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An efficient method for synthesis, characterization and molecular docking study of new sulfamethoxazole derivatives as antibacterial agents

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Abstract: A new series of sulfamethoxazole derivatives bearing some biologically active heterocycles such as pyrazole (2), 3,4-dihydropyrimidin (3-7, 11, 12), pyrrole (9) and 1,3-dihydropyrimidin (10) rings were successfully synthesized. Identification of designed compounds was done by physicochemical properties and spectral measurements (^{1}H-NMR, ^{13}C-NMR and FT-IR). New prepared derivatives were assay for their (*in vitro*) antibacterial efficacy against four types of pathogenic bacterial isolates. Significant of the newly prepared compounds appeared promising activity comparison to the cephalexin standard drug. Most of the active compounds are docked into the effective site of tested bacterial enzymes obtained by crystal structure; results reveal the binding template to enzymes of bacteria, which closely related to the laboratory results.

Keywords: Sulfamethoxazole, synthesis, characterization, molecular docking, antibacterial.

INTRODUCTION

One of the main challenges regarding human health is microbial infections. The antimicrobial treatments have been developed widely in recent years (Yao *et al.,* 2011). However, resistance to these drugs is the major drawback that makes them ineffective. This means that research is continuous to overcome resistance caused by various pathogens (Moellering *et al.,* 2011; Buffet *et al.,* 2012). The use of heterocyclic rings in new drug design is widely used. This may be due to their diversity and their occurrence in nature as well as human body such as nucleic acids and sugars (Li *et al.,* 2007; McAllister *et al.,* 2012). Sulfamethoxazole is one of the sulphonamides antibacterial agents (Harrold *et al.,* 2013). It is used as a combination with trimethoprim known as co-trimoxazole (Beale *et al.,* 2010). It has good activity against several types of bacteria such as *Staphylococcus aureus* (Grim *et al.,* 2005), *Haemophilus influenzae* (Karpanoja *et al.,* 2008) and *Escherichia coli* (Petri *et al.,* 2011). It acts by binding with dihydropteroate synthetase leading to stop the change of para-aminobenzoic acid (PABA) to dihydropteroate which is the tetrahydrofolic acid precursor, and hence inhibiting nucleic acid synthesis (Patrick 2017). In addition, it may block the glutamic acid transport which is a key step in folic acid synthesis (Whalen 2018). Antibacterial agents are associated with many side effects in addition to resistance. Therefore, various derivatives based on these drugs were synthesized to get rid of these effects (Frieri *et al.,* 2017). Derivatization on the aromatic amino group is one of these approaches (Mojica *et al.,* 2017). There are various heterocyclic scaffolds with different biological activities. Pyrimidine- and pyrazole-containing compounds were found to have different antimicrobial activities against bacteria (Pugh 2019), parasites (Kumar *et al.,* 2014), fungi (Figarella *et al.,* 2018), viruses (Zaki *et al.,* 2020) in addition to anticancer properties (Mohamed *et al.,* 2018; Hafez *et al.,* 2016). Owing to the fact that resistance shown by pathogenic bacteria to several antibiotics we took this into consideration in our research to develop new sulfamethoxazole derivatives containing heterocyclic moieties and test its potential vital effectiveness against various strains of bacteria.

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MATERIALS AND METHODS

The starting raw material sulfamethoxazole (SMZ), *p*-amino-*N*-(5-methylisoxazol-3-yl) benzene sulfonamide was received from the wadi al-rafidain for pharmaceutical products factory (Iraq). Other chemical materials and reagents applied in this research are purchased from commercial suppliers with no extra purification. Proton and carbon thirteen spectra were measured with a spectrometer Bruker (400 MHz) in deuterated methyl sulfoxide as solvent utilization tetramethyl silane (TMS) as an interior standard. FT-IR spectra were registered on a Shimadzu-8400 Spectrophotometer using potassium bromide disc. Melting points recorded on a (*STUART* SMP30) digitally melting point device and have not been corrected. Biological efficacy measurements Conducted laboratory in the educational laboratory of the Biology Department, University of Baghdad.

Synthesis of 4-{[(2,2-dicyanovinyl)amino]-N-(5-methylisoxazol-3-yl)}benzene sulfonamide (1).

A composition of 4-amino-*N*-(5-methylisoxazol-3-yl) benzenesulfonamide (SMZ) (0.01mol, 2.53gm) triethylorthoformate (0.01mol, 1.66ml.), malononitrile (0.01mol, 0.66gm.) and acetic acid (1ml.) in methanol

(20ml.) was heated under reflux for (7hrs.). The cooled reaction mixture then filtered. The filtrated solid was purified to give compound (1).

Synthesis of 4-{[(3,5-diamino-1-phenyl-1,5-dihydro-4H-pyrazol-4-ylidene)methyl] amino)-N-(5-methylisoxazol-3-yl)}benzenesulfonamide (2).

Compound (1) 4-{[2,2-dicyanovinyl)amino]-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide (0.005mol, 1.64gm) was blended with phenyl hydrazine (0.01mol, 1.08gm) in dioxane as solvents (20ml.) and then heated under reflux for (7hrs.). Ice water was used to cool the reaction mixture. Formed precipitate was filtered and purified to give compound (2).

Synthesis of 3,4-dihydropyrimidin derivatives (3-7).

