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SYNTHESIS AND MOLECULAR DOCKING STUDIES OF NEW SERIES OF BIS-SCHIFF BASES THIADIAZOLES DERIVED FROM DISULFIDES AND THIOETHERS WITH POTENT ANTIBACTERIAL PROPERTIES

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ABSTRACT:

Two new series of various heterocyclic bis-Schiff bases of Thiadiazoles disulfides and thioethers are synthesized. The first series was the synthesis of bis-Schiff of thiadiazole disulfides (**4a-h**) synthesized from their corresponding Schiff bases after oxidation by hydrogen peroxide. The second series was the synthesis of bis-Schiff base thiadiazole thioethers (**7a-h**). It was obtained from the reaction of bis-thiadiazole diamine (**6**) with different aldehyde. The diamine **(6)** was generated by reacting thiadiazole with dibromoethane in dimethylformamide and triethylamine as a catalyst. All of the synthesized compounds were analyzed by using FT-IR, ^{1}H-NMR, ^{13}C-NMR, and C-APT techniques. The synthesized compounds were screened against two gram-positive bacteria (*Staphylococcus and streptococcus*), and two strains of gram-negative (*E. coli and klebsiella*) in comparison to two standard strong antibiotic drugs (ciprofloxacin and vancomycin). Generally, all the synthesized compounds showed highly antibacterial properties, particularly, compounds (4b,4f, and 7b) which observed potent anti-bacterial properties against both gram- (positive, negative) bacteria were even higher than the standard drugs. Lastly, molecular docking studies were also applied to find the binding affinity and active sides of the products with the target proteins.

KEYWORDS: Thiadiazole, Schiff base, Molecular docking study, Biological activity, and Antibacterial resistance.

1. INTRODUCTION

1,3,4-thiadiazoles is an important and most biologically active family of 5-membered nitrogen-containing heterocyclic rings. This is due to their extensive applications in medicinal chemistry, materials science, and chemical synthesis (Niu et al., 2015), as well as in biological functions such as anti-diabetic and anti-epileptic drugs (Mousa, 2017), Antifungals (Zhang et al., 2017), analgesics, anthelmintic (Mathew *et al*., 2006), herbicides (Wang et al., 2011), and antivirals (Mahmoud *et al*., 2013) are just a few examples of the many biological activities that highlight these compounds. Furthermore, the 1,3,4-thiadiazole derivatives may affect DNA replication mechanisms, and they can stop the growth of cancerous cells (Matty *et al*., 2009; Janowska *et al*., 2020).

The interesting antibacterial activities of thiadiazole compounds and the need for new antibiotic drug candidates have attracted the interest of organic chemists to synthesize these compounds and become the starting material core structure for producing more new compounds.

The antibiotic resistance to the available antibiotic drugs is the serious issue of the century, each year, infections caused by AMR (antimicrobial resistance) claim the lives of millions of people. For infections caused by resistant microorganisms, treatment requires greater dosages of antimicrobial treatments or other therapies that may be more harmful than the original therapy. These strategies may also be more expensive. Therefore, the humanity is in urgent need to new antibiotic. Thiadiazole compounds might be promising future drugs to solve this issue.

The common synthesis of thiadiazole requires the reaction of hydrazine with carbon disulfide (Losanitch, 1922), and also oxidation of Bithioureas by hydrogen peroxide (Guha, 1922). Thiadiazoles could be also prepared from the reaction of thiosemicarbazide with carbon disulfide in the presence of Na_2CO_3 (Al-Badrani *et al*., 2010).

The synthesis of thiadiazole Schiff bases has also received a lot of interest because it is well-known that Schiff bases containing heterocyclic rings are cytotoxic and that they act against microorganisms and are anti-cancer and anti-fungal, malarial, viral, and bacterial infections. They are also known to be antidepressant enzyme inhibitors (Durmuş et al., 2017), analgesics, anthelmintic, growth regulators of plants (SALIH, 2008), and anti-pyretic (Kapadiya et al., 2020). Some Schiff bases are also utilized in ion sensors and electrochemical sensors to improve the selectivity and sensitivity of detection (Mobinikhaledi *et al*., 2011).

Recently, molecular docking as a computational approach aided the synthesis of many biologically important compounds. This finds the ligand that matches the protein's binding site of the bacteria energetically and geometrically (Azam *et al*., 2013). In particular, protein-ligand docking has a distinct position in the docking area because of its medical uses (Muegge *et al*., 2001; Vijesh *et al*., 2013). Nowadays, it has become a vital component of many drug development initiatives (Kitchen et al., 2004). This work aimed to synthesize and determine the antibacterial properties of the new bis-Schiff bases of thiadiazoles, molecular docking and the effect of disulfide and thioether groups on the antibacterial properties were also studied.

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2. EXPERIMENT SECTION

All the chemicals were purchased from Sigma-Aldrich, Fluka, and Merck and used without further purification. Melting points were determined on an electro thermal melting point apparatus (BUCHI Germany Model.B-540 at Koya University). Thin-layer chromatography (TLC) reaction monitoring was carried out using aluminum plate pre-coated SiO_2 gel (HF254, 200 mesh). n-hexane and methyl acetate was used as eluent in different ratios. Iodine and $KMNO_4$ were used as a stain, and a hand-held UV lamp was also used, which was supplied by genetic center at Koya University. FT-IR spectroscopy was conducted on the Thermo Scientific Spectrometer (Nicolet I S 10 at Raparin University and Shimadzu 1 S at Genetic center-Koya University).
^{1}H-NMR, ^{13}C-APT NMR (Attached-Proton-Test) and ^{13}C-NMR spectra for all the prepared compounds were recorded on (Bruker 400 MHz NMR spectrometer) at Firat University/Turkey and Basra University/Iraq). DMSO-d_6 was used as a solvent, and chemical shift expressions were reported in ppm representing multiplicity in standard expressions [singlet (s), doublet (d), triplet (t), coupling constant (J), Hertz (Hz)]. A micro plate reader at Koya technical Institute-Erbil polytechnic University was used to detect antimicrobial activity of the synthesized compounds.

2.1 Synthesis of 2-amino-5-mercapto-1,3,4 thiadiazole (1)

(1.36 g, 0.015 moles) of thiosemicarbazide and (1.59 gm, 0.015 moles) anhydrous sodium carbonate Na_2CO_3 were dissolved in, (15 mL) absolute ethanol. Carbon disulfide (3mL) was added, and the reaction mixture was refluxed for (8 hrs.). The reaction mixture was allowed to cool down at room temperature to form precipitation, which was isolated by filtration, and washed with (15 mL) of warm water. The filtrate was acidified with cold concentrated HCl to give a pale product, which then was filtrated and washed with cold water to remove excess of acid. The crude product was recrystallized from hot water to give the desired product, (60%), m.p (230-232) C^0 (Petrow *et al*., 1958; Samir, Ali *et al*., 2017).

2.2 Synthesis of Schiff base thiadiazole (3a-h)

Compound **(1**) (0.01 mole, 1.33 gm) in (10 mL) absolute ethanol was added dropwise to the stirred solution of appropriate aromatic aldehydes (**2a-h**) (0.01 mole) with (2-3) drops of glacial acetic acid. The reaction mixture was refluxed at 70 ^{0}C for (7 hrs.), The reaction was monitored with TLC to completion. The reaction mixture was cooled in an ice bath to give a precipitate, which was then filtrated off and washed several times with cold ethanol. The product was recrystallized in ethanol to give a pure product of Schiff bases (**3a-h**). IR υ_{max} (cm^{-1}) 3014-3090 (C-$H_{Ar.streching}$), 2867-2965 (C-H_{aliph}), 2453-2520(S-H $_{stretching}$), 1602-1627(CH=N) $_{stretching\ imine}$ and (1568-1586) for (C=$C_{Ar.stretching}$). ^{1}H-NMR(DMSO-d_6) ppm: δ_H 6.80-7.80 (d,4H,C-$H_{Ar.}$), 8.42-8.80 (s,1H,C-H) of Schiff base, and 13.18-14.20(s,1H,S-H) thiol. ^{13}C-APT (DMSO-d_6) ppm: δ exhibited following signal at (111-132) for (C-H_{Ar}), (121-155) due to ($C_{Ar.}$) and (160-170) due to (C=N) Schiff base.

Table 1: Physical properties of the compound **(3a-h)**

Comp.	R	Chemical formula	M.Wt	Yield %	M.P	Color
a	p-$N(CH_3)_2$	$C_{11}H_{12}N_4S_2$	264.30	89.1	223	Orange
b	2-OH , 3-OCH_3	$C_{10}H_9N_3O_2S_2$	267.35	85.7	226-228	Deep yellow
c	p-H	$C_9H_7N_3S_2$	221.30	82.4	228-230	Pale yellow
d	p-OH	$C_9H_7N_3OS_2$	237.30	83	223-225	Yellow-orange
e	p-Cl	$C_9H_6ClN_3S_2$	255.75	69	224	Yellow
f	p-NO_2	$C_9H_6N_4O_2S_2$	266	45	197-199	Yellow
g	p-OCH_3	$C_{10}H_9N_3OS_2$	251	80	208-210	Yellow
h	p-Br	$C_9H_6BrN_3S_2$	299.9	62	227-229	Yellow

2.3 Synthesis of 5,5- disulfanediyl-1,3,4-thiadiazole-2-amine (4a-h)

To the Schiff base compounds (3a-h) (0.02 mole) in 10 mL of absolute ethanol 6 drops of hydrogen peroxide 50% is added carefully dropwise with continuous stirring at room temperature. The stirring was continued for 4 hrs. Until the completion of the reaction in which it was monitored with TLC. The reaction mixture was filtrated off to collect the precipitate and was washed with cold ethanol to remove excess of hydrogen peroxide. The product was recrystallized with ethanol to give a pure target compound.

Table 2: Physical properties of the compound **(4a-h)**

Comp.	R	Chemical formula	M.Wt	Yield %	M.P ^{0}C	Color
a	p-$N(CH_3)_2$	$C_{22}H_{22}N_8S_4$	526..7	92	206-208	Deep brown
b	2-OH , 3-OCH_3	$C_{20}H_{16}N_6O_4S_4$	532.6	94	210-212	Pale yellow
c	p-H	$C_{18}H_{12}N_6S_4$	440.6	90	213-215	Yellow
d	p-OH	$C_{18}H_{12}N_6O_2S_4$	472.6	88	205-207	Yellow
e	p-Cl	$C_{18}H_{10}Cl_2N_6S_4$	509.5	87	202-204	Pale yellow
f	p-NO_2	$C_{18}H_{10}N_8O_4S_4$	530.6	81	178-180	Pale yellow
g	p-OCH_3	$C_{20}H_{16}N_6O_2S_4$	500.6	89	180-182	Yellow
h	p-Br	$C_{18}H_{10}Br_2N_6S_4$	598.4	84	204-207	Yellow

2.3.1 *5,5'disulfanediylbis (N-(4-(Dimethyl amino) benzylidene)-1,3,4-thiadiazole -2-amine* **(4a)**: Deep brown color solid 92% M. P=206-208 ^{0}C FT-IR υ_{max} (cm$^-$) 3046(C-H$_{Ar\text{-}str.}$), 2938,2867(C-H$_{aliph.str.}$),1634 (C=N$_{str.imine}$), 1582(C=C$_{Ar.str.}$). ^{1}H-NMR(DMSO-d$_6$) ppm: δ_H 3.34 (s,12H, 2(CH$_3$)$_2$), 6.81-6.83(d,4H, C-H$_{Ar.}$), *J*=7.9 Hz) 7.80-7.82(d,4H, C-H$_{Ar.}$) *J*=8.1 Hz), 8.43(s,2H,CH=N). ^{13}C-NMR (DMSO-d$_6$) ppm: δ_C 40.26(4C,4CH$_3$), 111.55(4C,C-H$_{Ar.}$), 132(4C,C-H$_{Ar.}$), 125.02 (2C,N=CH-C), 154.48(2C,C-N(CH$_3$)$_2$), 161.94(2C,2C-S$_{thiadiazole}$), 186.20(2C,2C-N$_{thiadiazole}$), 167.60(2C,2CH=N).

