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Synthesis, Characterization Of 1, 3-Thiazine Derivatives Using Chalcones, And Evaluation As Antimicrobial

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Abstract

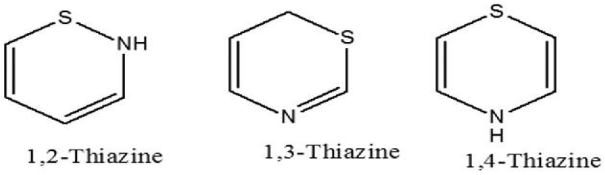
This study is concerned with the synthesis and characterization of the **1, 3-thiazine derivatives**. These compounds **(d-f)** were prepared by reacting of Thiosemicarbazide with the appropriate chalcones **(d-f)** in the presence of sodium hydroxide in ethanolrefluxed on water bath for 6 hrs, and the completion of the reaction was emphasize by thin layer chromatography (TLC), and the formed compounds were identified by NMR, IR,. The proposed reaction for their formations , And testing these organic compounds as antimicrobials

.

Key word : Chalcone , NMR , IR ,thiazine .

Introduction :

Thiazine is an organic compound containing a ring of four carbon, one nitrogen and one sulfur atom. There are three isomers of thiazine, 1,2-thiazine, 1,3-thiazine, and 1,4-thiazine, which differ by the arrangement of the nitrogen and sulfur atoms in the ringSynthetic heterocyclic compounds especially containing heteroatoms like N, S, O have massive potential primarily as drugs[1]. Thiazine is a heterocyclic compound having one(N)and (S) atom at varied positions in the six membered ring exist as 1,2; 1,3; 1,4-thiazines and subsequently their derivatives[2].



1,2-Thiazine 1,3-Thiazine 1,4-Thiazine

Structure of 1, 3- thiazines possesses an N-C-S linkage that is assent to be very useful units in the fields of medicinal and pharmaceutical chemistry. 1,3- thiazines and its derivatives have been reported to exhibit a variety of biological activities like Antibacteria[3] , Antifunga[4] , Antitubercular[5] , Antiinflammatory[6] , Analgesic[7] , Sedative-hypnotic[8] , Immunosuppressive agents[9] , Herbicida[10] .General synthesis involves synthesis of chalcones by ClaisenScmidth condensation reaction[10,11] followed by cyclo condensation of chalcones in the presence of thiosemicarbazide(N-Aminothiourea)giving 1, 3-Thiazines. The constitution of derivatives has been supported by elemental analysis, IR, NMR and Mass spectral data.

The Experimental

All solvents were distilled / dried prior to use.All solvent were dried over anhydrous sodium sulphate unless other wise specified. **^{13}C NMR; ^{1}HNMR** Spectroscopywere recorded using Bruker DRX system AL 500 (500 MHz). . **IR spectra** were recorded, usingshimadzu FT-IR affinity spectrophotometer in the Department of Chemistry, College of Science, Thi-Qar University, Iraq, as KBr disks. Only principal absorption bands of interest are reported and expressed in cm^{-1}.

1 : Prepare of Chalcones[12,13]

Chalcones can be prepared by an aldol condensation between benzaldehyde and acetophenone in the presence of sodium hydroxide as a catalyst.

1-Prepare of (E)-3-(4-bromophenyl)-1-(4-nitrophenyl)prop-2-en-1-one (a) :

To equimolar mixture (0.01 moles) of 1-(4-nitrophenyl)ethanone and 4-bromobenzaldehyde in ethanol (50 ml), 2% NaOH solution (1 ml) was added and stirred for 10 hrs at room temperature and then refluxed for 6 hrs on water bath. The excess solvent was distilled off under vacuum and poured into ice cold water, filtered and recrystallized from ethanol.

2 - Prepareof (E)-1-(4-(dimethylamino)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (b) :

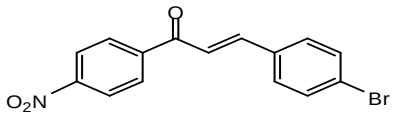
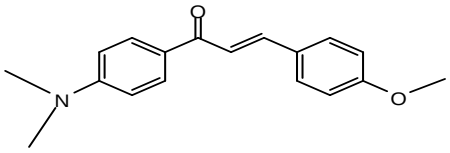
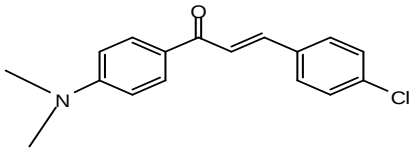
To equimolar mixture (0.01 moles) of 1-(4-(dimethylamino)phenyl)ethanone 4-methoxybenzaldehyde in ethanol (50 ml), 2% NaOH solution (1 ml) was added and stirred for 10 hrs at room temperature and then refluxed for 6 hrs on water bath. The excess solvent was distilled off under vacuum and poured into ice cold water, filtered and recrystallized from ethanol.

3 - Prepareof (E)-3-(4-chlorophenyl)-1-(4-(dimethylamino)phenyl)prop-2-en-1-one (c) :

To equimolar mixture (0.01 moles) of 1-(4-(dimethylamino)phenyl)ethanone4-chlorobenzaldehydein ethanol (50 ml), 2% NaOH solution (1 ml) was added and stirred for 10 hrs at room temperature and then

refluxed for 6 hrs on water bath. The excess solvent was distilled off under vacuum and poured into ice cold water, filtered and recrystallized from ethanol.

Table(1)

symbol	chalcone derivatives
a	
b	
c	

2 : Prepareof 1, 3-thiazine derivatives

1 - Prepareof 4-(4-bromophenyl)-2-hydrazinyl-6-(4-nitrophenyl)-6H-1,3-thiazine (d) :

To equimolar mixture (0.008 moles) of compound **(a)** and Thiosemicarbazide in ethanol (30 ml) was refluxed on water bath for 6 hrs. The reaction mixture was concentrated under vacuum, cooled and poured into ice cold water. The solid separated was filtered, dried and recrystallized from ethanol.