A mixture of compound (1) 4-{[2,2-dicyanovinyl)amino]-*N*-(5-methylisoxazol-3-yl)benzene sulfonamide (0.005mol. 1.64gm) and the suitable aryl isothiocyanate (0.005mol.) was refluxed for (12hrs.) in (EtOH/EtONa) mixture (15ml.). The reaction mixture was cooled with ice water, sour with diluted hydrochloric acid. Solid material formed was filtered and purified using suitable solvents to give compounds (3-7) respectively.

Synthesis of 4-[(2-acetyl-3-oxobut-1-en-1-yl) amino]-N-(5-methylisoxazol-3-yl)benzene sulfonamide (8).

A mixture of *p*-amino-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide(SMZ) (0.01mol, 2.53gm) triethylorthoformate (0.01mol, 1.66ml.) acetylacetone (0.01mol, 1.02ml.) and acetic acid (1ml.) in methanol (25 ml.) refluxed about (7hrs.). After reaction completing the mixture was filtered subsequently filtered solid was purified to give compound (8).

Synthesis of 4-(3-amino-2,4-dicyano-1H-pyrrol-1-yl)-N-(5-methylisoxazol-3-yl) benzenesulfonamide (9).

Compound (1) 4-{[2,2-dicyanovinyl)amino]-*N*-(5-methylisoxazol-3-yl)benzene sulfonamide (0.005mol, 1.64gm) was blend with stirring to chloro acetonitrile (0.005mol, 0.377gm) in dioxane (20ml.) with catalytic drops of triethylamine and then heated under reflux for (12hrs.) until TLC showed no stating material remained. The mixture was cooled and has been poured out chili water. Formed precipitate solid was infiltration and purified from to produce compound (9).

Synthesis of 4-{[(4,6-diamino-2-thioxopyrimidin-5(2H)-ylidene)methyl]amino}-N-(5-methylisoxazol-3-yl)benzenesulfonamide (10)

A mixture of compound (1) 4-{[2,2-dicyanovinyl)amino]-*N*-(5-methylisoxazol-3-yl)benzene sulfonamide (0.005mol, 1.64gm) and thiourea (0.01mol, 0.38gm) was refluxed for (10hrs.) in (EtOH/EtONa) mixture (15ml.). The cooled reaction mixture was poured onto ice water then dilutes hydrochloric acid used for acidifying. The solidify product was infiltration and crystallized to give compound (10).

Synthesis of 3,4-dihydropyrimidin drivatives (11, 12)

Compound (3) 4-(5-cyano-4-oxo-3-phenyl-2-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-*N*-(5-methylisoxazol-3-yl) benzenesulfonamide (0.005 mol, 2.32gm) was mixed with stirring to suitable hydrazines (0.01mol.) in dioxane (25ml.) and refluxed about (12hrs.). The mixture of reaction was standing overtime cool it down, pouring it on the crushed snow. The favorite solid precipitated derivatives were filtered and purified to produce compounds (11) and (12) respectively.

STATISTICAL ANALYSIS

Mean was taken using GraphPad Prism 8 and this was mentioned in table (5).

RESULTS

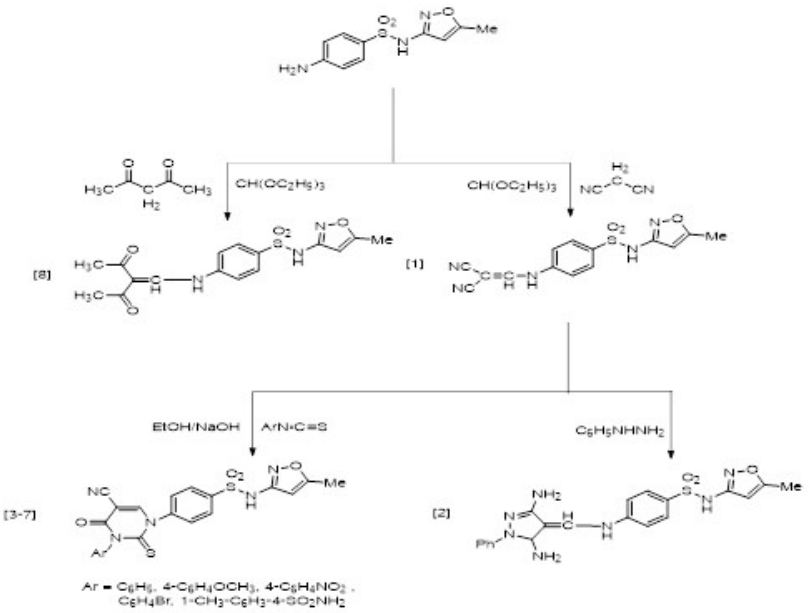
Chemistry

The general preparing pathways to the required sulfamethoxazole derivatives bearing different heterocyclic rings (1-12) are described in Scheme (1, 2 and 3) respectively.

