ORIGINAL ARTICLE

Synthesis, characterization and antibacterial evaluation of new 1,2,4- triazole-3-thiol derivatives

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HIGHLIGHTS

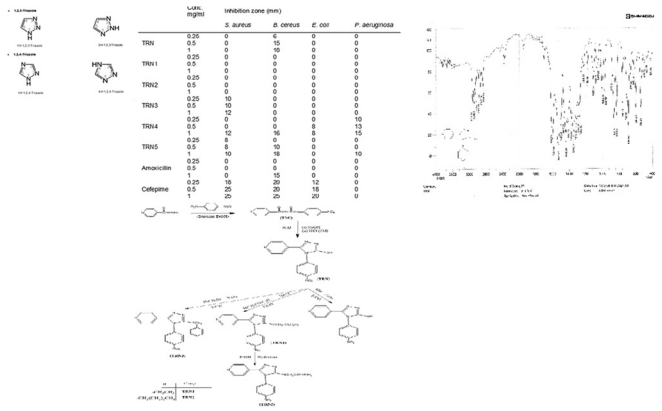
- New triazole compounds were prepared
- Use disc diffusion method to obtain biological activity
- New triazole compounds were characterized by different analytical methods.

Abstract

Context: In this manuscript, evaluation of the individual antibacterial effect of new synthesized 1,2,4-triazole-3-thiol derivatives against certain types of bacteria (Gram-positive and Gram-negative). **Methods:** Synthesize of some new 1,2,4-triazole derivatives and characterization of synthesized derivatives were characterized by Fourier transform-infrared spectroscopy, proton nuclear magnetic resonance, and elemental microanalysis (CHNS). The antibacterial effect of the synthesized derivatives was assessed by determining their inhibitory concentration whereby calculate their inhibition zone versus certain types of standard antibiotics, concentration ranging from 0.250, 0.500, and 1 mg/1 ml. **Results:** Most synthesized compound showed inhibition zone against Gram-positive and/or Gram-negative bacteria, compound (TRN4) showed moderate inhibition against resistant *Pseudomonas aerogenosa*, while standard reference drug (cefepime) did not show activity. **Conclusion:** These results indicate that the introduction of triazole -3-thiol moiety may produce antibacterial activity against certain types of bacteria and according to side chain group (beside thiol).

Key words: 1,2,4-triazole, antibacterial, heterocyclic compounds, triazol-3-thiol derivatives

GRAPHICAL ABSTRACT



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INTRODUCTION

Heterocyclic chemistry deals with heterocyclic compounds, which constitute about 65% of organic chemistry literature. Heterocyclic compounds are widely distributed in nature which is essential to life.[1] Heterocyclic chemistry is an integral part of organic chemistry and constitutes a considerable part of the syllabus for undergraduate and graduate students throughout the world.[2]

A large number of synthetic and natural heterocyclic compounds are pharmacologically active and are used in clinical applications such as letrozole, fluconazole, itraconazole, and miconazole.[3] Heterocyclic bases-pyrimidine and purines are also composed of genetic material DNA. Heterocyclic compounds are also chlorophyll-photosynthesizing and pigment-carrying hemoglobin-oxygen. Several heterocyclic compounds as insecticides, fungicides, herbicides, pesticides, etc., have applications in agriculture. In industrial compounds such as sensitizers, developers, antioxidants, and copolymers they also find applications. In the synthesis of other organic compounds, they are used as reagents.[4]

One of the most important groups in heterocyclic compounds is the five-membered ring, which contains one or more than one atom in the heteroatom system.[5]

Our goal in this research is to prepare compounds with three heteroatoms, called triazoles, which are nitrogen atoms. A triazole refers to one of the molecular formula $C_2H_3N_3$ heterocyclic compounds with a five-member aromatic ring composed of two carbon atoms and three nitrogen atoms. The triazole family's simplest form is triazole itself.[6] There are two set of isomers that differ according to position of nitrogen atoms. Each has two tautomers that differ by which nitrogen is bonded with hydrogen,[7] as shown in Figure 1, isomers of triazole.

1,2,4-Triazole and their derivatives constitute an important class of organic compounds with diverse agricultural, industrial, and biological activities, including antibacterial,[8] antifungal,[9] anticonvulsant,[10] antiviral,[11] antineoplastic,[12] analgesic,[13] antioxidant,[14] and anti-inflammatory.[15] In recent years, the synthesis of these heterocycles has received considerable attention for many researchers, which motivates us to prepare new derivatives of 1,2,4-triazole-S-thiol and evaluate it against certain types of bacteria, this triazole-3-thiol ring has been previously tested on some bacterial species based on other structures and has shown good activity, so our goal to test new synthesized triazoles compound on new (pathogenic) strains of bacteria for optimum activity.

