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Synthesis, Characterization and Biological Activity Study of Metronidazole- Thiadiazole derivatives

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Abstract : In this work the preparation of new compounds that contain both groups (metronidazole and 1,3,4-thiadiazole) have been carried out by the reaction of phenylenediamine with carbon disulfide and hydrazine in presence of potassium hydroxide the obtained compound was treated with chloroacetyle chloride to produce the 1,3,4-thiadiazole derivative, then two equivalent from metronidazole was added to both side of derivative moiety, in order to overcome resistance problem of metronidazole and to increase its spectrum of activity by synthesizing of new derivatives that have antibacterial activity against aerobic bacteria. Full characterization of the synthesized compounds was done by using of spectroscopic analysis such as FT-IR, ^{1}H-NMR, ^{13}C-NMR, mass and elemental microanalysis spectroscopies. The resultsshowed that the final compounds had higher antibacterial activity against gram positive and gram negative bacteria compared with metronidazole.
Keywords : Phenylenediamine,metronidazole, bis 1, 3, 4- thiadiazole, antibacterialactivity.

Introduction:

Metronidazole (1-[2-hydroxyethyl]-2-methyl-5-nitroimidazole) is a nitroimidazole drug active against anaerobic bacteria and certain parasites[1,2]. It is a widely used drug given its tolerability, high oral bioavailability, and capacity to penetrate into tissues well, including the central nervous system[3]. Reduction of the nitro group to the nitro radical anion by electron carriers in an anaerobic environment leads to decomposition to form toxic metabolites, which cause DNA damage and nonspecific macromolecular damage leading to cell death[4,5]. However, metronidazole resistance has been observed in both parasites[6] and anaerobic bacteria[7]. Over the last ten years metronidazole has also been extensively used to treat Clostridium difficile infection, an intestinal infection that causes life-threatening severe diarrhea, abdominal pain and fever[8]. Metronidazole is effective against mild to moderate anaerobic bacteria such as C. difficile infection, but, while not wide-spread, resistance has been observed in clinical isolates[9]. To this end, the development of next generation metronidazole analogues that can overcome resistance is therapeutically important.1,3,4, Thiadiazoles exhibit a broad spectrum of biological activities like anti-inflammatory[10], antibacterial[11], antidepressant[12], antifungal[13], anticancer[14] and anti-HIV[15,16]. Thus the aim of this research is to synthesize of some novel metronidazole derivatives containing 1,3,4 thiadiazole moiety by usingphenylenediamine as a spacer which could lead to new compounds with potential antibacterial activity and less resistance by bacteria.

Materials and methods:

All chemicals were supplied from different companies such as Fluka, Merck, BDH, SDI, Sinopharm, Sigma-Aldrich and Scharlau and used without further purification. Melting points are determined on an electro thermal melting point apparatus (Stuart, Germany), and they are uncorrected. Completion of reaction and purity of all compounds are checked on aluminum coated TLC plates 60 F245 (E. Merck) using Methanol: Benzene:

Chloroform: Ethanol (60:40:80: 20)as the mobile phase and visualized under iodine vapor. Determinations of infrared spectra were done and recorded as a KBr disks in the range of (500 -4000 cm^{-1}) using FTIR Shimadzu (Japan).The proton ^{1}H, ^{13}C-NMR spectra were recorded for the synthesized compounds using Bruker DMX-500 spectrophotometer (300 MHZ, solvent DMSO-$d_{6)}$ with TMS as internal standard[17]. Mass spectroscopy was done for compound [3] & [4] using mass spectrometer (Schimadzu). Powdered samples of compound [3] & [4] were analyzed by TGA to evaluate their thermal behavior during heating process (0-400 °C), where each sample was located separately in TG instrument pan to record the weight loss with increase temperature by subjecting them to a constant heating rate of 5 C°/min and air atmosphere with a gas (nitrogen) flow of 50 mL/min. As a result, thermal scan was recorded as plot of mass change versus temperature. Determinations of TGA were done using Thermo gravimetric analyzer (STA PT 1000 TG-DSC) Linsies Inc[18,19].

Synthesis of potassium 1,4-phenylene dicarbamodithioate[compound 1]

A round bottomed flask 250 mL in size was used to prepare compound [1]. (1 g, 9.25 mmol.) of *p*-phenelynediamine was put and dissolved in 25 mL ethanol with stirring, the solution was heated to reach the temperature of (60 ^{o}C). After 15 minutes a solution of (1g, 18.5 mmol.) KOH was dissolved in ethanol and added to the parent solution.

Carbon disulfide (1.2 mL, 18.5 mmol.) was added drop wise, and the mixture was refluxed for 6 hrs. at 70 ^{o}C with continuous stirring. The reaction was stopped, (60 mL) of brine solution (saturated solution of NaCl) was added, and then separated by DCM (40 mL three times) using separatory funnel. The organic layer was treated by rotary evaporator to remove the solvent, and then deep yellow precipitate was formed.

