ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/26apr1890

Design and Bioevaluation Assessment of Newly Designed Triazole-Based Analogues

Ajay Bagherwal[1]; Dr. Maya Sharma[2]; Dr. Mrunal K. Shirsat[3]

[1]Author, [2,3]Co-Author

[1]Associate Professor, College of Pharmacy Dr. Shri R.M.S. Institute of Science & Technology, Bhanpura, Distt. Mandsaur (M.P.)
[2,3]University/College, State: Pacific Academy of Higher education & research University Udaipur (Rajasthan) India

Publication Date: 2026/05/14

Abstract: A broad spectrum of triazole derivatives incorporating heterocyclic ring systems has been investigated for their potential as therapeutic agents, owing their biological properties, such as antifungal, antimicrobial, antibacterial, anti-inflammatory, anticonvulsant action. The growing challenge of antimicrobial resistance demands the discovery of new compounds with differentiated chemical structures.

In this study, a series of 2-(Dimethyl-amino)-N-(3-Mercapto-5-Phenyl-4H-1,2,4-triazol-4-yl) acetamide derivatives were synthesized. The synthetic pathway involved the conversion of aromatic acids into their corresponding esters, followed by reaction with hydrazine hydrate to form hydrazides. On further reaction with alcoholic KOH and carbon disulfide, the intermediates were converted into their corresponding potassium dithiocarbazinates. Subsequent Cyclization was carried out using hydrazine hydrate resulted in the formation of triazole derivatives. These compounds were then reacted with chloroacetyl chloride and finally with suitable amines to obtain the target molecules.

Structural elucidation of the compounds was performed by nuclear magnetic resonance, infrared, and mass spectrometric analyses.

The antimicrobial activities of the compounds (NJ-01, NJ-02, and NJ-03) were evaluated through the agar diffusion technique at concentrations spanning, from 1000 to 31.25 µg/ml, was employed through systematic dilution. Screening was carried out on Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) organisms using a disk diffusion assay.

The compounds showed strong antibacterial effect on Gram-positive organism's strains and promising antifungal activity effect on Candida *albicans.*

Keywords: *Triazole, Benzoic Acid, Antibacterial Activity, Antifungal Activity and Staphylococus Aureus, Candida Albicans.*

How to Cite: Ajay Bagherwal; Dr. Maya Sharma; Dr. Mrunal K. Shirsat (2026) Design and Bioevaluation Assessment of Newly Designed Triazole-Based Analogues. *International Journal of Innovative Science and Research Technology,* 11(4), 4473-4478. https://doi.org/10.38124/ijisrt/26apr1890

I. INTRODUCTION

A aromatic heterocyclic ring containing nitrogen atoms is known as triazole, which is also referred to S-triazole or pyrrodiazole. This ring system in the different tautomeric forms such as (1H-1,2,4 triazole) and (4H-1,2,4 triazole) both the compounds.

Fig 1 S-Triazole or 1, 2, 4 Triazole

ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/26apr1890

The triazole ring is a five membered heterocyclic system containing three nitrogen atoms and is commonly referred to as s-triazole or pyrrodiazole. This nucleus exists in different tautomeric forms, primarily 1H- and 4H-1,2,4 triazole, which contribute to its chemical versatility.

Compounds containing the triazole moiety have gained have attracted significant interest in medicinal chemistry because of their diverse pharmacological properties, such as including antifungal, antimicrobial, anticonvulsant, antitubercular effects. Several clinically used drugs, such as ribavirin (antiviral) and azole antifungals like fluconazole and itraconazole, incorporate the triazole scaffold, highlighting its pharmacological significance.

Despite the availability of these drugs, the increasing prevalence of microbial resistance and adverse effects limits their long-term effectiveness. Therefore, the design and development of structurally diverse triazole derivatives remain an important area of research for discovering new therapeutic agents.

In this study, a group of novel compounds 1,2,4-triazole derivatives were prepared through a multi-step synthetic approach and evaluated for their antimicrobial potential.

II. EXPERIMEIMENTAL WORKS

The present investigation, a group of triazole derivatives was synthesized & screened for biological action. The progress of reactions was performing by (TLC) Thin-Layer Chromatography using silica gel G plates (0.25 mm thickness). A solvent system of hexane: ethyl acetate (6:4) was employed and iodine vapor were used as the detecting agent.