2.3.2 *6,6'-(1E,1E') -((5,5'disulfanediylbis(1,3,5-thiadiazole-5,2-ditl) bis(azanylyidene)) bis(methanylilidene)) bis (2-methoxyphenol* **(4b)**: pale-yellow color solid 94% M.P=210-212 ^{0}C FT-IR υ_{max} (cm$^-$) 3255 (O-H$_{str}$), 3062(C-H$_{Ar\text{-}str.}$), 2951 and 2839(C-H$_{aliph.str.}$), 1637 (C=N$_{str.imine}$), 1492(C=C$_{Ar.str.}$). ^{1}H-NMR(DMSO-d$_6$) ppm: δ_H 3.85 (s,6H,2OCH$_3$), 6.93-7.27(6H,CH$_{Ar.}$), 8.68(s,2H,CH=N), 10.28 (s,2H,2OH). ^{13}C-NMR(DMSOd$_6$) ppm: δ_C 56.32(2C,OCH$_3$), 118.61-120.43(6C,C-H$_{Ar.}$), 122.80(2C,N=CH-C), 148.88(2C,C-OCH), 149.2 (2C,C-OH), 158.73(2C,2C-S$_{thiadiazole}$), 183.36(2C,C-N$_{thiadiazole}$), 168.41(2C,2CH=N).

2.3.3 *5,5'-disulfanediylbis (N-benzylidene-1,3,4-thiadiazole-2-amine)* **(4c)**: Yellow color solid 90% M. P= 213-115 ^{0}C FT-IR υ_{max} (cm^{-1}) 3062 (C-H$_{Ar\text{-}str.}$), 1635(C=N$_{str.imine}$), 1571(C=C$_{Ar.str.}$). ^{1}H-NMR (DMSO-d$_6$) ppm: δ_H 7.52-7.64(m,6H,C-H$_{Ar.}$), 7.86-7.88(d,4H, C-H$_{Ar.}$) *J*=7.8 Hz, 8.94(s,2H,CH=N). ^{13}C-NMR (DMSO-d$_6$) ppm: δ_C 128.71-131.2(10C,C-H$_{Ar.}$), 145.3(2C,N=CH-C), 148.44(2C,2C-S$_{thiadiazole}$), 173.72 (2C,C-N$_{Thiadiazole}$), 169.76 (2C, 2CH =N).

2.3.4 *4,4'-((1E,1'E)-((5,5'-disulfanediylbis(1,3,4-thiadiazole-5,2-diyl)) bis (azanylylidene bis(methanylylidene)) diphenol* **(4d):** Yellow color solid 88% M.P=205-207 ^{0}C FT-IR υ_{max} (cm^{-1}) 3271(O-H$_{str}$), 3070 (C-H$_{Ar\text{-}str.}$), 1643(C=N$_{str.imine}$), 1583(C=C$_{Ar.str.}$). ^{1}H-NMR (DMSO-d$_6$) ppm: δ_H 6.94-6.97(d,4H, C-H$_{Ar.}$) *J*=8.5 Hz) 7.75-7.78 (d,4H, C-H$_{Ar.}$) *J*=8.6 Hz), 8.17(s,2H, CH=N), 10.05(s,2H,2OH). ^{13}C-NMR (DMSO-d$_6$) ppm: δ_C 115.53(4C,C-H$_{Ar.}$), 131.3(4C,C-H$_{Ar.}$), 128.92(2C,N=CH-C), 159.48(2C,C-OH), 152.67(2C,2C-S$_{thiadiazole}$), 169.26(2C,2C-N$_{thiadiazole}$), 162.41 (2C,2CH=N).

2.3.5 *5,5'-disulfanediyl bis (N-(4-Chlorobenzylidene)-1,3,4-thiadiazole-2-amine)* **(4e)**: Pale-yellow color solid 87% M. P=202-204 ^{0}C FT-IR υ_{max} (cm^{-1}) 3056(C-H$_{Ar\text{-}str.}$), 1628(C=N$_{str.imine}$), 1574 (C=C$_{Ar.str.}$). ^{1}H-NMR (DMSO-d$_6$) ppm: δ_H 7.53-7.55(d,4H,C-H$_{Ar.}$) *J*=7.6 Hz), 7.78-7.80(d,4H,C-H$_{Ar.}$) *J*=7.6 Hz), 9.17(s,2H,CH=N). ^{13}C-NMR(DMSOd$_6$) ppm: δ_C 128.53(4C,C-H$_{Ar.}$), 132(4C,C-H$_{Ar.}$), 130.92 (2C,N=CH-C), 135.48(2C,C-Cl), 152.67(2C,2C-S$_{thiadiazole}$), 175.2 (2C,2C-N$_{thiadiazole}$), 167.48(2C,2CH=N).

2.3.6 *5,5'-disulfanediylbis (N-(4-nitrobenzylidene)-1,3,4-thiadiazole-2-amine)* (**4f**): Yellow color solid 81% M. P=178-180 ^{0}C FT-IR υ_{max} (cm^{-1}) 3067(C-H$_{Ar\text{-}str.}$), 1632(C=N$_{str.imine}$), 1572(C=C$_{Ar.str.}$), (1538, 1348) for (NO$_2$).^{1}H-NMR (DMSO-d$_6$) ppm: δ_H 7.90-7.92(d,4H,C-H$_{Ar.}$) *J*=7.5 Hz), 8.39-8.41(d,4H,C-H$_{Ar.}$) *J*=7.4 Hz), 9.48 (s,2H,CH=N). ^{13}C-NMR (DMSO-d$_6$) ppm: δ_C 128.53(4C,C-H$_{Ar.}$), 131 (4C,C-H$_{Ar.}$), 142.92(2C,N=CH-C), 153.48(2C,C-NO$_2$), 157.67 (2C,2C-S$_{thiadiazole}$), 179.26 (2C,2C-N$_{thiadiazole}$), 172.41(2C,2CH=N).

2.3.7 *5,5'-disulfanediylbis(N-(4-methoxybenzylidene)-1,3,4-thiadiazole-2-amine)* (**4g**): Pale-yellow color solid 89% M. P=180-182 ^{0}C FT-IR υ_{max} (cm^{-1}) 3061(C-H$_{Ar\text{-}str.}$), (2934,2854) (C-H$_{aliph.str.}$), 1613(C=N$_{str.imine}$), 1567(C=C$_{Ar.str.}$). ^{1}H-NMR(DMSO-d$_6$) ppm: δ_H 3.75(s,6H,2(OCH$_3$)), 7.12-7.13(d,4H, C-H$_{Ar.}$) *J*=7 Hz), 7.9-7.91(d,4H, C-H $_{Ar.}$) *J*=7.1 Hz), 9.88(s,2H, CH=N). ^{13}C-NMR (DMSO-d$_6$) ppm: δ_C 56.52(2C,O-CH$_3$), 114.53(4C,C-H$_{Ar.}$), 132(4C,C-H$_{Ar.}$), 128.92(2C, N=CH-C), 164.48(2C,C$_{Ar}$-OCH$_3$), 149.67(2C,2C-S$_{thiadiazole}$), 187.26 (2C,2C-N$_{thiadiazole}$), 173.41(2C,2CH=N).

2.3.8 *5,5'-disulfanediylbis(N-(4-Bromobenzylidene)-1,3,4-thiadiazole-2amine)* (**4h**): Pale -yellow color solid 84% M. P=204-207 ^{0}C FT-IR υ_{max} (cm^{-1}) 3052(C-H$_{Ar\text{-}str.}$), 1624(C=N$_{str.imine}$), 1562 (C=C$_{Ar.str.}$). ^{1}H-NMR (DMSO-d$_6$) ppm: δ_H 7.58-7.60(d,4H,C-H$_{Ar.}$) *J*=7.8 Hz), 7.82-7.84(d,4H,C-H$_{Ar.}$) *J*=7.8 Hz), 9.28(s,2H,CH=N). ^{13}C-NMR (DMSOd$_6$) ppm: δ_C 128.53(4C,C-H$_{Ar.}$), 131(4C,C-H$_{Ar.}$), 137.92 (2C,N=CH-C), 125.48(2C,C-Br), 151.67(2C,2C-S$_{thiadiazole}$), 178.26 (2C,2C-N$_{thiadiazole}$), 167.48(2C,2CH=N).

2.4 Synthesis of 5,5' (ethane-1,2-diylbis (sulfane di yl)) bis(1,3,4-thiadiazol-2-amine) (6)

To the Thiadiazole **(2)** (0.266 gm, 0.002 mole) in (5 mL) dimethylformamide, (1mL) of triethylamine was added with continuous stirring at room temperature. Dibromoethane **(5)** (0.187 gm, 0.001 mole) was added to the mixture dropwise and was refluxed at 70-80 ^{0}C for 6 hours until the T.L.C. showed the complete of the reaction. The mixture was cooled to room temperature and was poured into 10 mL of ice-cold water to produce the precipitate. The solid product was filtered and washed with cold water to give the crude product, which was then recrystallized from (n-hexane: ethyl acetate) (1:1) to give pure product as a pale brown precipitate (78% and M.P=178-180^{0}C) FT-IR υ_{max} (cm^{-1}) 3307 and 3267(N-H), 2941 and 2868(CH$_{aliph}$) ,1629 and 1614 (C=N$_{thiadiazole}$). ^{1}H-NMR(DMSO-d$_6$) ppm: δ_H 3.36-3.37(m,4H,2CH$_2$), 7.34(s,4H,2NH$_2$).^{13}C-NMR (DMSOd$_6$) ppm: δ_C 34 (2C,2CH$_2$), 149.2,149.5(2C,C-S$_{thiadiazole}$), 170.,170.7(2C,C-N$_{thiadiazole}$).

2.5 Synthesis of 5, 5- ethane-1, 2-diylbis (sulfanediyl)-1,3,4-thiadiazole-2-amine (7a-h)

Compound **(6)** (0.002 mole,0.584gm) in (10 mL) absolute ethanol was added dropwise to the stirred solution of appropriate aromatic aldehydes (**2a-h**) (0.004 mole) with a few drops of CH$_3$COOH. The reaction mixture was refluxed at 70 ^{0}C for 8 hours to complete the reaction in which it was monitored with TLC. The reaction mixture was cooled to give the ppt, which was then filtrated off and washed with cold ethanol to give the crude product. The product was recrystallized from a mixture of (n-hexane: ethyl acetate) (1:1) to give a pure product of Schiff bases (**7a-h**). The physical properties are shown in table (**3**).

