INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

SYNTHESIS, CHARACTERIZATION AND EVALUATION OF BIOLOGICAL ACTIVITY OF SOME HETEROCYCLIC COMPOUNDS CONTAINING 1,2,4- TRIAZOLE RING

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ABSTRACT

In this study, a new triazole derivatives were synthesized by many cyclization reactions. The malonichydrazide1 was synthesized by the reaction of ethyl malonate with hydrazine hydrate . The compound1 was react with CS_2 in asolution of alkali ethanol to give the salt2. The triazole3 was obtained from thecyclization of the salt2 with hydrazine hydrate derivative4 was prepared by the cyclization reaction of compound 3 with p-Bromophenacyl bromide, the compounds 7 and 8 were prepared according to the same procedures of the compound 3 and 4 using methyl benzoate instead of ethyl malonate. The biological activity of compounds 3,4,7 and 8 were studied against four types of bacteria two of them were gram negative (E.Coli and K.pneomonia) and the others were gram positive (S. aureus and E. faecalis). One type of fungi was used which was Candida albicans. The result showed that the mentioned above compounds possess high biological activity.

Keywords: Synthesis, Characterization, Biological activity, Antibacterial, 1,2,4-Triazole.

1-INTRODUCTION

In the past decades, the problem of multidrug resistant micro-organisms has reached on alarming level around the world, and the synthesis of new anti-infective compounds has become an urgent need for the treatment of microbial infections. The 1,2,4-triazole nucleus has been incorporated into a wide variety of therapeutically important agents, which mainly displaying antimicrobial activities[1,2].Organic compounds incorporating heterocyclic ring systems continue to attract considerable interest due to their wide range of biological activities. Among different five-membered heterocyclic systems 1,2,4-triazoles and 1,3,4-thiadiazoles and their derivatives have gained importance as they constitute the structural features of many bioactive compounds. It is known that triazole and thiadiazole rings are included in the structure of various drugs[3,4]. From these classes of heterocyclic compounds, the synthesis of new derivatives of 1,2,4-triazole-3-thiones and 2-amino-1,3,4-thiadiazoles has been attracting considerable attention because of various biological properties such as: antibacterial[3,5,6], antifungal[3,7], anti-tubercular[3,8,9], antiviral[3,10], antioxidant[3,11], antitumoral[3,12], anti-inflammatory[3,13,14], anticonvulsant[3,15] etc. In view of these facts and as a continuation of the research on the biological properties of 1,2,4-triazole and 1,3,4-thiadiazole containing derivatives[7], we have designed and synthesized triazole system, as antimicrobial agents.

2-Experimental

All starting materials and solvents were purchase from Fluca ,BDH and Thomas Baker companies ,used without further purification. Melting points were determined on electro thermal capillary apparatus and are uncorrected; FT-IR measurements were recorded on Shimadzu model FTIR-8400S.

^{1}HNMR spectra were obtained with Bruker spectrophotometer model ultra-shield at 300 MHz in DMSO- d6 solution with the TMS as internal standard.

2.1 synthesis of Malonohydrazide [1][16]

Ethylmalonate (0.12 mol, 19 ml)in 25 ml of ethanol absolute is taken in a round bottom flask. To that hydrazine hydrate (80 %) (0.24 mol, 14ml 12.16 g) was added and refluxed for 4 hour. The total volume of the solution is reduced to half and it was cooled in ice water. The solid is precipitated out and recrystallized from ethanol.

2.2 synthesis of bis-potassium dithiocarbazinate (2)[17]

Potassium hydroxide (0.03 mol, 1.68 g) was dissolved in absolute ethanol (25 mL). The solution was cooled in ice bath and the hydrazide (2) (0.01 mol, 1.32g) was added with stirring. To this carbon disulfide (0.05 mol, 5 ml) was added in small portions with constant stirring. The reaction mixture was agitated continuously for 18 h at room temperature. Cold ethanol (20 mL) and dry ether (20ml) was added to thesolution and then dried in vacuum. The potassium salt thus obtained was used in the next step without further purification.

2.3. Synthesis of 5,5'-methylenebis(4-amino-4H-1,2,4-triazole-3-thiol),[3][17]

A suspension of compound (2) (1 mmol, 0.3g) in water (5 mL) and hydrazine hydrate (80%, 3 mmol) was heated for 18– 20 h at 100 C with occasional shaking. The color of the reaction mixture changed to green with the evolution of hydrogen sulfide gas. A homogeneous reaction mixture was obtained during the reaction process. The reaction mixture was cooled to room temperature and diluted with cold water (20 mL). On acidification with HCl the required triazole was precipitated out, which was recrystallized with DMF–water mixture.

2.4 synthesis of bis(6-(4-bromophenyl)-7H-[1,2,4]triazolo[3,4 b][1,3,4]thiadiazin-3-yl)methane, [4][18]

suspension of triazole(3) (0.5mmol,0.3 g) and p-bromophenacyl bromide (1.5mmol,0.4 g) in absolute ethanol (10 mL) was heated under reflux for 3 hr. , then (1.5mmol,0.15 g) of anhydrous sodium acetate was added. The reaction mixture was heated for an additional 1hr., then cooled and poured onto ice-cold water. The solid product was crystallized from ethanol.

2.5. Preparation of benzohydrazide [5][16]

Methyl benzoate (1.36 ml, 0.01mol) in 25ml of ethanol is taken in a round bottom flask. To that hydrazine hydrate (0.70 ml, 0.15mol) is added and refluxed for 4 hours. The total volume of the solution is reduced to half and it was cooled in ice water. The solid is precipitated out and recrystallized from ethanol.

2.6 preparation of potassium dithiocarbazinate,[6][16]

To a solution of potassium hydroxide (8.5 g, 0.15 mol) in absolute ethanol (125ml), benzoic acid hydrazide (1.36 g, 0.1mol) and carbon disulphide (14.5 ml, 0.15mol were added and the mixture was stirred for 16 hrs. To the resulting solution anhydrous ether (250ml) was added and precipitated potassium dithiocarbazinate was collected by filtration, washed with diethyl ether and dried. The potassium salt obtained in quantitative yield was directly used without purification.

2.7 Synthesis of 4[amino]-5-phenyl-4H-1,2,4-triazole-3-thiol,[7][16]

A suspension of potassium salt (6) (4.44g, 0.02mol), hydrazine hydrate (2ml, 0.04mol) and water (80ml) was refluxed for 3 hrs. The color of the reaction mixture changed to green, hydrogen sulphide was evolved and a homogenous solution resulted. A white solid was precipitated by dilution with cold water (100ml) and acidification with concentrated hydrochloric acid. The product was filtered, washed with cold water (2×30 ml) and recrystallized from ethanol.

2.8 synthesis of 6-(4-bromophenyl)-3-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine[8][18]

A suspension of triazole(7) (1mmol, g) and p-bromophenacyl bromide (1mmol, 0.26 g) in absolute ethanol (10 mL) was heated under reflux for 3 hr. , then (1 mmol,0.1 g) of anhydrous sodium acetate was added. The reaction mixture was heated for an additional 1hr., then cooled and poured onto ice-cold water. The solid product was crystallized from ethanol. some physical properties of compounds (1-8) listed in table (1).

