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Synthesis, spectroscopic characterization of new heterocycles based on sulfamethoxazole as potent antimicrobial agents

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Abstract:

A series of sulfamethoxazole derivatives attached to heterocyclic ring such as 1,2-diazepane (3), 1,3,4-oxadiazole (4), two pyrazoles (5,6), 1,2,4-triazine (7), 1,3,4-oxadiazine (8) and four novel 1,3-oxazepines (14-17)were designed and synthesized in this research. The structures of the newly prepared compounds were confirmedbased on a comprehensive characterization of spectral data by applied (infrared, proton and carbon nuclear magnetic resonance spectroscopy). Physicochemical properties also determined for each synthesized derivatives. The finallyprepared compounds were tested for their anti-bacterial and fungal activity *in-vitro*. Four types of pathogenic bacteria with two types of yeast similar to fungi were used in the evaluation. Each screened compounds showed perfect antimicrobial activity comparable with sulfamethoxazole used as parent drug.

Key words: Synthesis, characterization, heterocycles, sulfamethoxazole, antimicrobial.

تحضير، تشخيص طيفي لحلقات غير متجانسة جديدة معتمدة على السلفاميثوكسازول كعوامل جيدة مضادة للمايكروبات

الخلاصة:

سلسلة من مشتقات السلفاميثوكسازول المتصلة بحلقات غير متجانسة مثل 2,1-دايازابان (3)،4,3,1-اوكسادايازول (4)، مركبين للبايرازول (6,5) ، 4,2,1-ترايازين (7) ،4,3,1-اوكسادايازين (8) ، اربع مركبات جديدة ل 3,1-اوكسازيبين(17-14) تم تحضيرها في هذ البحث. تراكيب المركبات المحضرة الجديدة تم اثباتها اعتمادا على بيانات التشخيص الطيفي (الاشعة تحت الحمراء،اطياف بروتون وكاربون الرنين النووي المغناطيسي) . الخواص الفيزيائية الكيميائية تم قياسها كذلكلجميع المشتقات المصنعة. كل المركبات النهائية تم اختبارها لفعاليتها المضادة البكترية والفطرية خارج جسم الكائن الحي. اربعة انواع من البكتريا المرضية ونوعين من الخمائر الشبيهة للفطر استخدمت في هذه الدراسة.جميع المركبات المختبرة اظهرت فعالية مثالية مقارنة بالسلفاميثوكسازول الذي استخدم كمادة اساس للمقارنة.

Introduction:

Sulfanilamides (sulfonamides) is very well known chemically as organic compounds composed of an aminobenzene derivatives with a sulfonamide attached group.[1] Pharmaceutically define as antibacterial agents and also used for treatment of some types of yeast infections. [2] Sulfamethoxazole (SMZ) is an example of a family of molecules containing these functional groups.

Sulfamethoxazole a formula analog of para-aminobenzoic acid, it was used widely for many bacterial infections. [3]In the recent years a great number of sulfamethoxazole derivatives were synthesized, characterized, tested and used for the treatment of bacterial diseases.[4]

Many derivatives currently were designed based on heterocyclic moieties are widely used in clinical medicine exhibits as pharmacological agents with a broad variety of biological actions such as anticancer, [5] antiviral agents,[6] anti-fungal,[7] herbicidal activities [8]and anti-tubercular applications.[9]

In view of the facts and to explore and developed the potential antimicrobial activities of sulfamethoxazole derivatives, a series of heterocyclic rings such as1,2-diazepane, 1,3,4-oxadiazole, 1,2-pyrazole, 1,2,4-triazine, 1,3,4-oxadiazine and four 1,3-oxazepines compoundsare designed and synthesized in the current research.

2. Experimental:

2.1. Materials and Methods

Sulfamethoxazole was supplied from the Iraqi factory wadi al-rafidain for pharmaceutical products. Other chemical reagents and solvents generally used and received from the commercial suppliers (Merck, Fluka, BDH, Sigma-Aldrich and Himedia companies).All melting points of the synthesized compounds were determined on a digital Stuart scientific apparatus (SMP30) in an open capillary tube and are uncorrected**.** FTIR spectra (ν, cm^{-1}) were designed in potassium bromide pellets on an 8400 infrared spectrophotometer (Shimadzu, Japan).Nuclear magneticspectra^{1}HNMR and ^{13}CNMR were recorded on a Bruker 300 MHz spectrometer in (DMSO-d^{6}) as a solvent, using TMS as internal reference and the chemical shifts (δ) are set in ppm by water, environment and arid regions research center, Al al-Bayt University (Jordon). The antimicrobial activities of the finally prepared compounds were done in consultant office at College of Science Baghdad University. Preliminary antibacterial and antifungal activities have been carried out according to well diffusion method.

2.2.Synthesis of ethyl (4-(N-(5-methylisoxazol-3-yl)sulfamoyl) phenyl)carbamate (1).

To a stirred solution of4-amino-N-(5-methylisoxazol-3-yl)benzenesulfonamide(sulfamethoxazole) (0.1 mol, 25.3g), potassium hydroxide (0.1 mol, 5.8 g) in (20 ml) absolute ethanol, an ethylchloroacetate (0.1 mol, 10.7 ml) was added drop by drop. The reaction carried out by refluxing the reaction mixture for (6 hrs.). The resulting solid product then it was filtered, dried and recrystallized fromethanol.[10]

2.3.Synthesis of N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)hydrazinecarboxamide (2).

A mixture of an ester ethyl (4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) carbamate (1) (0.01 mol, 3.25g) and hydrazine hydrate (0.01 mol, 0.5 ml) was refluxed for (2hrs.), absolute ethanol (15 ml) was added and the reaction mixture was refluxed for further (3hrs). The separated precipitate was collected, washed and recrystallized from chloroform.[11]

2.4. Synthesis ofN-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-3,7-dioxo-1,2-diazepane-1-carboxamide (3).

A mixture of a carbohydrazide N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)hydrazine carboxamide (2) (0.01 mol, 3.12g) and glutaric acid (0.01 mol, 1.32g) in (20 ml) absolute ethanol was heated under reflux overnight. The excess solvent was evaporated and the crude solid product was collected by filtration thendisred compound was obtained through recrystallization from ethanol.[12]

2.5. Synthesis of 4-(((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)amino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (4).

To a solution of carbohydrazide N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)hydrazine carboxamide (2) (0.01 mol, 3.12g) in absolute ethanol (25 ml), potassium hydroxide (0.01 mol, 0.58 g) and carbon disulfide (0.01 mole, 0.6 ml) were added respectively. The reaction mixture was refluxed about (20 hrs.) until the most of the formed hydrogen sulfide has been evolved and tested by litmus paper

exchange into red color. The residual solvent was evaporated in the vacuum; the separated desired solid was filtered and recrystallized from acetone.[13]

2.6. Synthesis of4-((2-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)amino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (5) and 4-((2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethyl)amino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (6).

A mixture of a carbohydrazideN-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)hydrazine carboxamide (2) (0.01 mol, 3.12g), appropriate ketones (ethylacetoacetate, acetylacetone) (0.01mol) respectively and absolute ethanol (15ml) was mixed carefully, reflexed for (10-12hrs.).The reaction mixture then concentrated and cooled with crushed ice to form the solid product, finally filtered and recrystallized fromchloroform as solvent.[14]

2.7. Synthesis ofN-(5-methylisoxazol-3-yl)-4-(((5-oxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)methyl)amino)benzenesulfonamide (7).