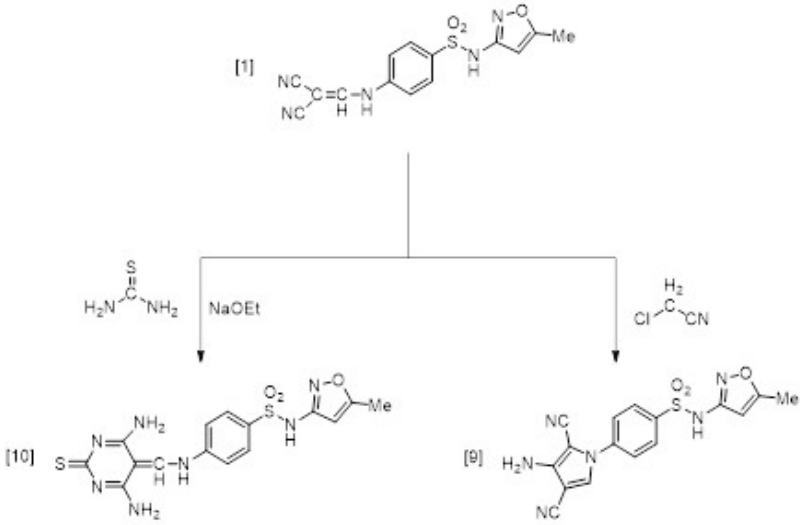
In this research, all newly sulfamethoxazole compounds with different moieties were designed and prepared successfully using the following methods. 4-{[2,2-dicyanovinyl)amino]-*N*-(5-methylisoxazol-3-yl)benzene sulfonamide (1) was provide by addition of malononitrile and acetic acid to a solution of *p*-amino-*N*-(5-methylisoxazol-3-yl) benzenesulfonamide and triethylorthoformate and refluxed to afforded the target compound.

Physicochemical behaviors of compound (1) and each other synthesized sulfamethoxazole derivatives are represented in table (1). Subsequently compound (1) was process with phenyl hydrazine in the existence of dioxane to give their identical 4-{[(3,5-diamino-1-phenyl-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl] amino}-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide(2). Series 4-(5-cyano-4-oxo-3-substituted benzene-2-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-*N*-(5-methylisoxazol-3-yl) benzenesulfonamide (3-7) derivatives was prepared by cyclization of compound (1) with different aromatic isothiocyanate in the presence of sodium ethoxide. Compound (1) was treated also with triethylorthoformate and acetylacetone in the presence of (CH_3CO_2H+MeOH) medium to give its 4-[(2-acetyl-3-oxo-1-buten-1-yl)amino]-*N*-(5-methylisoxazol-3-yl)benzene sulfonamide derivative (8) in good yield.

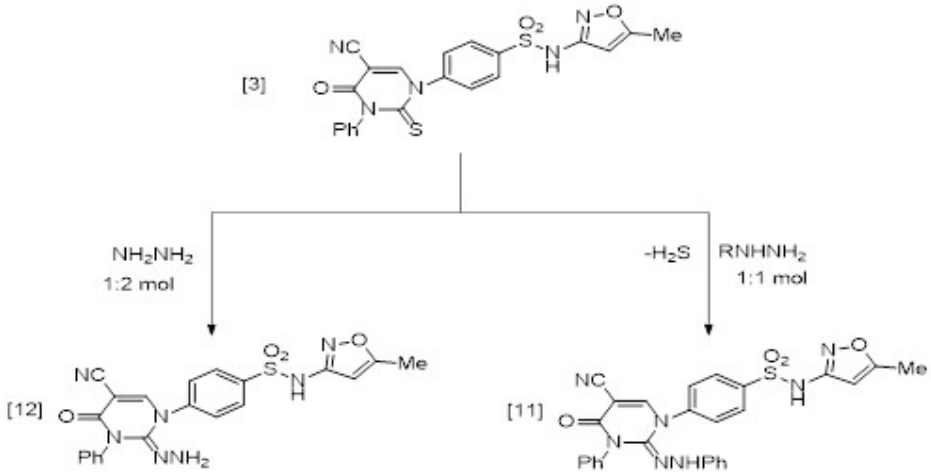
Another route that has been applied for synthesis of pyrrole (9) and 1,3-dihydropyrimidin (10) sulfamethoxazole derivaties by cyclization of compound (1) with either chloro acetonitrile in dioxane or thiourea in basic medium of sodium ethoxide respectively as clarify in Scheme (2).



Scheme (1)



Scheme (2)



Scheme (3)

Table 1: Physico-chemical parameters of synthesized sulfamethoxazole derivatives (1-12).

Compound no.	Empirical formulas	Yield (%)	m.p. (°C.)	Appearance	Solvent Recryst.
1	$C_{14}H_{11}N_5O_3S$	80	172-173	Off white powder	Ethanol
2	$C_{20}H_{21}N_7O_3S$	63	116-118	Bright red crystals	Ethanol
3	$C_{21}H_{15}N_5O_4S_2$	70	154-155	Light yellow powder	Ethanol: water (1:1)
4	$C_{22}H_{17}N_5O_5S_2$	78	161-162	Dark orange powder	Methanol
5	$C_{21}H_{14}N_6O_6S_2$	72	108-110	Dark yellow powder	Methanol
6	$C_{21}H_{14}BrN_5O_4S_2$	70	179-180	Orange powder	Methanol
7	$C_{22}H_{18}N_6O_6S_3$	75	149-151	Light orange powder	Methanol
8	$C_{16}H_{17}N_3O_5S$	79	169-170	Yellow fluffy powder	Ethanol-water (1:1)
9	$C_{16}H_{12}N_6O_3S$	55	124-125	Off white powder	Ethanol-water (1:1)
10	$C_{15}H_{15}N_7O_3S_2$	68	133-134	white fluffy powder	Ethanol
11	$C_{27}H_{21}N_7O_4S$	76	189-190	White powder	Ethanol
12	$C_{21}H_{17}N_7O_4S$	60	213-218	Yellow crystal	Ethanol

Table 2: Characteristic FT-IR ν(cm^{-1}) spectral data of synthesized compounds (1-12).