2.9-Biological study

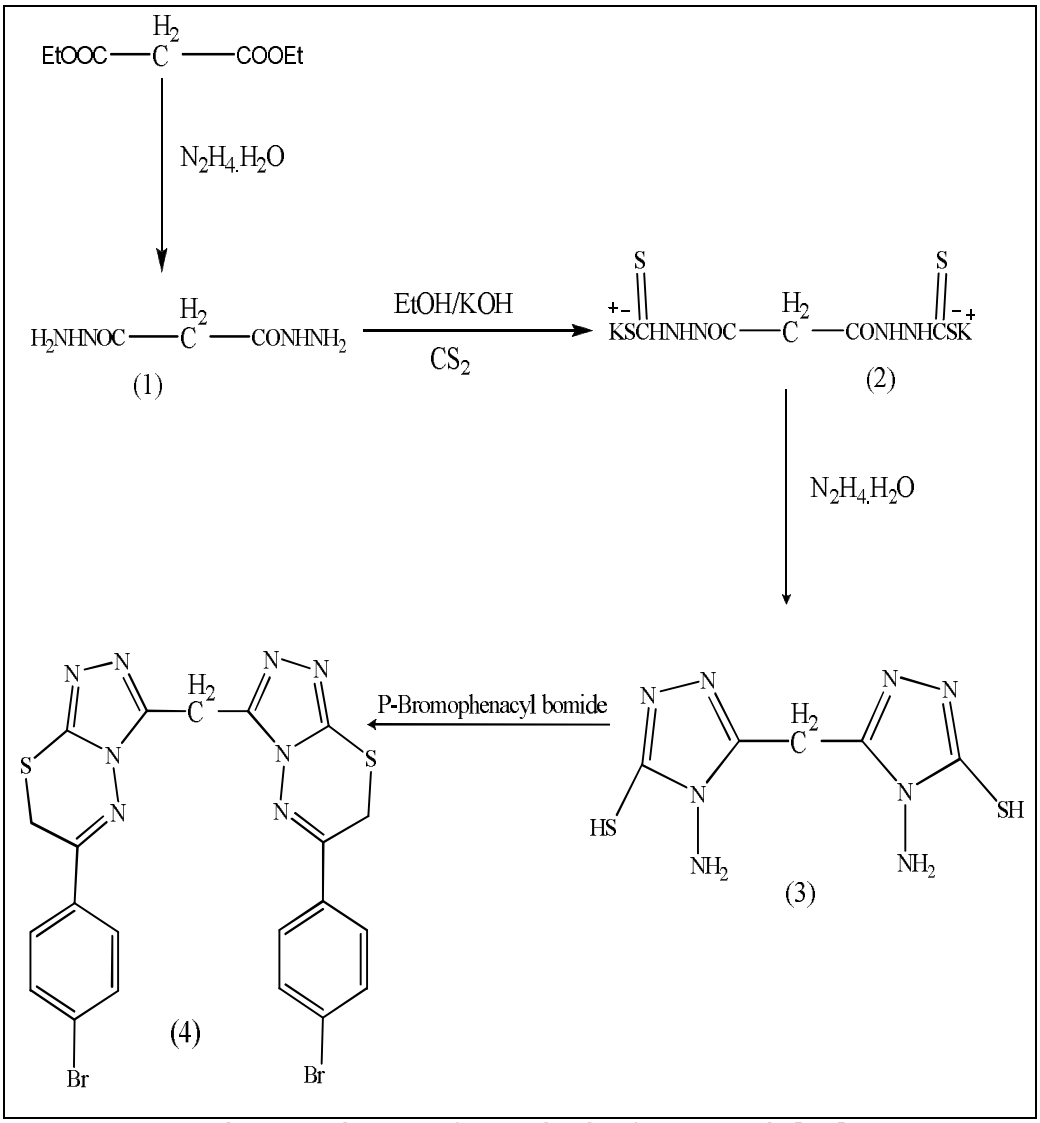
The antibacterial test was performed according to the disc diffusion method. Compounds ([3], [4], [7] and [8]) were assayed for their antimicrobial activity in vitro against four strains of bacteria (two of them were gram negative (*Escherichia coli*, *Klebsiellapneumoniae*) and the other were

gram positive (staphylococcus aureus, *Enterococcus faecalis*)). Prepared agar and petridishes were sterilized by autoclaving for15min at 121C°. The agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In the solidified medium suitably spaced apart holes were made all 6mm in diameter. These holes were filled with 0.1ml of the prepared compounds, four concentrations for each compound was prepared, (25, 50,100and 200 µg/ml), Amoxicillin and ceftriaxone were used as references antibiotic drugs, fluconazole was used as antifungal reference drug. DMSO was used as a solvent. One of these holes were filled with DMSO as control, to see the effect of solvent , These plates were incubated at 37C° for 24h .then experiment was retried using constant concentration of all compounds where was 25 µg/ml.

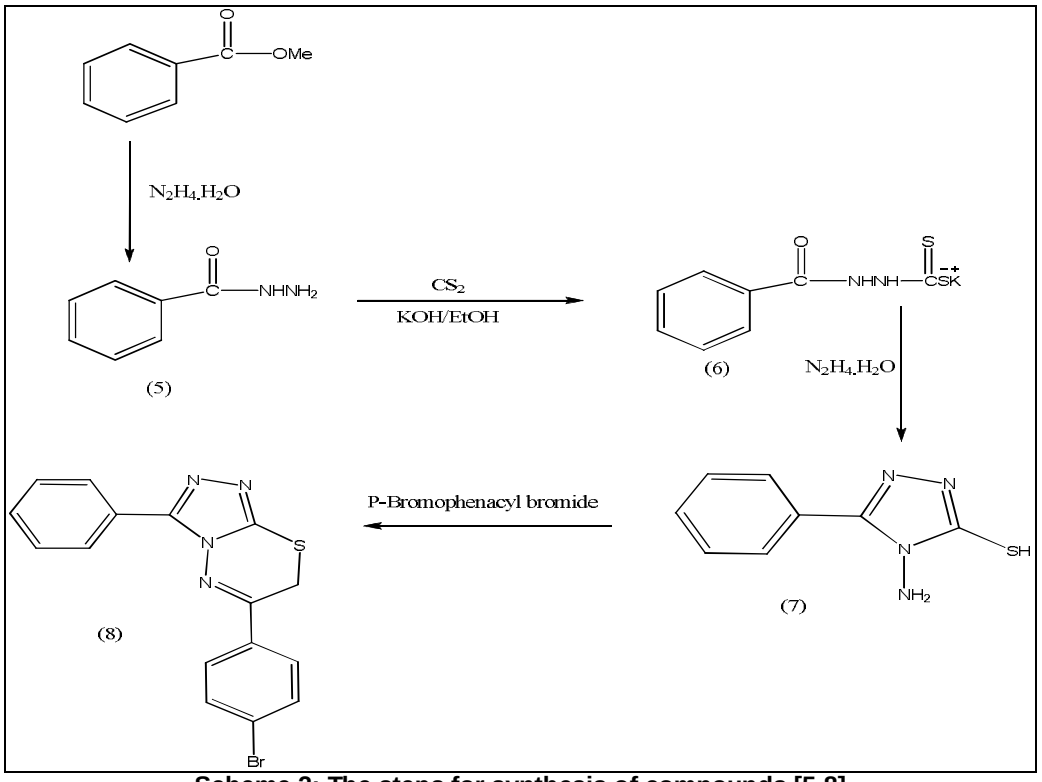
3-RESULT AND DISCUSSION

3.1-Chemistry

Compounds [1-8] were synthesized as shown in scheme 1 and scheme 2.some physical properties for this compounds were listed in table 1.