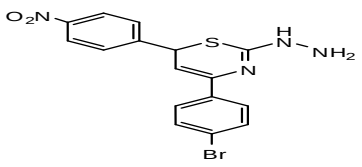
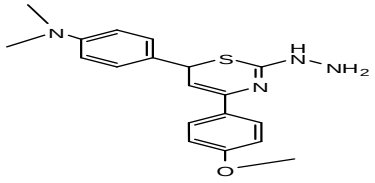
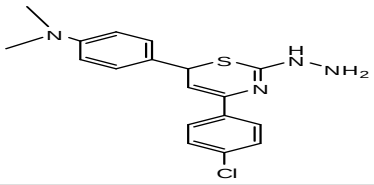
2 - Prepareof 4-(2-hydrazinyl-6-(4-methoxyphenyl)-6H-1,3-thiazin-4-yl)-N,N-dimethylaniline (e) :

To equimolar mixture (0.008 moles) of compound **(b)** and Thiosemicarbazide in ethanol (30 ml) was refluxed on water bath for 6 hrs. The reaction mixture was concentrated under vacuum, cooled and poured into ice cold water. The solid separated was filtered, dried and recrystallized from ethanol.

2 - 4-(6-(4-chlorophenyl)-2-hydrazinyl-6H-1,3-thiazin-4-yl)-N,N-dimethylaniline (f) :

To equimolar mixture (0.008 moles) of compound **(c)** and Thiosemicarbazide in ethanol (30 ml) was refluxed on water bath for 6 hrs. The reaction mixture was concentrated under vacuum, cooled and poured into ice cold water. The solid separated was filtered, dried and recrystallized from ethanol.

Table(1)

symbol	1, 3-thiazine derivatives
d	
e	
f	

Antimicrobial activity[14,15]

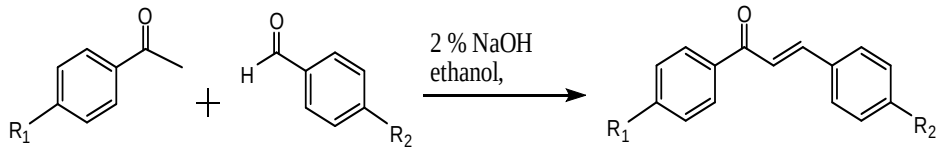
Different concentrations of the prepared compounds (0.1,0.2,0.3)mg/ ml were used to study their antimicrobial activity in vitro against two tested bacteria (Staphylococcus aureus)as gram positive and bacteria (E. Coli) as gram negative bacteria. Also Antifungal activity was screened against Candida albicans in Muller Hinton agar medium,preparation of nutrient broth, dilution and application were carried out using the same procedure as for antimicrobial testing.The plates were incubated at 30 °C for 48 hours (Fungi spp.) or 37 °C for 24 hours (bacteria) and the antimicrobial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the disc in mm.

Determination of cytotoxicity using Human erythrocytes[16]

Different amounts of solutions of1, 3-thiazine i.e., (0.2, 0.4, 0.6) mg/ml were prepared. serial dilutions of the compounds (d, e,f), were made in phosphate buffered saline. A total volume of 0.8 ml for each dilution was placed in an Eppendorf tube. A negative control tube (containing saline only) and a positive control tube (containing tap water) were also included in the analysis. human erythrocytes were added to each tube, to give a final volume of 1 ml. Solutions were incubated at 37°C for 30 min. The tubes were then examined for red blood cell decomposition.

Results and discussion

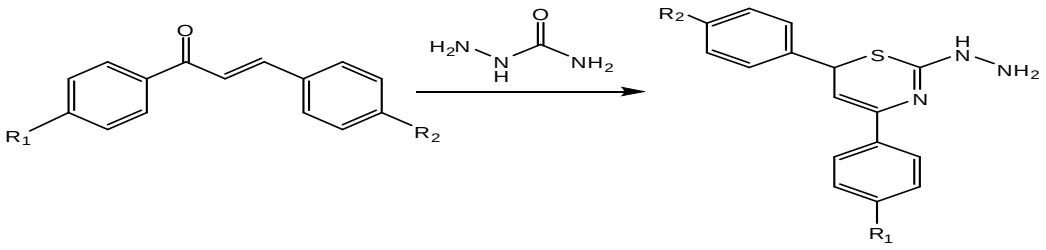
In this paper, the **1, 3-thiazine** were prepared, where in the first step the chalcone derivatives were prepared from the reaction of equal quantities of aldehydes derivatives and acetophenone derivatives (ketone) by condensation aldol.The proposed reaction for their formations was shown as below in (**scheme 1**).



chalcone derivatives

scheme (1)

1, 3-thiazine compounds were prepared from the reaction of chalcones with the semicarbazide compound, and the completion of the reaction was verified by thin layer chromatography (TLC), and the formed compounds were identified by NMR, IR, mass spectra. The proposed reaction for their formations was shown as below in (**scheme 2**).



1, 3-thiazine derivatives

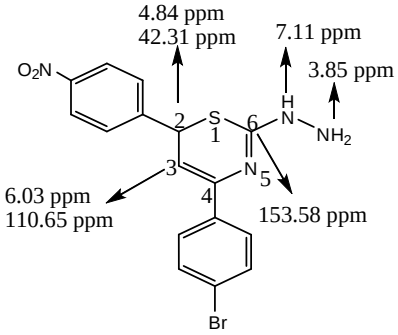
(**scheme 2**)

The structures of these Chalcones were established on the basis of spectral data, **IR**,spectraof these compounds **(a,b,c)** showed strong stretching absorption band at 1723,1738 and 1748 cm^{-1} ppm respectively for **(C=O)** as shown Figure(1-1), (1-2),(1-3) , **IR**,spectraof these compounds **(a,b,c)** showed stretching absorption band at 1597,1621 and 1629 cm^{-1}respectively for **(C=C)** as shown Figure(1-1), (1-2),(1-3).