A carbohydrazideN-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)hydrazine carboxamide (2) (0.01 mol, 3.12g) and chloroacetamide (0.01 mol, 0.93g) was mixed and dissolved in (20 ml) absolute ethanol and then refluxed under heating overnight.The solvent was vacuumed distilledand the solid product that separated was dried and recrystallized from ethanol.[15]

2.8. Synthesis ofN-(5-methylisoxazol-3-yl)-4-(((6-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl)methyl)amino)benzenesulfonamide (8).

A stirred mixture of N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)hydrazine carboxamide (2) (0.01 mol, 3.12g) and potassium hydroxide (0.01 mol, 0.58 g) was gently heating until the dissolving is complete. Chloroacetic acid (0.01 mol, 0.94g) and was added after cooling to temperature (25^{o} C). The reaction mixture was continuously refluxed for (12 hrs.). The separated crude solid was filtered and recrystallized from dioxane.[16]

2.9. Synthesis of4-((2-(2-formylhydrazineyl)-2-oxoethyl)amino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (9).

Formic acid (0.01 mol, 0.37ml) was added drop wise to a solution of carbohydrazide N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)hydrazine carboxamide (2) (0.01 mol, 3.12g) in absolute ethanol (25 ml). The reaction mixture afforded to heating under reflux for (16hrs.).The excess of remaining solvent was evaporated and thenthe formed solid was filtered off, recrystallized from acetone to give the final product. [17]

2.10. Synthesis ofN-(5-methylisoxazol-3-yl)-4-((2-oxo-2-(2-((substitutedphenylimino)methyl)hydrazineyl)ethyl)amino)benzenesulfonamide (10-13).

A mixture of 4-((2-(2-formylhydrazineyl)-2-oxoethyl)amino)-N-(5-methylisoxazol-3-yl) benzenesulfonamide (9) (0.003mol, 1.05g), appropriate amine (aniline, *p*-chloro aniline, *m*-nitro aniline, *o*-aminophenol)(0.003 mol), dry benzene (20 ml) and few drops of glacial acetic acid was heated under reflux about (4-6 hrs.). The remaining solvent has been steamed and each of the resulting imines was then crystallized from mixed solvents ethanol-water (1:1) for compounds (10, 12) and dioxane for compounds (11, 13) respectively.[18]

2.10. Synthesis of4-((2-(2-(1,5-dioxo-4-substituedphenyl-1,3,4,5-tetrahydrobenzo[1,3]oxazepin-3-yl)hydrazineyl)-2-oxoethyl)amino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (14-17).

To appropriate imine compounds (10-13) (0.003mol) in dry benzene (20 ml), as solvent,(0.003mol, 0.22g) phthalic anhydride was added. The reaction mixture then reflexed about (8-10 hrs.). The separated solid was filtered, dried and recrystallized from mixed solvents ethanol-water (1:1) for compound (14)dioxane for compounds (15,17) and chloroform forcompound (16) respectively to yield favorites oxazepine products. [19]

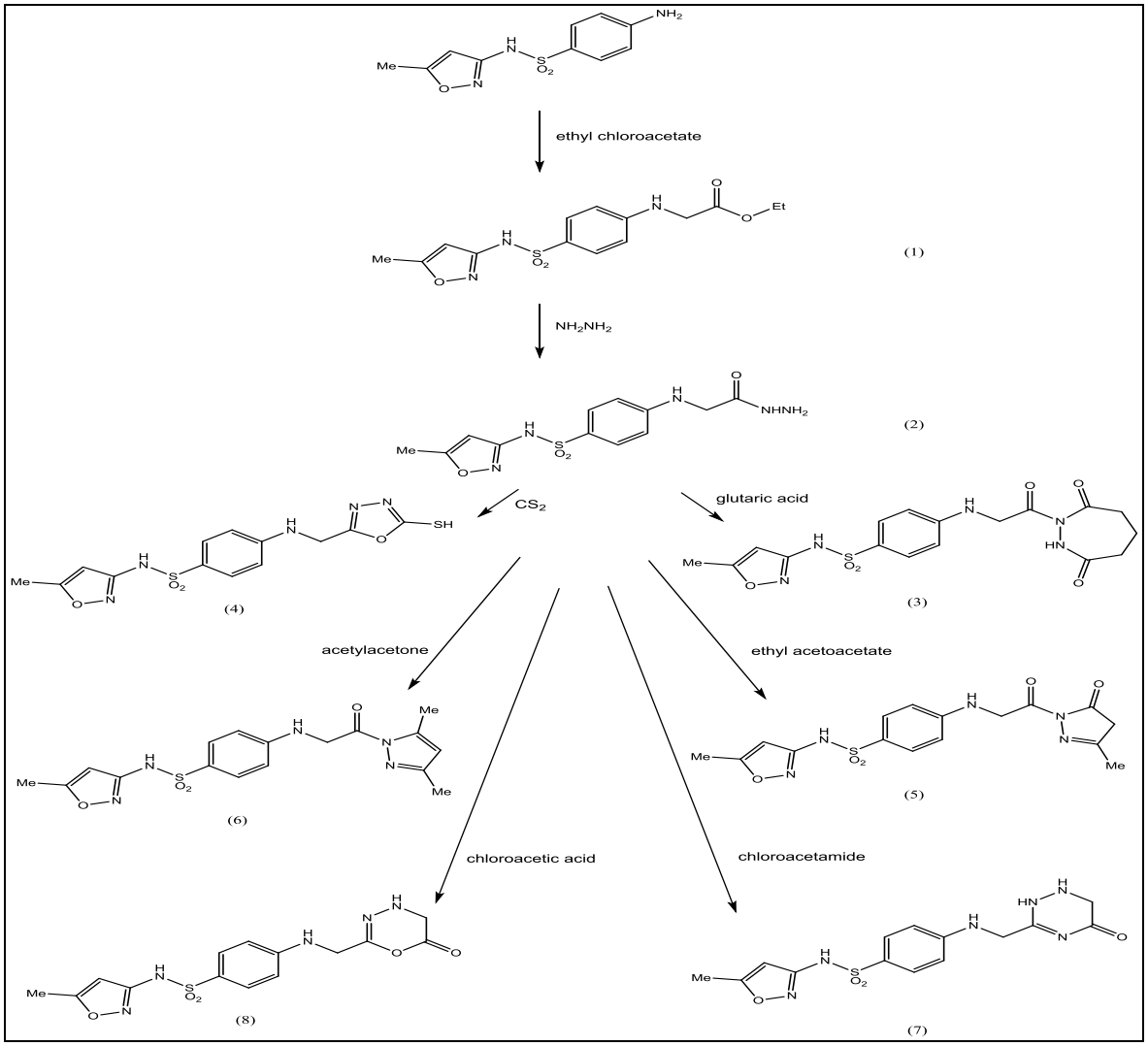
2.11. Antimicrobial study.