Table 3: Physical properties of the compounds **(7a-h)**

Comp.	R	Chemical formula	M.Wt	Yield %	M.P ^{0}C	Color
a	p-N(CH$_3$)$_2$	C$_{24}$H$_{26}$N$_8$S$_4$	554.8	78.2	192-194	Orange-brown
b	2-OH , 3-OCH$_3$	C$_{22}$H$_{20}$N$_6$O$_4$S$_4$	560.7	79.5	188-190	Yellow-brown
c	p-H	C$_{20}$H$_{16}$N$_6$S$_4$	468.6	70	158-160	Dep-Yellow
d	p-OH	C$_{20}$H$_{16}$N$_6$O$_2$S$_4$	500.6	72	180-182	Pale brown
e	p-Cl	C$_{20}$H$_{14}$Cl$_2$N$_6$S$_4$	537.5	68	198	Pale brown
f	p-NO2	C$_{20}$H$_{14}$N$_8$O$_4$S$_4$	558.6	65	172-174	Brown
g	p-OCH$_3$	C$_{22}$H$_{20}$N$_6$O$_2$S$_4$	528.7	78	178-180	Brown
h	p-Br	C$_{20}$H$_{14}$Br$_2$N$_6$S$_4$	626.4	64	194	Brown

2.5.1 *5,5'-(ethane-1,2-diylbis(sulfanediyl)) bis (N-(4 (dimethyl amino) benzylidene)-1,3,4-thiadiazole-2-amine* (**7a**): Orange-brown solid 78.2% M.P=192-194 ^{0}C FT-IR υ_{max} (cm^{-1}) 3084(C-H$_{Ar-str.}$), 2900 and 2821(C-H$_{aliph.str.}$), 1612(C=N$_{str.imine}$), 1572(C=C$_{Ar.str.}$). ^{1}H-NMR (DM SO-d$_6$) ppm: δ_H 2.75(s,12H,2(CH$_3$)$_2$), 2.92(s,4H,2(CH$_2$)), 6.80-6.83 (d,4H,C-H$_{Ar.}$) *J*=8.6 Hz), 7.69-7.72(d,4H,C-H$_{Ar.}$) *J*=8.6 Hz), 10.03 (s,2H, CH=N). ^{13}C-NMR (DMSO-d$_6$) ppm: δ_C 34.39(2C,2CH$_2$), 40.52 (4C, 4CH$_3$), 111.53(4C,C-H$_{Ar.}$), 132(4C,C-H$_{Ar.}$), 124.92(2C,N=CH-C), 149.4(2C,C-N(CH$_3$)$_2$), 154.67(2C,2C-S$_{thiadiazole}$), 170.26(2C,2C-N$_{thiadiazole}$), and 162 .8(2C,2CH=N).

2.5.2. *6,6'-((1E,1'E) -((5,5'-(ethane-1,2-dylbis(sulfanediyl)) bis (1,3,4-thiadiazole-5,2-diyl)) bis(azanylidene)) bis(methanylylidene)) bis(2-methoxyphenol)* (**7b**): Yellow-brown solid 79.5% M. P=188-190 ^{0}C FT-IR υ_{max} (cm^{-1}) 3235(O-H$_{str.}$), 3043(C-H$_{Ar-str.}$), 2937 and 2841(C-H$_{aliph.str.}$) 1615(C=N$_{str.imine}$), 1581(C=C$_{Ar.str.}$). ^{1}H-NMR (DMSO-d$_6$) ppm: δ_H 3.74(s,4H,2CH$_2$) 3.86(s,6H,2OCH$_3$), 6.92-6.97 (m,2H,C-H$_{Ar.}$), 7.24-7.27(d,2H,C-H$_{Ar.}$) *J*= 8.6 Hz), 7.37-7.4 (d,2H,C-H$_{Ar.}$) *J*=8.5 Hz), 9.10(s,2H,CH=N), 10.26(s,2H,2OH). ^{13}C-NMR (DMSO-d$_6$) ppm: δ_C 34.49(2C,2CH$_2$), 56.57(2C,OCH$_3$), 118.2-122.1(6C,C-H$_{Ar.}$), 119.5(2C,N=CH-C), 149.2(2C,C-OCH$_3$), 151.5 (2C,C-OH), 170.2(2C,2C-S$_{thiadiazole}$ and C-T$_{hiadiazole}$), and 169.11 (2C,2CH=N).

2.5.3. *5,5'-(ethane-1,2-diylbis(sulfanediyl)) bis (N-benzylidene-1,3,4-thiadizole-2, amine)* (**7c**): Deep yellow solid 70% M. P=158-160 ^{0}C FT-IR υ_{max} (cm^{-1}) 3084(C-H$_{Ar-str.}$), 2900 and 2821(C-H$_{aliph.str.}$), 1612(C= N$_{str.imine}$), 1571(C=C$_{Ar.str.}$). ^{1}H-NMR(DMSO-d$_6$) ppm: δ_H 2.90-2.91(s,4H,2CH$_2$), 7.37-7.42(m,4H,C-H$_{Ar.}$), 7.63-7.69(m,2H,C-H$_{Ar.}$), 7.92-7.94(d,4H,C-H$_{Ar.}$) *J*=8.5 Hz), 10.04(s,2H,CH=N). ^{13}C-NMR (DMSO-d$_6$) ppm: δ_C 34.4(2C,2CH$_2$), 129.7-135(10C,C-H$_{Ar.}$), 136.3(2C, N=CH-C), 149.4(2C,2C-S$_{thiadiazole}$), 170.2(2C,C-N$_{thiadiazole}$), and 168.7(2C, 2CH=N).

2.5.4. *5,5'-(ethane-1,2-diylbis(sulfanediyl)) bis (1,3,4-thiadiazole-5,2-diyl)) bis (azanylylidene) bis(methanylylidene)) diphenol* (**7d**): Pale brown solid 72% M.P=180-182 ^{0}C FT-IR υ_{max} (cm^{-1}) 3302(O-H$_{str.}$), 3089(C-H$_{Ar-str.}$), 2945 and 2857(C-H$_{aliph.str.}$), 1622(C=N$_{str.imine}$), 1571 (C= C$_{Ar.str.}$). ^{1}H-NMR(DMSO-d$_6$) ppm: δ_H 3.34(s,4H,2(CH$_2$)), 6.92-6.94 (d,4H, C-H$_{Ar.}$) *J*=7.9 Hz), 7.88-7.90(d,4H,C-H$_{Ar.}$) *J*=8 Hz), 8.73(s, 2H, CH=N), 9.79(s,2H,2OH). ^{13}C-NMR (DMSO-d$_6$) ppm: δ_C 34.4(2C, 2CH$_2$), 116.5(4C,C-H$_{Ar.}$), 132.24(4C,C-H$_{Ar.}$), 126.92 (2C,N=CH-C), 148.48(2C,C-OH), 164.5(2C,2C-S$_{thiadiazole}$), 170.26 (2C,2C-N$_{thiadiazole}$), and 161.78(2C,2CH=N).

2.5.5. *5,5'-(ethane-1,2-diylbis(sulfanediyl)) bis (N-(4-Chloro benzylidene)-1,3,4-thiadiazo le-2-amine* (**7e**): Pale brown solid 72% M.P=180-182 ^{0}C FT-IR υ_{max} (cm^{-1}) 3046(C-H$_{Ar-str.}$), 2937 and 2844(C-H$_{aliph.str.}$) 1612(C=N$_{str.imine}$), 1562(C=C$_{Ar.str.}$). ^{1}H-NMR (DMSO-d$_6$) ppm: δ_H 3.34(s,4H,2(CH$_2$)), 7.32-7.34(d,4H,C-H$_{Ar.}$) *J*=7.9Hz), 7.69-7.72(d,4H,C-H$_{Ar.}$) *J*=8 Hz), 8.97(s,2H,CH=N). ^{13}C-NMR (DMSO-d$_6$) ppm: δ_C 33.39(2C,2CH$_2$),118.53(4C,C-H$_{Ar.}$), 131(4C,C-H$_{Ar.}$), 124.9 (2C,N=CH-C), 149.5(2C,C-Cl), 154.7(2C,2C-S$_{thiadiazole}$), 171.3 (2C,2C N$_{thiadiazole}$), and 165.02(2C,2CH =N).

2.5.6. *5,5'-(ethane-1,2-diylbis(sulfanediyl)) bis (N-(4-nitro benzyl-idene)-1,3,4-thiadiazole-2-amine* (**7f**): Pale brown solid 72% M.P= 180-182 ^{0}C FT-IR υ_{max} (cm^{-1}) 3037(C-H$_{Ar-str.}$), 2937 and 2845(C-H$_{aliph.str.}$), 1618(C=N$_{str.imine}$), 1538(C=C$_{Ar.str.}$). ^{1}H-NMR (DMSO-d$_6$) ppm: δ_H 3.23 (s,4H,2(CH$_2$)), 7.10-7.12(d,4H,C-H$_{Ar.}$) *J*=7.9 Hz), 7.88-7.90(d,4H,C-H$_{Ar.}$) *J*=8 Hz), 8.77(s,2H,CH=N). ^{13}C-NMR (DMSO-d$_6$) ppm: δ_C 32.39 (2C,2CH$_2$), 117.53(4C,C-H$_{Ar.}$), 132(4C,C-H$_{Ar.}$), 124.92 (2C,N=CH-C) 149.48(2C,C-NO$_2$), 154.7(2C,2C-S$_{thiadiazole}$), 170.3 (2C,2C-N$_{thiadiazole}$), and 162.45(2C,2CH=N).

2.5.7. *5,5'-(ethane-1,2-diylbis(sulfanediyl)) bis (N-(4-methoxy benzylidene)-1,3,4-thiadiaz ole-2-amine* (**7g**): Pale brown solid 72% M.P=180-182 ^{0}C FT-IR υ_{max} (cm^{-1}) 3068(C-H$_{Ar-str.}$), 2935 and 2837 (C-H$_{aliph.str.}$), 1608(C=N$_{str.imine}$), 1566(C=C$_{Ar.str.}$). ^{1}H-NMR (DMSO-d$_6$) ppm: δ_H 3.75 (s,4H,2(CH$_2$)), 3.86(s,6H,2OCH$_3$), 7.11-7.13 (d,4H,C-H$_{Ar}$) *J*= 7.9 Hz), 7.98-7.99(d,4H,C-H$_{Ar.}$) *J*=8Hz), 8.82 (s,2H,CH=N). ^{13}C-NMR (DM SO-d$_6$) ppm: δ_C 33.66(2C,2CH$_2$), 56.16 (2C,O-CH$_3$), 115.27(4C,C-H$_{Ar.}$), 132.8(4C,C-H$_{Ar.}$), 127.6(2C,N=CH-C), 164.5(2C,C-O), 168.67(2C,2C-S$_{thiadiazole}$), 173.26(2C,2C-N$_{thiadiazole}$), and 163.4(2C,2CH=N).