The synthesized compounds were tested for solubility in various organic solvents at room temperature. Melting points were evaluated by the open capillary tube method and are reported un-corrected.

➢ *Synthesis of Compounds were Carried Out as Per Following Scheme*

The present investigation involved the synthesis of triazole derivatives compounds, which were subsequently assessed for their biological activity. Reaction progress was monitored by (Thin Layer Chromatography) using silica gel G coated plates 0.25 MM thickness. A mixture of hexane: ethyl acetate (6:4) served as the mobile phase, while iodine vapor was employed for spot detection. Visualization of the separated components was carried out in an iodine chamber.

The solubility behavior of the compounds was evaluated in different organic solvents at ambient temperature & Melting points was determined by the open capillary tube are reported without correction.

- *Step I: Synthesization of Aromatic Ester*

Substituted benzoic acids was refluxed with absolute alcohol in the presence of concentrated sulfuric acid. After completion, excess solvent was removed, and the reaction mixture was processed to obtain the corresponding esters, which were purified and confirmed by TLC.

- *Step II: Synthesis of Hydrazides*

The obtained esters were reacted with hydrazine hydrate under reflux conditions to form the corresponding hydrazides, which were isolated and recrystallized.

- *Step III: Formation of Potassium Dithiocarbazinates*

Hydrazides was treated potassium hydroxide (KOH) and carbon disulfide in ethanolic solution, resulting in Potassium dithiocarbazinates and subsequently employed without further purification.

- *Step IV: Cyclization to Triazole Derivatives*

Cyclization of the intermediates was achieved using hydrazine hydrate, leading to the formation of (5-aryl-4-amino-3-Mercapto-1-2-4-triazole)derivatives.

- *Step V: Chloroacetylation*

The synthesized triazole compounds were further reacted with chloroacetyl chloride in benzene to obtain chloroacetylated intermediates.

- *Step VI: Final Derivatives Formation*

These intermediates were then treated with suitable amines under reflux to yield the final target compounds.

III. RESULT AND DISCUSSION

➢ *Spectral Characterization of Synthesized Compounds*

- *Compound NJ-01*

✓ *{2-(Dimethyl-amino)-N-(3-Mercapto-5-Phenyl-4H-1,2,4-Triazol-4-yl)}acetamide*

The Infra-red spectrum revealed a strong absorption band at 3310 cm^{-1} corresponding to N–H stretching vibrations. The presence of aromatic C–H stretching was indicated at 3116 cm^{-1}, whereas aliphatic C–H stretching associated with methyl and methylene groups was observed within the region of 2962.6 cm^{-1} and 2928.8–2858.7 cm^{-1}. A distinct absorption at 2584.5 cm^{-1} confirmed the thiol (–SH) functional group. The carbonyl (C=O) stretching band appeared at 1660.6 cm^{-1}, while the azomethine (C=N) stretching of the triazole ring was detected at 1614.8 cm^{-1}. Additionally, aromatic C=C stretching was noted at 1580.0 cm^{-1}. Bands observed at 760.8 cm^{-1} and 670.0 cm^{-1} were attributed to out-of-plane C–H bending and C–S stretching vibrations.

Mass spectrometric analysis showed a molecular ion peak at m/z 277, which is consistent with the calculated molecular mass of the compound.

The ^1H NMR spectrum (400 MHz, DMSO-d_6) showed a singlet at δ 8.0 ppm corresponding to the NH proton. Aromatic protons appeared as multiplets in the region of δ 7.2–7.6 ppm. A singlet at δ 3.25 ppm was assigned to the

ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/26apr1890

methylene (–CH_2) group, while the thiol proton resonated at δ 3.1 ppm. The dimethyl-amino moiety gave a singlet at δ 2.3 ppm corresponding to six protons.

- *Compound NJ-02*

✓ *{2-(Dimethyl-amino)-N-(3-Mercapto-5-p-tolyl-4H-1,2,4-Triazol-4-yl)}Acetamide*

The IR spectrum exhibited a characteristic N–H stretching band at 3226.6 cm^{-1} along with aromatic C–H stretching at 3106.0 cm^{-1}. Aliphatic C–H stretching vibrations corresponding to methyl and methylene groups were recorded at 2958.0 cm^{-1} and within the range of 2928.8–2848.9 cm^{-1}. A distinct absorption band at 2584.5 cm^{-1} indicated the presence of the thiol (–SH) functionality. The carbonyl (C=O) stretching vibration was observed at 1662.6 cm^{-1}, whereas the C=N stretching band appeared at 1611.4 cm^{-1}. Additionally, aromatic C–H bending vibrations were noted at 787.8 cm^{-1}.