In order to measure antibacterial activity of newly prepared compounds (3-8), (14-17), a plate of two type of Gram positive bacterial strains [Enterococcus faecalis (EF),Staphylococcus aureus (SA)] and two type of Gram negative bacterial strains [Pseudomonas aeruginosa (PA), Klebsiella pneumonia (KP)]. The antifungal activity was assayed against two type of pathogenic yeast like fungi [Aspergillus niger (AN), Candida albicans (CA)].The activities were evaluated*in vitro* using the agar disc diffusion method. [20] Muller-Hinton agar (MHA) used for disc sensitivity. Sulfamethoxazolewere chosen as the basic drug. Stock solution of the synthesized sulfamethoxazoleattached toheterocyclicmoieties was prepared in dimethyl sulphoxide (DMSO) as solvent in a concentration of (100 $mg.L^{-1}$). The diameter and percentage of inhibition zone were noticed and recorded in (mm) after full inhibition of bacterial growth plates at (37 ^{O}C) for (24-48 hrs.). The tests were prepared in triplicates and the determination was repeated twice.

3. Results and Discussion:

3.1. Chemistry

Synthetic pathways for prepared compounds (1-8) are presented in Scheme(1).



Scheme (1)

Ethyl (4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) carbamate (1) was prepared by addition of ethylchloroacetate to a solution of KOH and 4-amino-N-(5-methylisoxazol-3-yl) benzenesulfonamide (sulfamethoxazole) and refluxed in absolute ethanol afforded the target ester.Physicochemicalproperties of compound (1) and each other synthesized compounds are listed in Table-1.

Table-1: Physicochemical data of the synthesized compounds (1-17).

Comp. No.	Molecular formula	Ar (substituents)	Yield (%)	m.p. °C.	Color	Recrystallization solvent
1	**$C_{13}H_{15}N_3O_5S$**	-	81	239-242	off white	ethanol
2	**$C_{11}H_{13}N_5O_4S$**	-	85	215-217	white crystals	chloroform
3	**$C_{16}H_{17}N_5O_6S$**	-	76	198-201	brown powder	ethanol
4	**$C_{14}H_{15}N_5O_5S$**	-	60	142-145	light orange	acetone
5	**$C_{16}H_{17}N_5O_5S$**	-	66	158-160	white	chloroform
6	**$C_{17}H_{19}N_5O_4S$**	-	72	115-117	light red	chloroform
7	**$C_{14}H_{16}N_6O_4S$**	-	64	188-191	white	ethanol
8	**$C_{13}H_{13}N_5O_4S_2$**	-	55	137-139	deep brown	dioxane
9	**$C_{13}H_{15}N_5O_5S$**	-	72	226-228	off white	acetone
10	**$C_{19}H_{20}N_6O_4S$**	**aniline**	63	124-127	brown	ethanol-water1:1
11	**$C_{19}H_{19}N_6O_4SCl$**	***p*-chloroaniline**	60	179-181	white	dioxane
12	**$C_{19}H_{19}N_7O_6S$**	***m*-nitro aniline**	58	165-168	dark yellow	ethanol-water1:1
13	**$C_{19}H_{20}N_6O_5S$**	***o*-aminophenol**	65	154-156	white	dioxane
14	**$C_{27}H_{24}N_6O_7S$**	**aniline**	70	147-149	gray	ethanol-water1:1
15	**$C_{27}H_{23}N_6O_7SCl$**	***p*-chloroaniline**	69	133-136	off white	dioxane
16	**$C_{27}H_{23}N_7O_9S$**	***m*-nitro aniline**	73	211-213	pale yellow	chloroform
17	**$C_{27}H_{24}N_6O_8S$**	***o*-aminophenol**	79	169-172	dark brown	dioxane

FTIR spectrum for ethyl (4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) carbamate (1) showed clear stretching bands at (3214 cm^{-1})were assigned to the v(N-H) stretching frequency. Besides the appearances of v(C=O) stretching band attributable to ester group at (1730 cm^{-1}) and stretching band at (1218 cm^{-1}) attributed to v(C-O-C) ester are best proof for the structure give to intended compound as listed in Table-2.

Ethyl (4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) carbamate (1) was allowed to react with hydrazine hydrate in

ethanol to give the desired acetohydrazideN-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)hydrazine carboxamide(2). The structure of the produced compound was confirmed by measuring its main physical properties and FT-IR spectral data. FTIR spectrum of hydrazine carboxamide showedremarkable stretching bands at (3321 cm^{-1}) and (3256 cm^{-1}) which were assigned to the v(-$NHNH_2$) group stretching frequency. On the other hand, the disappearance of v(C=O) stretching band attributable to ester group at (1730 cm^{-1}) with the appearance of bands at (1690 cm^{-1}) of amide proved the formation of compound (2)as shown in Table-2.

Table-2: FTIR v(cm^{-1}) data for the synthesized compounds (1-17)

Comp. No.	(N-H)	(C-H) Ar.	(C-H) Aliph.	(C=N) isoxazole	(C=C) Ar.	(SO_2) Asym.	(SO_2) sym.	Others
1	3214	3067	2944	1609	1566	1363	1184	1730 (C=O)ester, 1218 (C-O-C) ester.
2	3256	3088	2921	1612	1556	1391	1160	3321 ($NHNH_2$), 1690 (C=O) amide.
3	3231	3059	2911	1604	1542	1375	1153	1695 (C=O) amide.
4	3225	3055	2943	1613	1533	1373	1169	1183 (C=S), 1121 (C-O-C) oxadiazole.
5	3261	3071	2952	1610	1588	1377	1148	1721(C=O) pyrazolone, 1652 (C=O) amide.
6	3251	3092	2957	1620	1567	1386	1166	1666 (C=O) amide.
7	3262	3047	2936	1608	1581	1380	1147	1668 (C=O) triazine,
8	3242	3029	2974	1611	1539	1388	1138	1644 (C=O) oxadiazine.
9	3227	3061	2968	1605	1548	1366	1145	1712 (C=O) aldehyde, 1685 (C=O) amide.
10	3255	3085	2951	1601	1573	1369	1172	1648 (C=O) amide.
11	3219	3066	2981	1603	1531	1378	1182	1677 (C=O) amide, 861 (C-Cl).
12	3247	3041	2944	1612	1575	1390	1139	1659 (C=O) amide. 1512(NO_2)Asym. 1322 (NO_2)sym.
13	3252	3058	2959	1614	1583	1366	1162	3215 (O-H), 1691 (C=O) amide.
14	3217	3075	2938	1617	1544	1362	1156	1732 (C=O) oxazepine, 1672 (C=O) amide.
15	3258	3033	2973	1613	1563	1370	1148	1723 (C=O) oxazepine, 1646 (C=O) amide, 849 (C-Cl).
16	3266	3084	2942	1605	1591	1372	1135	1739 (C=O) oxazepine 1680 (C=O) amide. 1517(NO_2)Asym. 1308 (NO_2)sym.
17	3236	3053	2966	1607	1593	1383	1167	3209 (O-H), 1741 (C=O) oxazepine, 1675 (C=O) amide.

An acetohydrazideN- (4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)hydrazine carboxamide (2) and glutaric acid were refluxedovernight in absolute ethanol to afforded the target N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-3,7-dioxo-1,2-diazepane-1-carboxamide (3).