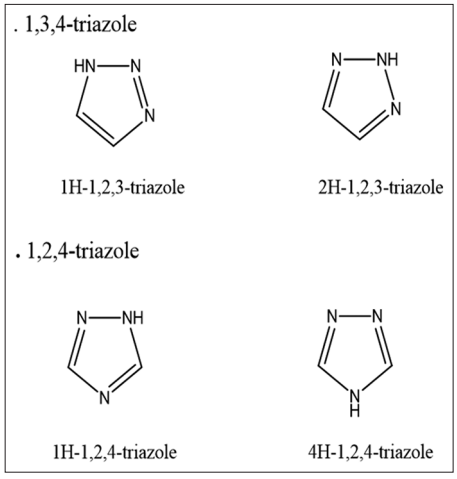


Figure 1: Isomers of triazole

Objective of the Study

The objective of the study was to evaluate the individual antibacterial effect of new synthesized 1,2,4-triazole-3-thiol derivatives against certain types of pathogenic isolate bacteria (Gram-positive and Gram-negative).

EXPERMENTAL

Chemicals

Isonicotinic acid hydrazide (INH), nitrophenyl isothiocynate, ethyl bromide, butyl bromide, benzyl bromide, absolute ethanol, methanol, and bromo ethyl acetate were produced from Sigma-Aldrich Pvt., Ltd., Bengaluru, India. Sodium hydroxide and hexane were produced from Sigma-Aldrich, Germany. Sodium acetate aswas produced from Fluka, Switzerland. Sodium hydroxide was produced from Merck, Germany. Hydrazine hydrate 99.5% were produced from ALPHA, India, most chemicals were purchased from a local distributor of Sigma-Aldrich, Baghdad, Iraq (OMA Company), others from local laboratory stock of pharmaceutical chemistry department, college of pharmacy, Basrah.

Instrumentation

Fourier transform-infrared (FT-IR) spectrometry

FTIR spectra for all studied compounds were measured as KBr discs using FTIR 8400S SHIMADZU (Japan).

Proton nuclear magnetic resonance (^{1}HNMR) spectra

The studied compounds were measured by use of 500MHz NMR (INOVA Switzerland). DMSO-d_6 was used as a solvent and tetramethylsilane as an internal standard.

Elemental analysis

The CHNS analysis measurements for all synthesized compounds were performed using CHNS elemental analyzer flash EA 1112 series, (Thermo Finnigan).

Synthesize of Compounds

Synthesis of thiosemicarbazide (TSC)

To a solution of INH (1.37 g) in a mixture of dioxane: ethanol (2:1), 1.8 g of nitrophenyl isothiocyanate dissolved in 10 ml of dioxane was added at 60°C. The reaction mixture was stirred at 60°C for 1 h, and then at room temperature for 30 min. The separated crystals were filtered, washed with cold ethanol, and dried at room temperature.[16,17] The yield (2.9 g) was crystal powder in shape with brown color and melting point 208–211°C.

Synthesis of 1,2,4-triazole-3- thiol (TRN)

A solution of 1.58 g of TSC and 0.2 g of sodium hydroxide in 100 ml of water were heated under reflux for 2 h. The resulting yellow solution was filtered and acidified with a cold solution of 2 M hydrochloric acid to pH 4.[16] The crystals were separated, filtered, and washed with water. The products were recrystallized from methanol and dried at room temperature.