Synthesis of *N,N′*-(1,4-phenylene)*bis*- (hydrazinecarbothioamide)[compound2]:

Compound [1] (3g, 8.91 mmol), was dissolved in (30ml) of ethanol in a round bottomed flask, then the reaction mixture was stirred at 60ºC for 15 min. A solution of (0.9 g, 17.98 mmol.) of hydrazine hydrate (80%) was added slowly in a drop wise manner to the mixture and then refluxed for 4 hrs. After the reaction has been finished, the solvent was evaporated under reduced pressure and the product was filtered and dried.

Synthesis of *N1,N4-bis*(5-(chloromethyl)-1,3,4-thiadiazol-2-yl) benzene-1,4-diamine [compound 3]:

Compound [2] (3 g, 11.7 mmol.) was dissolved in 30 ml absolute ethanol, the solution was stirred up to ten minutes, then a solution of chloroacetylchloride (2.64 g, 23.4 mmol.) was placed in dropper funnel and added to the solution of compound [2] as drop wise, the HCl gas releasing from reaction was observed by the white vapor breakdown in the first time of reaction, the mixture was refluxed with stirring for ten hours at 70 ^{o}C, the precipitate was formed in the bottom of the round flask, the solvent was removed via reducing the pressure of rotary evaporator, and the product was collected and dried with room temperature.

Synthesis of N1,N4-bis(5-(3-(2-methyl-5-nitro-1H-imidazol-1-yl)propyl)-1,3,4-thiadiazol-2-yl)benzene-1,4-diamine [compound 4]:

Metronidazole (0.92g, 5.38 mmol.) was dissolved in 20 ml of DMF and added slowly as drop wise to a solution containing (1g, 2.69 mmol.) of compound [3] in 25 ml of DMF, the mixture was stirred for 15 minutes, then 6 drops of glacial acetic acid were added as a catalyst, and let the mixture to reflux at 70 ^{o}C for 3 hours. A pale brown precipitate was obtained; the solvent was released under vacuum with rotary evaporator to yield the final product.

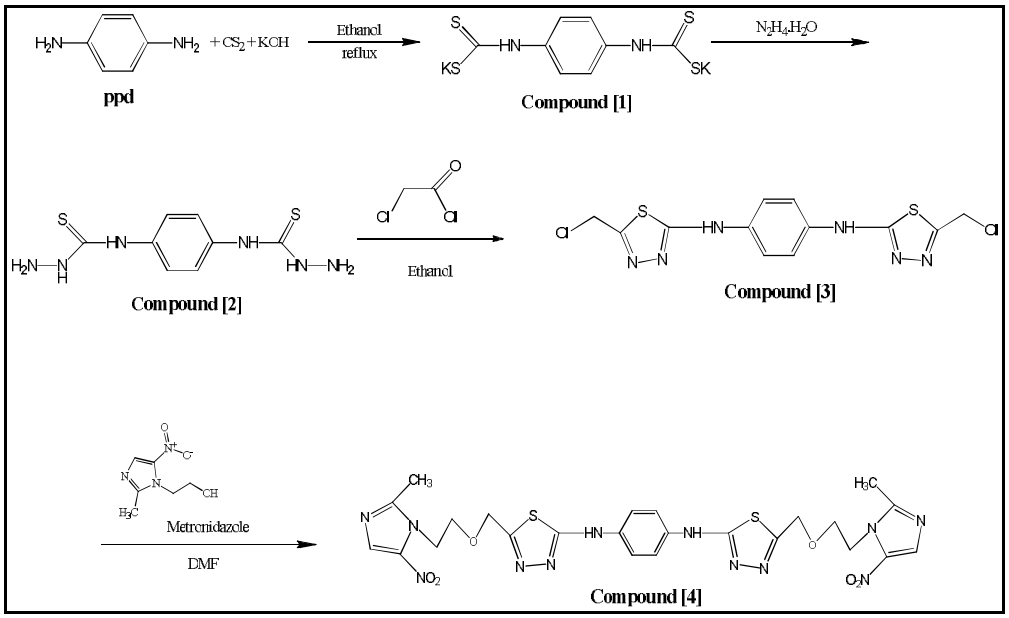
Antibacterial activity test

The antibacterial activity of compound [3] & [4] was done as well as MTZ. Each sample was tested against three types of Gram +ve bacteria (*Staphylococcus aureus, Streptococcus pyogen* and *Streptococcus viridance*) and three types of Gram –ve bacteria (*Providencia sp*, *Serratiamarcescens*, and *Enterobacter cloacae*) using a concentration of 50 µg/ml of pure MTZ and an equivalent concentration of compound [3] & [4]. The samples were dissolved using dimethylsulfoxide (DMSO) as a solvent and cultured in Muller Hinton agar for 24 h at 37°C[20,21,22].