Mass spectral analysis revealed a molecular ion peak at m/z 291, which is in accordance with the expected molecular mass and supports the proposed structure.

The ^1H NMR spectrum displayed a singlet at δ 8.0 ppm corresponding to the NH proton. Aromatic protons were observed as multiplets in the region of δ 7.0–7.2 ppm. The methylene (–CH_2) group appeared as a singlet at δ 3.25 ppm, while the thiol proton resonated at δ 3.1 ppm. Two additional singlets at δ 2.3 ppm and δ 2.1 ppm were attributed to methyl protons.

- *Compound NJ-03*

✓ *{2-(Dimethyl-amino)-N-(3-Mercapto-5-(4-Methoxyphenyl)-4H-1,2,4-Triazol-4-yl)}Acetamide*

The IR spectrum showed an N–H stretching band at 3310 cm^{-1}. Aliphatic C–H stretching vibrations due to methyl and methylene groups were observed at 2952.6 cm^{-1} and in the range of 2928.8–2858.7 cm^{-1}. The presence of the thiol group was confirmed by a band at 2584.5 cm^{-1}. A strong absorption at 1660.6 cm^{-1} corresponded to the carbonyl (C=O) group, while the C=N stretching vibration of the triazole ring was identified at 1614.8 cm^{-1}. Aromatic C=C stretching was observed at 1585.0 cm^{-1}. Bands at 773.4 cm^{-1} and 614.5 cm^{-1} were assigned to out-of-plane C–H bending and C–S stretching vibrations, respectively.

The mass spectrum displayed a molecular ion peak at m/z 307, consistent with the calculated molecular weight of the compound.

In the ^1H NMR spectrum, a singlet at δ 8.0 ppm was assigned to the NH proton. Aromatic protons appeared as multiplets within δ 6.7–6.9 ppm. A singlet at δ 3.7 ppm confirmed the presence of the methoxy (–OCH_3) group. The methylene protons resonated at δ 3.25 ppm, while the thiol proton was observed at δ 3.1 ppm. A singlet at δ 2.3 ppm corresponded to the methyl group.

➢ *In-Vitro Antimicrobial Activity*

The potential activities of the compounds were assessed using the cup plate method by measuring zones of inhibition. Bacterial activity was evaluated against selected Gram-positive and Gram-negative bacterial strains, whereas fungal activity was examined against representative fungal species. Standard reference drugs, namely ampicillin for anti-bacterial activity and griseofulvin for anti-fungal activity, were included for comparison, along with an appropriate solvent control.

The experimental results (Tables 2 and 3) demonstrated that the 1,2,4 triazole derivatives exhibited moderate antimicrobial activity. Differences in activity among the compounds were observed, which can be attributed to the nature of substituent's present on the phenyl ring. In particular, derivatives bearing electron-donating groups such as methyl and methoxy-substituent's showed relatively enhanced activity compared to unsubstituted analogs.

These findings indicate that structural variations within the 1,2,4 triazole framework significantly influence antimicrobial efficacy. Therefore compounds may serve as potential lead molecules for in addition structural optimization and development of more potent antimicrobial agents.

IV. CONCLUSION

The present investigation reports the successful preparation of a library of novel 1,2,4-triazole derivatives, which were structurally characterized using spectroscopic techniques including infrared (IR), proton nuclear magnetic resonance (^1H NMR), and mass spectrometry. The spectral results confirmed the proposed molecular structures of the prepared compounds.

The synthesized molecules were subsequently evaluated for their in vitro antimicrobial activity against selected bacterial and fungal strains using the cup plate technique. The results demonstrated that the obtained derivatives

Exhibited moderate antibacterial as well as antifungal activity in comparison with standard reference drugs, including ampicillin and griseofulvin.

Among the tested derivatives, compounds containing molecules with electron-donating groups (e.g., methyl and methoxy) exhibited relatively better antimicrobial activity, indicating the influence of substituents on the biological profile of the triazole nucleus.

Overall, the study highlights that 1,2,4,-triazole scaffold is a promising Pharmacophore for the design of new antimicrobial compounds. Further structural modifications, detailed pharmacological studies, and toxicity evaluations are recommended to enhance the activity and explore their potential for therapeutic applications.

ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/26apr1890

REFERENCES

[1]. Tsukuda T, Shiratori Y, Watanabe M, Ontsuka H, Hattori K, Shirai M, et al. Modeling, synthesis and biological activity of novel antifungal agents (1) Bioorg Med Chem Lett. 1998; 8:1819–24.

[2]. Küçükgüzel I, Güniz Küçükgüzel S, Rollas S, Otük-Sanis G, Ozdemir O, Bayrak I, et al. Synthesis of some 3-(arylalkylthio)-4-alkyl/aryl-5-(4-aminophenyl)-4H-1,2,4-triazole derivatives and their anticonvulsant activity. Farmaco. 2004; 59:893–901.

[3]. Husain MI, Amir M. Synthesis of some new substituted thiosemicarbazides and triazoles as possible anticonvulsants. J Indian Chem Soc. 1986; 63:317–9.

[4]. Valentina P, Ilango K, Deepthi M, Harusha P, Pavani G, Sindhura KL, et al. Antioxidant activity of some substituted 1,2,4-triazo-5-thione Schiff base. J Pharm Sci Res. 2009; 1:74–7.

[5]. Sahin G, Palaska E, Kelicen P, Demirdamar R, Altinok G. Synthesis of some new 1-acylthiosemicarbazides, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazole-3-thiones and their anti-inflammatory activities. Arzneimittelforschung. 2001; 51:478–84.

[6]. Shivarama Holla B, Sooryanarayana Rao B, Sarojini BK, Akberali PM, Suchetha Kumari N. Synthesis and studies on some new fluorine containing triazolothiadiazines as possible antibacterial, antifungal and anticancer agents. Eur J Med Chem. 2006; 41:657–63.

[7]. Fridkin SK, Jarvis WR. Epidemiology of nosocomial fungal infections. Clin Microbiol Rev. 1996; 9:499–511.

[8]. Alexander BD, Perfect JR. Antifungal resistance trends towards the year 2000. Implications for therapy and new approaches. Drugs. 1997; 54:657–78.

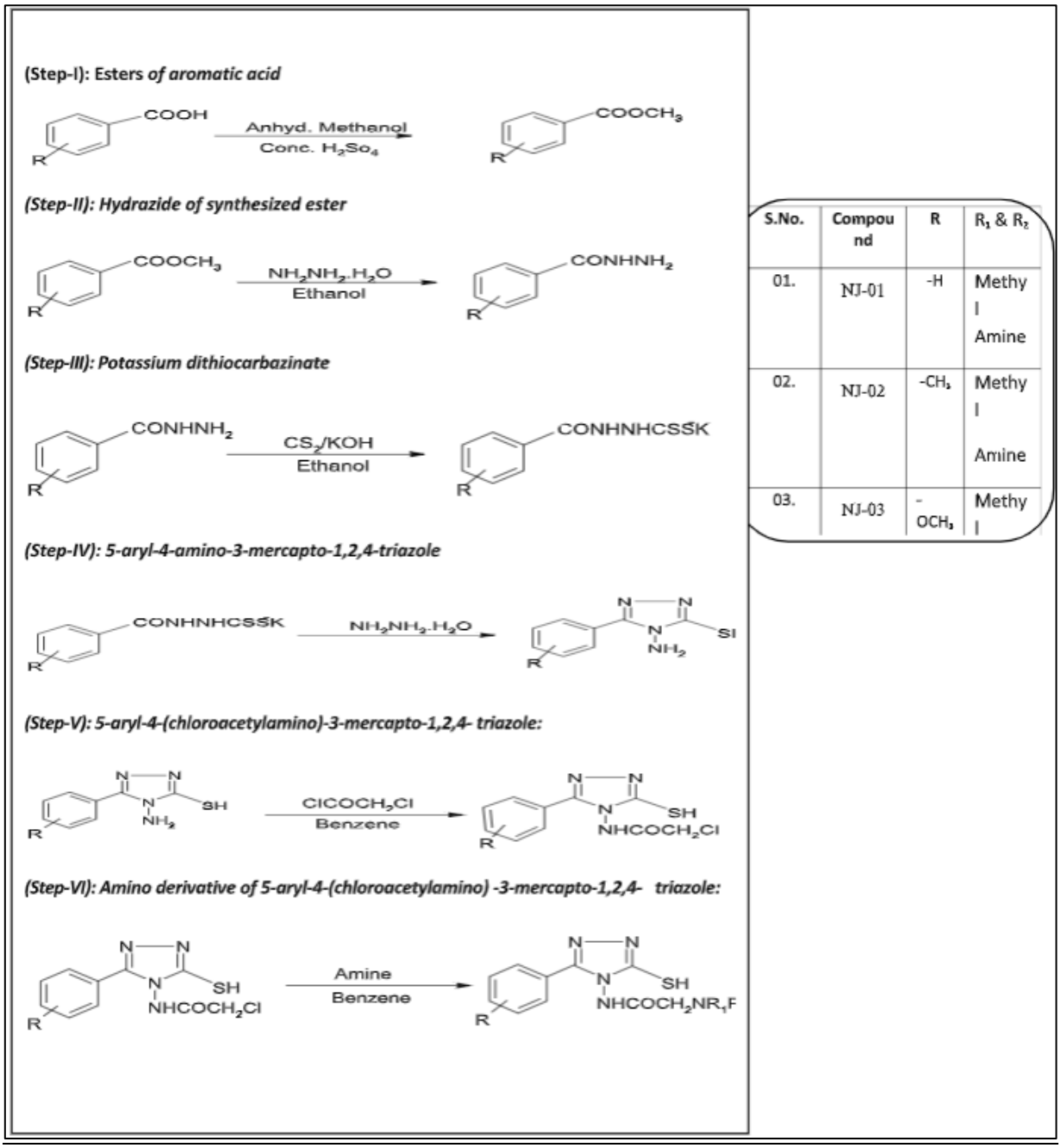
[9]. Sauleau A, Sauleau J. Phosphorus Sulfur Silicon. 2000; 167:219.

