromatography Technique[18] :

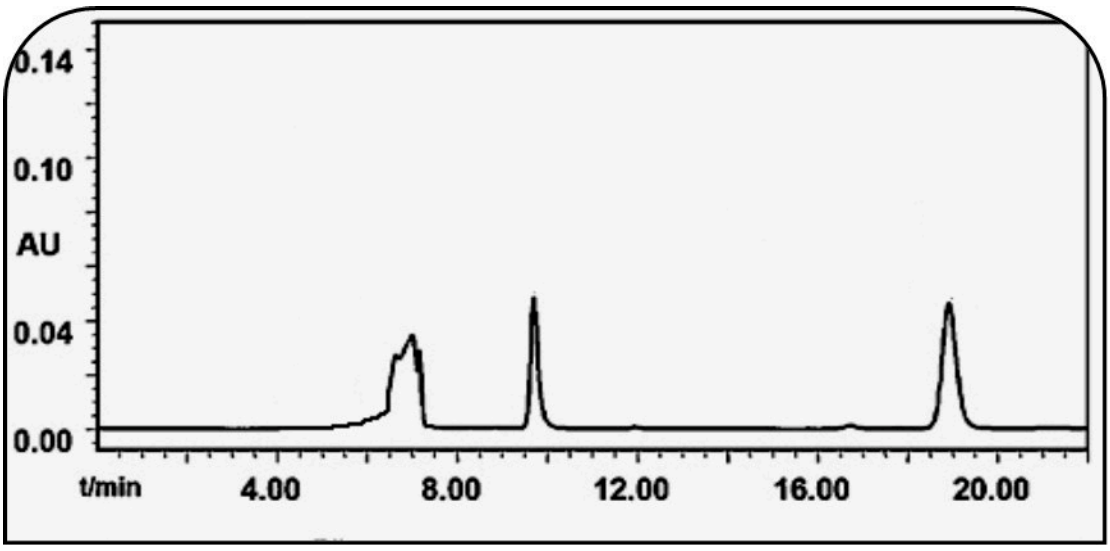
Preparation of diluted concentrations ((concentration of 1ppm for vehicles)) from compounds [1 , 3 , 4 , 6, 8] after dissolved with ethanol ,with shaking continuous., injected models by using a syringe(Hamilton) with a capacity of 10ml individually and then injected the mixture, and then install the measurement conditions through the use of nitrogen a gas flow of 25ml/min bus speeds and injection temperature was 25Cº degrees higher than the temperature separation column and then use a flame ionization detector is 50Cº higher than the temperatures of the column either column temperature programmed gradual increase of of(90-160)Cº, taking into consideration the maximum temperature to avoid damage to the column ., all data are shown in figures (12-16)



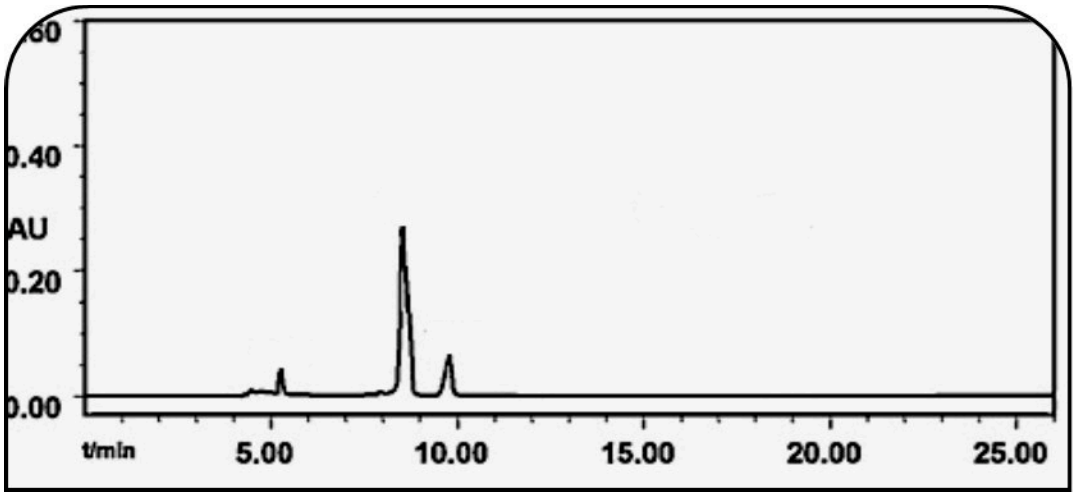
Fig(12): Chromatogram of Compound [1]