Results and discussion

The synthesis of compounds carried out *via* the direct reaction among the starting materials, the first step included the synthesis of compound [1] through the reaction between the *p*-phenylenediamine with CS_2 in

presence of potassium hydroxide (KOH) in ethanoic media, the resulted compound [1] was reacted with two equivalents of hydrazine hydrate to obtain compound [2] in title, Compound [3] was formed through the reaction of compound [2] with two equivalents of chloroacetylchloride in ethanol. The reaction of two equivalents of Metronidazole with one equivalent of compound [3] will give the final derivative [compound 4]. The overall reaction was summarized in **Scheme (1)**[23]



Scheme 1, synthesis route of compounds 1-4

The characterization and the purity of the intermediates and the targets (melting points, yielded percentages and Rf values) were summarized in **Table (1).**The FT-IR spectra of the synthesized compounds showed a characteristic bands of absorption which were in consistence with the proposed structure of the compounds. The values of the characteristics bands of these spectra were discussed according to the literature survey of similar compounds and references. The functional groups of the starting materials and the synthesized compounds were identified using FT-IR spectroscopy as shown in **Table (2).**The chemical structures were confirmed using elemental microanalysis (CHNS) as presented in **Table (3)** , the results were found agree with the corresponding calculated values.The mass spectra of compounds [1] & [2] were recorded, the parent ion (m/z^+) were shown corresponding to the expected molecular mass of the compounds. **Tables (4)** summarized the suggested fragmentation with the abundance and formula for each fragment. The ^1H, ^{13}C-NMR analysis was used to identify the synthesized compounds. The spectra were recorded using DMSO-d^6 solvent the values of the characteristics of the chemical shiftwere discussed according to the literature survey of similar compounds and references. These data were summarized in **Tables (5)** to **(8)** and **Figures (1) to (4)**[24,25].

Table (1): Physical Properties of Synthesized Compounds (1-4)

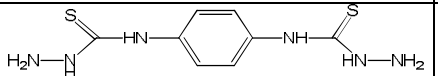
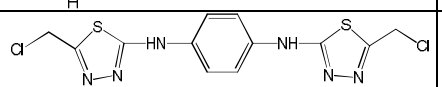
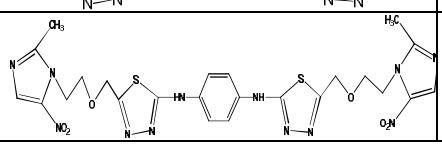
Comp	structure	M.Wt	Yield %	Color, physical appearance	M.P/ ^oC	Rf value
[1]		336.60	83	deep yellow precipitate	248-250	0.80
[2]		256.35	87	dark olive color precipitate	255-257	0.83
[3]		373.28	89	dark brown precipitate	171-173	0.75
[4]		642.67	65	pale brown precipitate	245-247	0.78

Table (2): FT-IR Spectral data for the starting and synthesized compounds(cm^{-1}, KBr disk)

	***p*-phenylenediamine**
3350, 3300	ν **(N-H)** asymmetrical and symmetrical stretching vibration of primary amine groups
3009	ν **(C-H)** aromatic stretching
1629	ν **(C=C)** aromatic stretching of benzene ring
1311, 1263	ν **(C-C)** stretching of aromatic ring
1128	ν **(C-N)** stretching vibration
923	Structural band of aromatic ring bending
	Compound [1]
3230, 3148	ν **(N-H)** secondary amine in different environments due to the fluctuation phenomena of molecule
1533	ν **(C=C)** aromatic stretching vibration
1406	ν **(C-C)** aromatic stretching vibration
1263	ν **(C=S)** stretching vibration
1139	ν **(C-N)** stretching vibration
927	ν **(C-S)** stretching vibration
837	ν **(C=C)** bending vibration
792	ν **(=C-H)** bending vibration
	Compound [2]
3398, 3306	ν **(N-H)**asymmetrical and symmetrical stretching vibration of primary amine groups
3211, 3149	ν **(N-H)** stretching vibration for two groups of secondary amine in two different positions
3099	ν **(C-H)** stretching of aromatic benzene ring
1624	ν **(C=C)** stretching vibration
1514	ν **(C-N)** stretching vibration
1128	ν **(C=S)** stretching vibration
	Compound [3]
3320	ν **(N-H)** stretching vibration
3050	ν **(C-H)** aromatic stretching vibration
2947, 2814	ν **(C-H)** aliphatic stretching vibration
1622	ν **(C=C)**aromatic stretching vibration
1541, 1506	ν **(C=N)** thiadiazole ring stretching in different positions
1311	ν **(C-C)** bending vibration
1118	ν **(C-H)** bending vibration
827	Structural band of benzene ring
	Compound [4]
3196	ν (**N-H)** stretching vibration of secondary amine
3050	ν (**C-H)** aromatic stretching vibration
2960, 2954	ν (**C-H)** asymmetrical and symmetricalstretching vibration for methyl groups
2920, 2823	ν (**C-H)** asymmetrical and symmetricalstretching vibration for methylene groups
1681	ν (**C=C)** aromatic stretching of benzene ring vibration
1583, 1506	ν (**C=N)** stretching vibration of thiadiazole and metronidazole rings
1369, 1535	ν **(NO_2)**stretching vibration
1267	ν **(C-O)** stretching
678	ν **(C-S)** stretching vibration

Table (3): Elemental microanalysis data (%) of the synthesized compounds (C.H.N.S.)