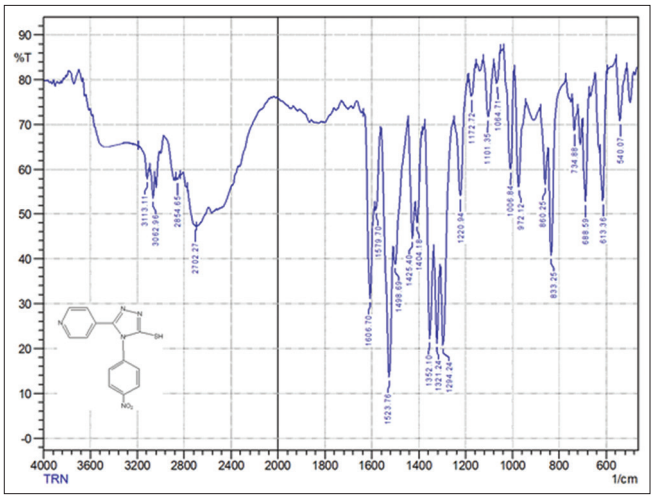


Figure 2: Infrared spectrum of TRN

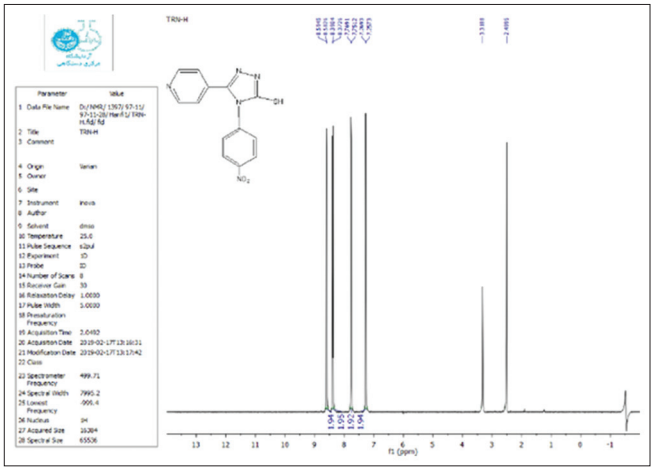


Figure 3: Proton nuclear magnetic resonance spectrum of TRN

Synthesis of mercaptoalkyl derivatives

The compounds, 4-(5-(ethylthio)-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl)pyridine (TRN1) and 4-(5-(butylthio)-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl)pyridine (TRN2), were synthesis by same procedure.

A mixture of 0.015 mole of TRN, 0.018 mole of ethyl bromide or butyl bromide, and 0.02 mole of sodium acetate in 100 ml of ethanol were heated under reflux for 3 h, then allowed to cool, and poured into 150 ml of cold water containing ice. The solid product was collected and recrystallized from ethanol.

Synthesis of mercapto benzyl derivative

The compounds, 4-(5-(benzylthio)-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl)pyridine (TRN3), were synthesized as follows.

To a solution of 1.5 g of TRN and 1.23 g of sodium acetate in 50 ml of absolute ethanol, 0.6 ml of benzyl bromide was added. The reaction mixture was refluxed for 4 h. The content was then poured into crushed ice and a solid mass which separated was filtered and recrystallized from ethanol.

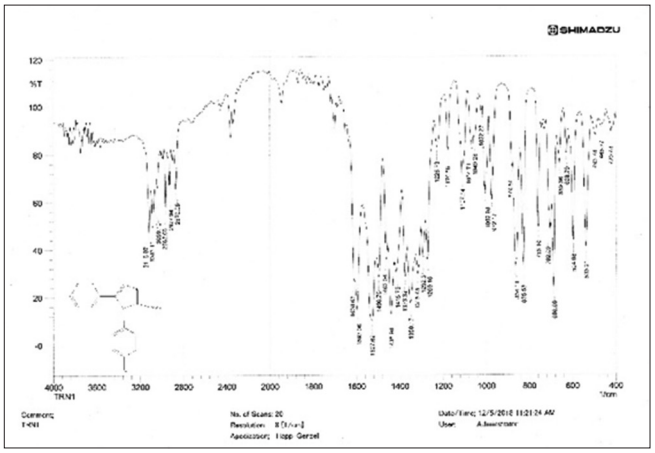


Figure 4: Infrared spectrum of TRN1

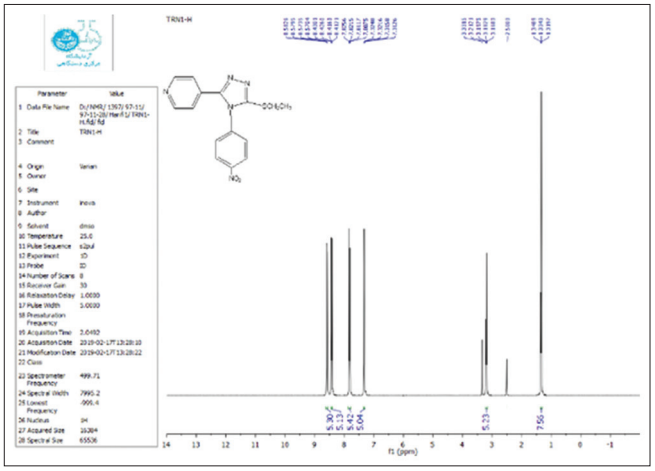


Figure 5: Proton nuclear magnetic resonance spectrum of TRN1

Synthesis of mercapto ethoxycarbonylmethyl derivative

The compounds, ethyl 2-((4-(4-nitrophenyl)-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)acetate (TRN4), were synthesized as following.

To a solution of 1 g of TRN and 0.36 g of sodium acetate in 50 ml of absolute ethanol, 0.45 ml of bromo ethyl acetate was added. The reaction mixture was refluxed for 3 h.[18] The content was then poured into crushed ice and a solid mass which separated was filtered and recrystallized from ethanol.

Synthesis of mercapto hydrazine carbonyl methyl derivative

The compounds, 2-((4-phenyl-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)acetohydrazide (TRN5), were synthesized from TRN4.

