Synthesis, Characterization and Evaluation of Biological Activity of New Heterocyclic Compounds Containing 1,2,4- Triazoleand 1,3,4-Thiadiazole Rings

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Abstract

In this study, a new Triazole derivative was synthesized by many cyclization reactions. Compound (1) was cyclized by the reaction of hydrazine hydrate with carbon disulfide. The compound (1) was reacted with chloroethyl acetate in presence of alkali ethanol to give the ester (2). The compound (3) was obtained from the reaction of compound (2) with hydrazine hydrate. The salt (4) was formation by reacting of compound (3) with CS_2inalkali ethanol. The cyclization of salt (4) with hydrazine hydrate was involved formation of compound (5). The last step of this study was prepared the derivative (6) by the cyclization reaction of compound (5) with p-Bromophenacyl bromide. The biological evaluated of compound (5) and (6) were studied. The result showed that the compounds (5) and (6) possess high biological activity.

Keywords: Synthesis, Characterization, Antibacterial, Antifungal, 1, 2, 4-Triazole, 1, 3, 4-Thiadiazole.

1-Introduction

In the past decades, the problem of multi drug 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 our research on the biological properties of 1,2,4-Triazole and 1,3,4-Thiadiazole containing derivatives[7], we have designed and synthesized Triazole and Thiadiazole systems, as antibacterial agents.

2-Experimental

All starting materials and solvents were purchase from Fluka, 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 2,5-dimercapto-1,3,4-thiadiazole(1)[16]:

A mixture of (80%) hydrazine hydrate (0.1 mol, 5g, 4.5 ml) and carbon disulfide (0.2 mol,15g ,20ml) with dry pyridine (30 mL) was refluxed for 5 hrs.

Then the excess solvent was then distilled off, and the resulting solid was separated out by adding (25 mL) of water and (5 mL) of hydrochloric acid. The mixture was then filtered and the solid was recrystallized from ethanol. m.p = (162-164) C°, yield=77.6 %

2.2-synthesis of diethyl 2,2`-(1,3,4-thiadiazole-2,5-diyl)bis(sulfanediyl)diacetate(2):[17]

To a solution of 2,5-dimercapto-1,3,4-thiadiazole (0.1 mol ,15 g) in 20 ml of absolute ethanol , (0.2 mol , 12g) of potassium hydroxide was added. The solution was stirred for 30 min., then ethyl chloroacetate (0.2mol, 25ml) was added drop wise to the solution. The reaction mixture was refluxed for 4-5 hrs. Then cooled to room temperature, poured in 100 ml of ice water. The precipitated was filtered off, washed with water and recrystallized from ethanol.m.p: (47-48) C°, yield 79%

2.3- Synthesisof 2,5-bis(mercapto-acetichydrazide)-1,3,4-thiadiazole(3).

A Suspension of compound [2] (0.02 mol ,6.4 g) in 20 ml of absolute ethanol was stirred for 15 min at 40 °C until the ester was dissolved , (0.04 mol, 2.2ml , 2 g) of hydrazine hydrate (80 %) was added and the solution was stirred for 30 min , cooled , filtrated ,washed and recrystallized from ethanol.m.p=(140-142)C°, yield=74%

2.4- Synthesis of bis-potassium dithiocarbazinate(4) [18]

Potassium hydroxide (0.03 mol, 1.68 g) was dissolved in absolute ethanol (25 mL). The solution was cooled in ice bath and the compound (3) (0.01 mol, 2.94 g) 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 the solution and then dried in vacuum. The potassium salt thus obtained was used in the next step without further purification. Yield 73%.

2.5-Synthesis of5,5'-(1,3,4-thiadiazole-2,5 diyl)bis(sulfanediyl)bis(methylene)bis(4-amino-4H-1,2,4-triazole-3 thiol)(5)[18]

A suspension of compound (4) (3mmol,1.56g) in water (4 mL) and hydrazine hydrate (80%, 9 mmol,0.45ml) was refluxed for 18– 20 hr. 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 (5mL). On acidification with Conc. HCl the required triazole was precipitated out, which was recrystallized from DMF–water mixture. m.p= (198-200) C°, Yield= 52%

2.6-synthesis of 2,5-bis((6-(4-bromophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)-1,3,4-thiadiazole(6)[19]

A suspension of compound(5) (0.5mmol, 0.4 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. m.p = (192-194) C°, yield =71%