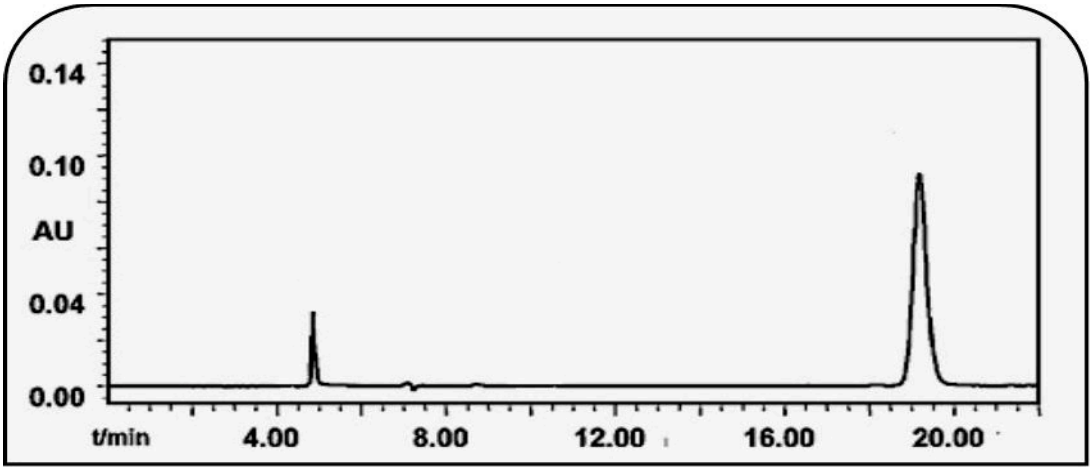
Fig(13): Chromatogram of Compound [3]



Fig(14): Chromatogram of Compound [4]



Fig(15): Chromatogram of Compound [6]



Fig(16): Chromatogram of Compound [8]

In the past decades, various methods developed and reported in the past studies for compounds analysis. The trend is to develop multi-compounds analysis methods which are simple and easy to separation.

The use of hetero cycles in compounds covered a wide area application in bio chemistry field or pharmaceutical field and analytical field like chromatography .

The results gave evidence that all compounds separated according to molecular weight and interaction between active groups in the compounds in separation column of chromatography technique.

Analytical Behavior - Effect of Using Solvents:

The interaction of monomers were tested in various solvents according to polarity type of solvents , the results are summarized in Table (4).

Table (4) : Interaction of Compounds in Different Solvents.

Compounds	Solvents					
	C_2H_5OH	CH_3OH	CH_2Cl_2	Ether	CCl_4	DMSO
[1]	+	+	-	-	-	+
[2]	+	+	-	-	-	+
[3]	+	+	-	-	-	+
[4]	+	+	-	-	-	+
[5]	+	+	-	-	-	+
[6]	+	+	-	-	-	+
[7]	+	+	-	-	-	+
[8]	+	+	-	-	-	+
[9]	+	+	-	-	-	+
[10]	+	+	-	-	-	+

The interactions of synthesized compounds depend on solubility and activity of functional group and

terminal groups (polarity of group) in solvents which cause interaction represented in : (OH - group , NH-group) or any other active groups.

Microbial Assay:

Antibacterial:

The biological activities of synthesized compounds have been studied for their antibacterial by agar via biological methods[1]. The antibacterial activities were done at three concentration (2 , 6 , 8) mg/ml concentrations in DMSO solvent , but concentration (8 mg/ml) gave higher activity than other concentrations with two types of bacteria (Gram – Positive) like :(***Bacillus subtilis* , *Streptococcus pyogenes***) and two types of bacteria (Gram – Negative) like :(***Klebsiella Pneumonia and Proteus vulgaris)*** . These bacterial strains were incubated for 24 hr at 37^{o}C .

Bio – Chemical Studying of Compounds:

All compounds [1-10] were tested according to their action against bacteria are described tables (5 , 6) and figures (17 , 18) . The presence of heterocyclic ring like five membered ring , benzo thiazole are reported to posses antibacterial effect may enhance or increase the antimicrobial activity of the thiazole derivatives

The antibacterial results are summarized in tables (5 and 6)., which appeared that the results of antibacterial studies it was found to be potentially activity against all types of bacteria ,which gave evident from the results that the biological activity of all compounds have high biological activity which inhibit the growth of bacteria .

The compounds [7 ,8 , 6 ,10] have higher activity than other compounds due to thiazol nuclei in [7 ,8, 6] and formazan compound in [10] in their structures.