Element	**Calculated**	**Observed**	**Element**	**Calculated**	**Observed**
Comp. [1] ($C_8H_6N_2S_4K_2$)			**Comp. [3] ($C_{12}H_{10}N_6S_2Cl_2$)**		
C	28.55	28.21	C	38.61	38.33
H	1.80	1.66	H	2.70	2.65
N	8.32	8.38	N	22.51	22.81
S	38.10	37.91	S	17.18	17.40
Comp. [2] ($C_8H_{12}N_6S_2$)			**Comp. [4] ($C_{24}H_{26}N_{12}O_6S_2$)**		
C	37.48	37.00	C	44.85	44.96
H	4.72	4.55	H	4.08	3.98
N	32.78	33.01	N	26.15	26.48
S	25.02	25.32	S	9.98	9.76

Table (4): Mass spectroscopy data for compound [3] & [4]

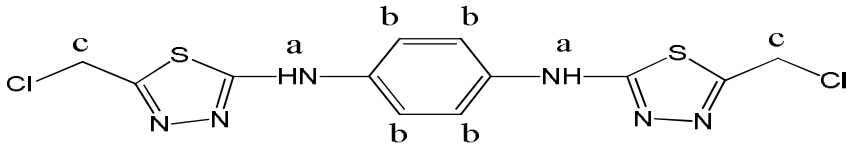
compound [3]

Fragments ions	Mass/Charge (m/	Abundance (%)
(C12H10Cl2N6S2)+	373	18
(C11H9ClN6S2)+	322	5
(C12H12N6S2)+	305	18
(C10H8N6S2)+	277	18
(C10H10N6S)+	248	7
(C11H11ClN4)+	236	25
(C8H10N3)+	148	50
(C7H9N)+	108	100
(C7H8)+	92	15

compound [4]

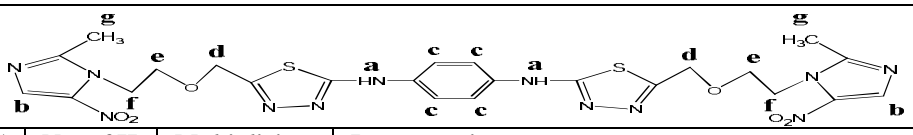
Fragments ions	Mass/Charge (m/z)	Abundance (%)
(C24H26N12O6S2)+	642	9
(C21H25N9O2S2)+	499	13
(C12H12N6OS2)+	322	15
(C6H9N3O3)+	171	25
(C6H8N2O)+	124	62
(C7H9N)+	108	100
(C4H5N2)+	81	55

Table (5): ^{1}H-NMR data and their interpretation of compound [3]



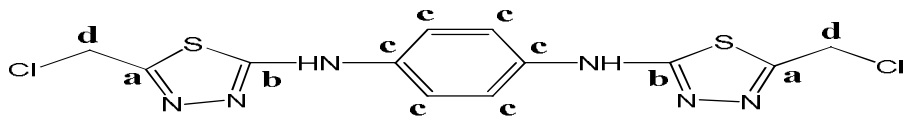
Signal	*(ppm)*	*No. of H*	*Multiplicity*	*Interpretation*
a	7.8	2H	Singlet	Protons of the secondary amine
b	7.2-7.5	4H	Doublet	Aromatic protons
c	4.1-4.3	4H	Singlet	Attributed to the methylene group was shifted to higherfield due to the neighboring withdrawing group (Cl)

Table (6): ^{1}H-NMR data and their interpretation of compound [4]



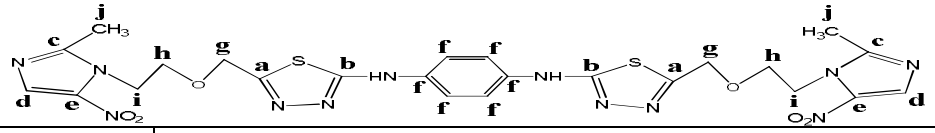
Signal	*(ppm)*	*No. of H*	*Multiplicity*	*Interpretation*
a	8.2	2H	Singlet	Protons of the secondary amine
b	7.8	2H	Singlet	Proton of imidazole ring neighboring to nitrogen atom
c	7.0-7.3	4H	Doublet	Protons of benzene ring
d	4.2	4H	Singlet	Protons of methylene adjacent to O atom & thiadiazole ring
e	3.6	4H	Triplet	Protons of methylene adjacent to O atom
f	3.5	4H	Triplet	Protons of methylene adjacent to N atom
g	2.2	6H	Singlet	Protons of terminal methyl group

Table (7):^{13}C-NMR data and their interpretation of compound [3]



Signal	***(ppm)***	***Functional groups***
a	170	(-C=N-) of thiadiazole ring (terminal)
b	161	(N-C=N-) of thiadiazole ring (internal)
c	121	Carbons of benzene ring
d	60	Carbon of methylene adjacent to Cl atom

Table (8):^{13}C-NMR data and their interpretation of compound [4]