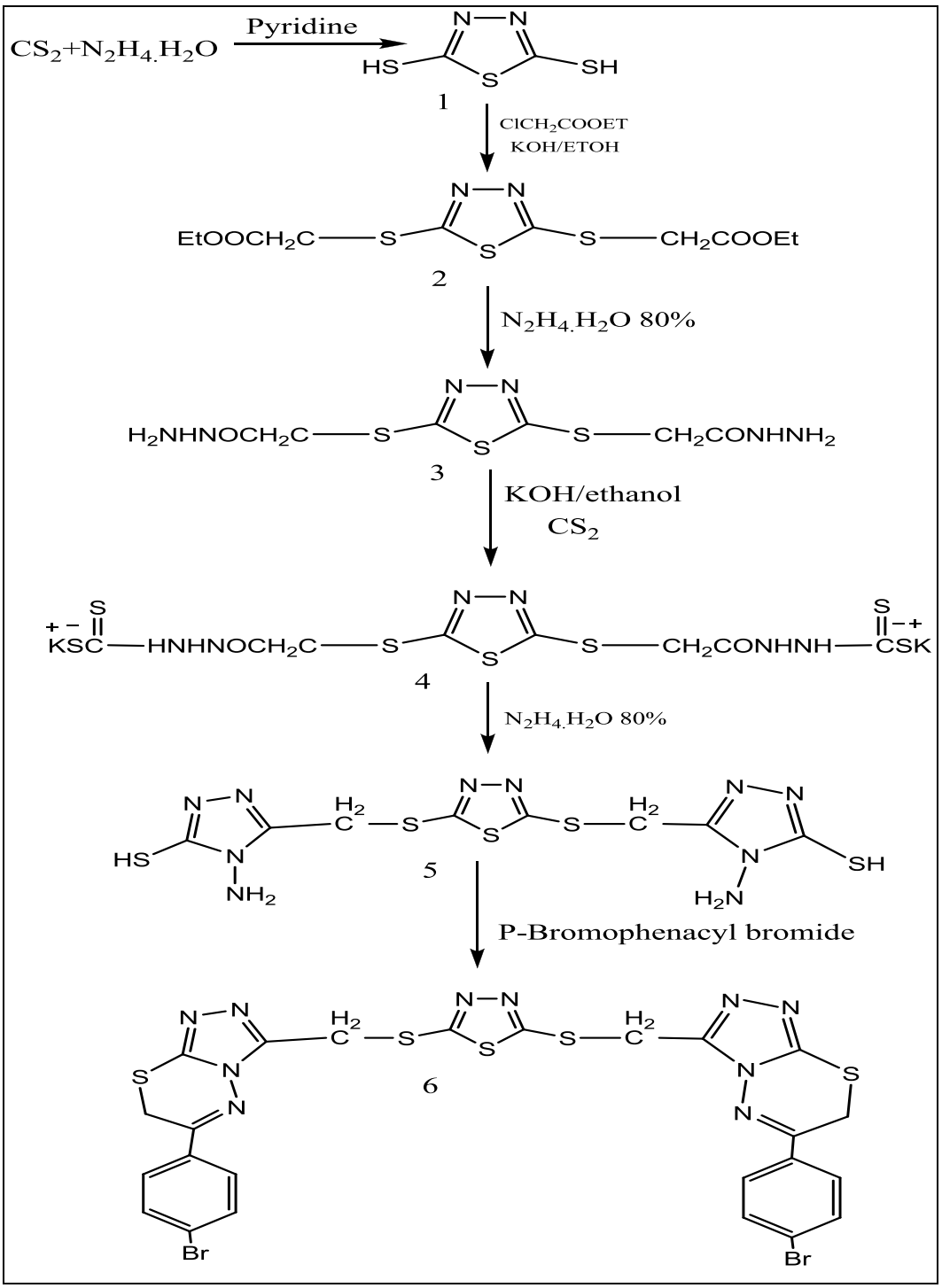
2.7-Biological study

The antibacterial test was performed according to the disc diffusion method. Compounds ([5], [6],) were assayed for their antimicrobial activity in vitro against four strains of bacteria (two of them were gram negative (*Escherichia coli*, *Klebsiella pneumoniae*) and the other were gram positive (staphylococcus aureus, *Enterococcus faecalis*)). Prepared agar and petri dishes 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 were prepared, (25, 50, 100 and 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

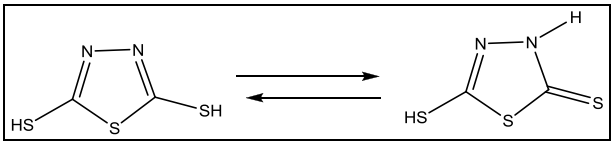
3.1-Chemistry

Compounds [1-6] were synthesized as shown in scheme 1.Some physical properties for this compounds were listed in table 1.