Scheme.1: The steps for synthesis of compounds [1-4]



Scheme 2: The steps for synthesis of compounds [5-8]

FTIR spectrum of acid hydrazide (1) shows characteristic absorption bands at 3300 cm^{-1} for N-H and (3220.60-3132) cm^{-1} for (NH_2) group ,and absorption band at 1666cm^{-1} due to carbonyl group .(Fig 1).

FTIR spectrum of triazole (3) showed the disappearance of the absorption band for carbonyl group, in the spectrum there are two other characteristic bands at (3210-3265) cm^{-1} and 2790 cm^{-1} due to (N-H_2) and (S-H) stretching vibrations, respectively. Fig(2)

the ^{1}HNMR spectrum shows a singlet signal at 3.868 ppm due to SCH_2 , singlet signal at 5.27 ppm due to NH_2 , singlet signal at 13.3 ppm due to S-H and singlet signals at 2.50 ppm and 3.32 ppm due to the solvent DMSO-d6 and water dissolved in DMSO-d6 respectively fig.(3).

FTIR spectra for compound (4) showed that disappearance of NH_2 bands and S-H band fig(4) .

The ^{1}HNMR shows a singlet signal at 3.856 ppm due to (CH_2) , singlet signal at 4.375 ppm for (SCH_2) in thiadiazoline ring ,doublet signal at (7.83 -7.90) belongs to (2H) in benzene ring and doublet signal at (7.22 -7.39) belongs to (2H) in benzene ring ortho to bromine and singlet signals at 2.50 ppm and 3.3 ppm due to the solvent DMSO-d6 and water dissolved in DMSO-d6 respectively. Fig.(5)

Compound(5) was characterized by its melting point which was (111-113) C° and reported (112-114) $C^{\circ(113)}$ and also by FTIR analysis , the FTIR spectrum showed appearances of two stretching bands of NH_2 asymmetric and symmetric at (3301 and 3214cm^{-1}), also the FTIR spectrum showed carbonyl of Amide appeared at 1661 cm^{-1} fig (6).

FTIR spectrum for compound (6) showed shifting in carbonyl group and appearances of C-S band at 600 Cm^{-1} fig (7)

compound (7) was characterizes by its melting point which was (195-197) °C , reported (198-200) $C^{\circ(16)}$, and its FTIR spectrum ,the FTIR spectrum showed absorption band at (3200-3118) C° due to NH_2 and another characteristic band at 2720 cm^{-1} belong to S-H. fig (8).

The FTIR spectrum for compound (8) showed that disappearance of absorption band for NH_2 and S-H.(fig 9).

3.2 Biological activity

The inhibition zones caused by the various compounds were examined. (**25** µg/ml concentration for all of these compounds). The results are listed in Table (2) and table (3).

4-CONCLUSION

From the result We conclude that the compounds3,4,7 and 8 have a good biological activities against four type of bacteria two of these were gram negative and the others were gram positive. Compound (8) showed the highest biological activity against *E.coli*and *E.faecalis* higher than the references Antibiotics (Amoxicillin & ceftriaxone), this activity may Attributed to Thiadizoline and triazole rings, whereas compounds 3,4 and 7 showed biological activity higher than Amoxicillin and less than ceftriaxone against these four bacteria, the activity of compound 3 and 7 came from the triazole ring and S-H groupas shown in fig (10).

Compounds 3,7,8 shows biological activity as antifungal agents against *Candida Albicans* higher than fluconazole this may attributed to S-H for compounds 3 , 7 and thiadiazoline ring for compound 8.Compound 4 showed biological activity against *candidaalbicans* a little less than *candida albicans* (fig. 11).

ACKNOWLEDGMENT

we are very grateful to Dr. Ivan H. R. Tomi for helpful about doing the ^{1}HNMR spectra and Dr. Zyad H.J. Al-Qaisi (Al-Mustansiriya University, Chem. Dept.) about doing the FT-IR spectra. Also we are very grateful to Dr. HaifaaHasany, Mr. HassanMajeed and Mr. AliMoed (Baghdad University, Biology. Dept.) for helping in the biological experiments. We ask Allah to bless all of them.

Table 1: some physical properties for compounds (1-8)

Compound Number	Chemical formula	M.wt g mol-1	color	m.p ◦C	yield %
1	$C_3H_8N_4O_2$	132.12	white	133-135	76.5
2	$C_3H_6K_2N_4S_4$	304.56	yellow	-	74
3	$C_5H_8N_8S_2$	244.30	white	190-193	52
4	$C_{21}H_{14}Br_2N_8S_2$	602.33	yellow	158-160	69
5	$C_7H_8N_2O$	136.15	white	111-113	71
6	$C_8H_7KN_2OS_2$	250.38	yellow	>300	66
7	$C_8H_8N_4S$	192.24	white	195-197	60
8	$C_{16}H_{11}BrN_4S$	371.25	yellow	213-215	72