Signal	***(ppm)***	***Functional groups***
A	170	(-C=N-) of thiadiazole ring (terminal)
B	165	(N-C=N-) of thiadiazole ring (internal)
C	153	(-N=C-) of imidazole ring
D	135	(=N-C=) of imidazole ring
E	134	(=C-NO_2) of imidazole ring
F	119-120	Carbons of benzene ring
G	61	(-O-C-) adjacent to thiadiazole ring
H	60	(O-C-) of methylene group
I	44	(-N-C-) of methylene adjacent to imidazole ring
J	18	Carbon of methylene attached to imidazole ring (terminal)

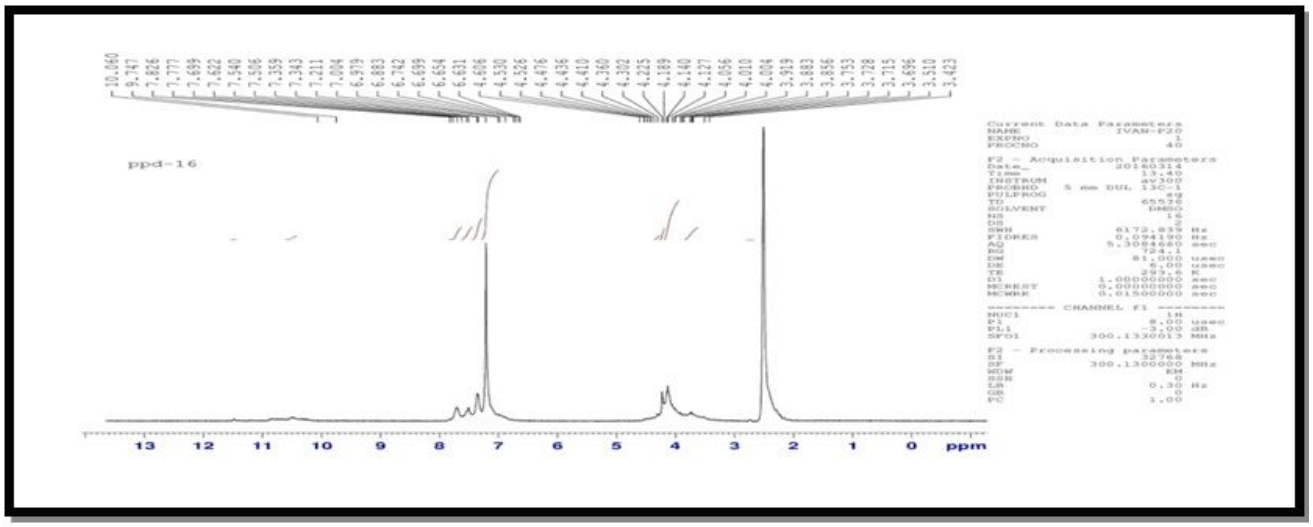


Figure (1): ^{1}H-NMR spectrum of compound [3]

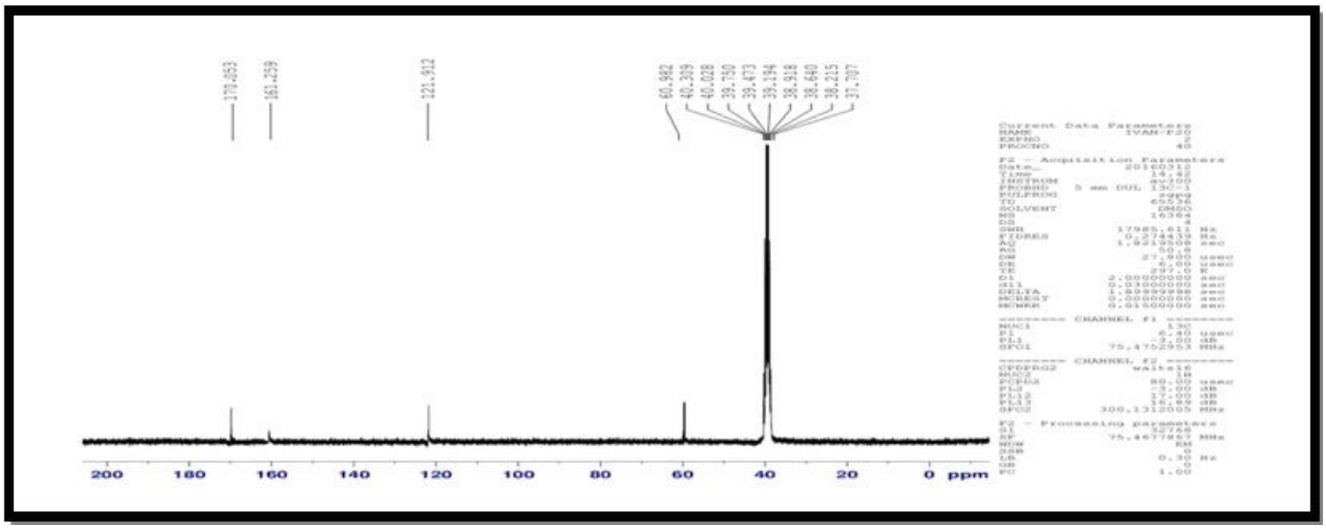


Figure (2): ^{13}C-NMR spectrum of compound [3]