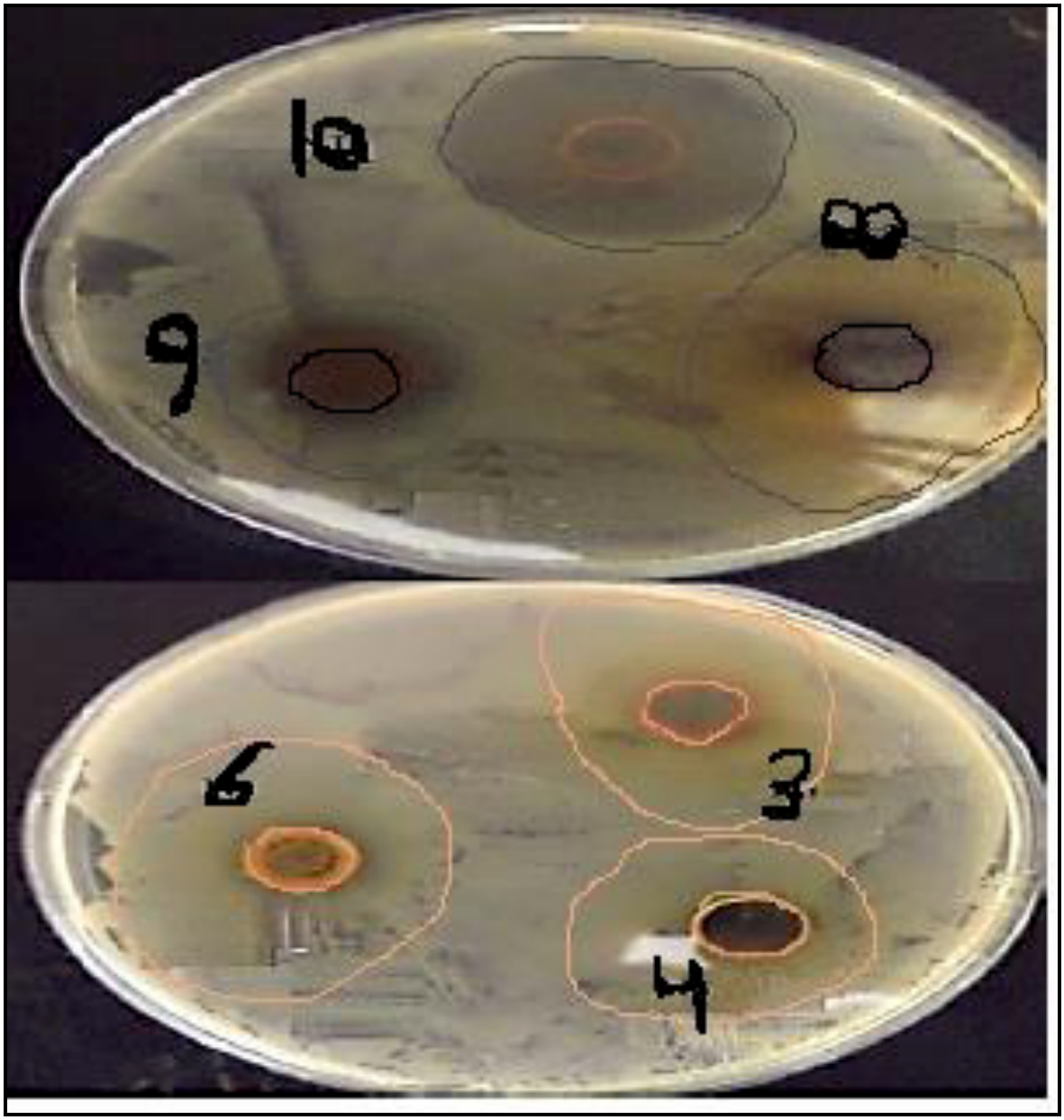
Acids which shown to inhibit cellular protein and RNA synthesis, they included some groups with sulfur atoms and hence inhibit the bacterial growth.

Furthermore, the mechanism of action of the compounds may involve the formation of hydrogen bond with the active centers of the cell constituents resulting in the interference with the normal cell process.

Hydrogen bonding and the anti metabolite action of the compound may be an important factor in antimicrobial activity., the prepared compounds gave excellent activity against bacteria .