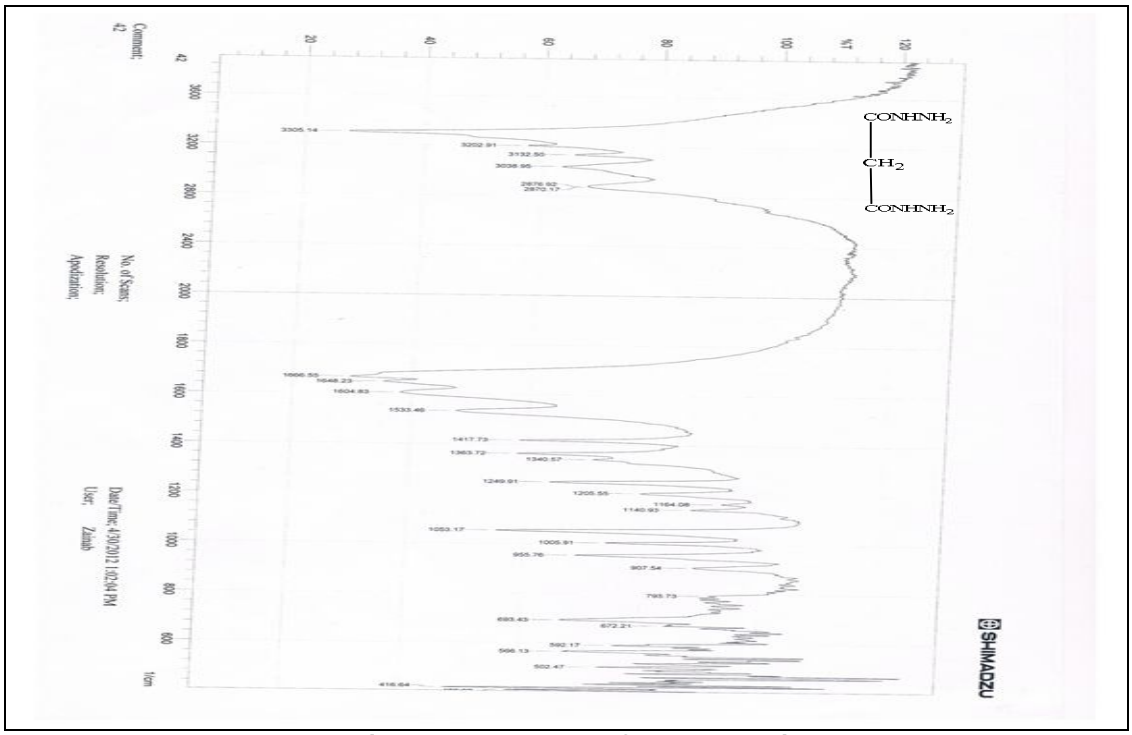


Fig. 1: FTIR Spectrum for compound 1.