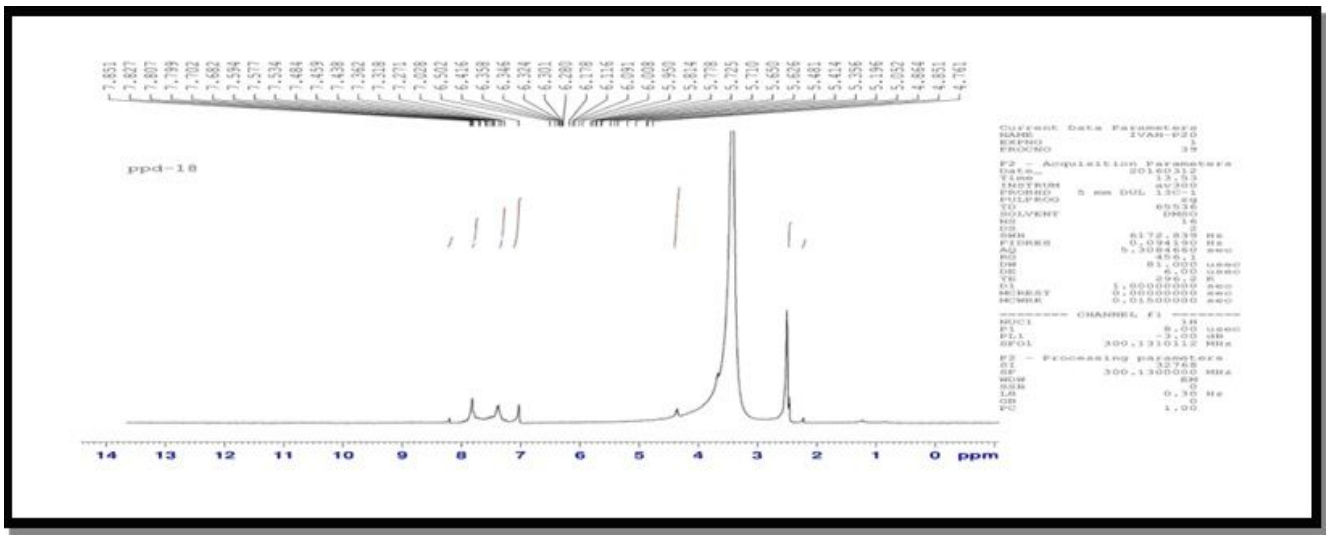


Figure (3): ^{1}H-NMR spectrum of compound [4]

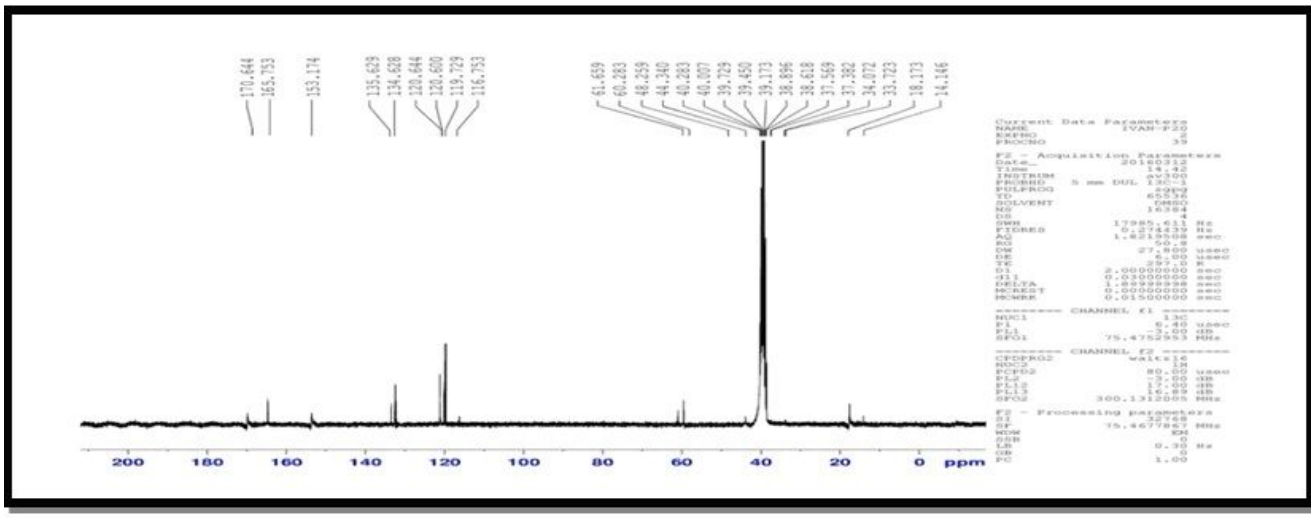


Figure (4): ^{13}C-NMR spectrum of compound [4]

The results of TGA for both compound [3] & [4] were obtained as a spectrum that demonstrate the mass change of the compound with the constant elevation of temperature(0-400 °C at a rate of 5 C°/min) which reflect the thermal stability behavior of that compound. However **figures (5)** and **figure (6)** shows the TGA spectrum of compound [3] & [4] respectively while **Table (9)** discusses their results[26].