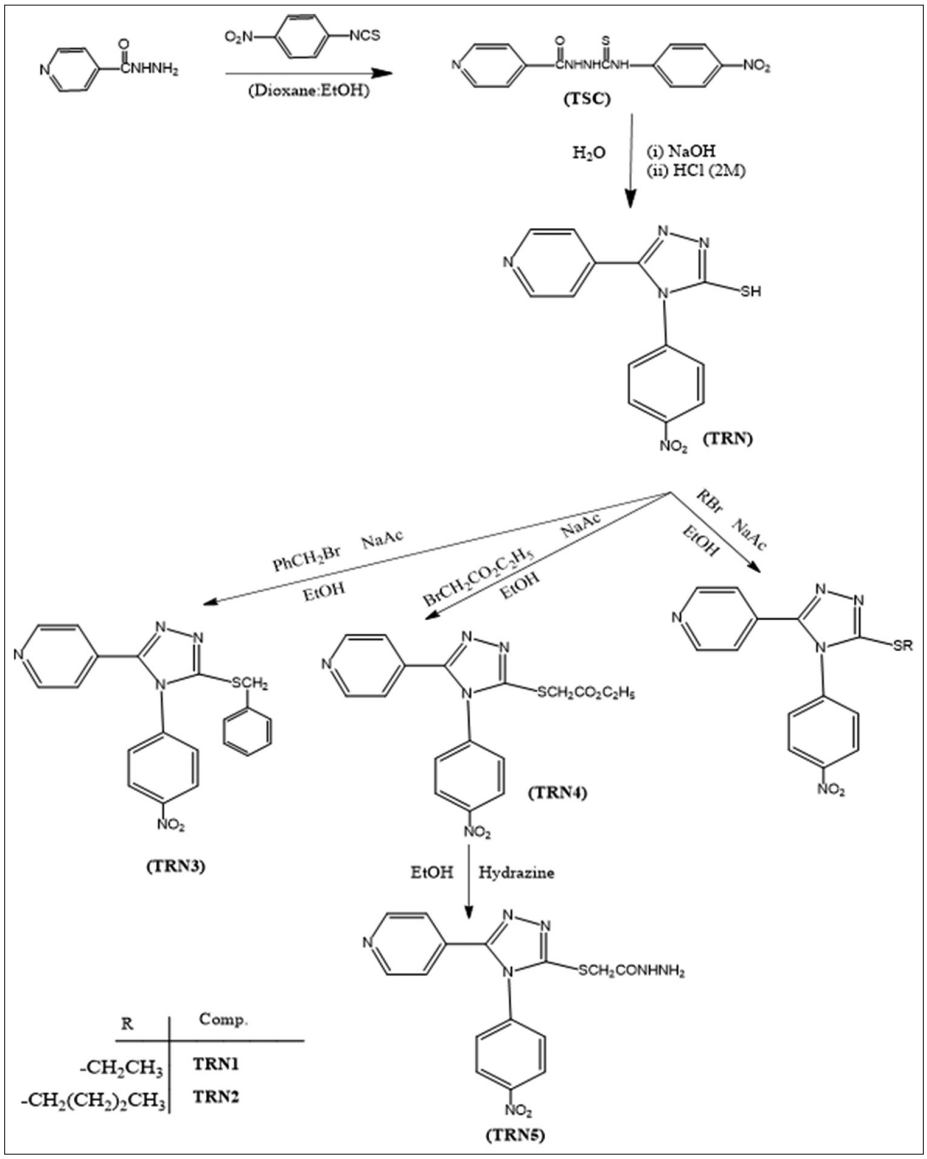
To solution of 1 g of TRN4 in 50 ml of absolute ethanol, 0.25 ml of hydrazine hydrate (99.5%) was added. The reaction mixture was refluxed for 6 h.[19] The content was left for 1 day to dry then crystals was separated, filtered and recrystallized from ethanol: dioxane (1:1), as shown in Scheme 1.

RESULTS

Spectral Data

4-(4-nitrophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (TRN)

Yellow fine powder, (M.P 295–296°C, yield 67%). IR(KBr, cm^{-1}): 2702 (S-H), 3062 (C-H) aromatic system, 1581 m, 1523 s, 1500 m (C=C) aromatic system, 1608 s (C=N),



Scheme 1: Synthesis pathway for 1,2,4-triazole compounds

1296 s (C-N), 686 m (C-S); ^{1}HNMR (500 MHz, DMSO-d_6): 8.6 d, 7.8 d (C-H) pyridine ring, 8.4 d, 7.3 d (C-H) nitro-benzene ring; elemental analysis (%Calc./Found): 52.17/51.60 (C), 3.03/3.07 (H), 23.40/23.17 (N), 10.71/10.56 (S) [Figures 2 and 3].