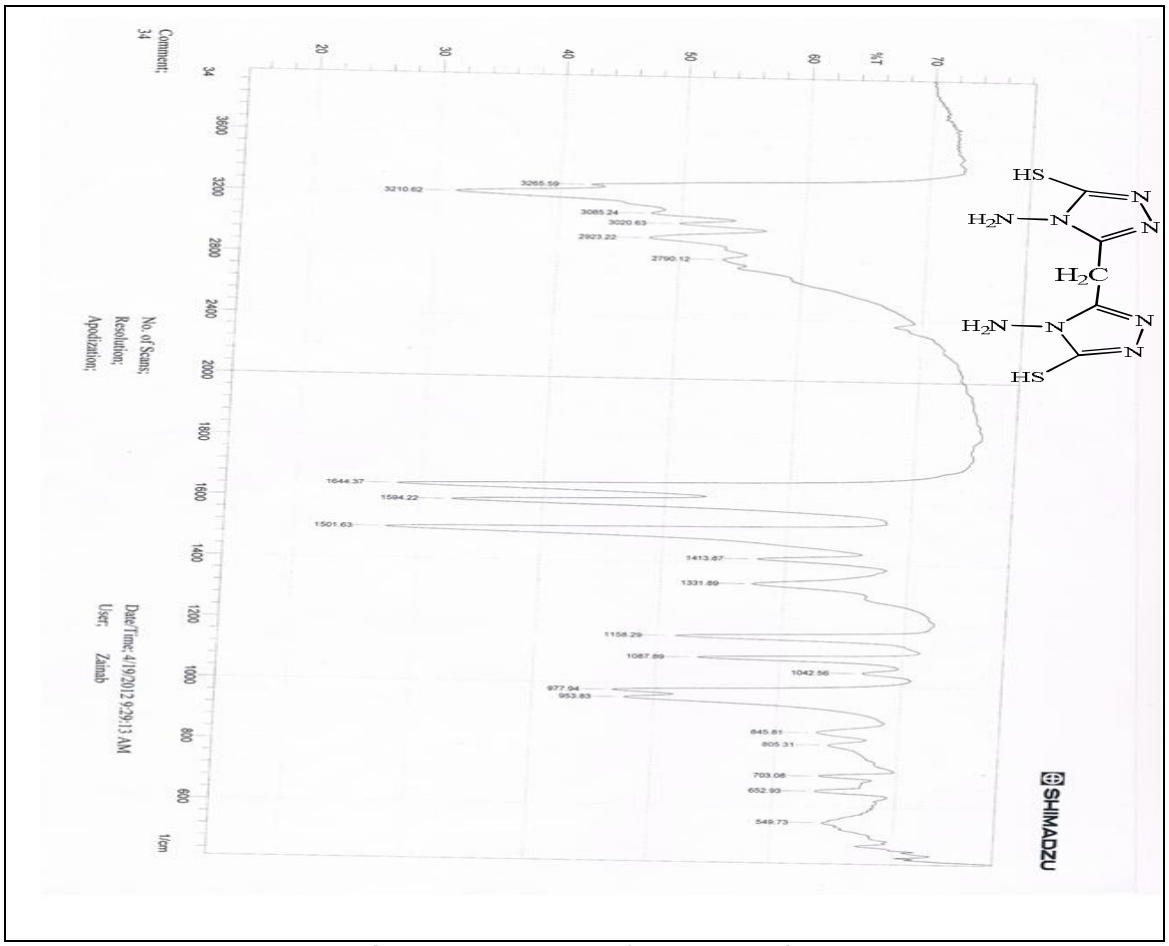


Fig. 2: FTIR Spectrum for compound 3

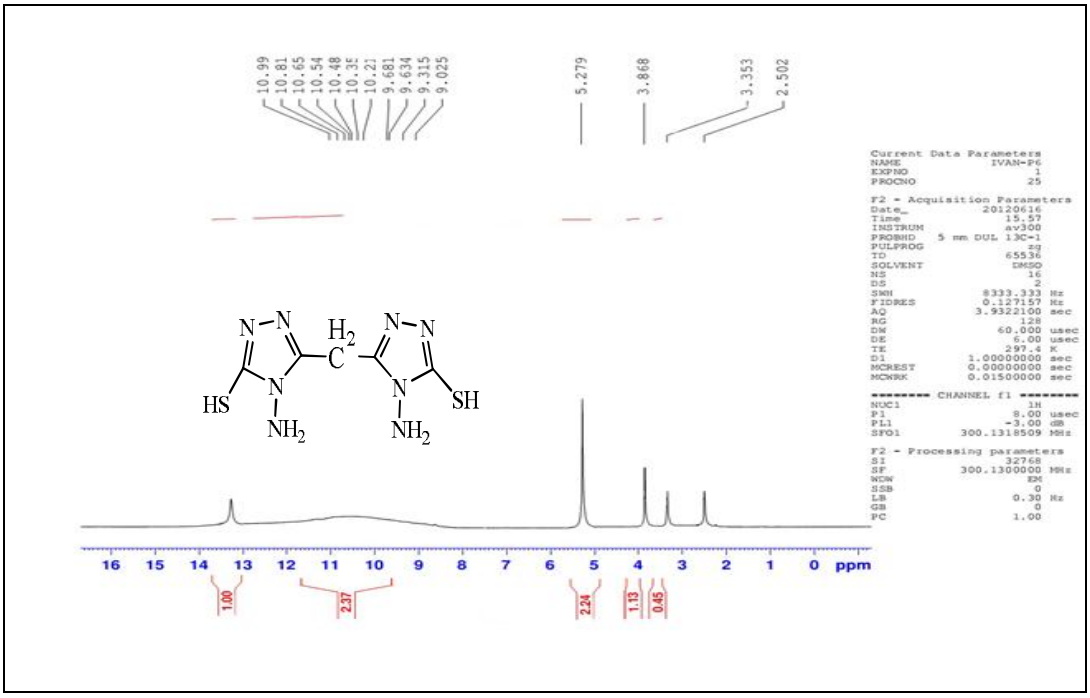


Fig. 3: ^{1}HNMR Spectrum for compound 3

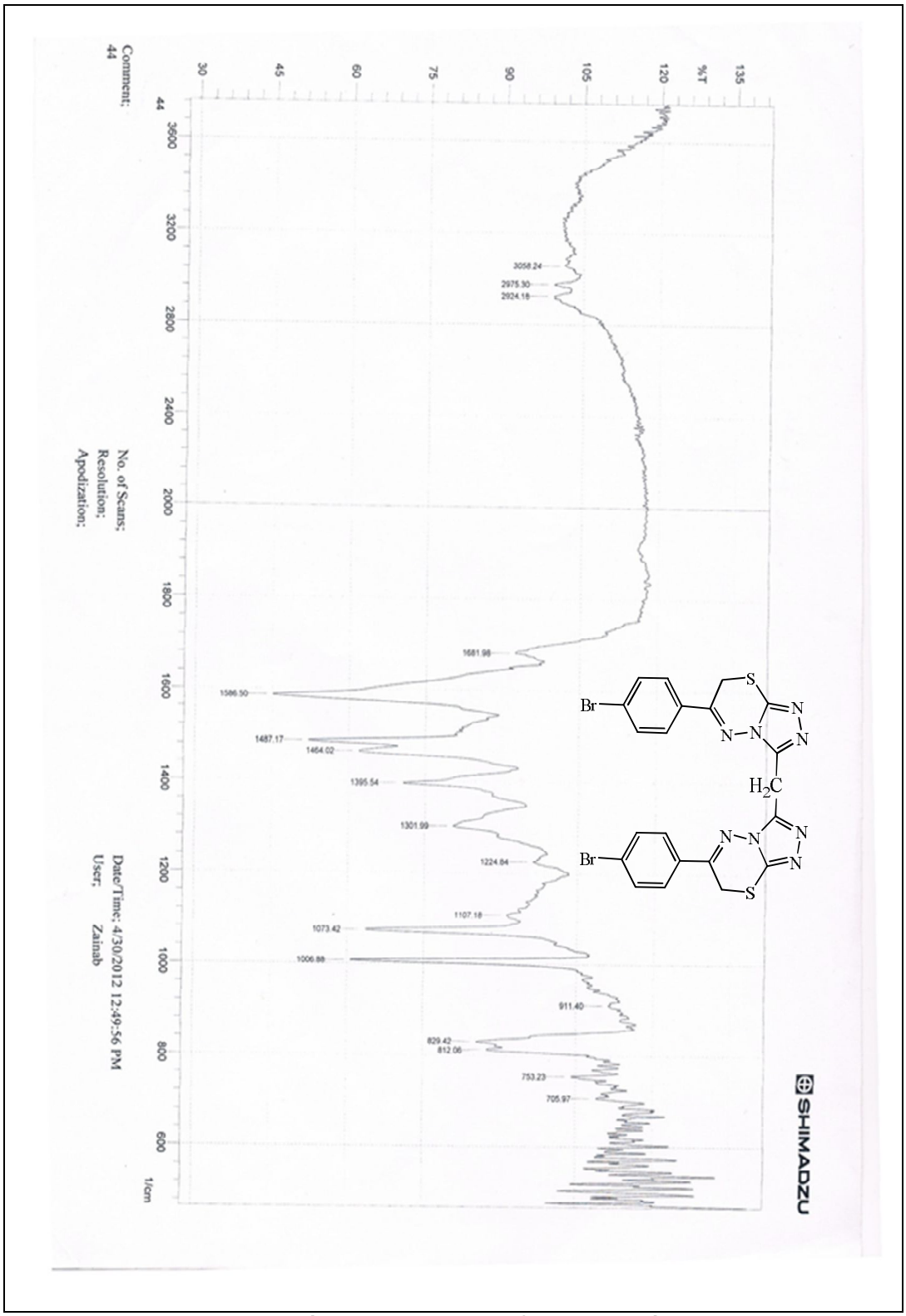