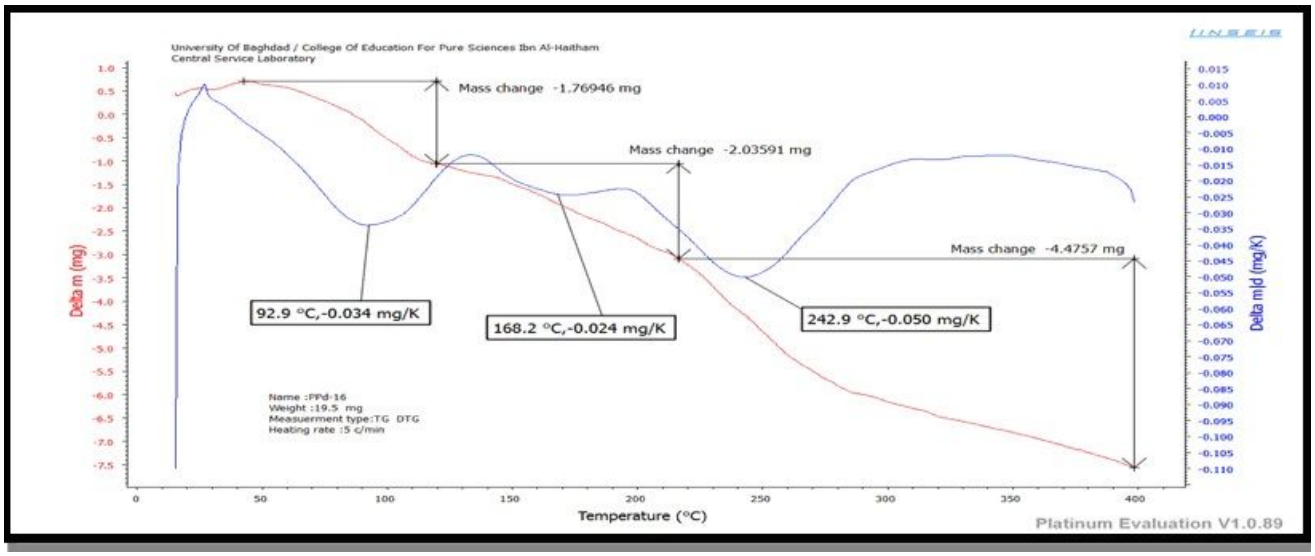


Figure (5): Thermo gravimetric Analysis of compound [3]