2.5.8. *5,5'-(ethane-1,2-diylbis(sulfanediyl)) bis (N-(4-bromo benzylidene)-1,3,4-thiadiazole-2-amine* (**7h**): Pale brown solid 72% M.P=180-182 ^{0}C FT-IR υ_{max} (cm^{-1}) 3042(C-H$_{Ar-str.}$), 2952 and 2858 (C-H$_{aliph.str.}$), 1612(C=N$_{str.imine}$), 1552(C=C$_{Ar.str.}$). ^{1}H-NMR (DMSO-d$_6$) ppm: δ_H 3.18(s,4H,2(CH$_2$)), 7.15-7.17(d,4H,C-H$_{Ar.}$) *J*=7.7 Hz), 7.82-7.84(d,4H,C-H$_{Ar.}$) *J*=8 Hz), 8.88(s,2H,CH=N). ^{13}C-NMR (DMSO-d$_6$) ppm: δ_C 34.39(2C,2CH$_2$), 118.5(4C,C-H$_{Ar.}$), 131.45 (4C,C-H$_{Ar.}$), 128.9(2C,N=CH-C), 149.5(2C,C-Br), 151.7(2C,2C-S$_{thiadiazole}$), 172.3 (2C, 2C-N$_{thiadiazole}$), and 164.58(2C,2CH=N).

2.6 In vitro antibacterial assay.

The compounds (**4a-h**) and (**7a-h**) were evaluated for their antibacterial activities against two gram-positive strains (*Staphylococcus and streptococcus*), and two gram-negative (*E. coli and klebsiella*) of bacterial. In a typical prescription, ciprofloxacin and, vancomycin was utilized as standard medications. The standard procedure was used. Prepared four different concentrations of all synthesized compounds and screened with bacteria. After 24 hours, they recorded an inhibition zone of them (Bakht et al., 2011).

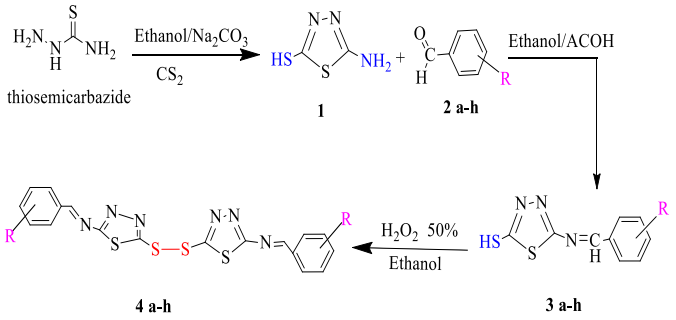
2.7 Molecular docking studies

PubChem (https://pubchem.ncbi.nlm.nih.gov/) and ChemDraw software programs were applied to design the synthesized (ligands) in the form of SDF file format. Data Bank of Protein was found in (http://www.rcsb.org/) for downloading the protein. The zinc (http://zinc.docking.org/) website was applied for the design and download of standard drugs (ciprofloxacin and vancomycin). PyRx (AutoDock-1.5.6) and BIOVIA Discovery Studio 2020 were employed to imagine and adapt ligand-protein structures (Sangar Ali Hassan *et al*., 2021).

3. RESULTS AND DISCUSSION

3.1 Chemistry

3.1.1 Synthesis of 5,5- disulfanediyl-1,3,4-thiadiazole-2-amine (4a-h): The synthesis route for the preparation of symmetrical Bis-Schiff base compounds that was illustrated in (**Scheme 1**). The starting material 2- amino-1,3,4- thiadiazole-5, thiol **(1)** was synthesized by cyclization of thiosemicarbazide with carbon disulfide and small amount of Na_2CO_3 in ethanol (Samir *et al*., 2017). The thiadiazole compound (**1**) was used to synthesize Schiff base derivatives **(3a-h)** via the reaction with different aromatic aldehydes **(2a-h)** and a few drops of glacial (CH_3COOH) as a catalyst. In addition, a new series of 5,5'-disulfanediylbis Schiff base thiadiazole derivatives **(4a-h)** were synthesized by oxidation Schiff bases **(3a-h)** with fresh hydrogen peroxide in ethanol.



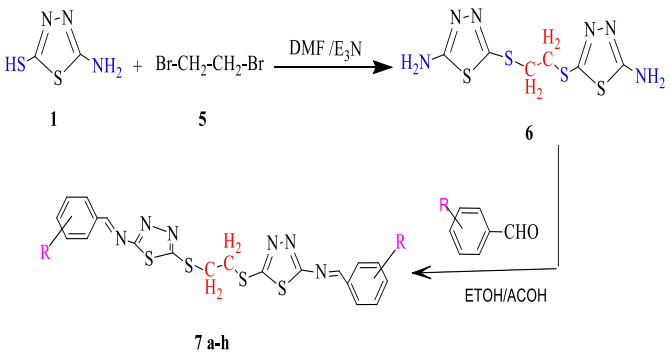
R: a=p-N(CH$_3$)$_2$, b=2-OH,3-OCH$_3$, c=p-H, d=p-OH, e=p-Cl, f=p-NO$_2$, g=p-OCH$_3$, h=p-Br

Scheme 1 Synthesis of series of symmetrical bis-Schiff bases disulfide derivatives

The synthesized compounds were characterized by FT-IR,^{1}H-NMR, and C-APT NMR spectroscopy. The FT-IR spectra of the Schiff bases compounds (**3a-h**) showed the diagnostic band of C=N group at (1600-1635) cm^{-1}. The disappearance of the bands around 3200 cm^{-1} for the NH$_2$ group of thiadiazole compound and appeared of C=O group at 1700 cm^{-1} for an aldehyde is another evidence to confirm that the production of Schiff bases (**3a-h)** was successful. The ^{1}H-NMR spectra of the Schiff base derivatives (**3a-h**) appeared as a singlet peak of the proton of (CH=N) at (8.44-8.89) ppm. Two distinct doublets at the respective chemical shift of δ = 6.81 and 7.83 ppm are attributed to four aromatic protons. Another important peak was a singlet peak of the proton of (SH) in regions (13.18-14.23) ppm which is another evidence to confirm the complete of the reaction. C-APT NMR spectra of the compounds **(3a-h**), appeared a signal of carbon imine group (CH=N) in region (161-169) ppm, which also means the formation of Schiff base compounds were successful.

The FT-IR absorption bands of 5,5'-disulfanediylbis Schiff base thiadiazole derivatives (**4a-h**) observed common characteristic IR bands, while the disappearance band of SH signal means the oxidation of the S-H bond and formation of disulfide bond bis-Schiff base. The ^{1}H-NMR chemical shifts for the compounds (**4a-h**) also showed the disappearance of the proton of S-H signal in the region (**13-14**) ppm. This evidence confirmed the conversion of thiadiazole to the disulfide bis-Schiff bases. The C-APT NMR spectra for the synthesized compounds (**4a-h**) were similar to the starting material chemical shifts, and only minor shifts were noticed. The main C-APT NMR characteristic peaks for the compounds (**4a-h**) are the thiadiazole ring in region (149-187) ppm, aromatic carbon peaks (111-132) ppm, and (**CH=N**) in region (162-179) ppm.

3.1.2 Synthesis of 5, 5- ethane-1, 2-diylbis (sulfanediyl)-1, 3, 4-thiadiazole-2-amine: 2-amino-1,3,4-thiadiazole-5, thiol compound (**1**) was used as the starting material to synthesize the target compound as shown in (**Scheme 2)**. Firstly, the reaction of the starting material (**1**) with dibromoethane **(5)** in the presence of triethylamine as a catalyst in dimethylformamaide as a solvent is to prepare a symmetrical bis-amine compound **(6)**. In which, later it was used to synthesize a new series of symmetrical 5,5'-(ethane-1,2-diylbis (sulfanediyl)) derivatives **(7a-h)** via the reaction with different aromatic aldehydes **(2a-h),** aldehydes **(2a-h)** and (2-3) drops of glacial acetic acid as a catalyst.



R: a=p-N(CH$_3$)$_2$, b=2-OH,3-OCH$_3$, c=p-H, d=p-OH, e=p-Cl, f=p-NO$_2$, g=p-OCH$_3$, h=p-Br

Scheme 2: Synthesis of series of symmetrical bis-Schiff bases sulfanediyl derivatives

The whole synthesized compounds were characterized by FT-IR,^{1}H, and C-APT NMR spectroscopies. The FT-IR spectra of bis-amine (**6**) exhibited different absorption bands. The spectrum band of NH$_2$ group appeared in (**3307 and 3267**) cm^{-1}. The most important evidence in spectrum is the disappearance of the SH band in product, and it existed in the starting material, due to the conversion of SH bond to thioether. The signal band of C-H aliphatic appeared in the region (**2972-2825**) cm^{-1} due to the (CH$_2$-CH$_2$) thioether which confirmed the formation of the compound (**6**). The ^{1}H-NMR spectra of the compound (**6**) showed the singlet peak at (2.72) ppm which belongs to the CH$_2$ thioether, the disappearance of the signal of (**SH**) is important evidence that confirmed the conversion of thiadiazole to the bis-amine thiadiazole. The C-APT NMR spectra for the synthesized compounds **(6)** showed the signal of the (**CH$_2$**) group in region (**33.39**) ppm. This evidence improves converting the starting material to the compound **(6)**.

The FT-IR spectra of 5,5'-(ethane-1,2-diylbis(sulfanedyl))-1,3,4-thiadiazole-amine derivatives **(7a-h)** exhibited common characteristic bands. An important band around (1600-1635) cm^{-1} due to the stretching vibration of (**C=N**) groups in the Schiff base compounds. Furthermore, the disappearance of the bands at 3200 and 1700 cm^{-1} for the NH$_2$ and C=O groups in starting material and substituted Benz aldehydes respectively are good evidence for producing Schiff bases (**7a-h**). The ^{1}H-NMR spectra of the Schiff bases derivatives **(7a-h)** exhibited singlet peak attributed to the proton of (**CH=N**) at (8.44-8.89) ppm, while the aromatic protons have shown in a region (6.81-7.83) ppm due to the formation of Schiff base. In C-APT spectra of the Schiff base compounds derivatives **(7a-h)**, a signal of carbon imine group (**CH=N**) appeared in region (161-169) ppm, the thiadiazole ring in region (154-173) ppm, and signals of the carbon aromatic in region (110-135) ppm, which also confirms the formation of Schiff base target compounds.

3.2 Biological activity

All synthesized compounds (**4a-h**) and (**7a-h**) were evaluated in vitro antibacterial activities against gram-positive strains, *Staphylococcus* aureus and *streptococcus* and gram-negative strains, *Escherichia coli*, *klebsiella*. As determined by the inhibition zone, the antibacterial activity of the synthesized compound is determined by inhibition zone bacteria (mm), and the findings were shown in (**Tables 3,4**).

The antibacterial properties of the synthesized compounds (**4a-h**) were tested against different bacterial strains, and the results showed high antibacterial activity in comparison to strong broad spectrum standard drugs such as ciprofloxacin and vancomycin. Interestingly, the synthesized compounds showed high activity against both gram-

positive and gram-negative strains, while the standard drugs are active only against either the gram-positive or gram-negative strains.

Particularly, the compounds **(4b, 4f)** showed potent antibacterial properties against *Escherichia coli* and *Staphylococcus aureus*, while the compounds (**4a,4g**) showed lower activity than others **(Table 3)**.

It was also noticed that, the bis-Schiff bases disulfides (**4a-h**) showed higher activity than the thiol Schiff base (**3a-h**), some results have been previously reported by (Samir et al., 2017). Interestingly our reported compounds showed good activity against *Escherichia coli*, while the thio Schiff base (Samir et al., 2017) had no activity against the same bacteria. This might be the effect of introducing the disulfide bridge within our reported structures.