Fig. 4: FTIR Spectrum for compound 4

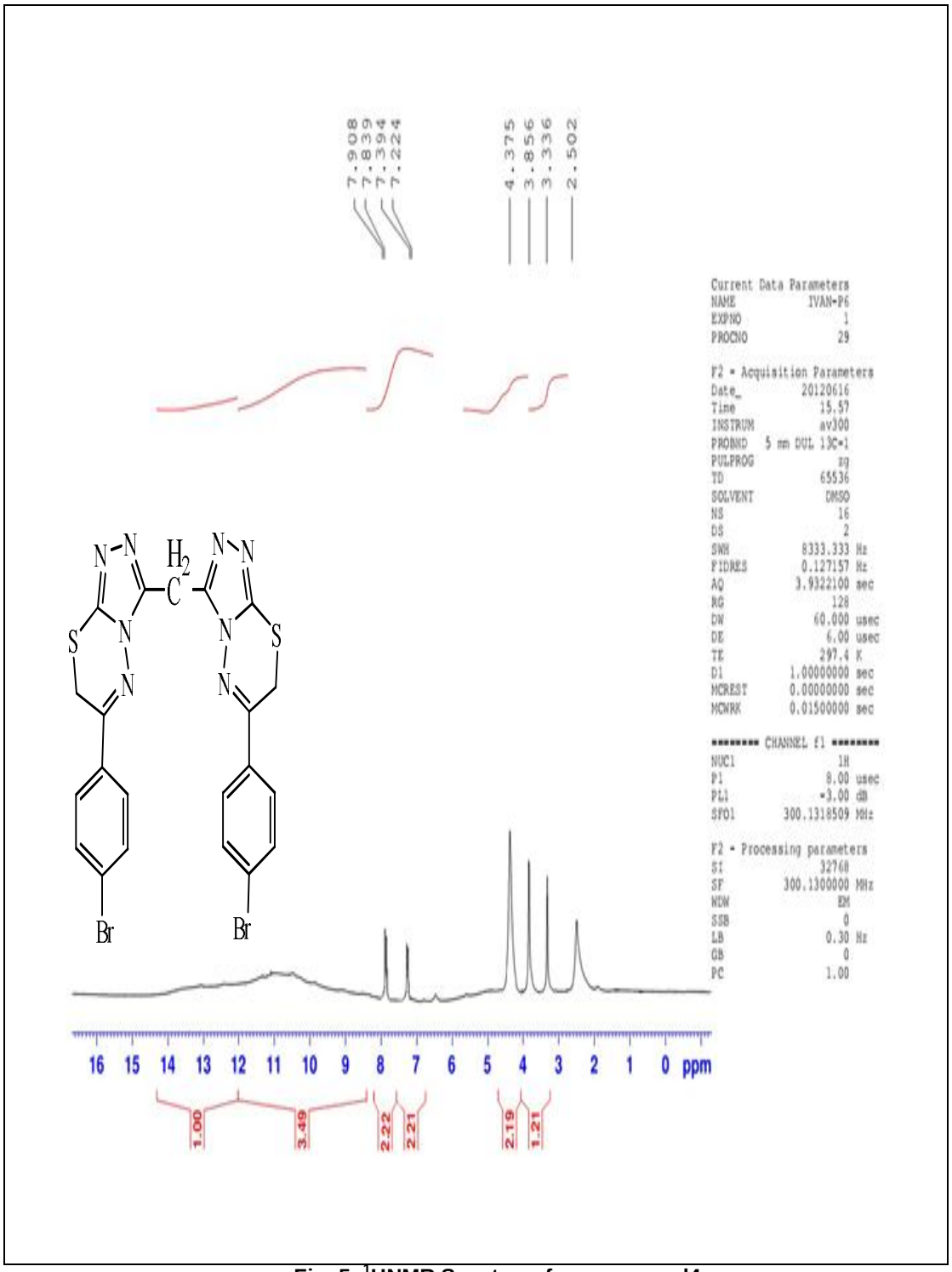


Fig. 5: ^{1}HNMR Spectrum for compound4

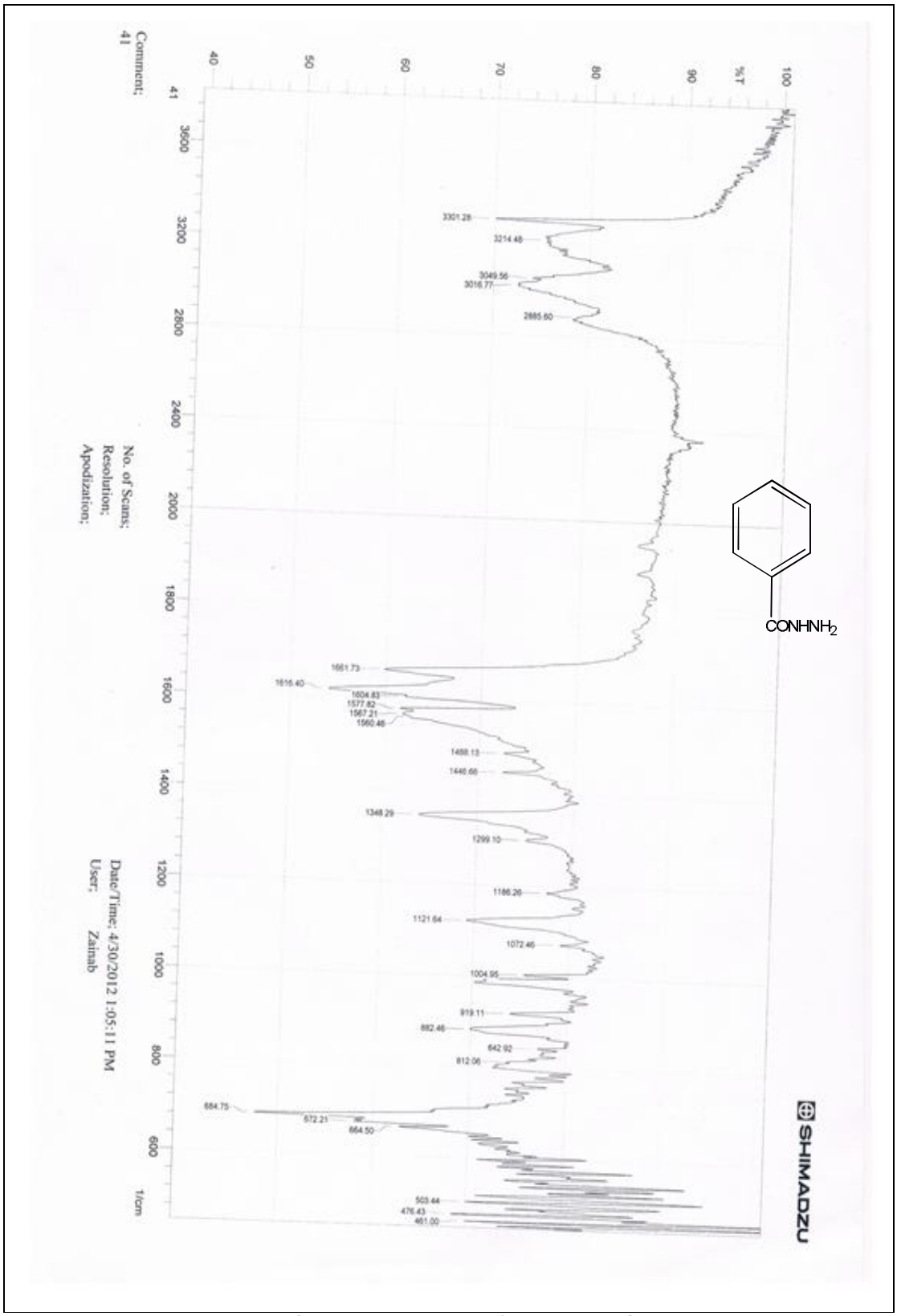


Fig. 6: FTIR Spectrum for compound 5.