Structure of the synthesized diazepanecompound was assigned by its melting point, FT-IR, ^{1}H-and ^{13}C-

NMR.FT-IR spectrum of diazepane compound (3) shows the distinguishedstretching bands at (3231 cm^{-1}), (3059 cm^{-1}), (2911 cm^{-1}) and (1695 cm^{-1}) assignable for v(N-H), v(C-H) aromatic, v(C-H) aliphatic and v(C=O) amide groups respectively. Other characteristic bands are listed in the Table-2.

^{1}H-NMR spectrum of diazepane compound (3), figure (1), shows the important characteristic chemical shifts (DMSO-d_6, ppm). Itdisplayed signals attributed to three protons of methyl group attached to isoxazole ring, six protons for three methylene groups of diazepane ring, two protons of methylene group (NH-$\underline{CH_2}$-CO), one proton of (CH) isoxazole ring, four aromatic ring protons, one proton of secondary amine (NH), one proton of amine group attached to SO_2 and one proton for aminegroup of diazepane ring respectively aslisted in Table-3.

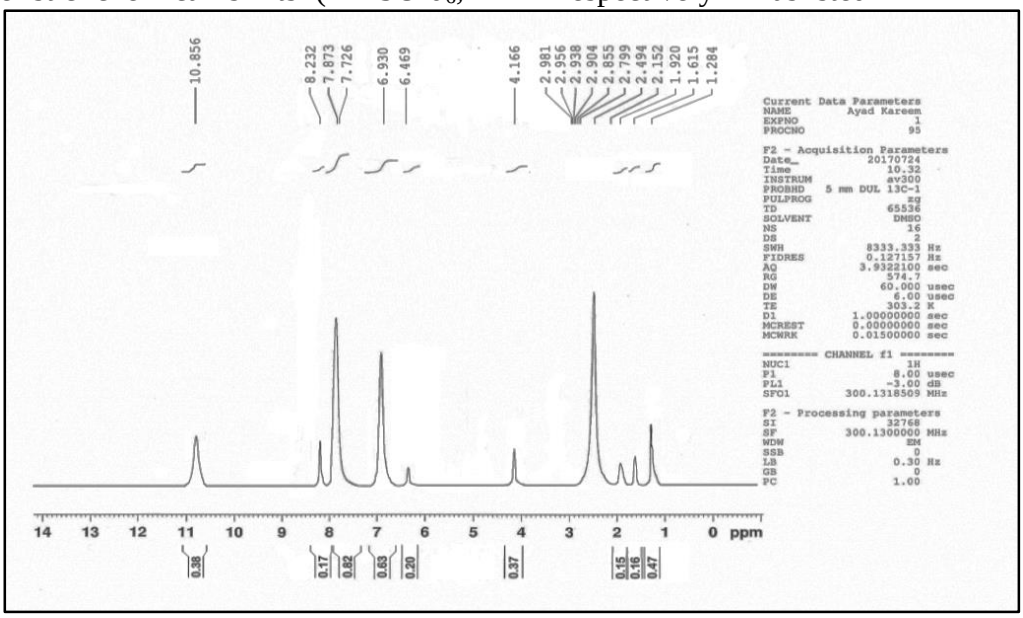


Figure -1: ^{1}H-NMR Spectrum for N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-3,7-dioxo-1,2-diazepane-1-carboxamide.

^{13}C-NMR spectrum of diazepane compound (3), figure (2), appears the following characteristic chemical shift (DMSO-d_6, ppm). The signals belongs to carbonsof methyl group (-CH_3) attached to isoxazole ring, three methelene groups (-CH_2-)of diazepane ring,methylene group of (NH-$\underline{CH_2}$-CO),(CH) of isoxazole ring, aromatic ring carbons, two carbon(C) of isoxazole ring, carbonyl group of (CH_2-$\underline{CO}$-N)and two carbonyls of diazepane ring respectively as recorded in Table-4.

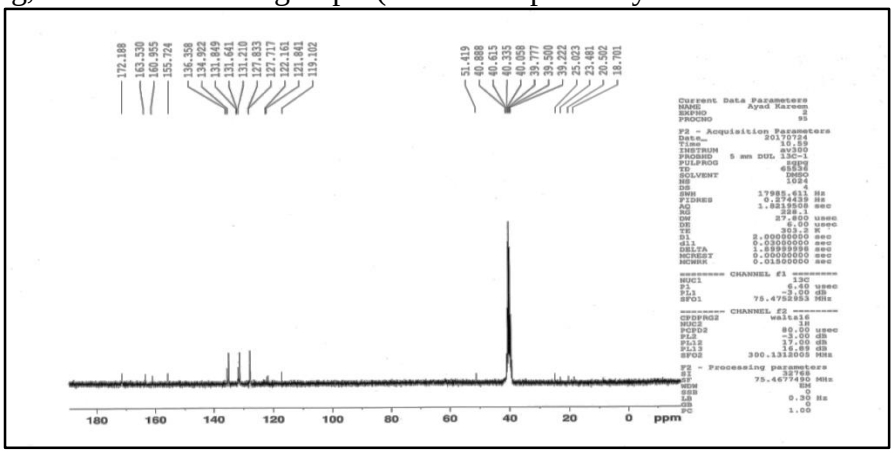
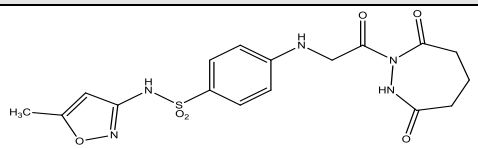
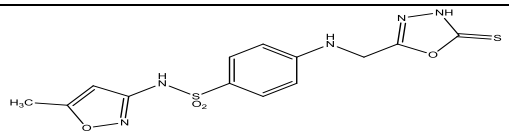
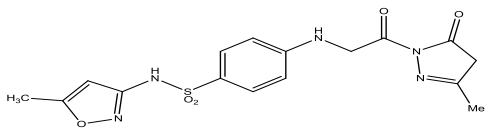
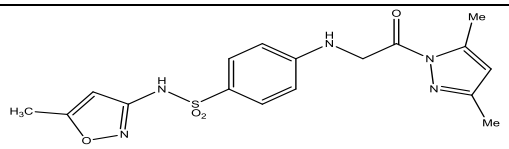
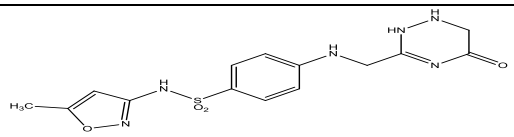
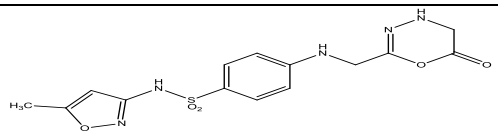
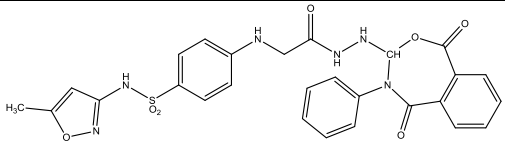
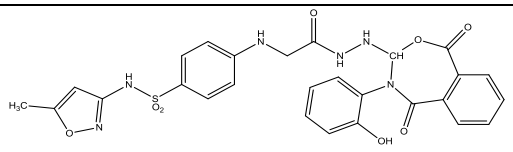


Figure (2): 13C-NMR Spectrum for N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-3,7-dioxo-1,2-diazepane-1-carboxamide.