Table 4: The results of antibacterial assay of the compounds **(4a-h)**

Entry	Inhibition zone of bacteria-mm			
	Bacterial strains			
	E. Coli	Klebsiella	Staphylococcus	Streptococcus
A	07	03	09	06
B	16	12	20	15
C	15	10	16	06
D	13	09	13	14
E	12	04	11	12
F	18	15	18	14
G	11	13	12	11
H	13	12	13	11
Ciprofloxacin	18	15	2	3
Vancomycin	4	4	16	14

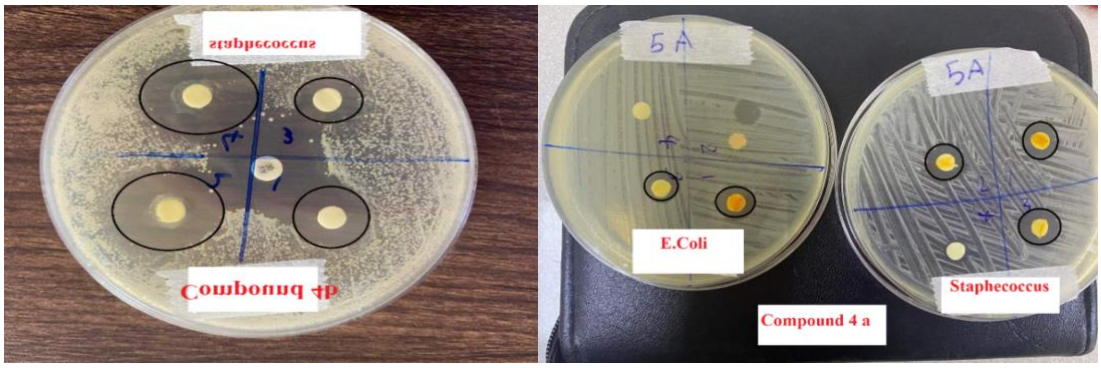


Figure 1. Inhibition zone of compound **4a** (Right) and Compound **4b** (Left)

In addition, the compounds (**7a-h**) showed good activity as they were compared with two strong standard drugs such as ciprofloxacin and vancomycin. Compounds (**7b,7e,7f**) exhibited the most elevated antibacterial activity with all bacteria, especially with Escherichia coli and streptococcus compared with two other bacteria. While, compounds (**7a,7g**) had the lowest antibacterial activity in opposition to staphylococcus and streptococcus. Table 4 illustrates the anti-bacterial activity of compounds (**7a-h**).

Table 5: The result of antibacterial assay of the compounds **(7a-h)**

Entry	Inhibition bacteria- mm			
	Bacterial strains			
	E. Coli	Klebsiella	Staphylococcus	Streptococcus
a	10	05	08	07
b	18	06	10	20
c	16	12	11	17
d	14	10	14	15
e	16	11	11	14
f	17	10	13	16
g	12	04	11	10
h	13	03	09	11

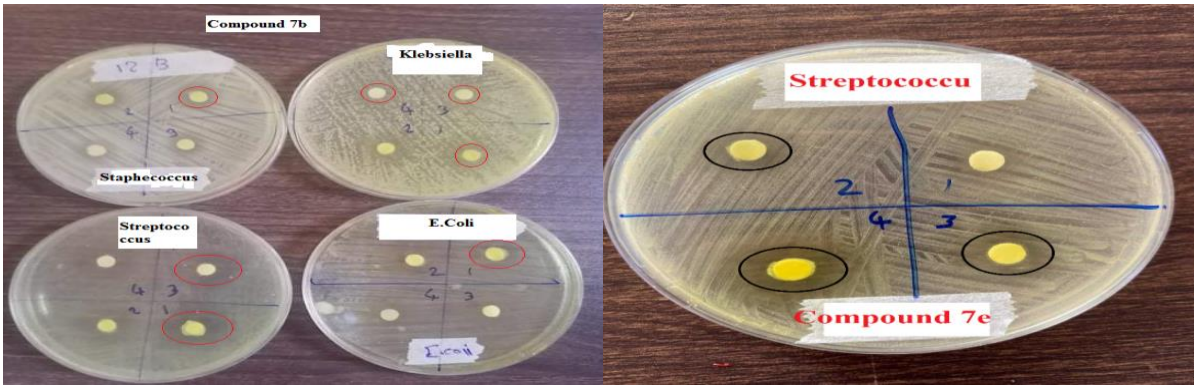


Figure 2. Inhibition zone of compound 7b (Left) and compound 7e (Right) against four bacteria

3.3 Molecular docking studies

Molecular docking analysis was applied to investigate the binding affinities and interactions between produced compounds and the target protein to provide vitro antibacterial activity of the generated compound. For this purpose, the target proteins of E. coli were selected to be a likeable target for molecular docking (Sangar A Hassan et al., 2021). The stability of the ligand with receptors depends on the energy affinity to bind them (Sangar Ali Hassan *et al*., 2021). Drug-drug and drug-receptor interactions may be screened by using the most recent drug computational research (Sangar Ali Hassan, 2022). As compared to the pharmacological references (ciprofloxacin and vancomycin), docking experiments demonstrated that the synthesized compounds showed good interactions and binding energies.

Regarding the (**4a-h**) compounds, the binding affinity range was between (-7.4 to -10.5) and the binding affinity and residues interaction were shown in Figures (**1**) and Table (**5**). The compounds 4f, 4c, and 4b had the minimum binding affinity value of -10.5, -10.2, and -10.1 Kcal/mole respectively. It was also noticed that the substituted nitro, hydroxy and methoxy groups were further involved in binding interactions and reduced the minimum binding affinity compared to other substituted groups. The position of hydroxy and methoxy was a key role in biological activity. In addition to their wide variety of orientations, the halogen group interacted with critical residue active sites.

Table 6: Molecular docking studied data of synthesized Schiff base disulfide **(4a-h)** applied with E.coli.

Comp.	ΔG bind (kcal/mol)	Interaction
4a	-7.4	**Van der Waals**: ALA A:147, ARG A:225, ASN A:83, GLY A:142, GLY A:146, GLY A:237, MET A:150, HIS A:271, PHE A:274, PRO A:141, GLN A:241. **Conventional hydrogen bond:** TYR A:187. **Carbon hydrogen bond**: GLN A:229, SER A:82, SER A:288, GLU A:308. **Pi-Cation:** ARG A:242. **Pi-alkyl**: ALA A:154, ILE A:140, ARG A:188.
4b	-10.1	**Van der Waals**: ALA A:147, ARG A:225, GLY A:79, GLY A:142, GLY A:145, GLY A:146, GLY A:237, ILE A:192, ILE A:140, LEU A:197, LEU A:98, TYR A:77, TYR A:42, TYR A:149, CYS A:236, PHE A:274, SER A:115, SER A:143, SER A:235, VAL A:198, VAL A;199. **Conventional hydrogen bond:** ASN A:83, GLY A:142**,** VAL A:199, SER A:82. **carbon hydrogen bond**: TYR A:149. Pi-sulfur: MET A:150. **Pi-Pi stacked:** TYR A:149. Pi-Alkyl: ALA A:152, PRO A:141
4c	-10.2	**Van der Waals**: ALA A:152, ARG A:225, ARG A:310, GLY A:79, GLY A:81, GLY A:142, GLY A:145, GLY A:146, GLY A:237, ILE A:88, ILE A:140, ILE A:192, LEU A:78, LEU A:197, MET A:150, SER A:143, TYR A:77, PHE A:174, VAL A:198, VAL A:199. **Conventional hydrogen bond**: ASN A:83, SER A:82, PRO A:141**. carbon hydrogen bond:** TYR A:77, TYR A:149. **Pi-Pi T-Stacked**: TYR A:149. **Pi-alkyl**: PRO A:141.
4d	-9.6	**Van der Waals**: ALA A:147, ALA A:152, ASN A:80, ASN A:83, GLY A:79, GLY A:81, GLY A:142, ILE A:140, MET A:150, GLN A:241, PHE A:240, PRO A:141. **Conventional hydrogen bond**: GLY A:237, SER A:82, SER A:143, SER A:238, ARG A:188. **Unfavorable Donor-Donor**: ARG A:242. **Pi-Anion**: GLU A:308. **Pi. Donor hydrogen bond**: TYR A:187. **Amide-pi stacked**: GLY A:146. **Pi-alkyl** : ARG A:188.
4e	-9.7	**Van der Waals**: ALA A:152, ASN A:80, ASN A:83, GLY A:142, GLY A:146, GLY A:237, SER A:143, GLU A:308, ARG A:310, TYR A:77, GLN A:241, ILE A:84, ILE A140, MET A:150, PHE A:240. **Conventional hydrogen bond**: SER A:82, SER A:238, PRO A:141. **Pi-Donor Hydrogen bond**: TYR A:187. **Pi-Pi stacked**: TYR A:149. **Pi-alkyl**: ARG A:188, ARG A:242, TYR A:149.
4f	-10.5	**Van der Waals**: ALA A:147, ALA A:152, ASN A:80, GLY A:79, GLY A:81, GLY A:142, GLY A:146, GLY A:237, ILE A:140, LEU A:78, LEU A:231, MET A:150, SER A:143, SER A:238, TYR A:42, TYR A:77, TYR A:149, ARG A:168, ARG A:225, GLN A:229. **Conventional hydrogen bond**: ASN A:83, SER A:82, SER A:235, PRO A:141, ARG A:310. **Pi-Pi Stacked:** PHE A:274. **Pi-alkyl**: PRO A:141, ILE A:84.
4g	-9.0	**Van der Waals**: ALA A:147, ALA A:152, ARG A:242, GLY A:142, GLY A:146, GLY A:237, ILE A:140, GLU A:308, PHE A:240, SER A:148, ASN A:83, GLN A:241. **Conventional hydrogen bond:** ARG A:310, PRO A:141**,** SER A:82, SER A:238. **Pi-Donor hydrogen bond**: TYR A:77, TYR A:187. **Pi-Pi stacked:** TYR A:149. **Pi-Alkyl**: ILE A:84, MET A:150, ARG A:188.
4h	-9.2	**Van der Waals**: ASN A:83, GLY A:142, SER A:143, SER A:238, GLU A:229, ARG A:225, ARG A:310, TYR A:77, TYR A:155. **Conventional hydrogen bond**: GLY A:146, GLY A:153, SER A:82, PRO A:141. **Pi-Cation**: ARG A:188. **Pi-Pi stacked**: TYR A:149. **Pi-Sulfur**: MET A:150. **Pi-alkyl**: ALA A:152, ALA A:154, ILE A:84, ILE A:140, LYS A:228, TYR A:149.