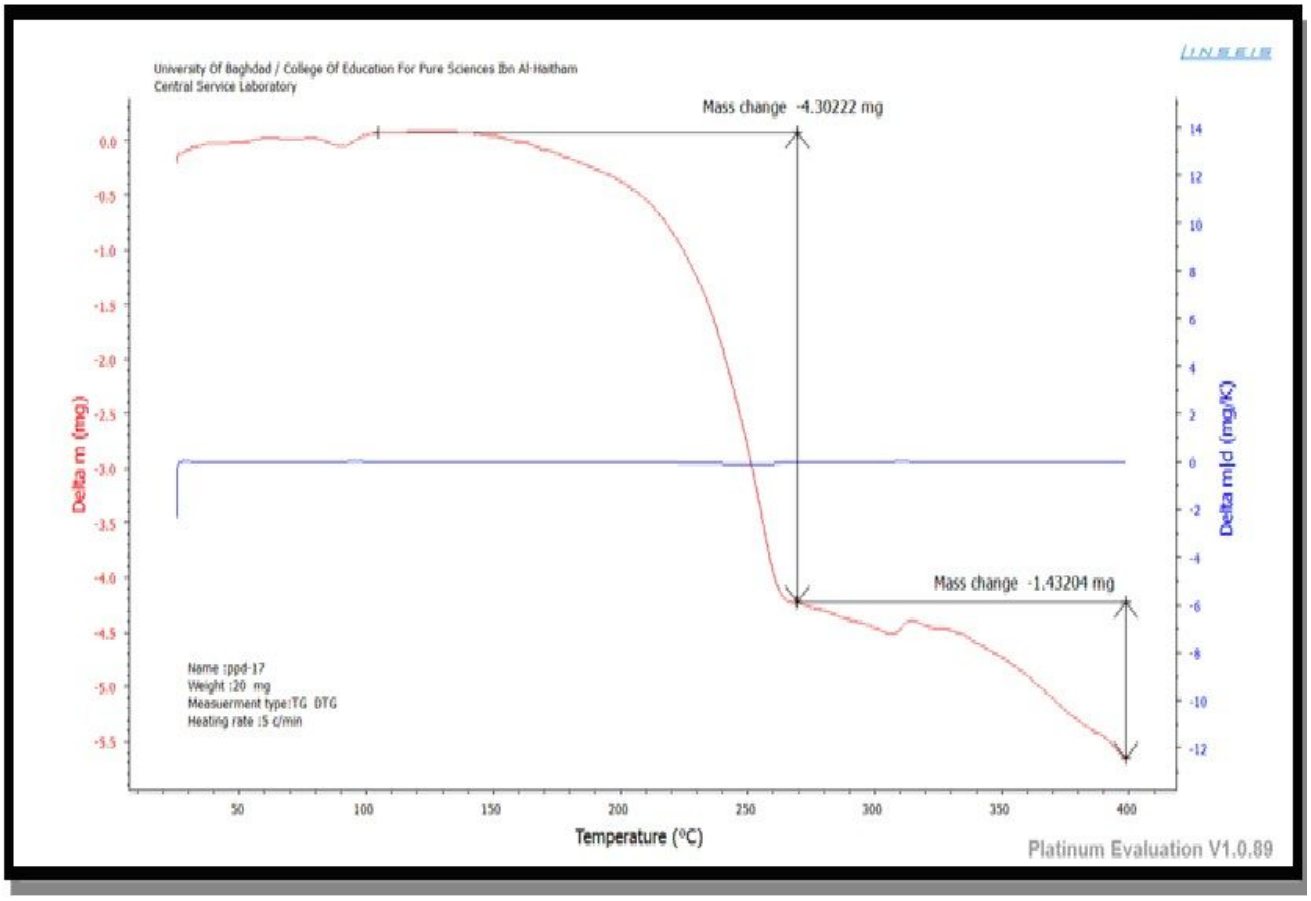


Figure (6): Thermo gravimetric Analysis of compound [4]

Table (9): Thermal Graphical Analysis (TGA) data and their interpretation of compound [3&4]

Compound	Interpretation
Comp. [3]	The thermal graphical analysis of compound [3] **Fig. (?)** displays the losing weight and stability behavior of compound with increasing the temperature, since the sample shows the change at 92.9 ^{o}C with weight loss 1.76 mg can be attributed to the Cl losing comparison with the beginning sample weight is 19.5 mg, the second change shows the characteristic change in the mass with 2.03 mg losing weight at 168.2 ^{o}C indicating the stability of compound in the room temperature compared with the melting point of the prepared compound which that shows the decomposition at 171-173 ^{o}C shows the more stability up to 400 ^{o}C. The signal shows the highly weight losing equal to 4.5 mg at 242.9 ^{o}C.
Comp. [4]	The TGA of compound [4] **Fig. (?)** displays the stability of drug derivative at room temperature to 400 ^{o}C. The gradually increased temperature did not affect the prepared compound and it is stable to 269 ^{o}C shows the first change with mass change 4.30 mg referred to the high stability of prepared compound.

Commercial reference strains of Gram-positive bacteria (*Staphylococcus aureus, Streptococcus pyogen* and *Streptococcus viridance*) and Gram-negative bacteria (*Providencia sp*, *Serratia marcescens*, and *Enterobacter cloacae*) were selected to assess the potential antibacterial effect of the prepared compounds. The bacterial species chosen are of biological importance to represent a good range of bacteria that is the causative factor for many infectious diseases. The assessment of antibacterial activity was based on the dimension of the inhibition zone diameter which is formed around the well. The results show that MTZ has no antibacterial activity against all the tested bacteria (this is a predicted result because MTZ active against anaerobic bacteria only, while all the selected bacteria were aerobic ones). From the inhibition zones it appears clearly that compound [4] is more active than compound [3] against the tested bacteria, these results confirm the expected antibacterial activity of compound [4] due to the presence of the biologically important 1,3,4-thiadiazole group in addition to the parent nitroimidazole group. All the results were summarized in **Table (10)**[27]**.**

Table (10): Antibacterial activity (inhibition zone in mm) for MTZ, compound [3], and compound [4] against tested bacteria

Gram positive bacteria:			
Compound	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Streptococcus viridance*
MTZ	_	_	_
Compound [3]	20	_	20
Compound [4]	27	15	30
Control (DMSO)	_	_	_
Gram negative bacteria:			
Compound	*Providencia sp.*	*Serratia marcescens*	*Enterobacter cloacae*
MTZ	_	_	_
Compound [3]	15	_	22
Compound [4]	25	16	22
Control (DMSO)	_	_	_

Conclusions

The using of *p*- phenylenediamine as a spacer molecule which attached to 1,3,4-thiadiazole on both sides of the molecule by using carbon disulfide and hydrazine hydrate results in the formation of two thiadiazole rings which contain N & S atoms that makes the molecule more biologically active compound. The attachment of MTZ molecules, which classified as antibacterial (anaerobic bacteria) and antiprotozoal agent, with the derived compound resulted in the formation of a new compound. The resulted compounds were characterized by the available techniques such as C.H.N.S. micro-elemental analysis, FT-IR, ^{1}H, ^{13}C-NMR, and mass spectroscopies also the biological activity of the prepared compounds were tested against G+ve and G-ve bacteria. The spectroscopic studies proved the formation of compounds in title, and the surprise of the current work shows the biological activity results; the activity of MTZ derivative is more than the parent compounds and wider spectrum.

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