Scheme1 .the steps for synthesis of compounds [1-6]

Compound (1) was characterized by its melting point and by FTIR spectrum, melting point was recorded (162-164) C° and the reported m.p was (164-166) C° [18]. The FTIR spectrum of compound (1) showed a medium intensity band at 1624 cm^{-1} that could corresponds with (C=N) bond in the vicinity of 1,3,4-thiadiazole ring. In this spectrum there are two other characteristic bands at 3055.35cm^{-1} and 2733.22 cm^{-1} due to (N-H, thion form) and (S-H) stretching vibrations, respectively. That means compound 1 can exist in the thiol and thion form.

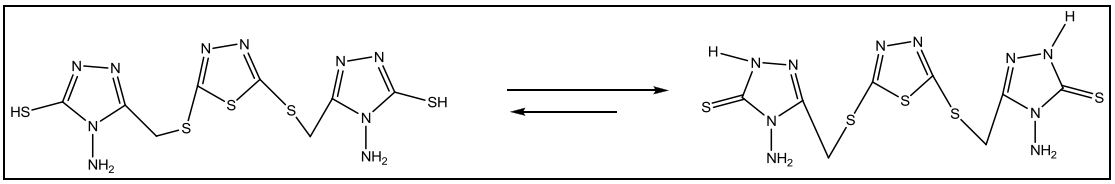


Compound 2 was characterized by FTIR and ^{1}HNMR spectroscopy, FTIR spectrum shows strong absorption band at 1741.78 cm^{-1} due to carbonyl group for ester and bands at 2987.84 cm^{-1} and 2939.61 cm^{-1} for aliphatic (CH_2) group. Disappearance of S-H and N-H absorption bands was also detected.

^{1}HNMR spectrum of compound (2) showed triplet signal at δ=(1.163 -1.211) ppm belong to (CH_3),quartet signal at δ =(4.09-4.16)ppm belong to (CH_2), singlet signal at δ=4.215 ppm belong to (SCH_2) and singlet signal at δ =2.50 ppm and 3.32 ppm due to the solvent DMSO-d6 and water dissolved in DMSO-d6 respectively.(Fig 1).

FTIR spectrum of compound(3) shows characteristic absorption bands at 3319 cm^{-1} for N-H and (3292.60-3269.45)cm^{-1} for (NH_2) group ,and shifting in carbonyl group to 1693.56 cm^{-1}.(Fig 2).

FTIR spectrum of compound (5) shows the disappearance of the absorption band for carbonyl group, in the spectrum there are two other characteristic bands at 3456.55 cm^{-1} and 2804.59 cm^{-1} due to (N-H) and (S-H) stretching vibration bands, respectively. That indicates that the compound (5) exists in the thiol and thion form.



the ^{1}HNMR spectrum shows a singlet signal at 4.265 ppm due to SCH_2 , singlet signal at 5.27 ppm due to NH_2 , singlet signal at 12.8 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
The formation of compound (6) is indicated by disappearance of NH_2bands, NH band and S-H band in FTIR spectrum. The ^{1}HNMR shows a singlet signal at 4.158 ppm due to (SCH_2) , singlet signal at 4.808 for (SCH_2) in thiadiazoline ring doublet signal at (7.78 -7.80) belongs to (2H) in benzene ring and doublet signal at (7.12 - 7.29) belongs to (2H) in benzene ring ortho to bromine and singlet signals at 2.50 ppm and3.3 ppm due to the solvent DMSO-d6 and water dissolved in DMSO-d6 respectively.(Fig.4)

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). From the results we conclude that the compounds 5 and 6 have a biological activity as antibacterial agents against four type of bacteria two of these were gram negative and the others were gram positive, compound 5 shown strong biological activity against *E.Coli* more than ceftriaxone and amoxicillin, whereas compound 6 shown biological activity more than Ceftriaxone and Amoxicillin against *E.faecalis* as shown in(fig 5) Compound 6 and compound (5) shows biological activity as antifungal agents against *Candida Albicans* higher than fluconazole at the same concentration which was 25 µg/ml, (fig 6explainsthe biological activity of compound 5 and 6 and compare these compounds with fluconazole against *Candida Albicans*).