Comp.	ν(N-H)	ν(C-H)Ar.	ν(C-H)Aliph.	ν(C=N)	ν(C=C)Ar	ν(SO_2) Asym.	Others
1	3328	3061	2945	1601	1558	1370	ν(CN) 2251
2	3331	3056	2962	1606	1555	1331	ν(NH_2) 3415
3	3329	3059	2988	1597	1542	1365	ν(CN) 2238,ν (C=O) amide 1687.
4	3321	3070	2943	1599	1549	1338	ν(CN) 2243,ν (C=O) amide 1688.
5	3325	3044	2951	1598	1539	1342	ν(CN) 2248,ν (C=O) amide 1674.
6	3327	3074	2953	1604	1543	1361	ν(CN) 2241,ν (C=O) amide 1675.
7	3316	3072	2955	1602	1552	1369	ν(CN) 2245,ν (C=O) amide 1781.
8	3336	3048	2981	1603	1549	1355	ν (C=O) carbonyl 1731
9	3318	3062	2953	1596	1557	1334	ν(NH_2) 3418,ν(CN) 2236.
10	3320	3053	2937	1612	1538	1346	ν(NH_2) 3418
11	3312	3048	2972	1599	1529	1341	ν(CN) 2259,ν (C=O) amide1674.
12	3322	3035	2966	1610	1537	1348	ν(NH_2) 3411, ν(CN) 2258, ν(C=O) amide 1671.

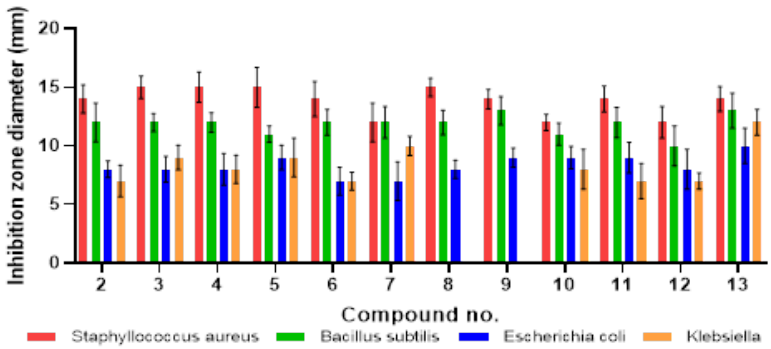


Fig. 1: Antibacterial activity of sulfamethoxazole derivatives against bacterial strains.

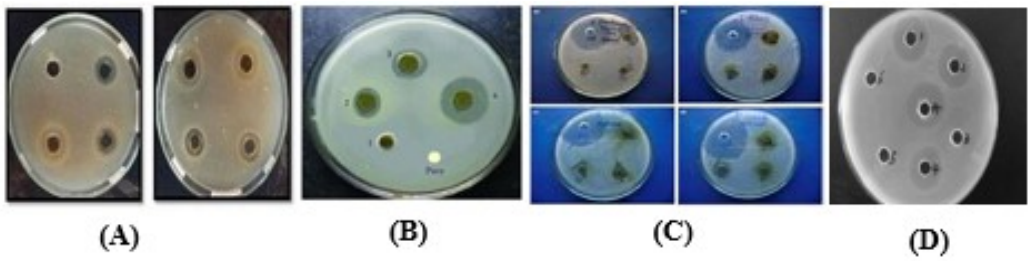
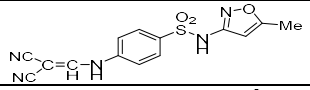
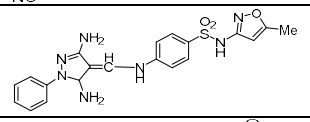
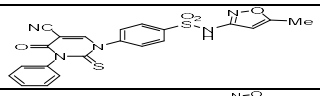
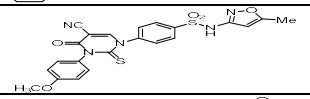
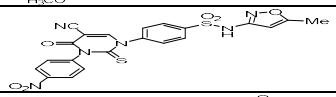
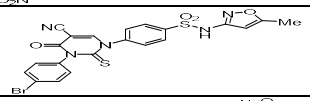
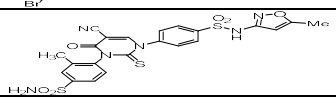
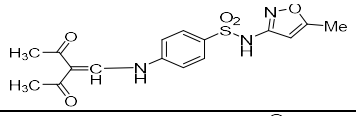
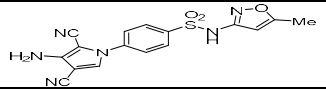
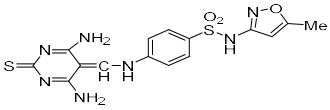
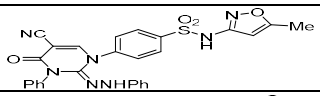
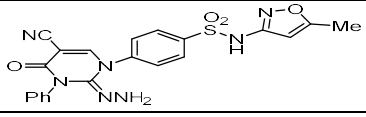


Fig. 2: Effect of compounds on some bacterial isolates (A) *Staphylococcus aureus* (B) *Bacillus subtitle* (C) *Escherichia coli* (D) *Klebsiella*

Table 3: ^{1}H-NMR spectral data (δppm) of synthesized compounds (1-12).