The structures of these1,3 Thiazine were established on the basis of spectral data, **IR**,**^{1}H-NMR** , **^{13}C NMR**. **IR** spectraof these compounds **(d,e,f)** showed strong stretching absorption band at 1622,1621 and 1615 cm^{-1} ppm respectively for **(C=N)** as shown Figure(1-1), (1-2),(1-3) , **IR** spectraof these compounds **(a,b,c)** showed stretching absorption band at (3430-3347), (3417-3334) and (3340-3281) cm^{-1} respectively for **(NH_2)**, also **IR** spectraof these compounds **(d,e,f)** showed stretching absorption band at 3239,3221 and 3176 cm^{-1} ppm respectively for **(NH)** as shown Figure(1-4), (1-5),(1-6) .

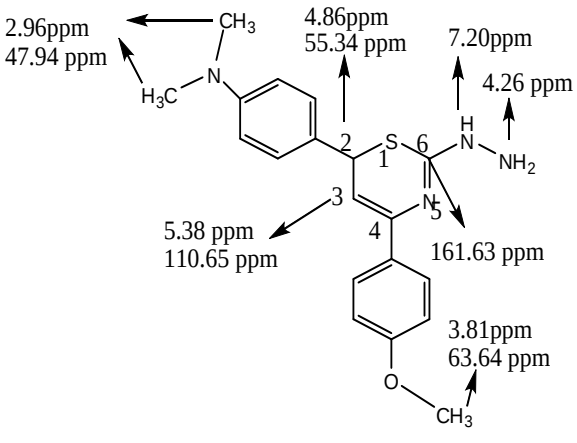
The **^{1}H-NMR** of **d**showedshowed singlet peak C_2-H δ(4.48) and C_3-H δ(6.03 ppm) The ^{1}H-NMR spectrum of **(d)** showed singlet peak NH_2 δ(3.85ppm) and NH at δ(7.11 ppm) . Finally , ^{1}H-NMR spectrum of **d** showed aromatic protons integrated 8H at δ(7.31- 8.22) ppm shown in Figure (1-1)

The **^{13}C NMR** spectrum of the **d**showed resonance between δ121.44-151.19 ppm which assigned to the carbon[30,31,32]group were belonged to the aromatic carbons.C-2 and C-3 were appeared at δ 42.31and 110 .65 ppm respectively , whereas the resonance at δ 153.58 ppm were assigned to the C-6 atom respecteivly figure (1-2).



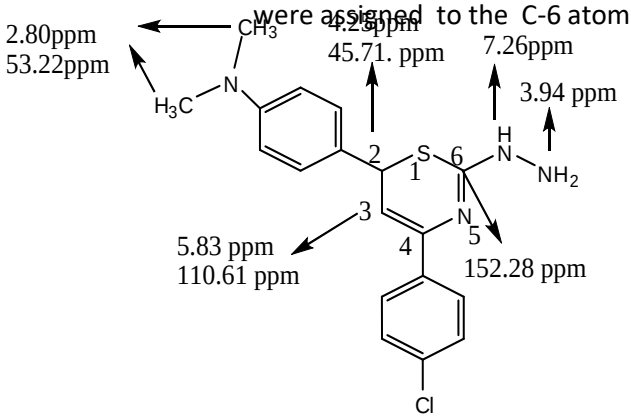
The **^{1}H-NMR** of **(e)**showedsnowed singlet peak C_2-H δ(4.86) and C_3-H δ(5.83 ppm). The ^{1}H-NMR spectrum of **(e)** showed singlet peak NH_2 δ(4.26 ppm) and NH at δ(7.20 ppm). The ^{1}H-NMR spectrum of **e** showed singlet peak for methoxy group(O-CH_3) at δ 3.81 ppmThe ^{1}H-NMR spectrum of **d** showed singlet peak (equivalent protons) for two methyl groups at δ 3.204ppm (s,6H,2CH_3-N)Finally , ^{1}H-NMR spectrum of **d** showed aromatic protons integrated 8H at δ(6.77- 7.44) ppm shown in Figure (1-3)

The **^{13}C NMR** spectrum of the **d** showed resonance between δ112.98-153.44ppm which assigned to the carbongroup were belonged to the aromatic carbons.C-2 and C-3 were appeared at δ 55.34and 110 .56 ppm respectively , .O-C in methoxy group were appeared at δ63. 46ppm , , The **^{13}C NMR** spectrum of the **f** showed resonance singlet peak (equivalent carbons) for two methyl groups(CH_3)$_2$-N at δ47. 94ppm, whereas the resonance at δ 161.36 ppm were assigned to the C-6 atom respecteivly figure (1-4).



The **^{1}H-NMR** of **(f)**showed singlet peak C_2-H δ(4.25) and C_3-H δ(5.83 ppm) . The ^{1}H-NMR spectrum of **(f)** showed singlet peak NH_2 δ(3.94ppm) and NH at δ(7.26 ppm). The ^{1}H-NMR spectrum of **d** showed singlet peak (equivalent protons) for two methyl groups at δ 2.80ppm (s,6H,2CH_3-N)Finally , ^{1}H-NMR spectrum of **d** showed aromatic protons integrated 8H at δ(6.67- 7.61) ppm shown in Figure (1-5)

The **^{13}C NMR** spectrum of the **f**showed resonance between δ111.95-147.91ppm which assigned to the carbongroup were belonged to the aromatic carbons.C-2 and C-3 were appeared at δ 45.71 and 110 . 61 ppm respectively, The **^{13}C NMR** spectrum of the **f**showed resonance singlet peak (equivalent carbons) for two methyl groups(CH_3)$_2$-N at δ 53. 22ppm, . , whereas the resonance at δ 161.36 ppm were assigned to the C-6 atom respecteivly figure (1-6).