4-Conclusion

From the result it can be concluded that the compounds (5) and (6) have a good biological activities against these microorganisms. compound (5) showed the highest biological activity against *E.coli* higher than the references Antibiotics (Amoxicillin & ceftriaxone) , this activity may Attributed to S-H group , compound (6) showed the highest biological activity against *E.faecalis* higher than the references Antibiotics this may Attributed to thiadiazoline ring whereas ceftriaxone shown its highest biological activity against *K. pneumonia* and *S.aurous(*Fig.5).

Compound (5) showed strong biological activity against fungi *Candida Albicans* higher than compound (6) and reference antifungal (fluconazole) (figure 6)

Table 1 Some physical properties for compounds (1-6).

Compound number	Chemical formula	Molecular weight gm/mol^{-1}	Color
1	$C_2H_2N_2S_3$	150.25	yellow
2	$C_{10}H_{14}N_2O_4S_3$	322.42	White
3	$C_6H_{10}N_6O_2S_3$	294.38	white
4	$C_8H_8K_2N_6O_2S_7$	522.84	yellow
5	$C_8H_{10}N_{10}S_5$	406.56	white
6	$C_{24}H_{16}Br_2N_{10}S_3$	700.45	Yellow

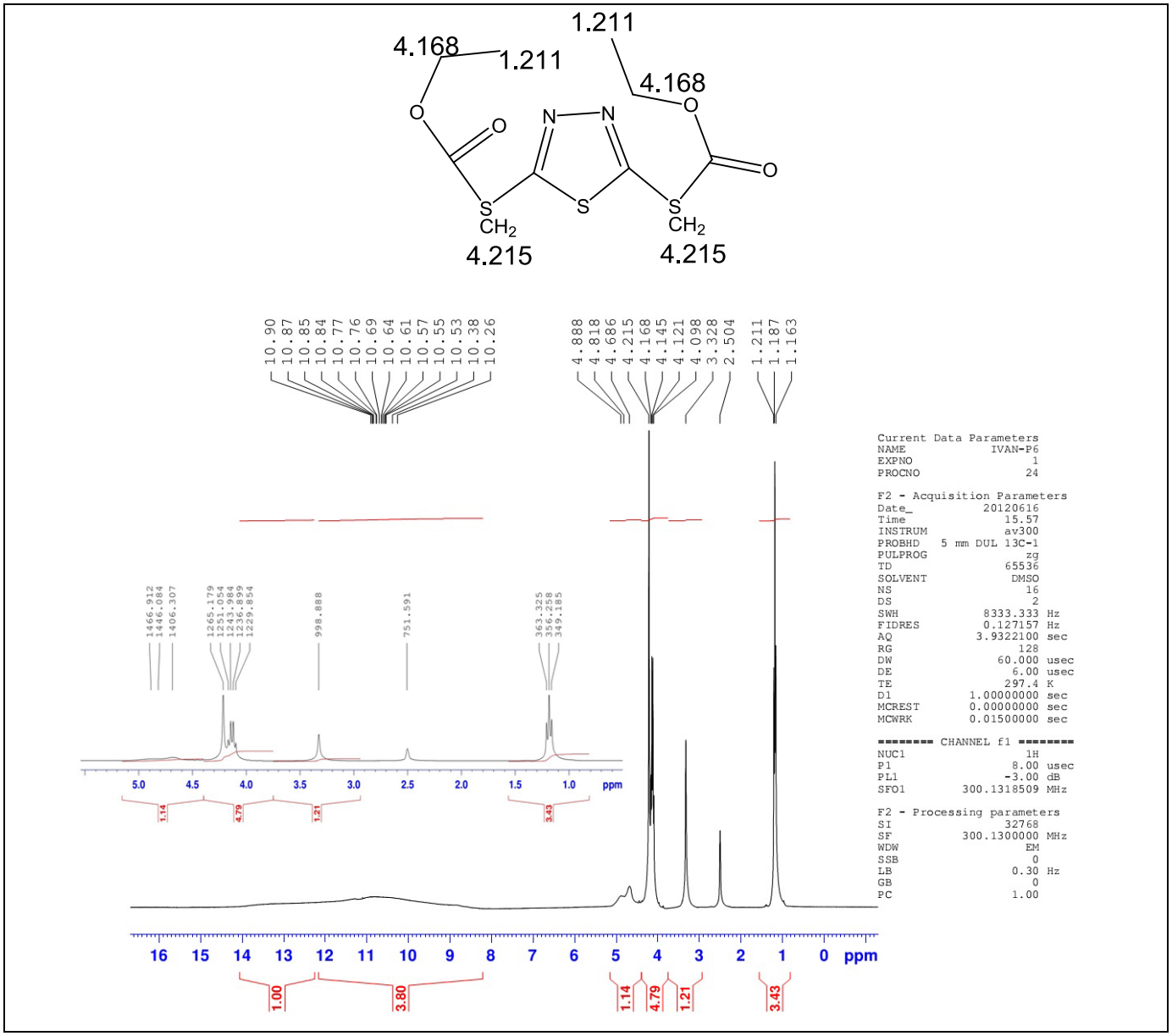


Fig.1^{1}HNMR for compound 2

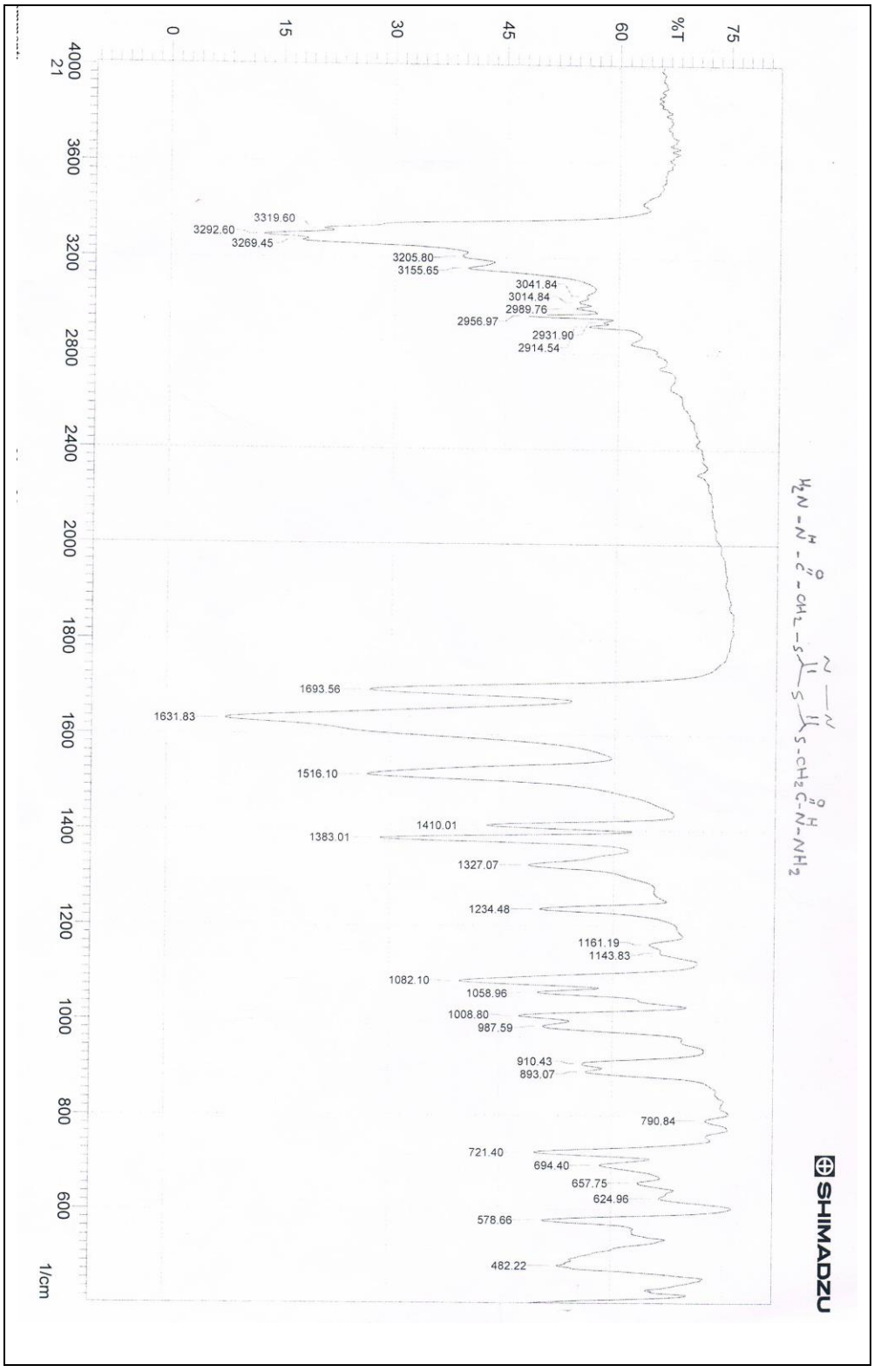


Fig.2 FT-IR spectrum for compound (3)