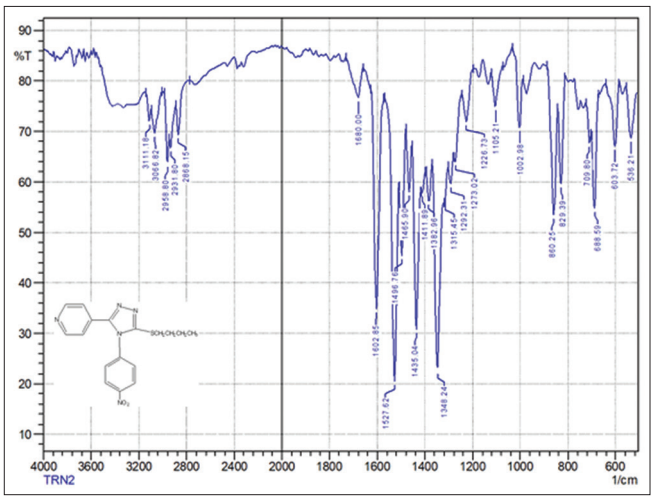


Figure 6: Infrared spectrum of TRN2

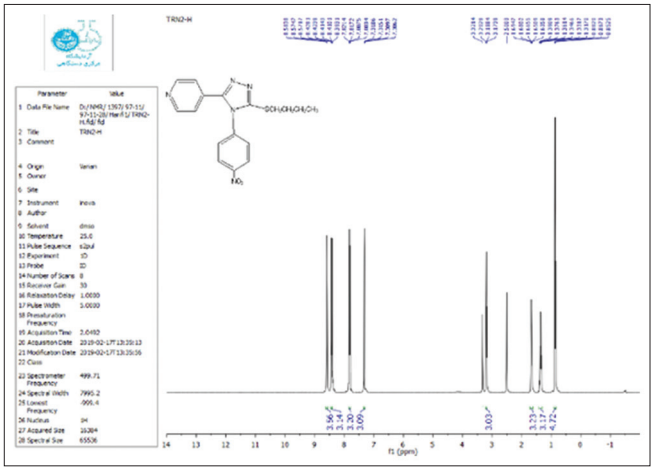


Figure 7: Proton nuclear magnetic resonance spectrum of TRN2

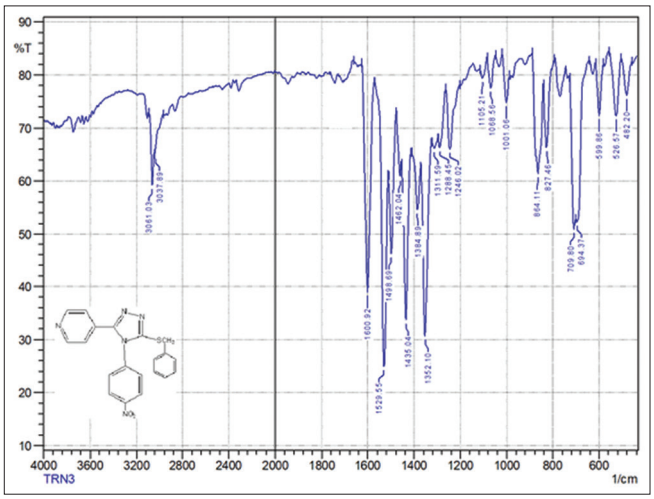


Figure 8: Infrared spectrum of TRN3

4-(5-(ethylthio)-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl)pyridine (TRN1)

Yellow crystal powder, (M.P 184–187°C, yield 56%). IR (KBr, cm^{-1}): 3086 (C-H) aromatic system, 2962 m, 2870 m