Comp No.	Comp. Structure	^{1}H-NMR parameters (δppm)
1		3.16 (s, 3H, CH_3 , methyl), 6.21 (s, 1H, CH, isoxazole ring), 6.89-7.78 m (4H, Ar-H), (1H, NH-CH=C-), 8.44 (s, 1H, NH, N-sulfonamide), 9.15 (s, 1H, NH sec. amine).
2		3.22 (s, 3H, CH_3 , methyl), 4.55 (s, 1H, CH, pyrazole ring), 6.31 (s, 1H, CH, isoxazole ring), 6.91-7.84 (m, 9H, Ar-H), (1H, NH-CH=C-), 8.50 (s, 1H, NH, N-sulfonamide), 8.71(s, 4H, NH_2 primary amine), 9.12 (s, 1H, NH sec. amine).
3		3.44 (s, 3H, CH_3 , methyl), 6.34 (s, 1H, CH, isoxazole ring), 6.90-7.88 m (9H, Ar-H), (1H, CH, pyrimidine ring), 8.37 (s, 1H, NH, N-sulfonamide).
4		3.37 (s, 3H, CH_3 , methyl), 3.90 (s, 3H, OCH_3, methoxy), 6.20 (s, 1H, CH, isoxazole ring), 6.85-7.91 m (8H, Ar-H), (1H, CH, pyrimidine ring), 8.57 (s, 1H, NH, N-sulfonamide).
5		3.35 (s, 3H, CH_3 , methyl), 6.41 (s, 1H, CH, isoxazole ring), 6.73-7.96 m (8H, Ar-H), (1H, CH, pyrimidine ring), 8.90 (s, 1H, NH, N-sulfonamide).
6		3.44 (s, 3H, CH_3 , methyl), 6.31 (s, 1H, CH, isoxazole ring), 6.79-7.94 m (8H, Ar-H), (1H, CH, pyrimidine ring), 8.92 (s, 1H, NH, N-sulfonamide).
7		3.40 (s, 6H, CH_3 , methyl), 6.29 (s, 1H, CH, isoxazole ring), 6.80-7.92 m (7H, Ar-H), (1H, CH, pyrimidine ring), 8.71(s, 2H, NH_2 sulfonamide),8.98 (s, 1H, NH, N-sulfonamide).
8		2.97 (s, 6H, $COCH_3$), 3.51 (s, 3H, CH_3 , methyl), 6.22 (s, 1H, CH, isoxazole ring), 6.87-7.59 m (4H, Ar-H), (1H, NH-CH=C-), 8.59 (s, 1H, NH, N-sulfonamide). 9.10 (s, 1H, NH sec. amine).
9		3.56 (s, 3H, CH_3 , methyl), 6.17 (s, 1H, CH, isoxazole ring), 6.89-7.83 m (4H, Ar-H), (s, 1H, pyrrole ring), 8.52 (s, 2H, NH_2), 8.91 (s, 1H, NH, N-sulfonamide).
10		3.29 (s, 3H, CH_3 , methyl), 6.18 (s, 1H, CH, isoxazole ring), 6.76-7.98 m (4H, Ar-H), (1H, NH-CH=C-), 8.40 (s, 4H, NH_2), 8.57 (s, 1H, NH, N-sulfonamide), 9.22 (s, 1H, NH sec. amine).
11		3.27 (s, 3H, CH_3 , methyl), 6.16 (s, 1H, CH, isoxazole ring), 6.85-7.81 m (14H, Ar-H), (1H, pyrimidine ring), 8.48 (s, 1H, NH, N-sulfonamide), 8.79 (s, 1H, NH sec. amine).
12		3.36 (s, 3H, CH_3 , methyl), 6.30 (s, 1H, CH, isoxazole ring), 6.14 (s, 2H, NH_2),6.68-7.97 m (7H, Ar-H), (1H, pyrimidine ring), 8.42 (s, 2H, NH_2), 8.58 (s, 1H, NH, N-sulfonamide), 8.93 (s, 1H, NH sec. amine).

These compounds and all synthesized derivatives displayed essentially characteristic infrared bands are listed in table (2).

The thio dihydropyrimidine compound (3) was successfully treated with either ($NH_2NH_2.2H_2O$) or ($Ph.NHNH_2$) respectively in dioxane medium to afford its hydrazono dihydropyrimidine derivatives (11, 12). Hydrogen sulfide elimination gives an indication of the reaction proceeding by using lead acetate paper. The preparatory tracks for compounds (11 and 12) are displayed in Scheme (3).

Proton and carbon nuclear magnetic resonance techniques were also applied to prove structure elucidation of all compounds as shown in tables (3 and 4) respectively were consistent with the suggested structures.

In-vitro Antibacterial screening

Anti-bacterial activity was tested for nearly all of newly synthesized sulfamethoxazole derivatives substituted pyrazole, 3,4-dihydropyrimidin, pyrrole and 1,3-dihydropyrimidin (2-12)were evaluated for the (*In Vitro*) antibacterial assay against several pathogenic bacterial strains i.e. (G+) bacteria: *Bacillus subtitles*, *Staphylococcus aurous* also for (G-) bacteria: *Escherichia coli*, *Klebsiella pneumonia* at concentrations (100mg/mL). The selected compounds in this screening were dissolved in dimethylsulfoxide. Inhibition areas caused by these compounds were determined in diameter (mm.) compared with the cephalexin used as reference drug showed interesting results as listed in table (5) and shown in figs. (1, 2) respectively.

Table 4: ^{13}C-NMR spectral data (δppm) for new synthesized derivatives (1-12).