Antimicrobial activity

The results showed that the prepared compounds had biological activity against, both Gram-positive and Gram-negative bacteria, and also these compounds had activity against fungi.based on measurement of the diameter of inhibition zone formed around the well, show that the zone of inhibition varied with the increasing of concentration of the tested compounds, as show in Table

Table. The antibacterial and antifungal activities of compounds

Variables		Antibacterial Activity		Antifungal
Sym.	Conc.	Gram positive bacterria Compd. No. Klebsilla pneumonia Staph.areus	Gram negative bacteria E.coli	C. albicans

		Gram positive bacteria staph.aureas		
d	0.1	14	12	7
	0.2	16	14	10
	0.3	19	17	11
e	0.1	18	19	10
	0.2	19	20	12
	0.3	21	22	14
f	0.1	13	11	9
	0.2	15	15	10
	0.3	18	17	11

Determination of cytotoxicity using Human erythrocytes

The results of cytotoxicity of the prepared compounds in the direction of human red blood cells showed that the compounds (d, e,f) do not carry any toxicity at the concentrations (0.2, 0.4, 0.6) mg/ml. Red blood cells have been used to detect the toxicity of prepared compounds because this method is inexpensive, easy to apply and quick results. Red blood cell decomposition depends on the concentration of the material, incubation period and temperature. The red blood cell crash is due to the breakdown of the red cell membrane due to the co-toxicity between the toxic substances and groups (1) found in the initial structure of the protein

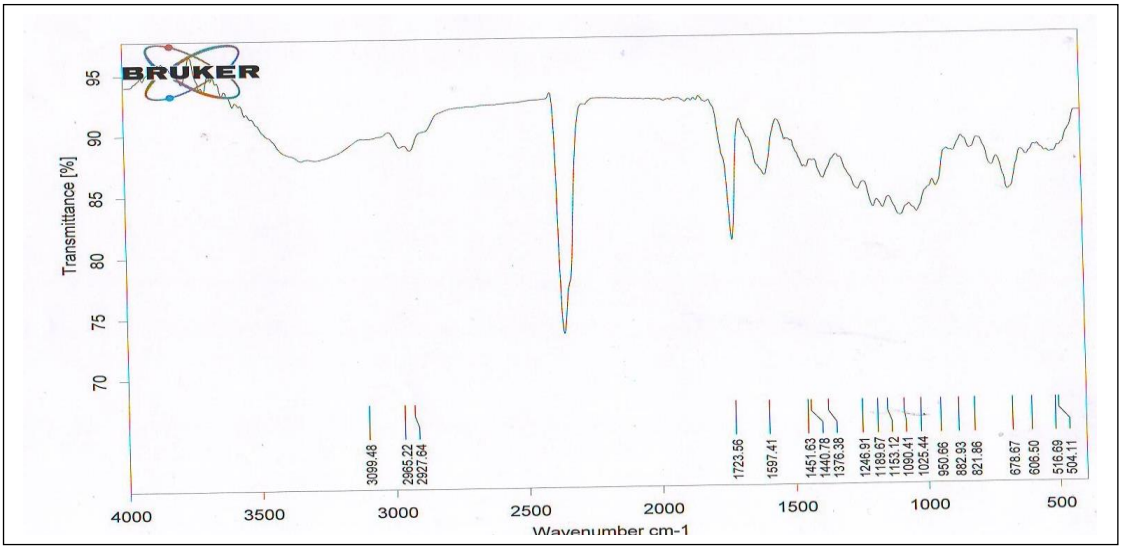


Figure (1-1) IR for (a)

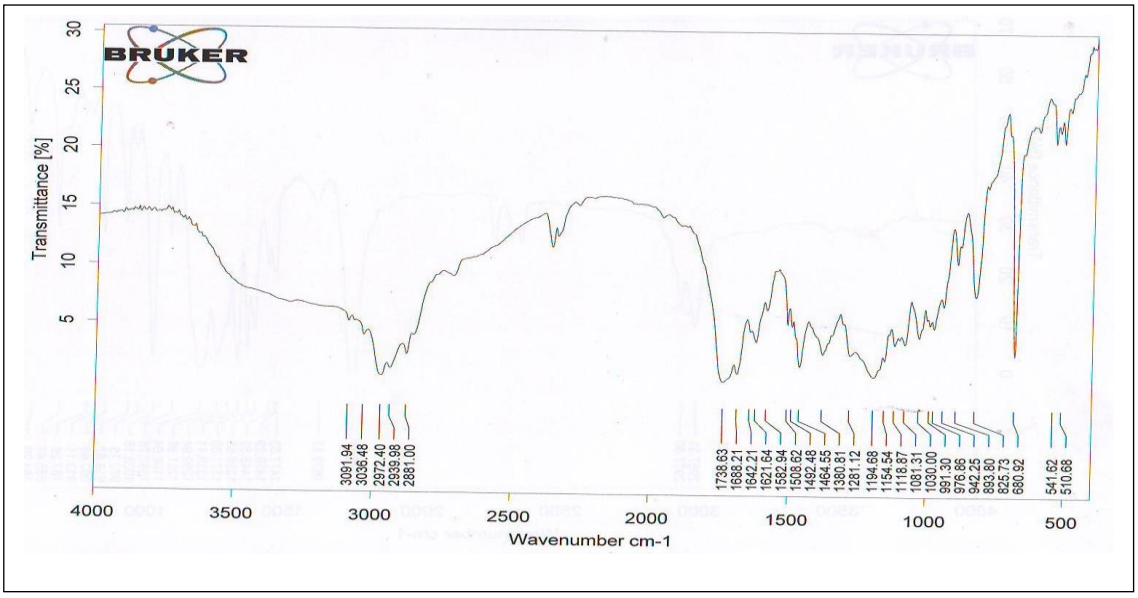


Figure (1-2) IR for (b)