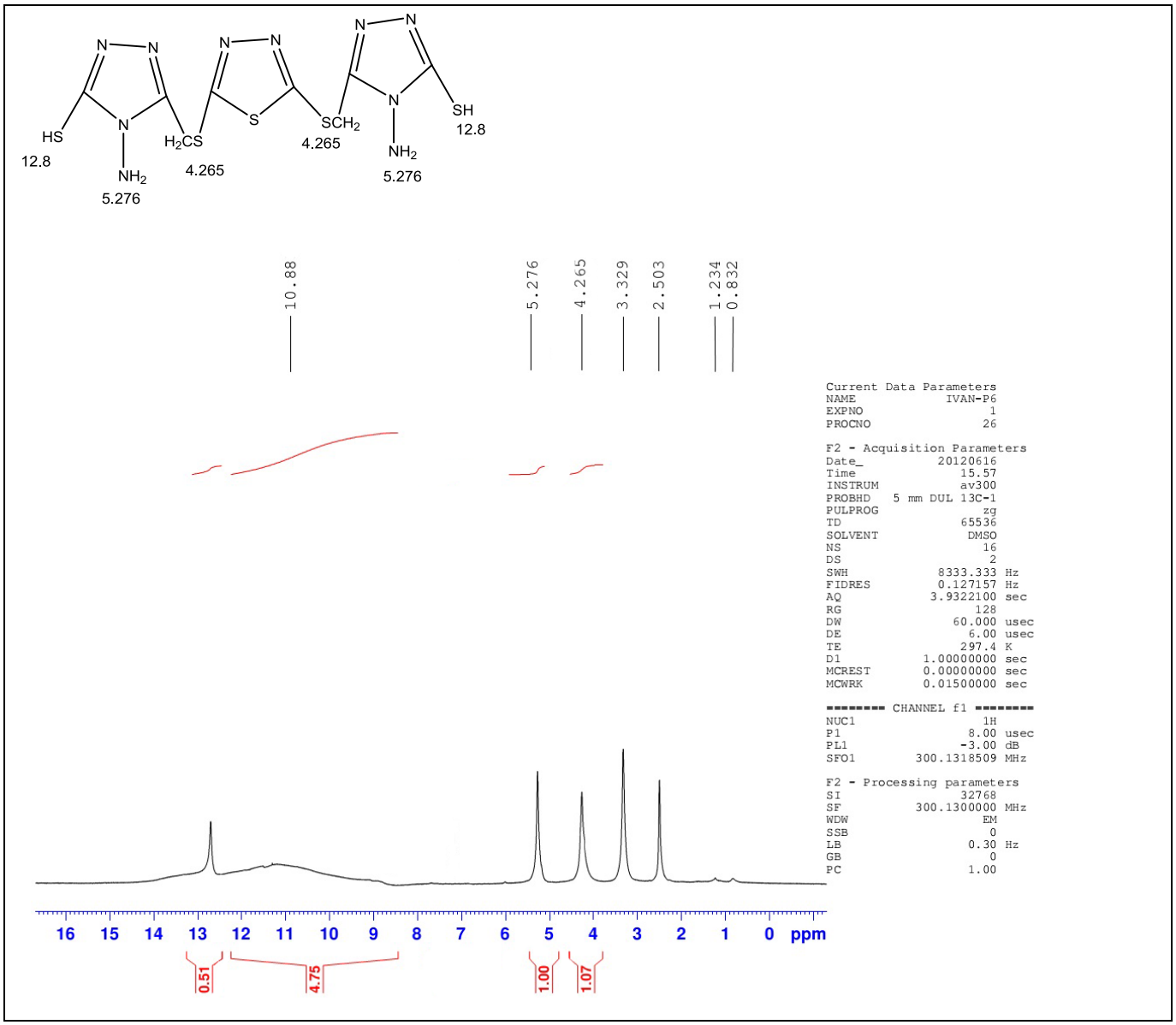


Fig. 3[1]HNMR spectrum for compound 5.