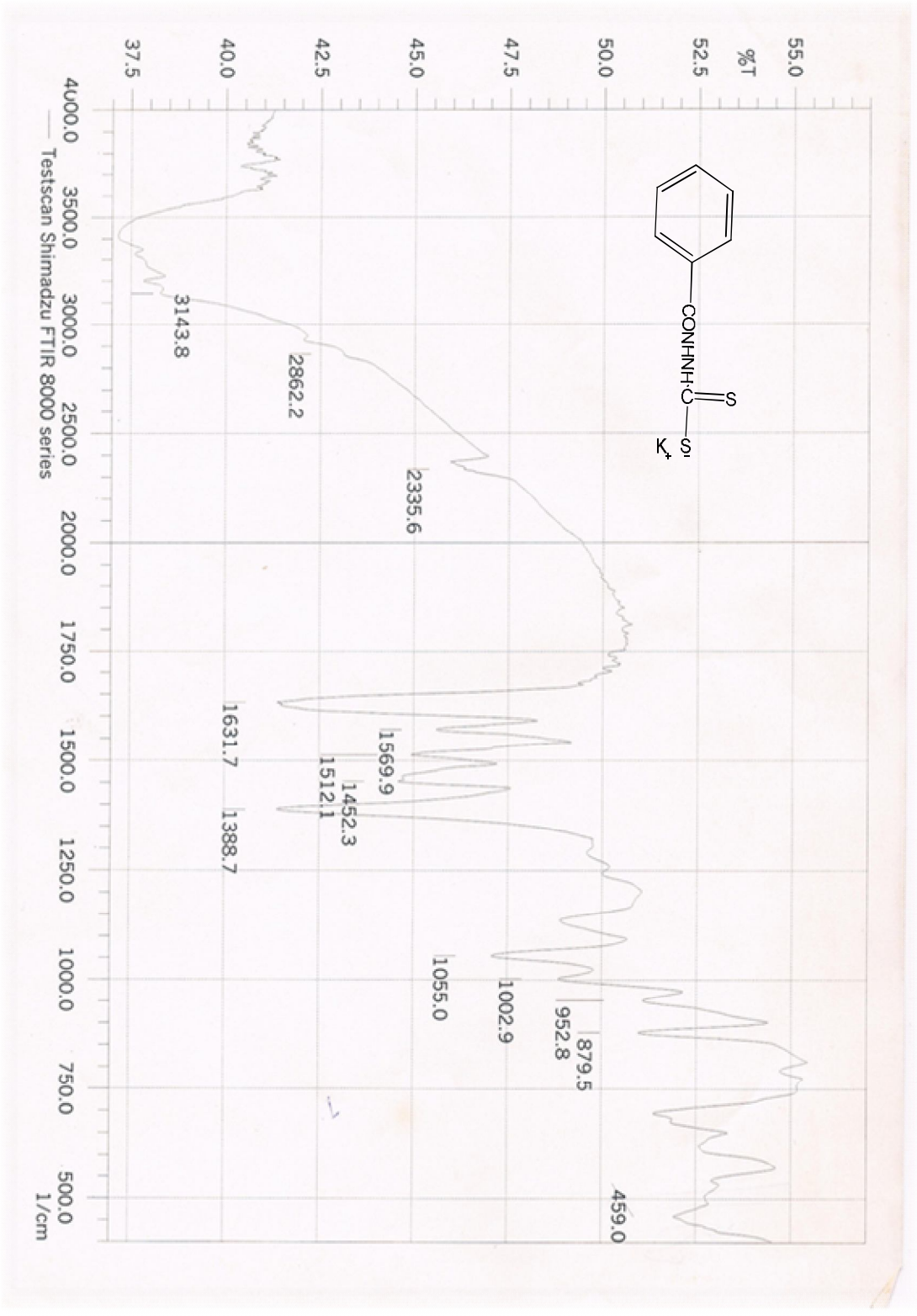


Fig. 7: FTIR Spectrum for compound 6

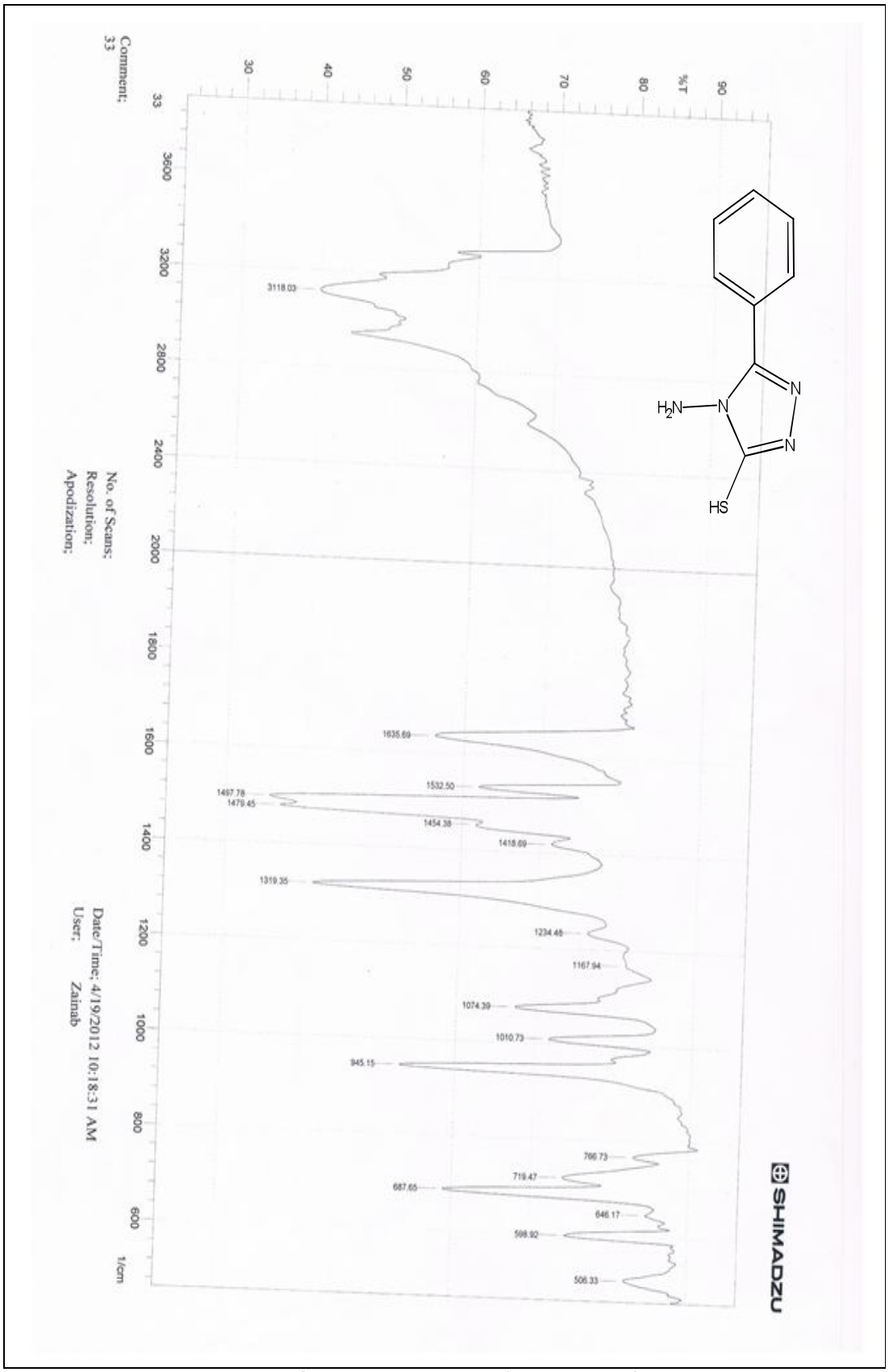


Fig. 8: FTIR Spectrum for compound 7

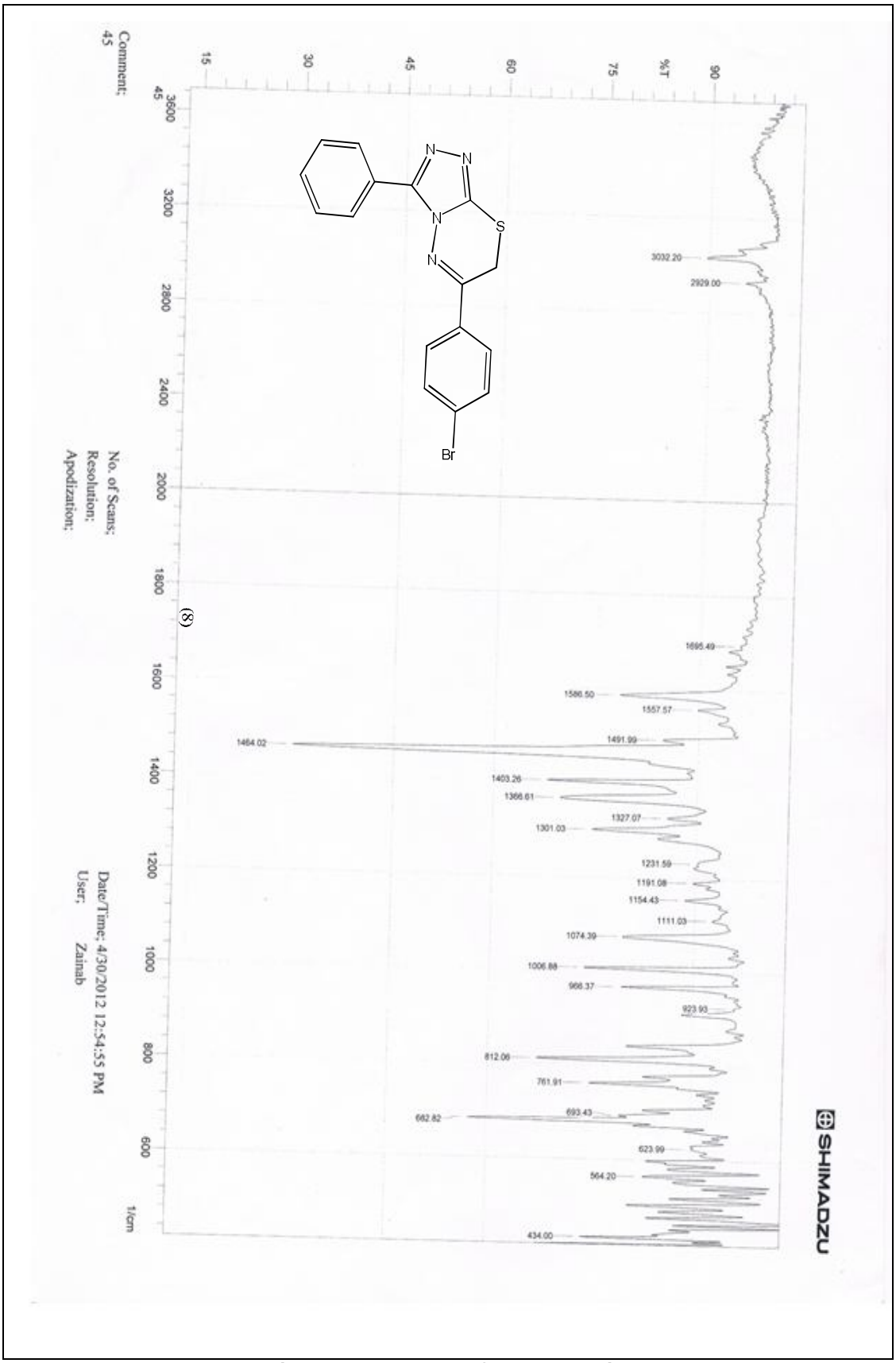


Fig. 9: FTIR Spectrum for compound 8

Table 2: Inhibition zones of compounds3,4,7,8 and the references antibiotics

compound	concentration mg/mL	inhibition zone in mm			
		gram positive		gram negative	
		E.faecalis	S.aureus	E.coli	K.pneumonia
3	25	15.8	18.94	18.9	17.2
4	25	16.77	17.58	15.39	16.17
7	25	15.5	17.9	16.22	15.9
8	25	19.3	18.26	23.77	16.8
ceftriaxon	25	18.2	21.3	20.28	20.93
Amoxicilline	25	14.1	13.4	15.11	12.9