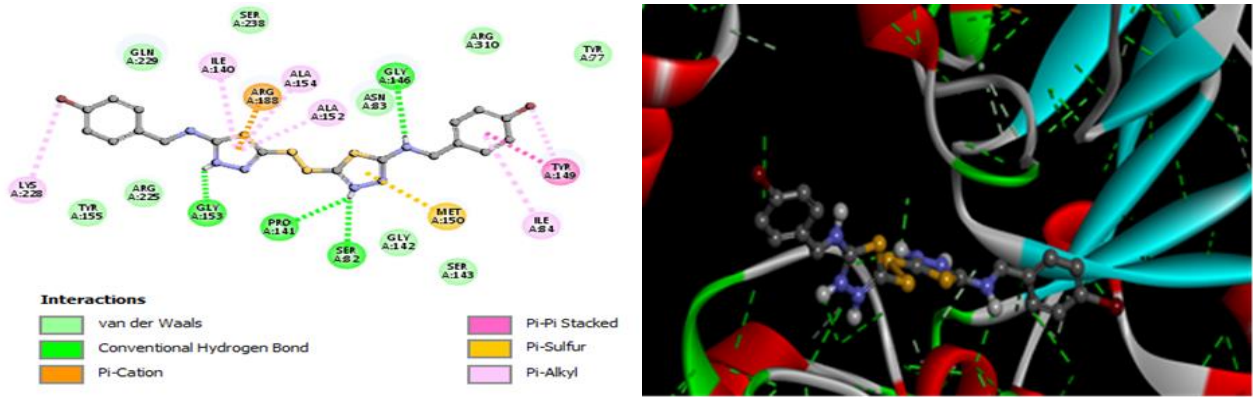


Figure 3: The three-dimension (left) and two-dimensions (right) binding compound (**4h**) applied with the active site E.coli-binding protein.

Furthermore, for compounds (**7a-h**), the binding affinity range was between (-9.1 to -10.2), and the binding and residues interaction for all synthesized compounds with ciprofloxacin were illustrated in figures **(2,3)** and table **(6).** The compounds **7c** and **7d** have displayed the maximum binding affinity value as (-10.2 and -10.1). The hydroxy group is good to enhance producing hydrogen bonds with amino acids more than other compounds. Also, Halogen group were a good orientation to produce binding and interaction. In conclusion, investigating the docking of compounds **4a-h** was near and better than **7a-h** because the two methylene groups between disulfide did not produce any binding with amino acid. All freshly synthesized compounds were further supported, designed, and analyzed using in silico computational research and theoretical conclusions near the experimental values with slight discrepancies. Usually, the theoretical calculations do not provide the exact values of the practically obtained ones from antibacterial assays, because the environment of the experiment, such as temperature, disc packing and its diameter, slightly affects the practical results (Sangar Ali Hassan, 2022).

Table 7: Docking studied data of all synthesized Schiff bases compounds **(7a-h)** applied with E.coli.

Comp.	ΔG bind (kcal/mol)	Interaction
7a	-9.1	**Van der Waals**: ALA A:147, ALA A:152, ARG A:188, ARG A:225, ARG A:310, ASN A:80, ASN A:83, GLY A:79, GLY A:81, GLY A:142, GLY A:146, GLY A:153, GLY A:237, ILE A:84, ILE A:192, LYS A:228, MET A:150, SER A:143, TYR A:77, HIS A:196, PHE A:274, PRO A:141, VAL A:198. **Carbon hydrogen bond**: GLN A:229, SER A:82, LEU A:197. **Pi-Pi Stacked:** TYR A:149, TYR A:155, GLU A:229. **Pi-alkyl**: ALA A:154, ILE A:140.
7b	-9.9	**Van der Waals**: ALA A:154, ARG A:225, ASN A:80, GLY A:79, GLY A:81, GLY A:142, GLY A:146, GLY A:153, GLY A:237, ILE A:84, LEU A:78, LEU A:231, MET A:150, TYR A:77, HIS A:196, PRO A:141. **Conventional hydrogen bond:** ARG A:310**,** GLN A:229, SER A:82, TYR A:42. **Carbon hydrogen bond**: SER A:235. **Pi-Pi T-Shaped:** ASN A:83, TYR A:149, PHE A:274. **Pi-cation:** ARG A:188. **Pi-alkyl**: ALA A:152, ILE A:140, PRO A:141.
7c	-10.2	**Van der Waals**: ARG A:225, ARG A:310, ASN A:80, GLY A:79, GLY A:81, GLY A:142, GLY A:145, GLY A:146, GLY A:237, ILE A:84, ILE A:192, LEU A:78, LEU A:231, MET A:150, SER A:115, SER A:143, SER A:235, TYR A:42, TYR A:77, VAL A:198. **Conventional hydrogen bond**: ASN A:83, SER A:82, PRO A:141**. Pi-Donor hydrogen bond :** TYR A:149 **Pi-Pi T-Shaped** :TYR A:149, PHE A:274 **Pi-alkyl** : ILE A:140, LEU A:98, VAL A:199, PRO A:141.
7d	-10.1	**Van der Waals**: ALA A:147, ALA A:152, ARG A:188, ARG A:225, ARG A:310, ASN A:80, ASN A:83, GLY A:79, GLY A:81, GLY A:142, GLY A:146, ILE A:84, ILE A:140, ILE A:192, LEU A:231, MET A:150, SER A:143, SER A:238, TYR A:77, TYR A:233. **Conventional hydrogen bond**: GLY A:237, SER A:82, SER A:235, TYR A:42, TYR A:141. **Pi-Pi T-Shaped** : TYR A:149, PHE A:274 **Pi-alkyl** : PRO A:141.
7e	-9.9	**Van der Waals**: ALA A:152, GLY A:142, SER A:143, GLU A:308, ARG A:310, TYR A:77, GLN A:241, ILE A:140, VAL A:239, PHE A:240. **Conventional hydrogen bond**: GLY A:146, GLY A:237, SER A:82, SER A:238, PRO A:141, ASN A:83, ARG A:188. **Pi-Donor Hydrogen bond**: TYR A:187, SER 238. **Pi-Pi stacked**: TYR A:149. **Pi-alkyl**: ARG A:188, ARG A:242, TYR A:149, TYR A:187, ILE A:84. **Pi-Sulfur**: MET A:150.
7f	-9.4	**Van der Waals**: ALA A:152, GLY A:142, GLY A:146, ILE A:140, ILE A:84, SER A:238, TYR A:77, TYR A:187, PHE A:240, PRO A:141. **Conventional hydrogen bond**: ASN A:83, SER A:82, ARG A:188, ARG A:242, ARG A:310, TYR A:187. **Carbon Hydrogen bond**: GLN A:241, SER A:238. **Unfavorable Donor-Donor:** ARG A:188. **Pi-Anion:** GLU A:308. **Pi-sulfur:** MET A:150. **Pi-Pi Stacked:** TYR A:149. **Pi-alkyl**: ARG A:188.
7g	-9.6	**Van der Waals**: ALA A:152, ARG A:242, ARG A:310, GLY A:79, GLY A:81, GLY A:142, GLY A:146, ILE A:84, ILE A:140, GLU A:308, PHE A:240, SER A:143, ASN A:80, GLN A:241, MET A:150, TYR A:77, HIS A:196. **Conventional hydrogen bond:** ARG A:188, PRO A:141**,** SER A:82, SER A:238, GLY A:237, ASN A:83. **Pi-Donor hydrogen bond**: TYR A:187. **Pi-Pi stacked:** TYR A:149. **Pi-Alkyl**: ARG A:188.
7h	-9.7	**Van der Waals**: ALA A:147, ALA A:152, ASN A:80, ASN A:83, GLY A:81, GLY A:142, GLY A:146, GLY A:153, SER A:82, SER A:143, SER A:235, SER A:238, ARG A:225, ARG A:188, ARG A:310, GLN A:229, TYR A:77, ILE A:84, ILE A:140, MET A:150. **Conventional hydrogen bond**: GLY A:237, PRO A:141. **Carbon hydrogen bond**: LEU A:231, GLY A:146. **Pi-Pi –T shaped**: TYR A:149, PHE A:274. **Pi-alkyl**: PRO A:141, PHE A:274, TYR A:42, TYR A:149. **Halogen :** PRO A:230

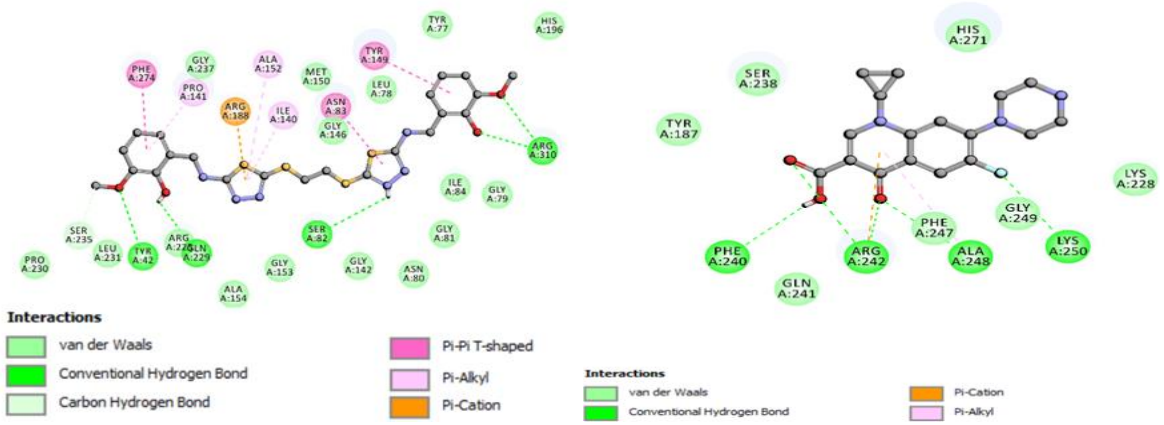


Figure 4. The two-dimension binding compound (**7b**) (left) and ciprofloxacin (right) applied with the active site ciprofloxacin-binding protein.

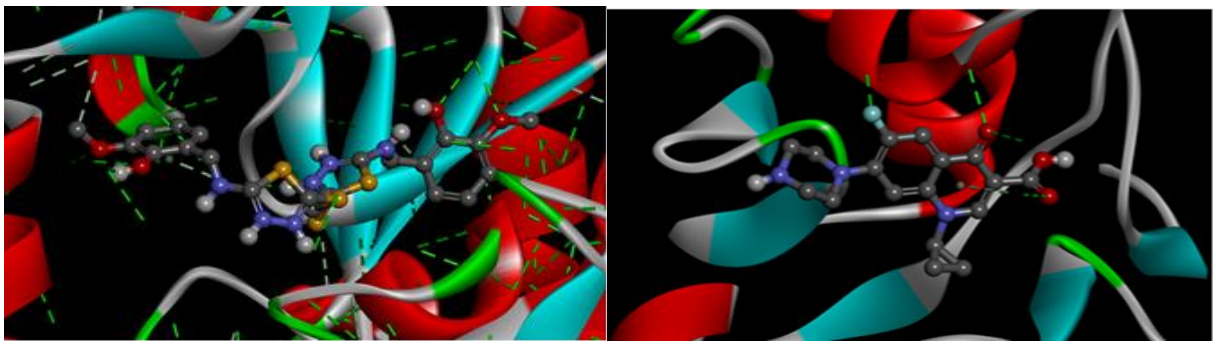


Figure 5. The three-dimension binding compound **(7b)** (left) and the ciprofloxacin (Right) applied into the active site ciprofloxacin-binding protein.

4. CONCLUSION

In conclusion, this study was able to synthesize two new series of biologically active bis-Schiff bases as disulfide derivatives (**4a-h**) and bis-Schiff base of thioether derivatives (**7a-h**) in good yields. The overcome of the challenge of making disulfides from their corresponding thiols is another achievement of this study, because such kinds of reactions may lead to over oxidation and the formation of byproducts such as sulphoxide and sulphones. The synthesized compounds were tested for antimicrobial activity and generally showed a good activity compared with standard drugs. The compounds **4b,4f, and 7b which** contain vanillin and nitro benzaldehyde showed the highest antibacterial activity against Escherichia coli and streptococcus, and less activity against Klebsiella. In addition, molecular docking was applied for this purpose, synthesized compounds showed good binding energy and interaction compared with standard drugs. The substituents (methoxy, hydroxy, nitro, and halogens) groups were in a good orientation to produce binding and hydrogen bonds. Finally, based on our preliminary tests, it is believed that the newly synthesized compounds are promising to be good antibacterial compounds and further testing might show other biological activity potentials of these compounds to become future drug candidates for the treatment of certain illnesses.