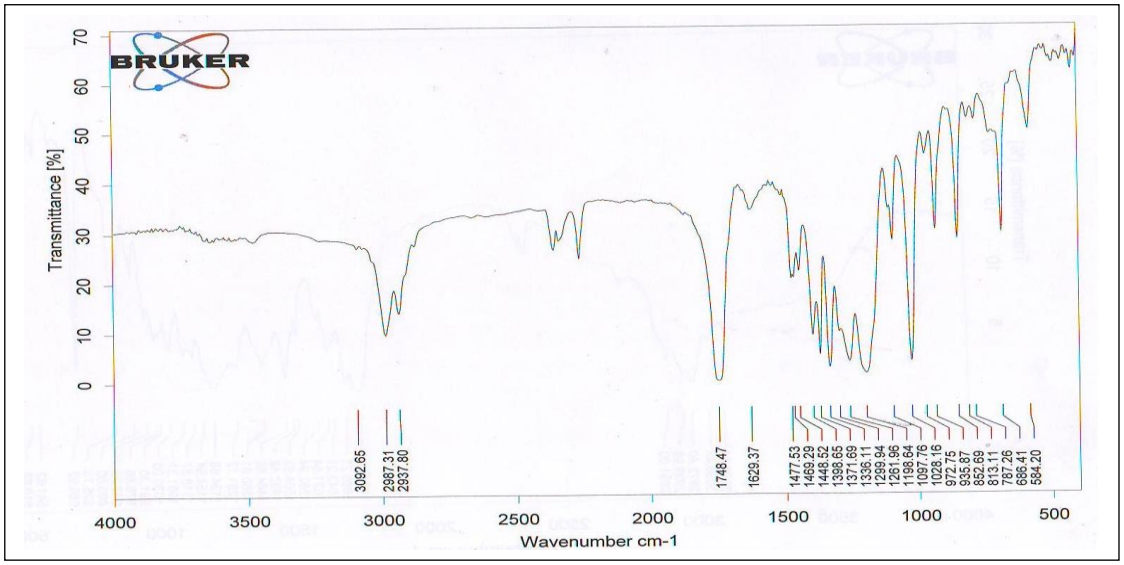


Figure (1-3) IR for (c)

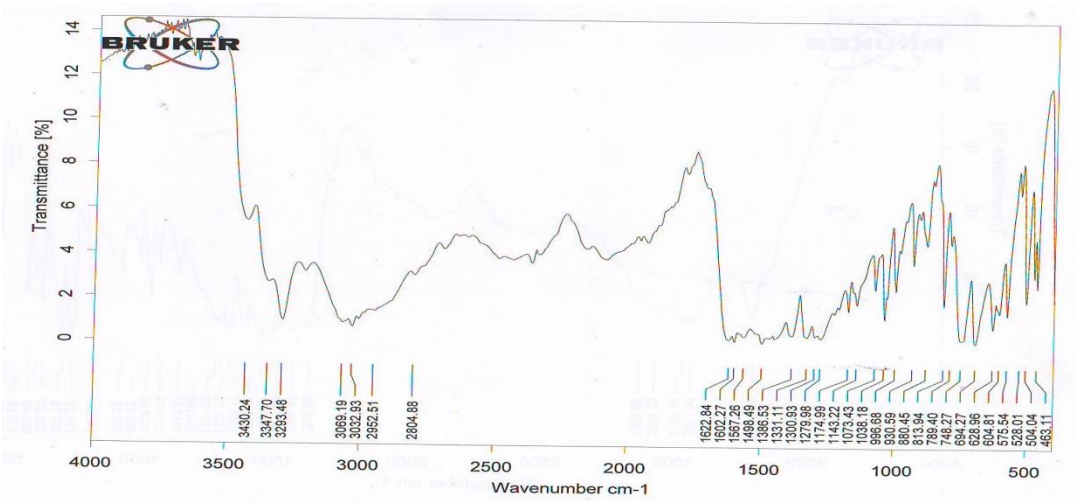


Figure (1-4) IR for (d)

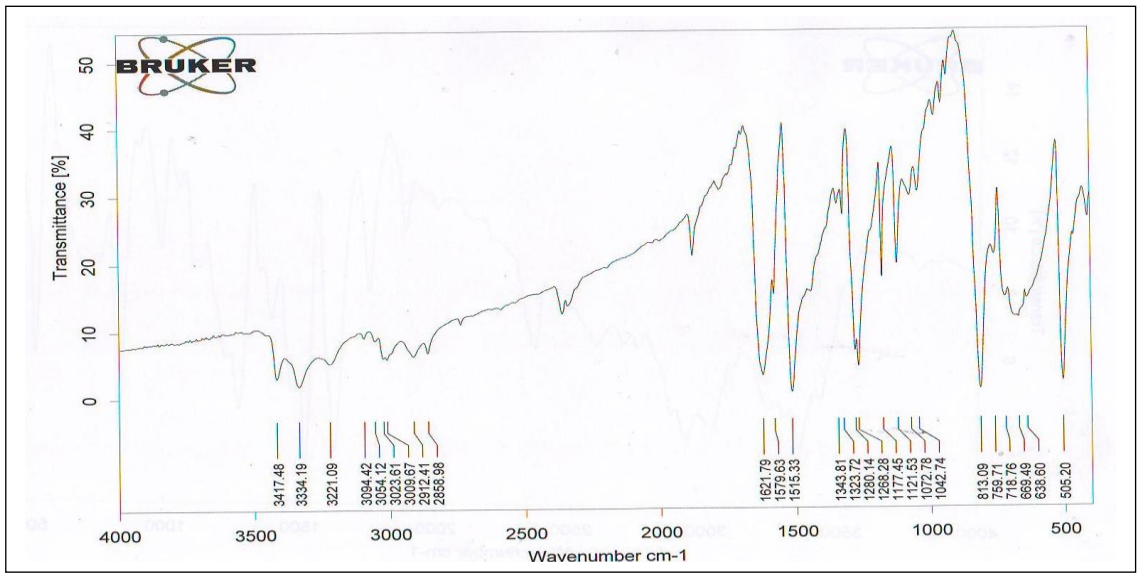


Figure (1-5) IR for (e)