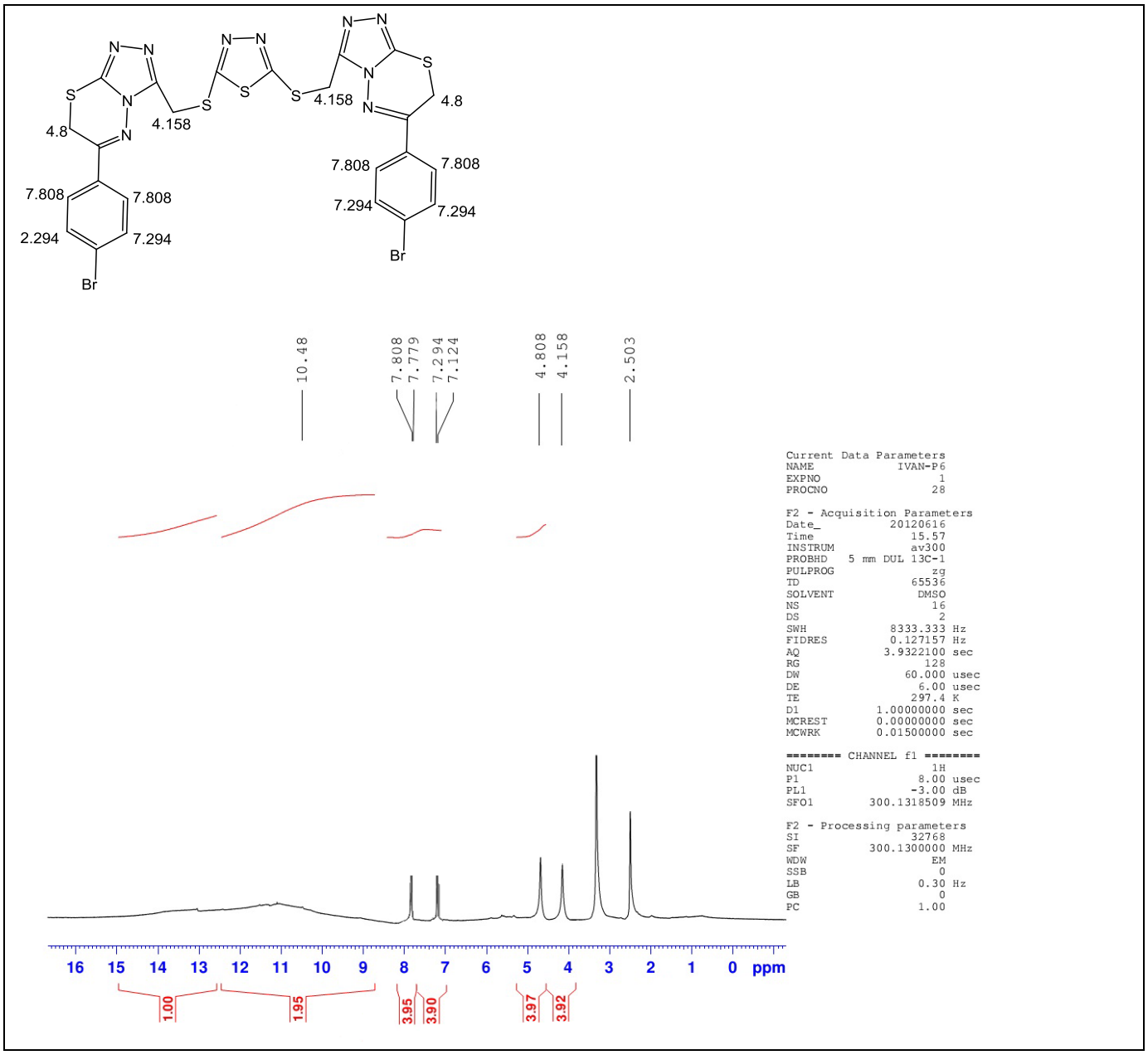


Fig.4 ^{1}H NMR spectrum for compound 6

Table 2. Inhibition zones of compound 5, 6 and the references antibiotics

Compound	Concentration mg/mL	Inhibition zone in mm			
		gram positive		gram negative	
		E. faecalis	S. aureus	E. coli	K. pneumonia
5	25	16.5	18.33	21.44	16.8
6	25	18.8	16.9	17.57	13.3
ceftriaxone	25	18.2	21.3	20.28	20.93
Amoxicillin	25	14.4	13.4	15.11	12.9
DMSO		-	-	-	-

Table3. Inhibition zones of compound 5,6 and the references antifungal

compound	concentration mg/mL	Inhibition zone in mm
		Candida Albicans
5	25	19.9
6	25	18.61
fluconazole	25	17.85
DMSO		-