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APPENDIX

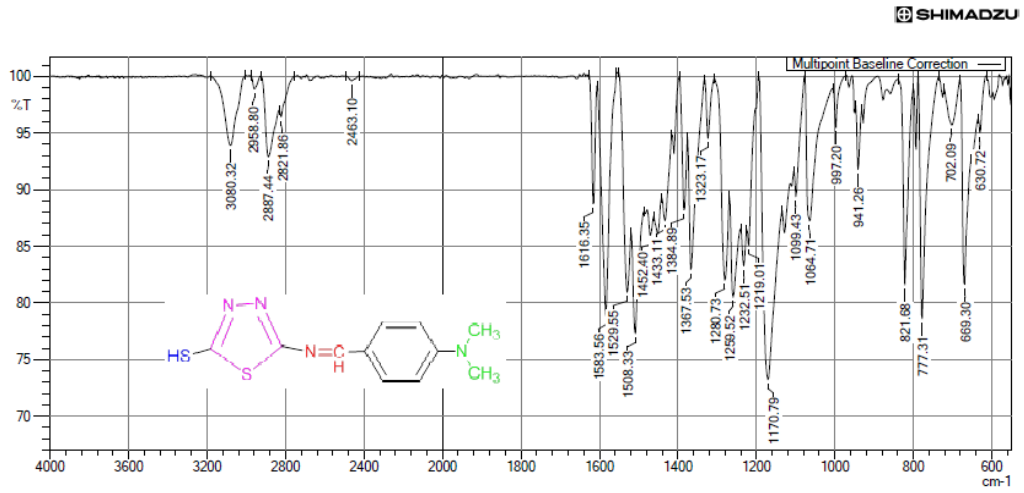

Figure 6. FT-IR spectrum of 5-((4-(dimethylamine) benzylidene) amino)-1,3,4-thiadiazole-2-thiol **(3a).**

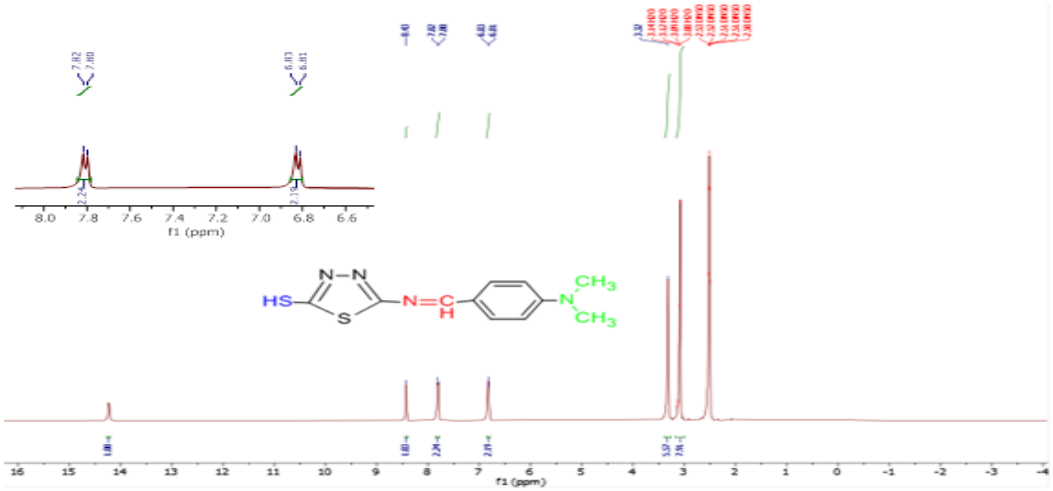

Figure 7.[1]H-NMR Spectrum of 5-((4-dimethylamino)benzylidene)amino)-1,3,4-thiadiazole-2-thuol **(3a)**

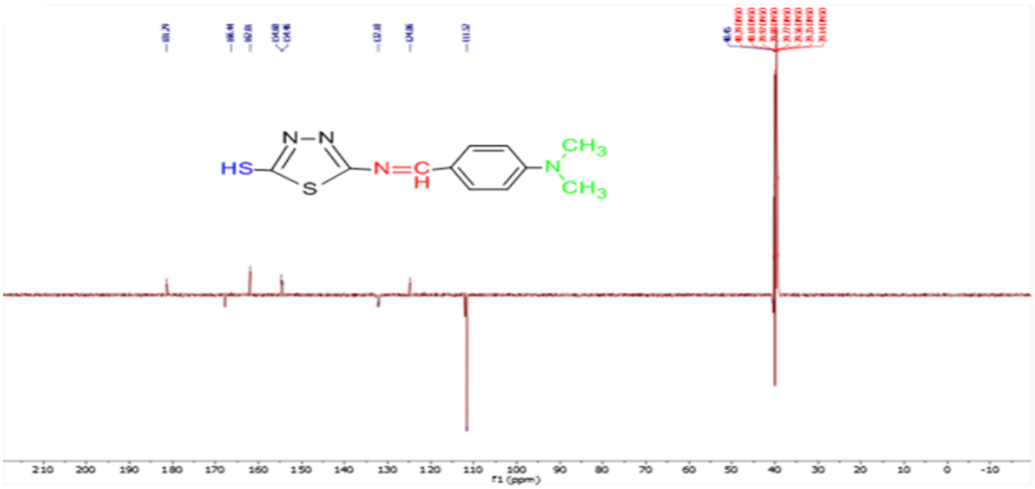

Figure 8.[13]C-APT spectrum of 5-((4-dimethylamino) benzylidene) amino)-1,3,4-thiadiazole-2-thuol **(3a)**

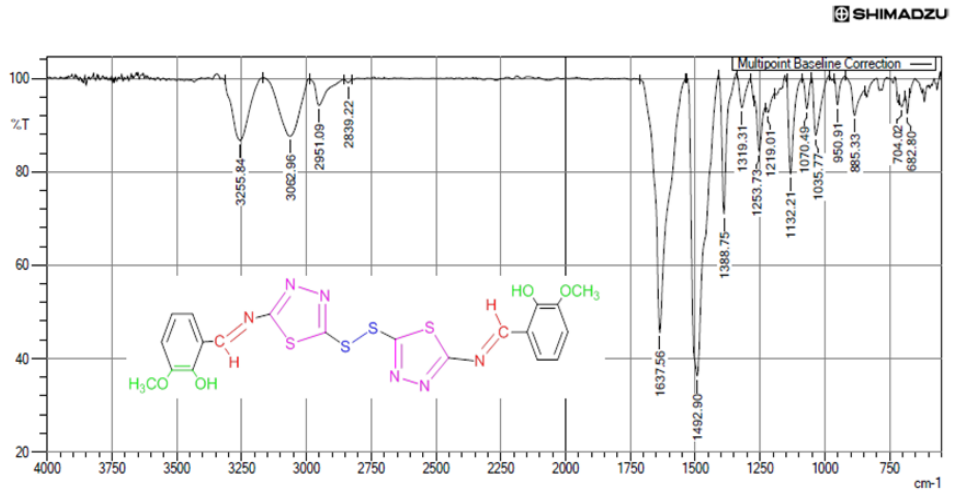

Figure 9.FT-IR spectrum of 6,6'-(1E,1E') -((5,5'disulfanediylbis(1,3,5-thiadiazole-5,2- ditl) bis (azanylyidene)) bis (methanylilidene)) bis (2-methoxyphenol **(4b)**

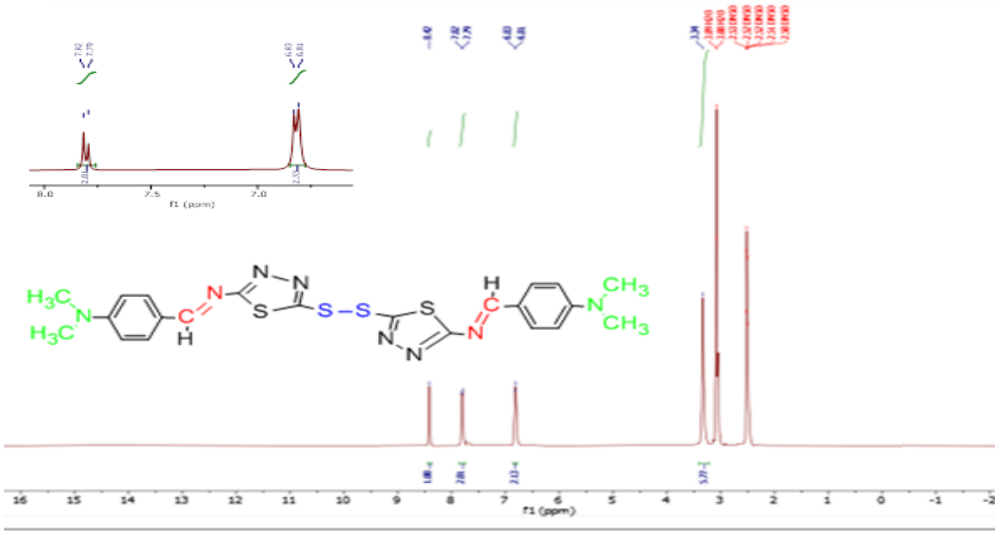

Figure 10.[1]H-NMR spectrum of 5,5'disulfanediylbis (N-(4-(Dimethyl amino) benzylidene)-1,3,4-thiadiazole -2-amine **(4a)**

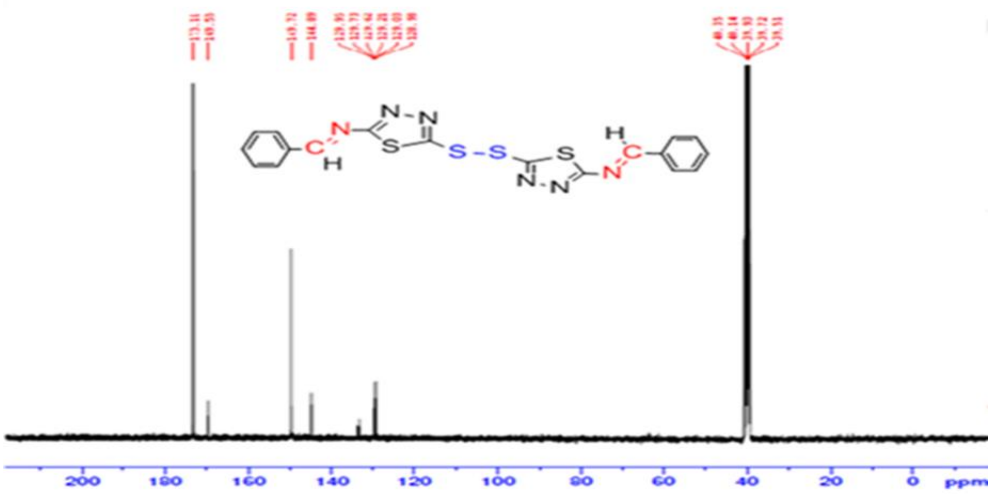

Figure 11.[13]C-NMR spectrum of 5,5'-disulfanediylbis (N-benzylidene-1,3,4-thiadiazole-2-amine) **(4c)**