Table-3: ^{1}H-NMR spectral data (δppm) for selected synthesized compounds.

Comp. No.	Compound structure	^{1}H-NMR parameters (δppm)
3		1.28 (s, 3H, CH_3 isoxazole),1.61 (m, 2H, CH_2 diazepane), 1.92 (t, 4H, $2CH_2CO$ diazepane), 4.16 (s, 2H, NH-CH_2-CO),6.46 (s, 1H, CH isoxazole), 6.93-7.87 (m, 4H, Ar-H), 8.23 (s, 1H, NH), 10.85 (s, 2H, NH-SO_2, N-NH-CO diazepane).
4		1.35 (s, 3H, CH_3 isoxazole), 4.52(s, 2H, NH-CH_2-C), 6.32 (s, 1H, CH isoxazole), 6.78-7.90(m, 4H, Ar-H), 8.39 (s, 1H, NH), 11.20 (s, 2H, NH-SO_2, N-NH-C oxadiazole).
5		1.23 (s, 6H, CH_3 isoxazole, CH_3 pyrazolone), 3.47 (s, H, CH_2 pyrazolone),4.67 (s, 2H, NH-CH_2-CO),6.11 (s, 1H, CH isoxazole), 6.81-7.63 (m, 4H, Ar-H),8.40 (s, 1H, NH),10.69 (s, 1H, NH-SO_2).
6		1.55 (s, 9H, CH_3 isoxazole, $2CH_3$ pyrazole), 4.22 (s, 2H, NH-CH_2-CO), 6.18 (s, 1H, CHisoxazole), 6.37 (s, 1H, CH pyrazole),6.94-7.79 (m, 4H, Ar-H), 8.11 (s, 1H, NH),10.93 (s, 1H, NH-SO_2).
7		1.41 (s, 3H, CH_3isoxazole), 3.79 (s, H, CH_2triazine), 4.08 (s, 2H, NH-CH_2-C),6.12 (s, 1H, CH isoxazole),7.01.-7.88 (m, 4H, Ar-H), 8.94 (s, 2H,NH, NH triazine), 11.57 (s, 2H, NH-SO_2, NH triazine).
8		1.43 (s, 3H, CH_3 isoxazole),3.29(s, H,CH_2 oxadiazine), 4.14(s, 2H, NH-CH_2-C), 6.53(s, 1H, CH isoxazole), 7.34-7.92(m, 4H, Ar-H), 8.20 (s, 2H, NH, NH oxadiazine), 10.91 (s, 1H, NH-SO_2).
14		1.37 (s, 3H, CH_3isoxazole), 4.29 (s, 2H, NH-CH_2-CO), 5.31 (s, 1H, CH oxazepine),6.04 (s, 1H, CH isoxazole), 6.71-7.98 (m, 13H, Ar-H), 8.29 (s, 3H, 3NH), 11.57 (s, 2H, NH-SO_2).

17		1.44 (s, 3H, CH_3isoxazole),4.20(s, 2H, NH-$\underline{CH_2}$-CO), 5.22 (s, 1H, CH oxazepine),6.10 (s, 1H, $\underline{CH}$ isoxazole), 6.53- 7.95 (m, 12H, Ar-H), 8.13 (s, 3H, 3NH), 10.15 (s, 1H, OH), 11.71 (s, 2H, $\underline{NH}$-SO_2).

The target 4-(((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)amino)-N-(5-methylisoxazol-3-yl) benzene sulfonamide (4) were synthesized from reacting the carbohydrazide (2) with carbon disulfide in absolute ethanol.

FT-IR spectrum showed disappearance of bands at (3321 cm^{-1}) due to ν (-$NHNH_2$) moiety of compound (2) with the appearance bands at 1121 cm^{-1}assignable due to υ(C-O-C) cyclic group of oxadiazole ring which are good evidence for the structure assigned to this compound. Further, the appearance of absorption band at (1183 cm^{-1}) due to υ (C=S),indicates the presence of tautomerism are shown in figure (3). All details of FT-IR spectrum for compound (4) are shown in Table-2.

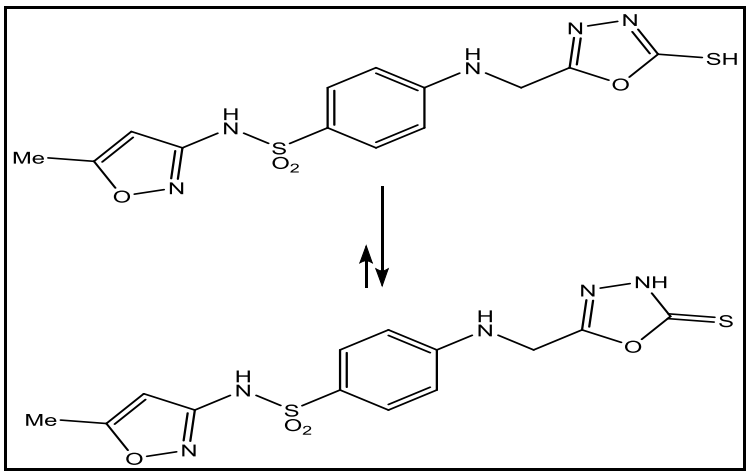
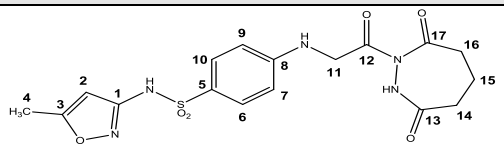
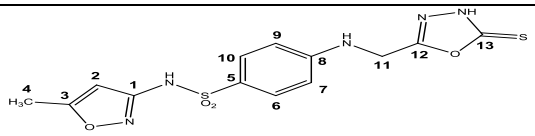
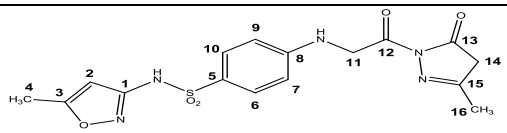
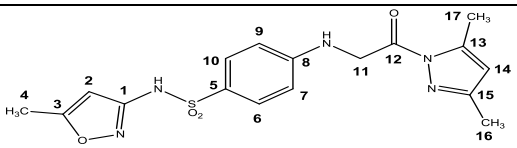
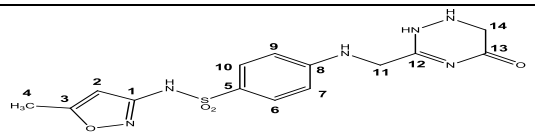
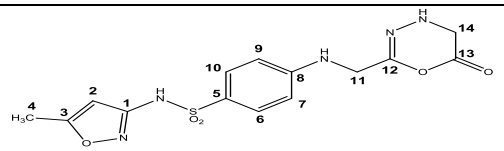
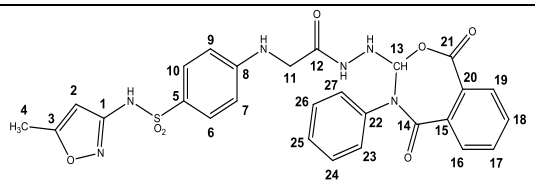
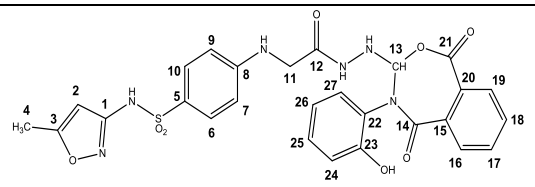


Figure -3: Tautomerism in 4-(((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)amino)-N-(5-methylisoxazol-3-yl) benzene sulfonamide

^{1}H-NMR spectrum ofoxadiazole-compound (4) displayed the basic characteristic signals due to three protons of methyl group attached to isoxazole ring, two protons of methylene group (NH-$\underline{CH_2}$-C), one proton of (CH) isoxazole ring, four aromatic ring protons, one proton of secondary amine (NH), one proton of amine group attached to SO_2 and one proton for aminegroup ofoxadiazole ring respectively aslisted in Table-3.