DMSO		-	-	-	-

Table 3: Inhibition zones of compounds 3,4,7,8 and the references antifungal

Compound	concentration mg/mL	inhibition zone in mm
		Candida Albicans
3	25	17.85
4	25	16.89
7	25	22.73
8	25	21.35
Fluconazole	25	17.58
DMSO	-	-

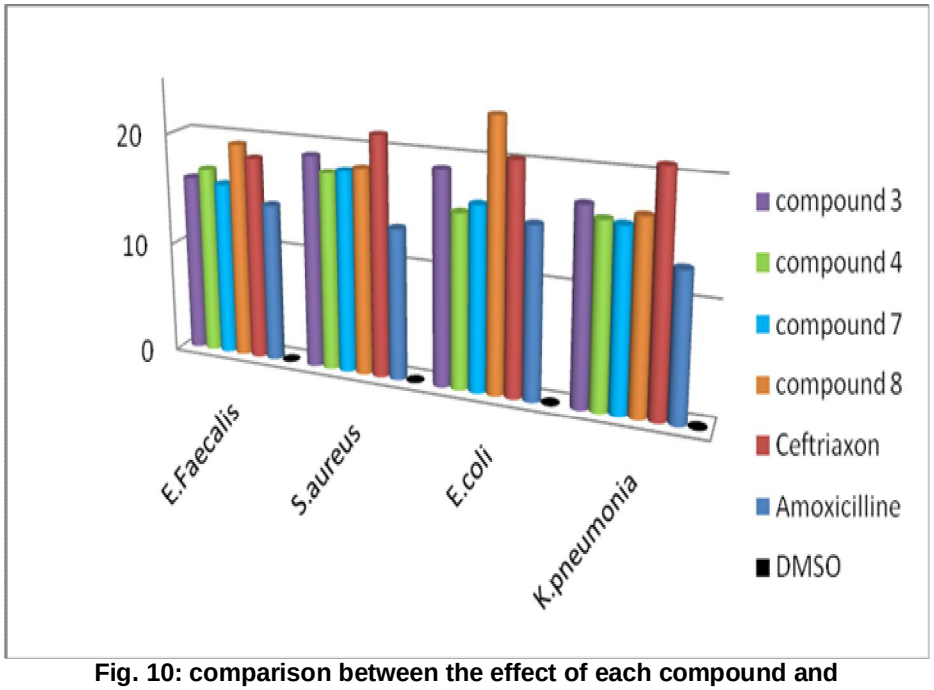


Fig. 10: comparison between the effect of each compound and reference antibiotics (Amox.&Cef.) for each type of bacteria

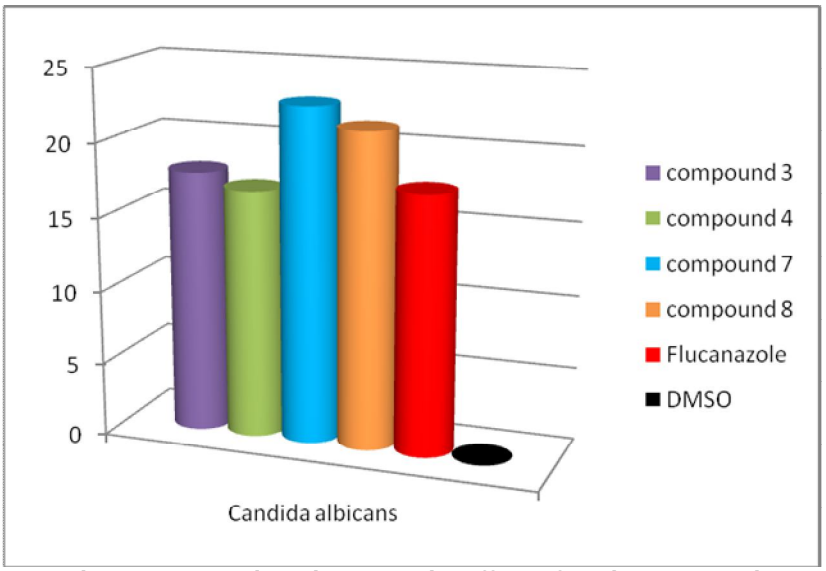


Fig. 11: Comparison between the effect of each compound and reference antifungal (fluconazole) against*candida albicans*

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