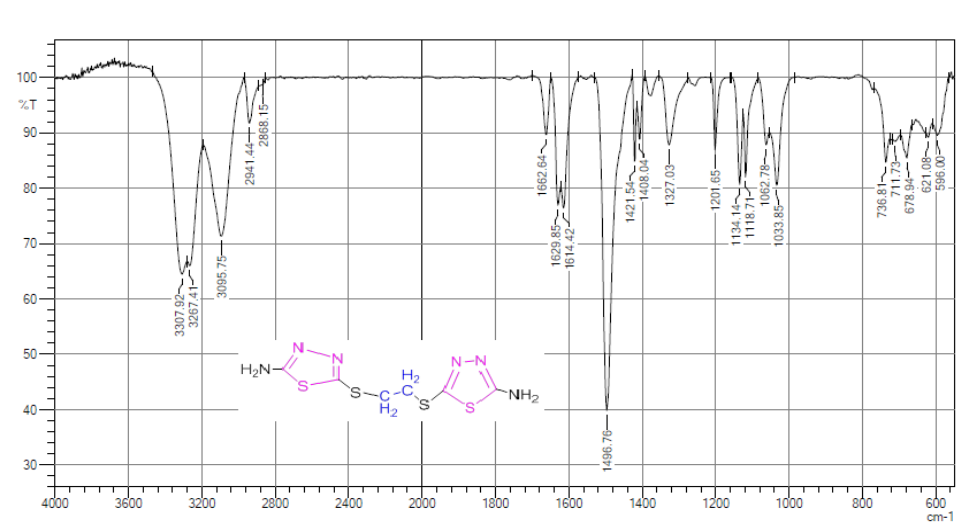

Figure 12.FT-IR spectrum of 5,5'(ethane-1,2-diylbis(sulfanediyl)) bis(1,3,4-thiadiazol-2-amine) **(6)**

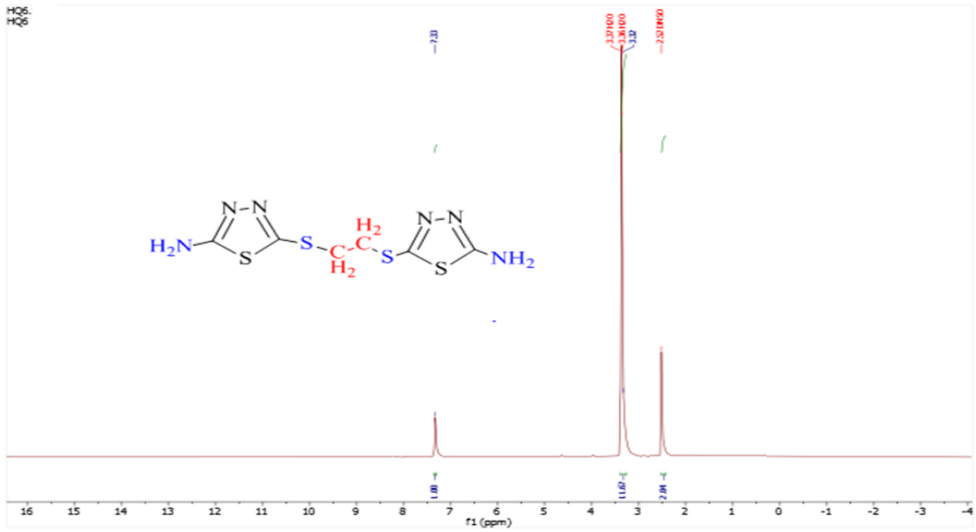

Figure 13.[1]H-NMR spectrum of 5,5 (ethane-1,2-diylbis(sulfanediyl))bis(1,3,4-thiadiazole-2-amine) **(6)**

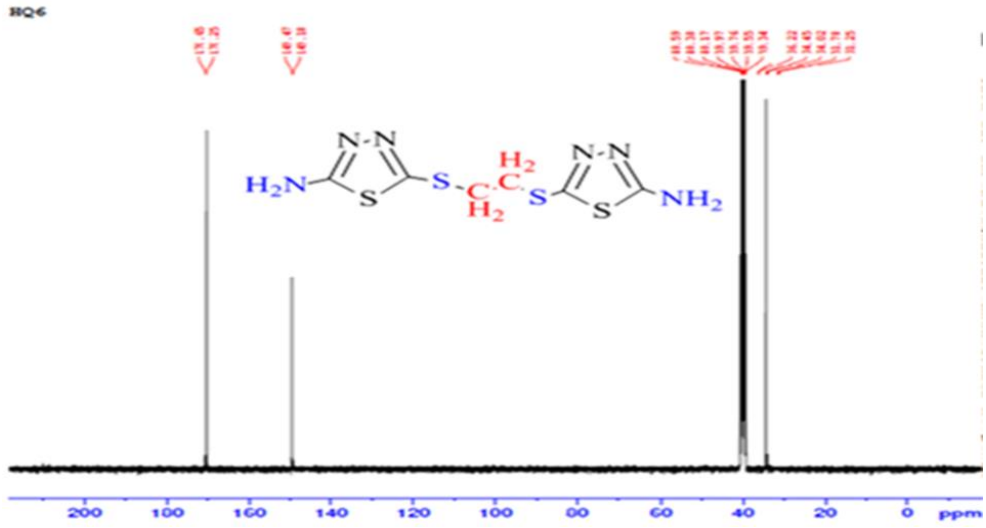

Figure 14.[13]C-NMR spectrum of 5,5 (ethane-1,2-diylbis(sulfanediyl))bis(1,3,4-thiadiazole-2-amine) **(6)**

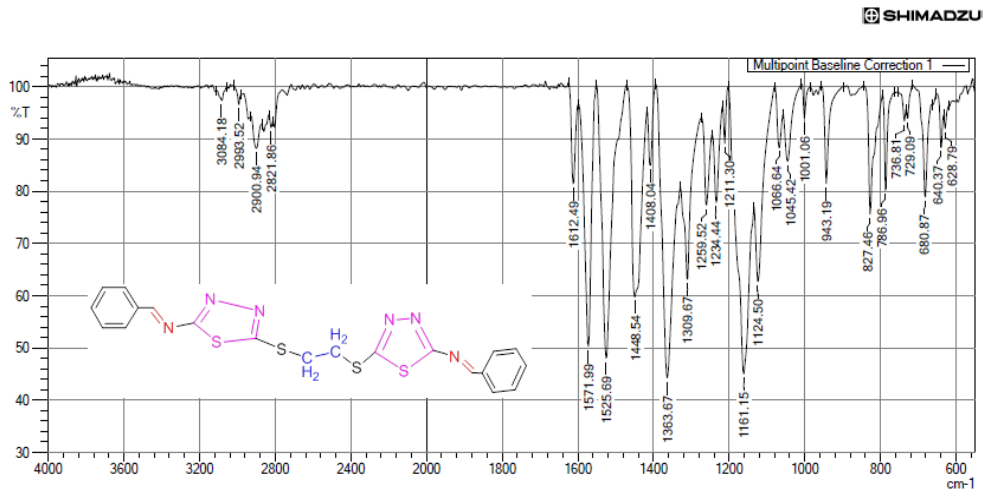

Figure 15.FT-IR spectrum of (NE, N'E)-5,5'-(ethane-1,2-diylbis(sulfanediyl)) bis (N-benzylidene-1,3,4-thiadizole-2, amine) **(7c)**

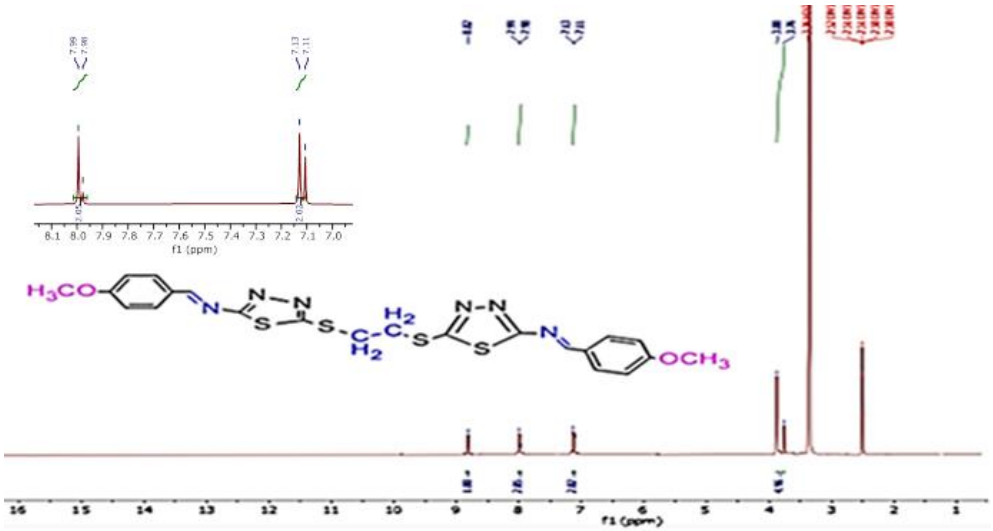

Figure 16.[1]H-NMR spectrum of 5,5'-(ethane-1,2-diylbis(sulfanediyl)) bis (N-(4-methoxybenzylidene)-1,3,4-thiadiaz ole-2-amine **(7g)**

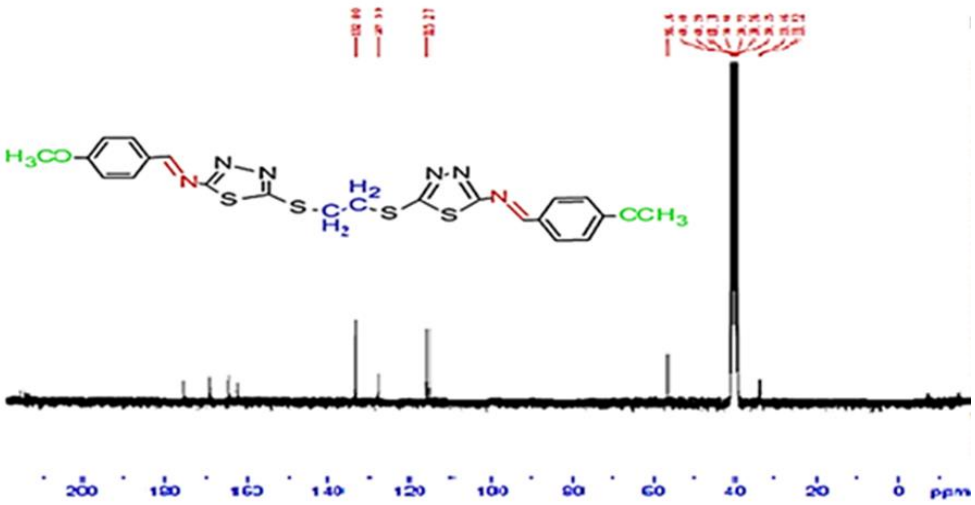

Figure 17.[13]C-NMR spectrum of 5,5'-(ethane-1,2-diylbis(sulfanediyl)) bis (N-(4-methoxybenzylidene)-1,3,4-thiadiaz ole-2-amine **(7g)**