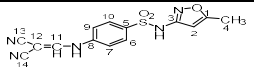
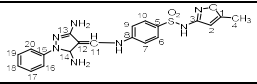
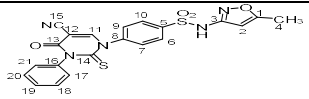
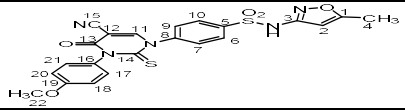
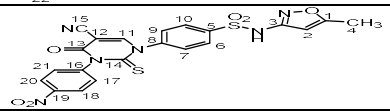
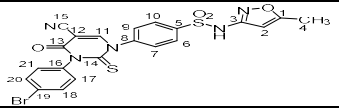
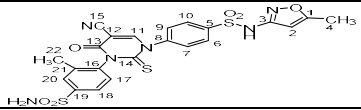
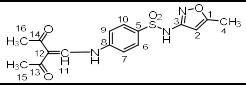
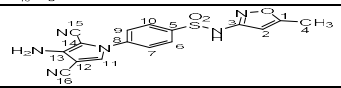
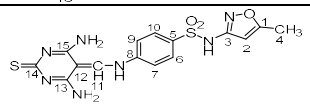
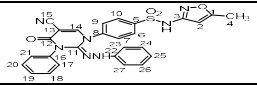
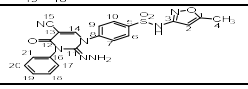
Comp No.	Compound structure with numbering of carbon atoms	^{13}CNMR Spectral Data (δ ppm)
1		19.81 (C_4), 61.59 (C_{12}), 98.73 (C_2), 117. 49 (C_{13},C_{14}), 121.68-138.31 (C_5-C_{10}), 157.22 (C_{11}), 159.11 (C_3), 163.37 (C_1).
2		19.66 (C_4), 98.12 (C_2),115.72 (C_{12}), 121.18-137.93 (C_5-C_{10}), (C_{15}-C_{20}), 145.61 (C_{13},C_{14}), 156.42 (C_{11}), 158.20 (C_3),164.44 (C_1).
3		19.38 (C_4), 96.23 (C_2), 99.30 (C_{12}), 115.19 (C_{15}),122.33-139.49 (C_5-C_{10}) (C_{16}-C_{21}), 159.62 (C_3), 161.74 (C_{11}), 164.11 (C_1), 167.33 (C_{13}), 169.51 (C_{14}).
4		19.91 (C_4), 64.79 (C_{22}), 97.43 (C_2),99.14 (C_{12}), 116.55 (C_{15}),122.72-140.22 (C_5-C_{10}) (C_{16}-C_{21}), 159.46 (C_3), 162.81 (C_{11}),165.30 (C_1), 168.73 (C_{13}),170.11 (C_{14}).
5		19.56 (C_4), 96.41 (C_2), 98.17 (C_{12}), 117.37 (C_{15}),121.66-141.43 (C_5-C_{10}) (C_{16}-C_{21}), 158.11 (C_3), 161.78 (C_{11}), 165.21 (C_1), 169.50 (C_{13}), 171.25 (C_{14}).
6		17.69 (C_4), 95.12 (C_2), 98.59 (C_{12}), 118.97 (C_{15}),122.90-139.22 (C_5-C_{10}) (C_{16}-C_{21}), 157.06 (C_3), 160.41 (C_{11}), 166.15 (C_1), 169.32 (C_{13}), 171.44 (C_{14}).
7		19.71 (C_4), 97.47 (C_2), 99.53 (C_{12}), 117.25 (C_{15}),121.62-138.79 (C_5-C_{10}) (C_{16}-C_{21}), 158.28 (C_3), 161.33 (C_{11}), 164.82 (C_1), 168.55 (C_{13}), 170.18 (C_{14}).
8		19.43 (C_4), 31.57 (C_{15},C_{16}), 96.81 (C_2), 117.25 (C_{12}), 120.87-139.11 (C_5-C_{10}), 157.72 (C_3), 160.52 (C_{11}), 163.61 (C_1), 170.18 (C_{13},C_{14}).
9		19.49 (C_4), 97.22 (C_2), 118.41 (C_{15}, C_{16}), 121.49-138.77 (C_5-C_{10}), (C_{11}-C_{14}), 158.72 (C_3), 165.21 (C_1).
10		18.93 (C_4), 92.67 (C_{12}), 97.31(C_2), 120.73-139.55 (C_5-C_{10}), (C11), 156.95 (C_3), 164.39 (C_{13}, C_{15}), 166.82 (C_1),171.22 (C_{14}).
11		19.90 (C_4), 95.66 (C_2), 98.42 (C_{13}), 116.55 (C_{15}), 121.33-141.38 (C_5-C_{10}), (C_{11}, C_{14}),(C_{16}-C_{27}), 154.41 (C_3), 166.69 (C_1), 168.57 (C_{12}).
12		18.56 (C_4), 96.81 (C_2), 99.35 (C_{13}), 117.62 (C_{15}), 120.18-140.71 (C_5-C_{10}), (C_{11}, C_{14}),(C_{16}-C_{21}), 155.49 (C_3), 167.34 (C_1), 169.39 (C_{12}).