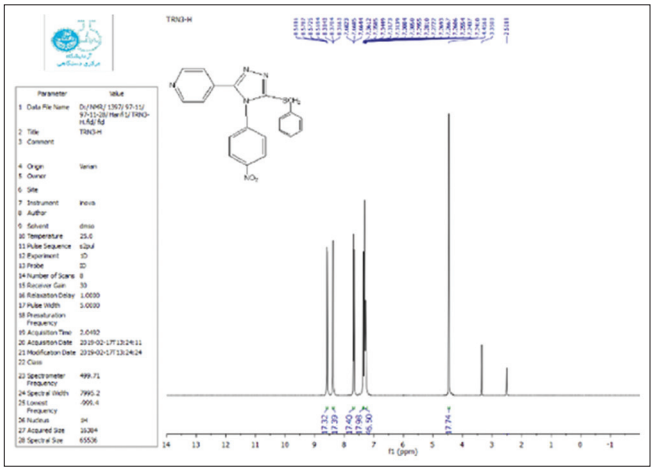


Figure 9: Proton nuclear magnetic resonance spectrum of TRN3

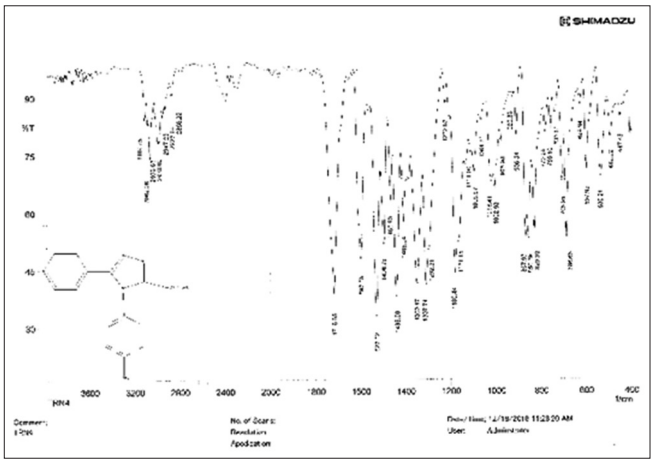


Figure 10: Infrared spectrum of TRN4

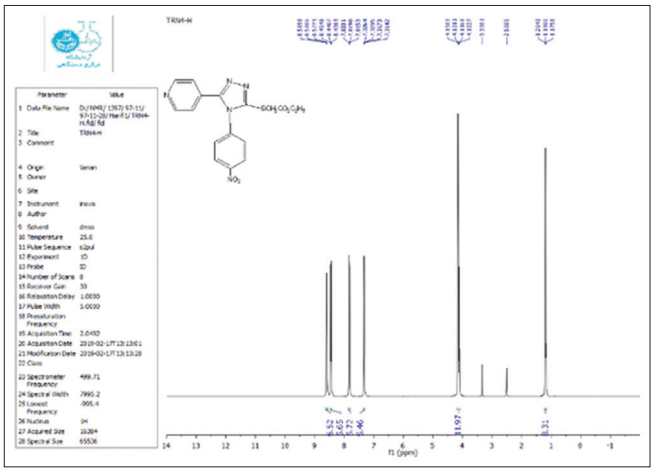


Figure 11: Proton nuclear magnetic resonance spectrum of TRN4

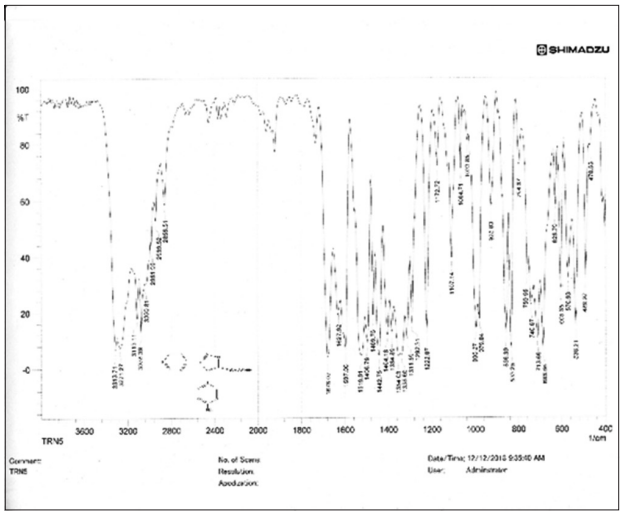


Figure 12: Infrared spectrum of TRN5

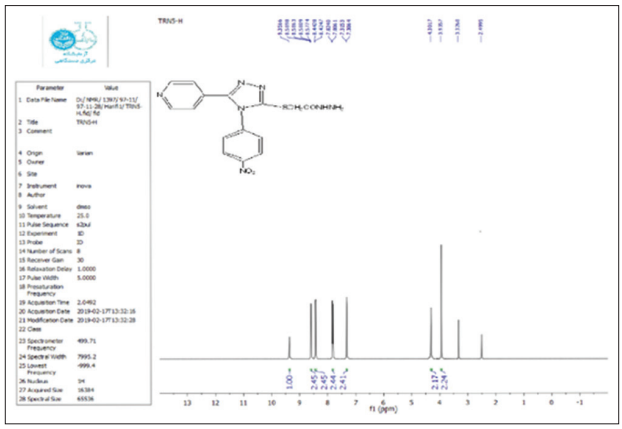


Figure 13: Proton nuclear magnetic resonance spectrum of TRN5

(C-H) aliphatic, 1597 m, 1527 m, 1496 m (C=C) aromatic system, 1608 s (C=N), 1292 s (C-N), 686 m (C-S); ^{1}HNMR (500 MHz, DMSO-d_6): 8.6 d, 7.8 d (C-H) pyridine ring, 8.4 d, 7.3 d (C-H) nitro-benzene ring, 1.3 t (-CH_3), 3.18 q (-CH_2); elemental analysis (%Calc./Found): 55.04/54.75 (C), 4.00/4.01 (H), 21.39/20.95 (N), 9.79/9.42 (S) [Figures 4 and 5].