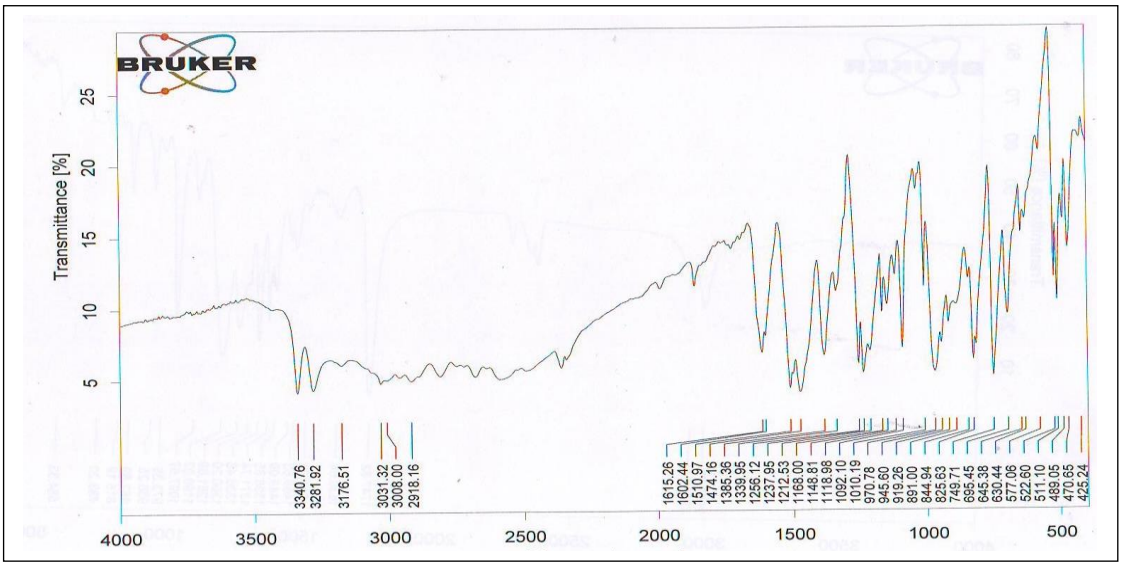


Figure (1-6) IR for (f)