Table 5: Antibacterial activity of selected sulfamethoxazole derivatives

Comp. No.	Inhibition zone diameter (mm)			
	Staphylococcus aurous	Bacillus subtitles	Escherichia coli	Klebsiella
2	14	12	8	7
3	15	12	8	9
4	15	12	8	8
5	15	11	9	9
6	14	12	7	7
7	12	12	7	10
8	15	12	8	-
9	14	13	9	-
10	12	11	9	8
11	14	12	9	7
12	12	10	8	7
Cephalexin[std.] (13)	14	13	10	12
DMSO	-	-	-	-

[Conc.]: 1mg/ml, Zone inhibition: (-) no inhibition zone

Table 6: Docking score of selected sulfamethoxazole derivatives

Comp. No.	Docking score in kcal/mol			
	Staphylococcus aurous	*Bacillus subtitles*	*Escherichia coli*	*Klebsiella*
2	-5.57	-2.37	-4.96	-5.84
3	-5.91	-1.22	-3.30	-6.07
4	-5.83	-2.05	-2.66	-5.99
5	-7.28	-3.19	-2.85	-5.46
6	-6.33	-1.88	-3.13	-5.50
7	-5.13	-2.53	-3.45	-1.97
8	-5.69	-3.20	-4.30	-5.33
9	-4.64	-3.83	-4.69	-3.15
10	-5.64	-3.19	-4.83	-3.12
11	-5.26	-3.34	-4.54	-5.79
12	-5.57	-1.52	-3.82	-6.02
Cephalexin[std.]	-5.36	-3.29	-6.45	-6.95

Table 7: Surrounding amino acids & interactions inside bacterial active sites for compounds (3,4,5 & 8).

Comp. No.	Surrounding Amino Acids & Interactions			
	Staphylococcus aurous	*Bacillus subtitles*	*Escherichia coli*	*Klebsiella*
3	THR46, PHE92, TYR98, ILE5, VAL6, ALA7, THR111, ASP27, LEU28, LEU20, ILE31, LYS32, ARG57, PRO55, LEU54, LYS52, ILE50. In addition H-bond (N-LYS52)	GLY106, ALA105, VAL104, THR16, ARG15, GLY14, HIS13, ARG11, THR9, SER76, HIE75, TYR74, GLU73, PRO72. In addition H-bond (O-HIE75), H-bond (O-THR16), H-bond (O-ARG15 & Pi-cation (ARG11-isoxazole ring)	VAL111, ARG136, ASP106, ASP105, PHE104, LYS103, GLY102, ASN46, ALA47, ASP49, GLU50, LEU52, ALA53	ILE35, GLY219, ASN220, LYS211, CYS208, VAL73, ASP124, GLN123, HIE122, GLU152. In addition two H-bonds (O-ASN220 & NH-GLN123)
4	LEU20, TRP22, ILE5, VAL6, ALA7, ASP27, LEU28, ILE31, LYS32, ARG57, ASN56, PRO55, LEU54, LYS52, ILE50. In addition H-bond (N-LYS52)	GLY110, LYS109, GLY108, GLY107, GLY106, ALA105, VAL104, THR16, ARG15, ARG11, PRO72, GLU73, TYR74, HIE75, SER76. In addition H-bond (O-SER76) & H-bond (O-ARG15)	HIS55, ALA53, LEU52, GLU50, ASP49, ALA47, ASN46, ILE94, PHE104, LYS103, GLY102. In addition H-bond (N-ARG76)	SER217, GLY219, ASN220, LYS211, VAL37, ASP124, GLN123, MET145, GLU152. In addition two H-bonds (O-ASN220) & (NH-GLN123)
5	GLN19, LEU20, PHE92, TYR98, ILE5, VAL6, ALA7, ASP27, LEU28, ILE31, LYS32, ARG57, PRO55, LEU54, LYS52 , ILE50, SER49, THR46. In addition Pi-cation (N^+-PHE92), H-bond (OH-ILE5), H-bond (O-LYS52)	GLY106, ALA105, ARG11, ALA81, GLY80, SER79, MSE78, SER76, HIE75, TYR74, GLU73, GLY111, GLY110. In addition H-bond (O-GLY80), H-bond HIE75 & H-bond (OH-GLU73)	SER112, ASP73, GLY75, ARG76, ILE78, PRO79, THR165, ASN46, ALA47, ILE94, ASP49, GLU50, LEU52, ALA53. In addition H-bond (N-ARG76) & H-bond (O-H_2O)	HIS189, ZN303, ZN304, LYS211, CYS208, HIS250, ILE35, ASP124, GLN123, HIE122. In addition four H-bonds (O-ASN220, O-LYS211, O-ASP124 & NH_3 as well as salt bridge (O^-LYS211 & ZN303)
8	THR111, LEU54, TYR109, ALA7, VAL6, ILE5, ASP27, LEU28, ILE31, PHE92, THR46, SER49, ILE50, HIS23, TRP22, LEU20, GLN19. In addition H-bond (O-LEU20) & H-bond (NH-SER49	ARG15, THR16, ARG11, PRO10, ALA81, GLY80, SER79, HIE75, TYR74, GLU73, PRO72 In addition H-bond (O-GLY80), H-bond (NH-GLU73) & 2 Pi-Pi stacking (TYR74 with isoxazole ring as well as benzene ring	VAL111, PRO79, ILE78, GLY77, ARG76, ASP73, VAL167, THR165, VAL43, ASN46, ALA47, ASP49, GLU50, ALA53. In addition 3H-nond (NH-PHE104), (O-ARG76), (O-ASN46) & Pi-cation (ARG76 with benzene ring	LYS211, ASN220, VAL73, ILE35, PRO68, MET67, ASP66, LEU65, HIE122, GLN123, ASP124, HIS189, ZN302, ZN303, HIS250
Cephalexin	ASP27, LEU28, ILE31, ALA7, VAL6, ILE5, PHE92, LEU54, LYS52, ILE50. In addition H-bond (NH_2-ASP27)	HIE75, TYR74, GLU73, PRO72, GLY106, ALA105, VAL104, THR16, ARG15, GLY14, ARG11, THR9. In addition 2 H-bond (O-ARG11) & (O-ARG15) as well as Pi-Pi stacking (TYR74 with benzene ring)	PRO79, ILE78, ARG76, GLY75, ASP73, VAL71, VAL43, ASN46, ALA47, ASP49, GLU50, GLY102, LYS103, PHE104, ASP105. In addition 3 H-bond (NH_2-ASP73, O-PHE104 & OH -GLU50)	HIS189, LEU218, GLY219, ASN220, GLY222, ASP223, ASP124, HIE122, GLU152. In addition one H-bond (OH-ASN220)