^{13}C-NMR spectrum of oxadiazole compound (4) offered the following special signals belong to carbonsof methyl group (-CH_3-) attached to isoxazole ring, methylene group of (NH-$\underline{CH_2}$-C), (CH) of isoxazole ring, aromatic ring carbons, carbon (C) of oxadiazole ring, two carbon (C) of isoxazole ring, thiocarbonyl group (C=S) of oxadiazolering respectively as listed in Table-4.

Table-4: ^{13}C-NMR spectral data (δppm) for selected synthsized compounds.

Comp. No.	Compound structure with numbering of carbon atoms	^{13}CNMR Spectral Data (δ ppm)
3		18.70 (C_4), 20.50 (C_{15}), 23.48(C_{14}), 25.20 (C_{16}), 51.41 (C_{11}), 119.10-136.35 (C_2, C_5-C_{10}),155.72 (C_1), 160.95 (C_3), 163.53 (C_{12}), 172.18 (C_{13}, C_{17}).
4		17.34 (C_4), 53.91 (C_{11}), 120.16-134-79 (C_2, C_5-C_{10}, C_{12}), 153.44 (C_1), 161.72 (C_3), 176.39 (C_{13}).
5		18.55 (C_4), 21.47 (C_{16}), 41.90 (C_{14}), 52.33 (C_{11}), 119.33-135.76 (C_2, C_5-C_{10}, C_{15}), 155.81 (C_1), 162.41 (C_3), 164.20 (C_{12}), 169.78 (C_{13}).
6		16.88 (C_4), 22.45 (C_{16}, C_{17}), 53.82 (C_{11}), 118.45-137.89 (C_2, C_5-C_{10}, C_{13}-C_{15}) 155.63 (C_1), 161.17 (C_3), 163. 49 (C_{12}).
7		17.11 (C_4), 54.07 (C_{11}), 63.25 (C_{14}), 121.10-136.94 (C_2, C_5-C_{10}, C_{12}), 157.25 (C_1), 163.46 (C_3), 170.06 (C_{13}).
8		16.45 (C_4), 49.29 (C_{11}), 61.72 (C_{14}), 117.10-137.51 (C_2, C_5-C_{10}, C_{12}), 152.86 (C_1), 160.27 (C_3), 168.33 (C_{13}).
14		18.23 (C_4), 52.38 (C_{11}), 119.33-139.57 (C_2, C_5-C_{10}, C_{15}-C_{20},C_{22}-C_{27}), 110.26 (C_{13}), 151.39 (C_1), 162.82 (C_3), 170.41 (C_{14}, C_{21}).
17		18.05 (C_4), 51.68 (C_{11}), 118.58-139.92 (C_2, C_5-C_{10}, C_{15}-C_{20},C_{22}-C_{27}), 113.41 (C_{13}), 151.85 (C_1), 161.59 (C_3), 171.30 (C_{14} , C_{21}).

4-((2-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)amino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (5) and 4-((2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethyl)amino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (6)was synthesized from refluxing suitable carbonyl compound (ethyl acetoacetate , acetylacetone) respectively with carbohydrazide hydrazide (2) in presence of absolute ethanol.

The FTIR spectrum of pyrazolone compound (5) and pyrazole compound (6) appears the disappearance of (NH_2) bands at (3321 cm^{-1}) of the starting carbohydrazide hydrazide (2) and appearance of additional bands at (1721cm^{-1})due to carbonyl of pyrazolone ring for compound (5).

^{1}H-NMR spectrum of pyrazolone compound (5)showed the signify characteristicchemical shifts were

appeared signals suggested the attribution of the three protons of methyl group attached to isoxazole ring, three protons for methyl group of pyrazolone ring, two protons of methylene group (NH-$\underline{CH_2}$-CO), one proton of (CH) isoxazole ring, four aromatic ring protons, one proton of secondary amine (NH), one proton of amine group attached to SO_2 respectively as shown in Table-3.

^{13}CNMR shows a specific signals for carbons of pyrazolone compound (5) linked to carbons of methyl group (-CH_3) attached to isoxazole ring, methyl group (-CH_3) attached to pyrazolone ring, methylene group (-CH_2-) of pyrazolone ring, methylene group of (NH-$\underline{CH_2}$-CO), (CH) of isoxazole ring, aromatic ring carbons, carbon (C) of pyrazolone ring, two carbon (C) of isoxazole ring, carbonyl group of (CH_2-$\underline{CO}$-N) and carbonyl group (C=O) of pyrazolone ring respectively as listed in Table-4.

On the other hand ^{1}-HNMR and ^{13}-CNMR spectral data of compounds (6) gives results confirmed the structure of the synthesized compound.^{1}H-NMR spectrum of pyrazole compound (6) shows the signals belong to three protons of methyl group attached to isoxazole ring, six protons for two methyl groups of pyrazole ring, two protons of methylene group (NH-$\underline{CH_2}$-CO), one proton of (CH) isoxazole ring, four aromatic ring protons, one proton of secondary amine (NH), one proton of amine group attached to SO_2 respectively as shown in Table-3.

While ^{13}C-NMR spectrum of pyrazole compound (6) afford the following characteristic signals belong to carbons of methyl group (-CH_3-) attached to isoxazole ring, methyl groups (-CH_3-) attached to pyrazole ring, methylene group of (NH-$\underline{CH_2}$-CO), (CH) of isoxazole ring, aromatic ring carbons, carbons of pyrazole ring, two carbon (C) of isoxazole ring and carbonyl group of (CH_2-$\underline{CO}$-N) respectively as listed in Table-4.

Chloroacetamide was refluxed with acetohydrazideN-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)hydrazine carboxamide (2) in absolute ethanol to yield the target triazine derivative N-(5-methylisoxazol-3-yl)-4-(((5-oxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl) methyl)amino)benzenesulfonamide (7).

FTIR spectrum of N-(5-methylisoxazol-3-yl)-4-(((5-oxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl) methyl)amino)benzenesulfonamide (7)specified bands at (3262 cm^{-1}) which assignable tov(N-H) stretching vibrations. The bands at (3047 cm^{-1}), (2936 cm^{-1}) and (1668 cm^{-1}) due to v(C-H) aromatic, v(C-H) aliphatic and v(C=O) amide in the triazine ring moiety respectively.