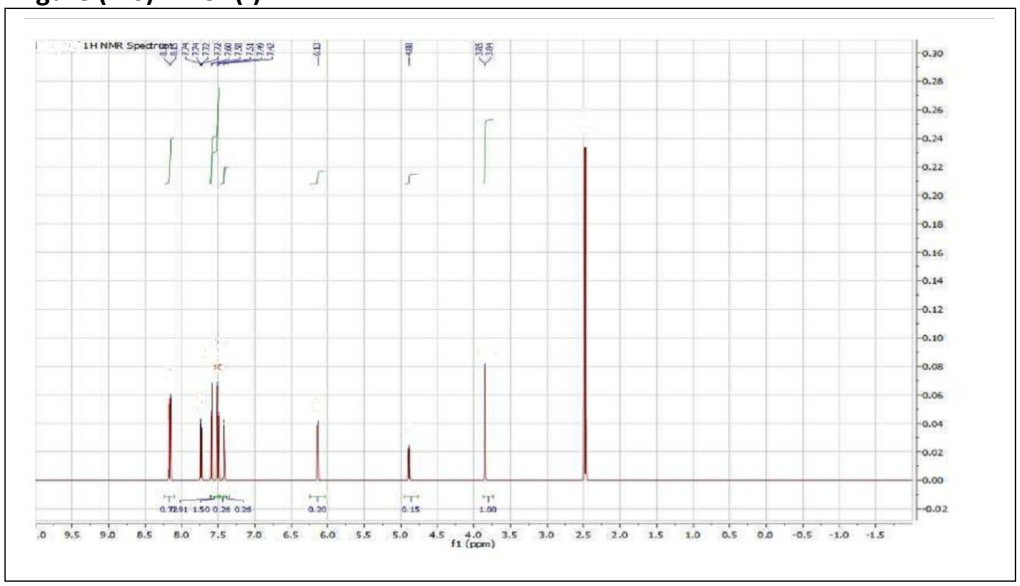


Figure (1-7) 1HNMR of d

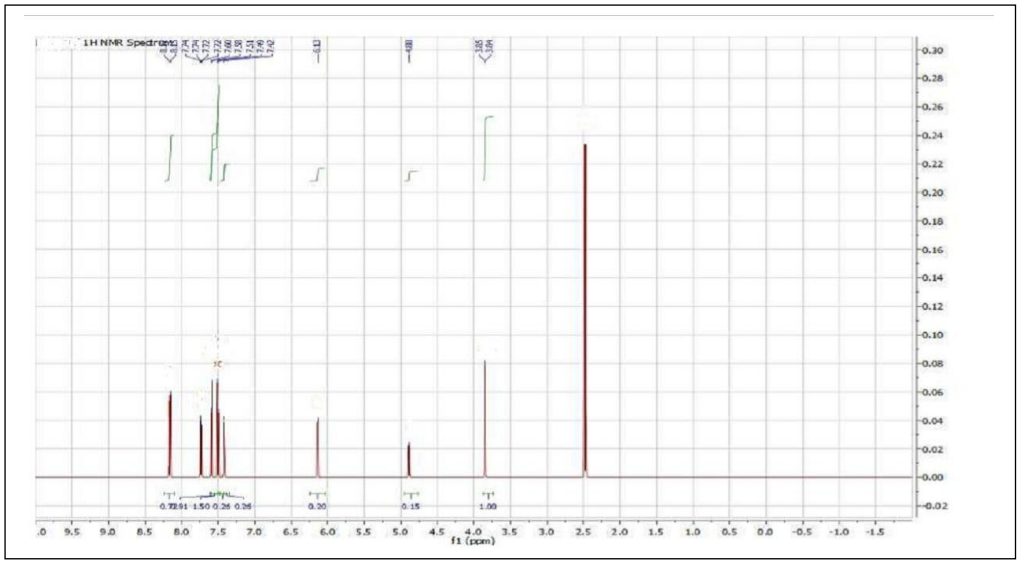


Figure (1-8) ^{13}CNMR of d

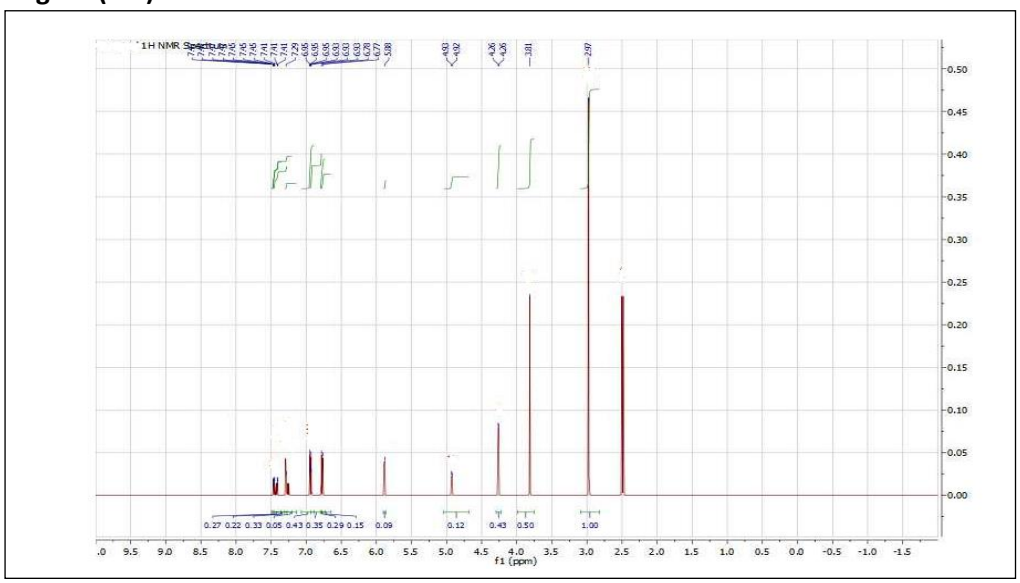


Figure (1-9) 1HNMR of (e)

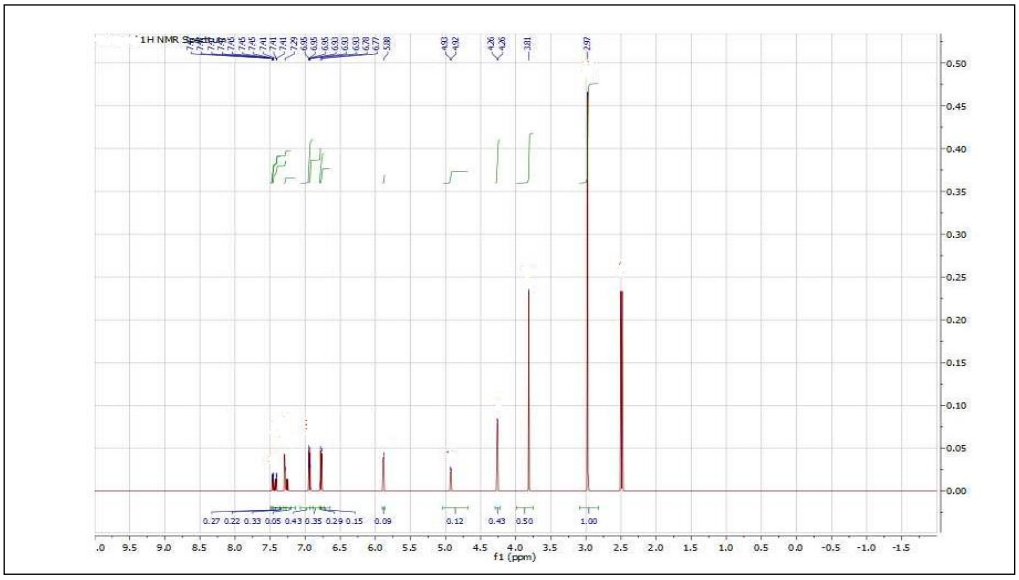


Figure (1-10) 13CNMR of (e)

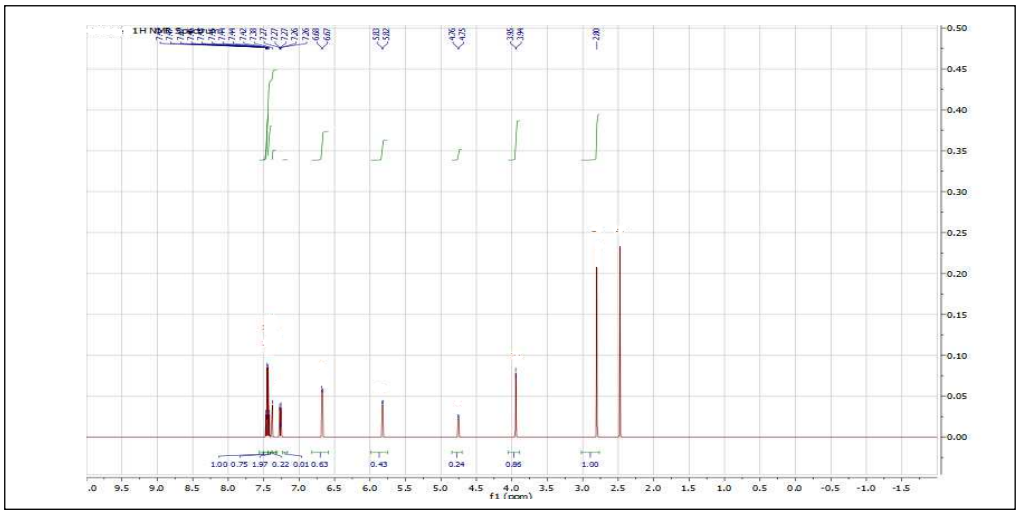


Figure (1-11) 1HNMR of (f)