DISCUSSION

The result displayed that some of the synthesized compounds offered were excellent activities against bacteria *Pseudomonas aeruginosa* and *Bacillus Subtitles* compared to reference drugs used. On the other hands these derivatives displayed moderate antibacterial activity against *Escherichia coli* while compounds [8 and 9] show no inhibition against *Klebsiella isolates*.

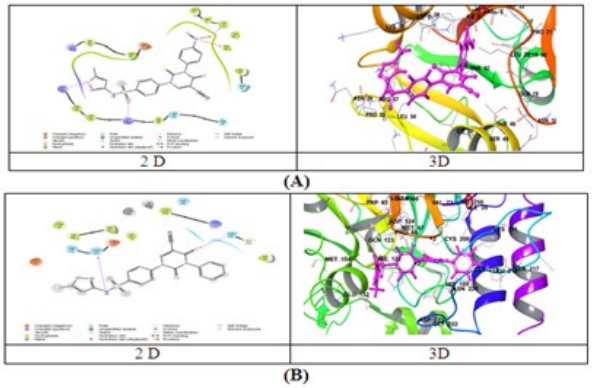


Fig. 3: selected compounds in 2D & 3D inside bacterial active site surrounding by amino acids and interactions (A) Compound (5) inside *Staphylococcus aurous* (B) Compound (3) inside *Klebsiella*.

Computational method

All sulfamethoxazole derivatives chemical structures drawn by Chem Draw 18.0 software. Next step, the geometry optimization applied by energy force field using Hyperchem software 8.0 and saved as mol file format with additional optimization by semi-empirical mechanics. The lowest geometrical conformation of all derivatives was kept as sdf file for by Spartan 14.0 program with Monte Carlo mechanics method. Next, docking evaluation study was done by Glide tool under Schrodinger Maestro 11.1 software. The crystal structures for each bacterium were obtained from Protein Data Bank under codes as following: 2W9S of Staphylococcus aureus with trimethoprim, 1NNI of Azobenzene Reductase from Bacillus subtilis, 3G7E of E. coli Gyrase B co-complexed with inhibitor, and 4HL2 of Metallo-beta-Lactamase-1 with Hydrolyzed Ampicillin. Ligands (sulfamethoxazole derivatives) preparation process was performed by using LigPrep tool before docking for accurate ionization, adding missing hydrogen and the achievement of lowest energy conformations. The receptor preparation done by using ProPrep tool by cleaning, optimizing and minimization. Then by same tool is used for filling of all missing loops and preparation with high receptor quality.

The grid box for docking study was adjusted to 1.2 Å with an atomic charge of 0.28 and obtained ligand docked back for validation process by applying flexibly XP method (Glide-extra precision) simulations for attached ligands while active site receptor pocket was kept rigid during all docking process. Finally, all obtained data was obtained as xlsx file and kept for future characterization. Docking score screening of all sulfamethoxazole derivatives inside active site of each corresponding bacterial pocket shows the activity related to positive control (Cephalexin) in a sequence similar to experimental activities sequence referring to the ability of docking result to estimate the activity action of sulfamethoxazole derivatives to inhibit the activity of each selected bacteria table (6).

In this research, docking simulator of the more effective synthesized compounds was achieved to check the binding areas and the quality of interactions. The docking simulation for compound (5) which offered the highest inhibitory efficacy against *Staphylococcus aurous* with Inhibition zone 15 diameter discloses that this compound can be hosted effectively within the site active gorge with Pi-cation (N+-PHE92), H-bond (OH-ILE5) as well as H-bond (O-LYS52) with free binding energy -7.28 kcal/mol) as shown in fig. (3). Compound (3) demonstrated the complete insertion and proper positioning of this inhibitor into the active site gorge of Klebsiella coli, this compound showed two strong hydrogen bonding interaction (O-ASN220 & NH-GLN123) in addition hydrophobic interactions with ILE35, GLY219, ASN220, LYS211, CYS208, VAL73, ASP124, GLN123, HIE122, GLU152. The free binding energy for this compound is (-6.07kcal/mol) as demonstrated in fig. (3). The rest Surrounding Amino Acids & Interactions inside bacterial active sites for Compounds 3, 4, 5 & 8 were listed in table (7) listed the interactions of highest active sulfamethoxazole derivatives compound (3,4,5 and 8) inside all bacterial active sites with surrounding amino acids.

CONCLUSIONS

A substituted pyrazole, 3,4-dihydropyrimidin, pyrrole and 1,3-dihydropyrimidin pharmacophore derivatives derived from amine group of a sulfamethoxazole were designed, synthesized and evaluated as anti-bacterial agents. Molecular docking analysis was used to identify the mode of binding of these compounds. It can be concluded from these results that designing new derivatives based on existing antibacterial agents may reveal enhanced antibacterial activity.

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