^{1}H-NMR spectrum of triazine compound (7) shows the following characteristic signals belong to three protons of methyl group attached to isoxazole ring, two protons for methylene group of triazine ring, two protons of methylene group (NH-$\underline{CH_2}$-C), one proton of (CH) isoxazole ring, four aromatic ring protons, one proton of secondary amine (NH), one proton of amine group attached to SO_2 and one proton for aminegroup of triazine ring respectively as shown in Table-3.

^{13}C-NMR spectrum of triazine compound (7) gives the following characteristic signals especial to carbons of methyl group (-CH_3) attached to isoxazole ring, methylene group of (NH-$\underline{CH_2}$-CO), methylene group (-CH_2-) of triazine ring, (CH) of isoxazole ring, aromatic ring carbons, carbon of triazine ring, two carbons (C) of isoxazole ring and carbonyl group of triazine ringrespectively as listed in Table-4.

Refluxing mixture of chloroacetic acid with acetohydrazideN-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)hydrazine carboxamide (2)in absolute ethanol affording the target oxadiazine derivativeN-(5-methylisoxazol-3-yl)-4-(((6-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-

2-yl)methyl)amino)benzenesulfonamide (8).

FTIR spectrum of N-(5-methylisoxazol-3-yl)-4-(((6-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl)methyl)amino)benzenesulfonamide (8) showed absorption bands at (3242 cm^{-1}) which belong to v(N-H) stretching vibrations. Other bands at (3029 cm^{-1}), (2974 cm^{-1}) and (1644 cm^{-1}) due to v(C-H) aromatic, v(C-H) aliphatic and v(C=O) amide of the oxadiazin ring moiety, respectively.

^{1}H-NMR spectrum of oxadiazine compound (8), figure (4) shows the following characteristic signals belong to three protons of methyl group attached to isoxazole ring, two protons for methylene group of oxadiazine ring, two protons of methylene group (NH-$\underline{CH_2}$-C), one proton of (CH) isoxazole ring, four aromatic ring protons, one proton of secondary amine (NH), one proton for aminegroup of oxadiazine ring and one proton of amine group attached to SO_2 respectively as shown in Table-3.

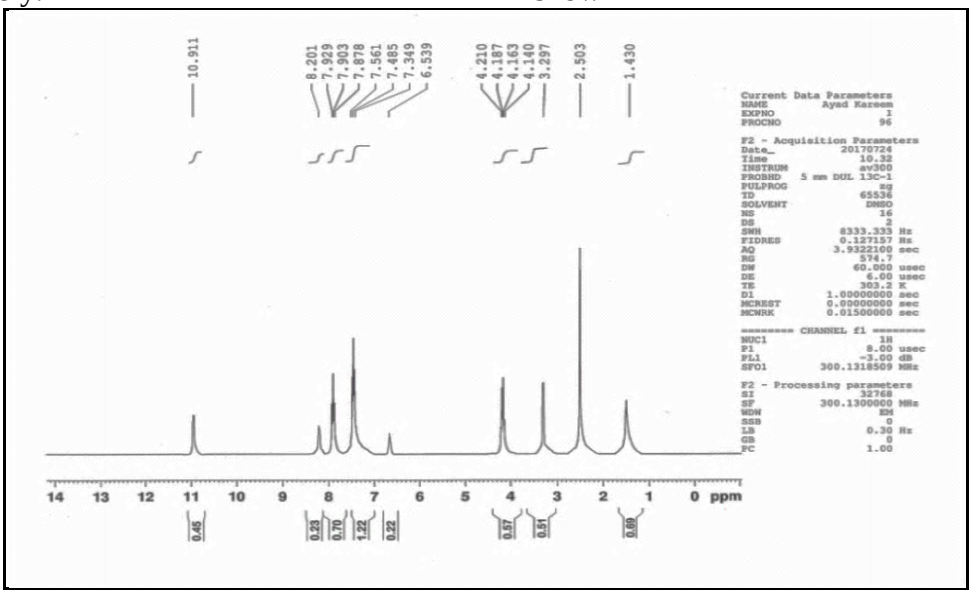


Figure-4: ^{1}H-NMR Spectrum for N-(5-methylisoxazol-3-yl)-4-(((6-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl)methyl)amino)benzenesulfonamide.

^{13}C-NMR spectrum of oxadiazine compound (8), figure (5) shows the main characteristic signals belong to carbons of methyl group (-CH_3) attached to isoxazole ring, methylene group of (NH-$\underline{CH_2}$-CO), methylene group (-CH_2-) of triazine ring, (CH) of isoxazole ring, aromatic ring carbons, carbon of oxadiazine ring, two carbons (C) of isoxazole ring and carbonyl group of oxadiazine ringrespectively as listed in Table-4.

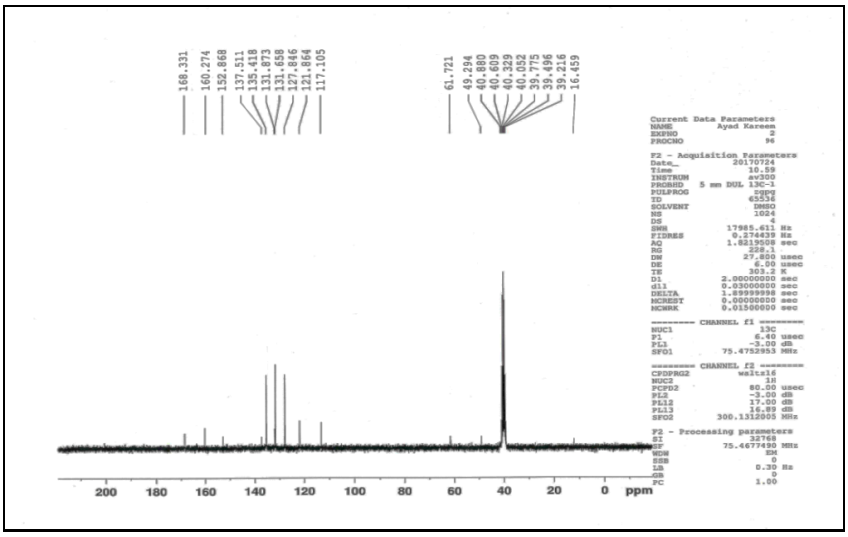
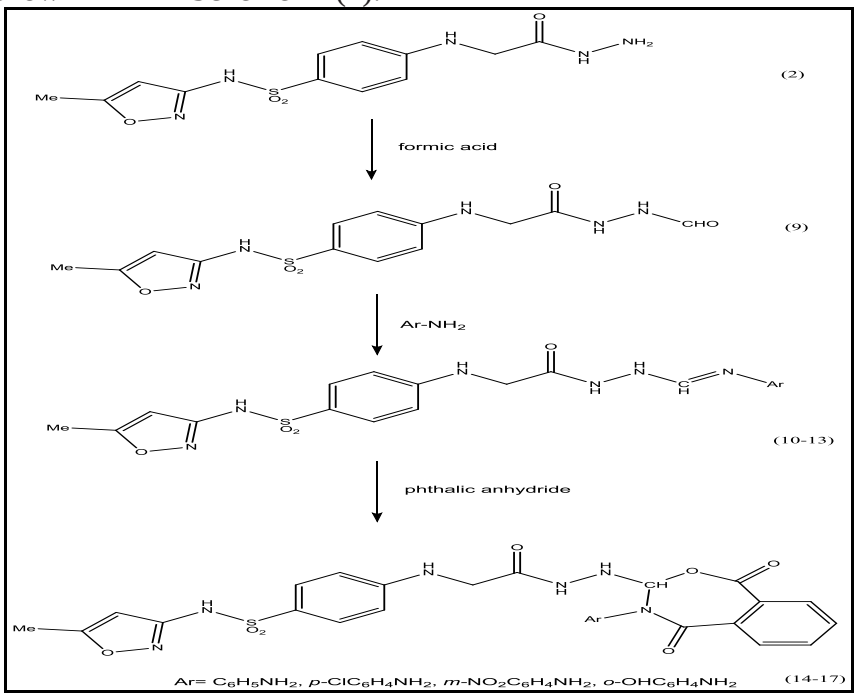


Figure -5: ^{13}C-NMR Spectrum for N-(5-methylisoxazol-3-yl)-4-(((6-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl)methyl)amino)benzenesulfonamide.