4-(5-(butylthio)-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl)pyridine (TRN2)

Dark yellow l powder, (M.P 78–81°C, yield 40%). IR(KBr, cm^{-1}): 3066 (C-H) aromatic system, 2958 m, 2873 m (C-H) aliphatic, 1597 m, 1535 m, 1527 m (C=C) aromatic system, 1608s (C=N), 1273 s (C-N), 686 m (C-S);^{1}HNMR (500 MHz, DMSO-d_6): 8.6 d, 7.8 d (C-H) pyridine ring, 8.4 d, 7.3 d (C-H) nitro-benzene ring, 0.9 t (-CH_3), 1.35 m (-CH_2), 1.65 m (-CH_2), 3.15 t (-CH_2) beside sulfur; elemental analysis (%Calc./Found): 57.45/56.98 (C), 4.82/4.82 (H), 19.71/19.35 (N), 9.02/8.95 (S) [Figures 6 and 7].

4-(5-(benzylthio)-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl)pyridine (TRN3)

Pale yellow powder, (M.P 189–192°C, yield 64%). IR(KBr, cm^{-1}): 3062 (C-H) aromatic system, 2997 m (C-H) aliphatic, 1597 m, 1554 m, 1527 m (C=C) aromatic system, 1608 s (C=N), 1292 s (C-N), 690 m (C-S); ^{1}HNMR (500 MHz, DMSO-d_6): 8.57 d, 7.66 d (C-H) pyridine ring, 8.35 d, 7.35 d (C-H) nitro-benzene ring, 7.31 m, 7.29 m, 7.24 m benzene ring, 4.45 s (-CH_2); elemental analysis (%Calc./Found): 61.68/61.65 (C), 3.88/3.79 (H), 17.98/17.60 (N), 8.23/8.01 (S) [Figures 8 and 9].

ethyl 2-((4-(4-nitrophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)acetate (TRN4)

Pale brown powder, (M.P 95–97°C,yield 72%). IR (KBr, cm^{-1}):1716 s (C=O) 3062 (C-H) aromatic system, 2993 m, 2866 m (C-H) aliphatic, 1527 m, 1496 m, 1465 m (C=C) aromatic system, 1597 s (C=N), 1307 s (C-N), 686 m (C-S); ^{1}HNMR (500 MHz, DMSO-d_6): 8.57 d, 7.66 d (C-H) pyridine ring, 8.35 d, 7.35 d (C-H) nitro-benzene ring, 1.19 t (-CH_3), 4.10 q (-CH_2) beside oxygen, 4.15 s (-CH_2) beside sulfur; elemental analysis (%Calc./Found): 52.98/52.73 (C), 3.92/4.00 (H), 18.17/17.74 (N), 8.32/7.97 (S) [Figures 10 and 11].

2-((4-(4-nitrophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)acetohydrazide (TRN5)

White crystalline powder, (M.P 230–233°C, yield 62%). IR (KBr, cm^{-1}):1678 s (C=O) amide, 3317 s (N-H), 3078 (C-H) aromatic system, 2981 m (C-H) aliphatic, 1597 m, 1519 m, 1496 m (C=C) aromatic system, 1627 m (C=N), 1292 m (C-N), 686 m (C-S); ^{1}HNMR (500 MHz, DMSO-d_6): 8.57 d, 7.66 d (C-H) pyridine ring, 8.35 d, 7.35 d (C-H) nitro-benzene ring, 3.93 s (-CH_2) beside sulfur, 9.3 s (-NH), 4.3 s (-NH_2); elemental analysis (%Calc./Found): 48.51/48.41 (C), 3.53/3.60 (H), 26.4/25.97 (N), 8.63/8.39 (S) [Figures 12 and 13].

Antibacterial Activity of Synthesized

Compounds

The antibacterial activities of the prepared compounds were investigated by filter paper disc diffusion method (Kirby–Bauer Method). A stock solution of each compound was prepared in dimethyl sulfoxide solvent and stored at 4–8°C until used. The agar plates (Mueller-Hinton agar) in the Petri dishes were inoculated by dipping a sterile cotton swab into the inoculum and evenly streaking the swab in three directions over the entire surface of the plates. Filter paper (6 mm diameter) discs were impregnated with a solution of tested compounds dried and placed on an agar plate containing lawn cultures of certain bacteria. The plates were incubated at an optimum growth temperature (37°C) for 24 h, and then the zone of microbial growth inhibition around the discs was measured (in mm) and compared with amoxicillin and Cefepime. Bacteria species were isolated pathogenic strains.