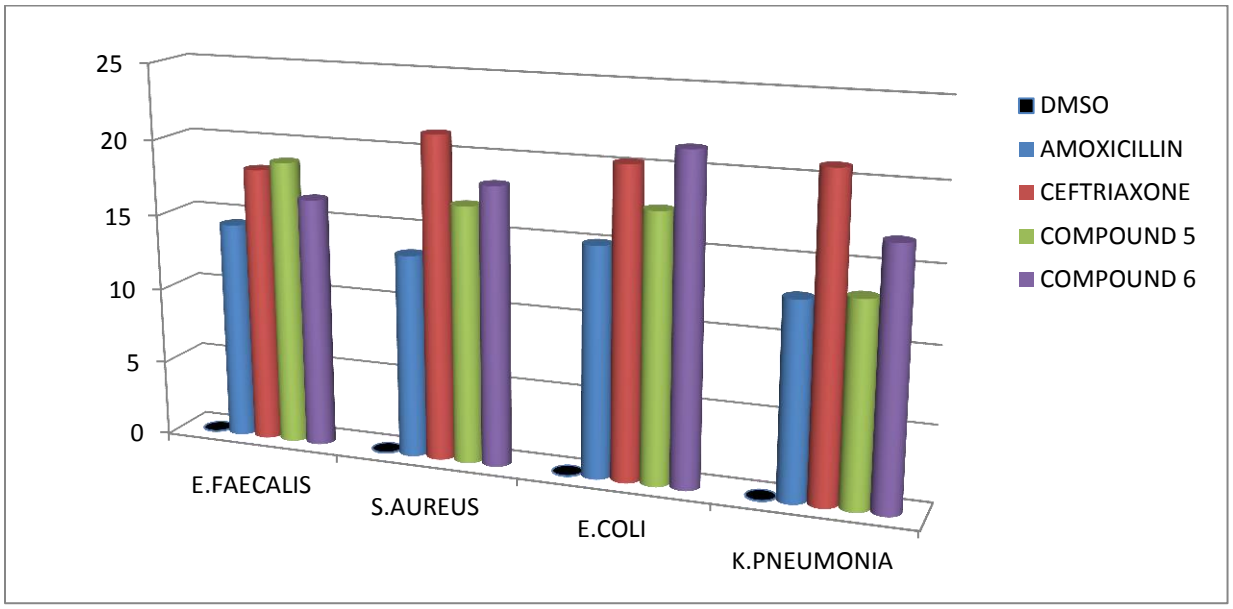


Fig 5.Comparison between the effect of each compound and reference antibiotics (Amox.&Cef.) for each type of bacteria

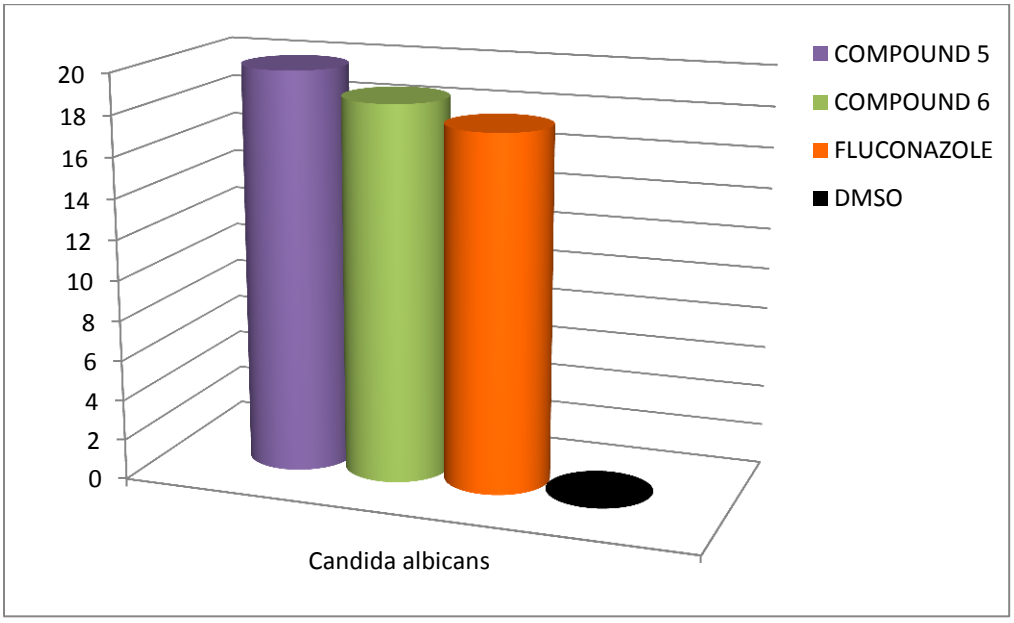


Fig 6. Comparison between the effect of each compound and reference antifungal (fluconazole) against*candida albicans* .

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