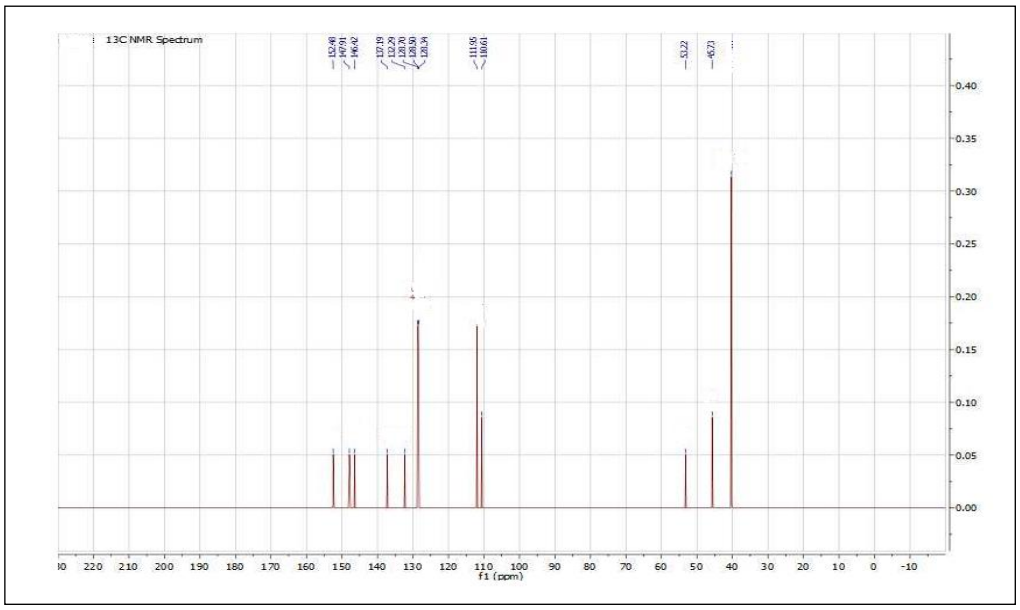


Figure (1-12) 1HNMR of (f)

References

1 - Barkenbus, Charles; Landis, Phillip S. (February 1948). "The Preparation of 1,4-Thiazine". Journal of the American Chemical Society. **70** (2): 684–685

2- Aurelija Urbanaite. Synthesis of 4H-thiazine Chemistry of heterocyclic compounds 2016; 52:1..

3. C. Sanjeeva Reddy and A. Nagaraj, Synthesis and Biological Study of Novel Bis-chalcones, Bis-thiazines and Bispyrimidines, J. Iran. Chem. Soc., Vol. 5, No. 2, June 2008, 262-267.

4. Mamoru Koketsu, Kohsuke Tanaka, Yuichi Takenaka, Cecil D. Kwong and Hideharu Ishihara , Synthesis of 1,3- thiazine derivatives and their evaluation as potential antimycobacterial agents, European J. Pharm. Sci., April 2002,Volume 15, Issue 3, Pg no: 307- 310.

5. R. Kalirajan, S.U.Sivakumar, S. Jubie, B. Gowramma and B. Suresh, R. Kalirajan, S.U. Sivakumar, S. Jubie, B. Gowramma and B. Suresh, Internl. J. Chem Tech Res., Jan – March 2009, Vol.1, No.1, 27-34.

6. Tojkoporan, Synthesis of some 1,2,4- triazolo[3,2-b]-1,3-thiazine-7-ones with potential analgesic and antiinflammatory activities, pubmed, 2002 Feb; 57(2), 145-52.

7. Li Fu, Li Fu, Ding Ye, Ying Li and Shufan Yin, Synthesis and calming activity of 6H-2-Amino-4-Aryl-6-(4-β-DAllopyranosyloxyphenyl)-1,3-Thiazine, Chemistry of natural products, vol.46, no.2, 169-172.

8. Zawisza, Syntheses and pharmacological analysis of new derivatives of tetrahydro-[1,3]-thiazine and 2-thiobarbituric acid, National center for biotechnology information, 1981; 29(2), 235-48.

9. Jin Guiyu Cao Chunyang, Synthesis and biological activities of substituted pyrazolo [4,5 e][1,3] thiazine derivatives, Chinese journal of pesticide science, 1981;29(2),Pg no:235-48.

10. Chetana B. Patil, S. K. Mahajan, Suvarna A. Katti, Chalcone: A Versatile Molecule, J. pharm. Sci and Res., Vol.1(3), 2009, 11-22

11-Sajda ,S. and Haider,A. Synthesis and characterization of a new Schiff base {N-(2-{[(4bromophenyl)imino]methyl}phenyl)acetamide}and its compolexes with some transition metal, J. Edu.Pur.Sci.2012. 2(4)

12 . Leusink, A.J; Noltes, J.G (1966). "Reaction of organotin hydrides with α,β-unsaturated ketones". Tetrahedron Letters. 7 (20): 2221–5.

13. Outirite, Moha; Lebrini, Mounim; Lagrenée, Michel; Bentiss, Fouad (2008). "New one step synthesis of 3,5-disubstituted pyrazoles under microwave irradiation and classical heating". Journal of Heterocyclic Chemistry. 45 (2): 503–5.

14- Ali, R. (2011) Synthesis of New Schiff's Bases Derived from4-Phthalimidyl Acetophenone ,Journal of Al-Nahrain,14(3):1-8.

.15-Teeba T.Kudar; Mohammed Hashim .Al-yasiri, Ahmed K.Atya (2021). "Characterization of pathogens community in women with vaginal infection" university of Thi-Qar journal of science,ISSN:2709-025,Vol 8,No. 1

16- Xian-gno , H . and Ursella , M . **(1994)** Antifungal compounds from saloum nigrescence, J. Ethnopharm.,43:173-177