Synthetic pathways for other series of prepared sulfamethoxazole derivatives (9-17) are shown in Scheme (2).



Scheme (2).

Compound (9), 4-((2-(2-formylhydrazineyl)-2-oxoethyl)amino)-N-(5-methylisoxazol-3-yl) benzene sulfonamide, prepared by reaction of formic acid with an acetohydrazide N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl) phenyl)hydrazine carboxamide (2) in absolute ethanol.

The structure of the synthesized compound (9) was assigned by its physicochemical properties and FT-IR spectral data. The FT-IR spectrum shows the following characteristics absorption bands at the range (3227 cm^{-1}) due to v(N-H) stretching vibration. Besides v(C-H) aromatic and v(C-H) aliphatic appear at (3061 cm^{-1}) and (2968 cm^{-1}) respectively. In addition to sharp band at (1712 cm^{-1}) due to v(C=O) of aldehyde moiety stretching vibration.

The new Schiff bases N-(5-methylisoxazol-3-yl)-4-((2-oxo-2-(2-((substitutedphenylimino) methyl)hydrazineyl)ethyl)amino)benzenes ulfonamide (10-13) were synthesized by the refluxing of equimolar quantities an aldehyde derivative (9) and appropriate aromatic primary amines such as (aniline, *p*-chloro aniline, *m*-nitro aniline, *o*-aminophenol) in dry benzene with some drops of glacial acetic acid.

Imine derivatives (10-13) were identified by their physicochemical as shown in Table-1 and by FT-IR absorption spectrum shows the disappearance of absorption bands (1712 cm^{-1}) due to v(C=O)for aldehyde derivative (9) and appearance of new absorption bands of aromatic primary amines substituents such(861 cm^{-1}) for v(C-Cl), (1512 cm^{-1}, 1322 cm^{-1}) for asym.,sym. v(NO_2)and (3215 cm^{-1}) for v(O-H) respectively. All details of FT-IR spectral data forimine derivatives (10-13) are listed in Table-2.

Oxazepine derivatives 4-((2-(2-(1,5-dioxo-4-substituedphenyl-1,3,4,5-tetrahydrobenzo [1,3]oxazepin-3-yl)hydrazineyl)-2-oxoethyl)amino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (14-17) have been synthesized by using a pericyclic reaction type [2+5] cycloadditionreaction between imine group (C=N) in compounds (10-13) as two membered component and phthalicanhydrides as five membered components to give seven-membered 1,3-oxazepine rings.

FT-IR spectrum of 1,3-oxazepine derivative (14-17) showed appearance of thestrong absorption bands at range (1723-1741 cm^{-1}) attributed to the v(C=O) forlactone structures inside1,3-oxazepine rings. Besides appearance of other absorption bands for aromatic primary amines substituents such v(C-Cl),asym.,sym. v(NO_2)and v(O-H) respectively. All details of FT-IR spectral data for imine derivatives (14-17) are listed in Table-2.

^{1}H-NMR spectrum of oxazepine compound (14) shows the essential characteristic signals belong to three protons of methyl group attached to isoxazole ring, two protons of methylene group (NH-$\underline{CH_2}$-CO), one proton for (CH) group of oxazepine ring, one proton of (CH) isoxazole ring, thirteen aromatic ring protons, three protons of secondary amine (NH) in different positions, one proton for amine group attached to SO_2respectively as shown in Table-3.

^{13}C-NMR spectrum of oxazepine compound (14) shows the following major signals due to carbons of methyl group (-CH_3) attached to isoxazole ring, methylene group of (NH-$\underline{CH_2}$-CO), (CH) of isoxazole ring, aromatic ring carbons, (CH) of oxazepine ring, two carbons (C) of isoxazole ring and carbonyls group of oxazepine ringrespectively as listed in Table-4.

^{1}H-NMR spectrum of oxazepine compound (17) gives the important characteristic signals related to three protons of methyl group attached to isoxazole ring, two protons of methylene group (NH-$\underline{CH_2}$-CO), one proton for (CH) group of oxazepine ring, one proton of (CH) isoxazole ring, thirteen aromatic ring protons, three protons of secondary amine (NH) in different positions, one proton for hydoxylgroup (-OH) and one proton of amine group attached to SO_2respectively as shown in Table-3.

On the other hand characteristic signals for ^{13}C-NMR spectrum of oxazepine compound (17) showed results in a similar manner to compound (14) are listed in Table-4.

3.2. The Antimicrobial Activity

The inhibition zone of the newly synthesized sulfamethoxazole derivatives (3-8) and (14-17) were observed and measured. The antibacterial activities of these compounds were performed against some types of pathogenic bacterial isolates while antifungal activities are evaluated against some yeast similar to fungi.The

obtained results of these study are summarized in Table-5.

Table-5: Antimicrobialactivities expressed by inhibition zone (mm) for some sulfamethoxazole derivatives.

Sample No.	EF (mm)	SA (mm)	PA (mm)	KP (mm)	AN (mm)	CA (mm)
3	14	18	15	16	7	9
4	15	12	16	14	8	8
5	16	19	17	18	9	7
6	15	18	14	17	12	11
7	18	15	16	15	13	10
8	14	16	14	15	7	8
14	17	16	15	14	14	12
15	18	19	18	20	13	12
16	17	16	14	16	10	11
17	14	15	17	16	12	10
S	16	20	18	20	9	8
C	-	-	-	-	-	-

EF: Enterococcus faecalis; SA: Staphylococcus aureus; PA: Pseudomonas aeruginosa; KP: Klebsiella pneumonia; AN: Aspergillus niger; CA: Candida albicans; S: Sulfamethoxazole (References drug), C: Control (Dimethyl sulfoxide)gives no inhibition.

The results showed that several of the synthesized sulfamethoxazole derivatives displayed notable antimicrobial activity. Derivative(15)displayed the highest both antibacterial and antifungal activity. Some of the novel derivatives are superior to parent sulfamethoxazole for their antifungal activity especially compounds (6,7 and 14-17).

In general, the compounds designed based on a sulfamethoxazole with 1,3-oxazepines skeleton (compounds 14-17) are further active than those obtained from other sulfamethoxazole with heterocycles analogues.

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