DISCUSSION

A new class of 1,2,4-triazoles was prepared in appropriate yields as demonstrated in Scheme 1, Table 1 and the

Table 1: Inhibition zone of tested compounds and standard drugs

Comp	Conc. mg/ml	Inhibition zone (mm)			
		S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*
TRN	0.25	0	6	0	0
	0.5	0	15	0	0
	1	0	18	0	0
TRN1	0.25	0	0	0	0
	0.5	0	0	0	0
	1	0	0	0	0
TRN2	0.25	0	0	0	0
	0.5	0	0	0	0
	1	0	0	0	0
TRN3	0.25	10	0	0	0
	0.5	10	0	0	0
	1	12	0	0	0
TRN4	0.25	0	0	0	10
	0.5	0	0	8	13
	1	12	16	8	15
TRN5	0.25	8	0	0	0
	0.5	8	10	0	0
	1	10	18	0	10
Amoxicillin	0.25	0	0	0	0
	0.5	0	8	0	0
	1	0	15	0	0
Cefepime	0.25	18	20	12	0
	0.5	25	20	18	0
	1	25	25	20	0

S. aureus: Staphylococcus aureus, B. cereus: Bacillus cereus, E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa

compounds were confirmed through elemental and spectral results are described in experimental procedures. The starting material INH is condensed with p-nitrophenyl isothiocyanate to give the open compound, TSC which was subjected to cyclization reaction in aqueous alkaline medium to give 1,2,4-Triazole-3-thiol (TRN). The compound (TRN) was reacted with diverse compounds to give mercapto-substituted -1,2,4-triazole. These reactions involved sulfur nucleophilic substitution (an attack by SH) at the alkyl carbon of different R-Br.[16-18] Compound (TRN5) was prepared by reaction of compound (TRN4) with hydrazine hydrate, the reaction includes nucleophilic substitution of hydrazine to form hydrazide (TRN5) compound.[19] The structure of compound (TRN) was declared by ^{1}HNMR spectral results. ^{1}HNMR spectra of the prepared triazole derivatives were performed in deuterated dimethyl sulfoxide as solvent and tetramethylsilane as an internal standard. All spectra showed a signal at 2.5 ppm, which was due to DMSO solvent and all spectra showed a sharp signal at 3.33 ppm due to dissolved water in DMSO.[20] The ^{1}HNMR spectra demonstrated that the C-H aromatic appeared as a doublet at δ 7.263, 7.760, 8.381, and 8.588 ppm while other compounds show in addition to these another signals of alkyl chain and according to R substituents. In compound (TRN5) alkyl chain signals disappeared and displayed a signal at 9.356 ppm as singlet related to N-H proton beside carbonyl (O=C-NH-NH_2), singlet signal at 4.301 ppm related to NH_2 protons, and singlet signal at 3.935 ppm related to methylene group beside sulfur (S-CH_2-).

In antibacterial activity, most of the synthesized compounds showed antibacterial activity against Gram positive and Gram negative bacteria, but some of them showed no activity against (*Staphylococcus aureus*) compound TRN, TRN1, TRN2, and standard amoxicillin at all tested concentration, compound TRN3, TRN4, and TRN5 show slight to moderate activities against *S. aureus* at different concentrations except TRN4 which exhibit activity at high concentration only 1 mg. For compounds TRN1 and TRN2 they did not exhibit any biological activity which they are with side alkyl chain (S-R) which are ethyl and butyl, respectively. Compound TRN, TRN4, and TRN5 exhibit moderate activity against *Bacillus cereus*, also compound TRN4 showed activity at high concentration only.

No compound exhibit activity against Gram-negative except TRN4 and TRN5, TRN4 slightly activity against *Escherichia coli* and moderate activity against *Pseudomonas aerogenosa*, while TRN5 shows moderate activity against *P. aerogenosa* only at high concentration (1 mg), while both standards used did not exhibit activity against *P. aerogenosa*, thus may be due to resistance of this bacteria against 4th cephalosporin AB, tested activity of TRN4 may be related to its polar side chain beside S,[21] TRN5 show slightly activity against this bacteria.[21] The new triazoles, TRN4 and TRN5 containing ester and hydrazide group, respectively, have promising antibacterial activity.[18] The type of substituents beside sulfur had great effect on its activity. However, further studies are required to screen them for cytotoxic effect.

CONCLUSION

The new triazoles, TRN4 and TRN5 containing ester and hydrazide group, respectively, have promising antibacterial activity. The type of substituents beside sulfur had great effect on its activity. However, further studies are required to screen them for cytotoxic effect.

ACKNOWLEDGMENTS

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