Table(5):Antibacterial Activity (Gram –Positive) of Compounds (Inhibition Zone in (mm)) and in Concentration (8 mg.ml^{-1})

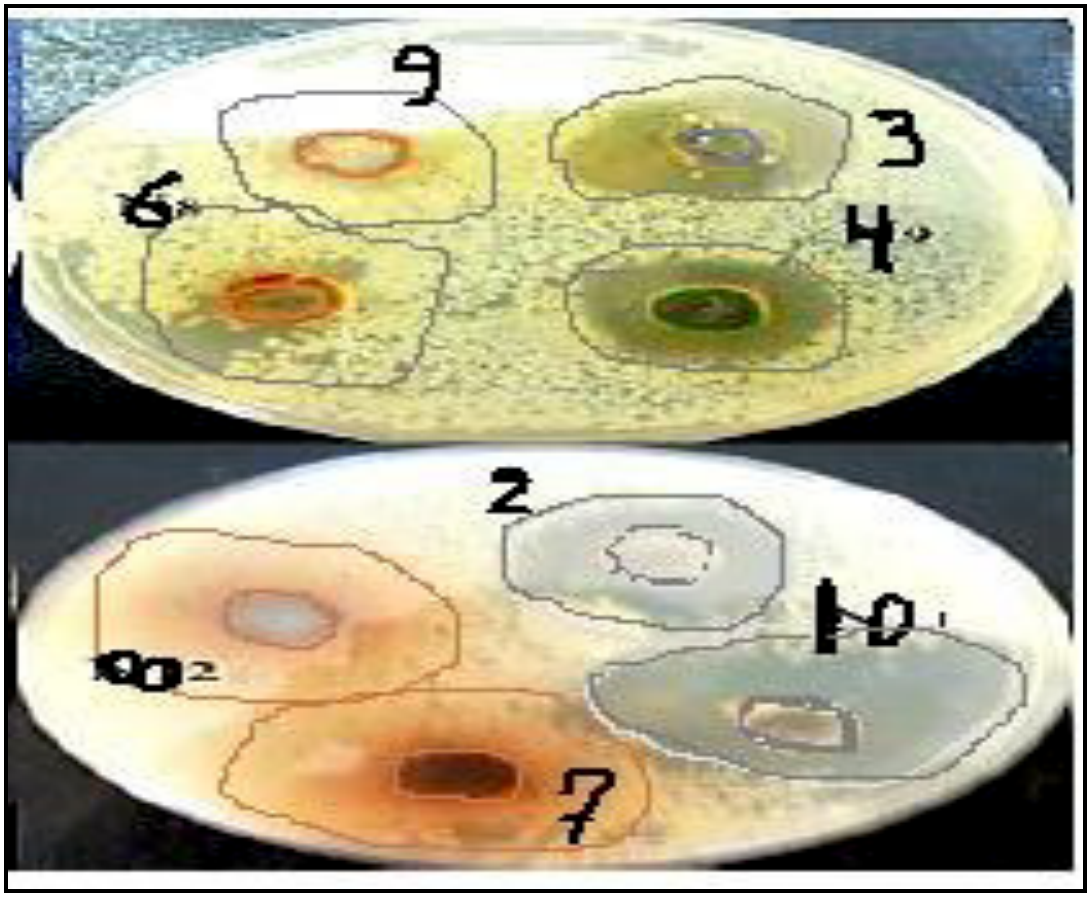
Compounds	*Streptococcus pyogenes*	*Bacillus subtilis*
[1]	10	6
[2]	14	12
[3]	16	14
[4]	14	12
[5]	12	10
[6]	18	14
[7]	26	20
[8]	22	18
[9]	14	12
[10]	16	14



Figure(17):Inhibition zone of compounds against *Streptococcus pyogenes*

Table(6):Antibacterial Activity (Gram –Negative) of Compounds (Inhibition Zone in (mm)) and in Concentration (8 mg.ml^{-1})

Compounds	*Klebsiella Pneumonia*	*Proteus vulgaris*
[1]	6	-
[2]	8	6
[3]	14	10
[4]	12	8
[5]	6	-
[6]	14	10
[7]	20	16
[8]	16	14
[9]	10	8
[10]	16	10



Figure(18):Inhibition zone of compounds against *Klebsiella Pneumonia*

Other Analytical Properties :

Some physical and chemical properties like R_f of TLC- Technique for following the reactions , solvent which are used in TLC – Plate , and products from reactions % , all data are listed in Table (7):

Table(7): Some Physical and Chemical Properties for Compounds.

Compounds	Products %	R_f	Solvents of (TLC)
[1]	72	0.68	Ethanol : dioxan
[2]	80	0.74	Ethanol : dioxan
[3]	82	0.64	Ethanol : dioxan
[4]	86	0.67	Ethanol : dioxan
[5]	76	0.72	Ethanol : dioxan
[6]	84	0.62	Ethanol : dioxan
[7]	82	0.72	Ethanol : dioxan
[8]	78	0.62	Ethanol : dioxan
[9]	86	0.80	Ethanol : dioxan
[10]	84	0.64	Ethanol : dioxan

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