[10]. Srivastava, S.K.; Srivastava S.; Srivastava, S.D., Ind. J. Chem. 2002, 41B, , 1937.

[11]. Mishra, R.K.; Tiwari, R.K.; Shrivastava, S.; Bahel, S.C., J. Indian. Chem. Soc., 1991, 68, 110-112.

[12]. Zamini, K.; Faghihi K.; Tofighi, T.; Shariatzadeh, M.R., Turk J. Chem., 2004, 28, 95-100.

ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/26apr1890



S.No.	Compound	R	R_1 & R_2
01.	NJ-01	-H	Methyl Amine
02.	NJ-02	$-CH_3$	Methyl Amine
03.	NJ-03	$-OCH_3$	Methyl

Fig 2 Synthetic Scheme for the Preparation of 1, 2,4-Triazole Derivatives (NJ-01 to NJ-03).

Table 1 Physical Parameters of Triazole Derivatives Synthesized Compounds

S.No.	S. No.	R	Mol. Form.	Rf value	Molecular weight	% Yield	M.P. (°C)
01.	NJ-01	H	$C_{12}H_{15}O_1N_5S_1$	0.56	277	56%	170-172
02.	NJ-02	CH_3	$C_{13}H_{18}O_1N_5S_1$	0.48	291	48%	158-160
03.	NJ-03	OCH_3	$C_{13}H_{18}O_2N_5S_1$	0.59	307	55%	212-214

Table 2 Data of Antimicrobial Activity of Synthesized 1,2,4-Triazole Derivatives

S. No.	Compound	Diameter of zone of inhibition (mm)				
		B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
01.	NJ-01	14	16	15	14	13
02.	NJ-02	15	18	16	15	14
03.	NJ-03	16	19	17	15	15
Ampicillin		16	20	18	15	-
Griseofulvin		-		-	-	16

ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/26apr1890

Table 3 Data of Antibacterial and Antifungal Activity of Synthesized 1, 2, 4-Triazole Derivatives

S. No.	Comp.	*B. subtilis*				*S. aureus*				*E. coli*				*P. aeruginosa*				*C. albicans*			
Dilution		I	II	III	IV-V	I	II	III	IV-V	I	II	III	IV-V	I	II	III	IV-V	I	II	III	IV-V
1	NJ-01	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
5	NJ-02	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
9	NJ-03	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
Ampicillin		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
Griseofulvin																		-	-	-	-

I-1000µg/ml, II-500µg/ml, III-250µg/ml, IV-125µg/ml, V-62.5µg/ml

(-) indicates absence of growth; (+) indicates presence of growth

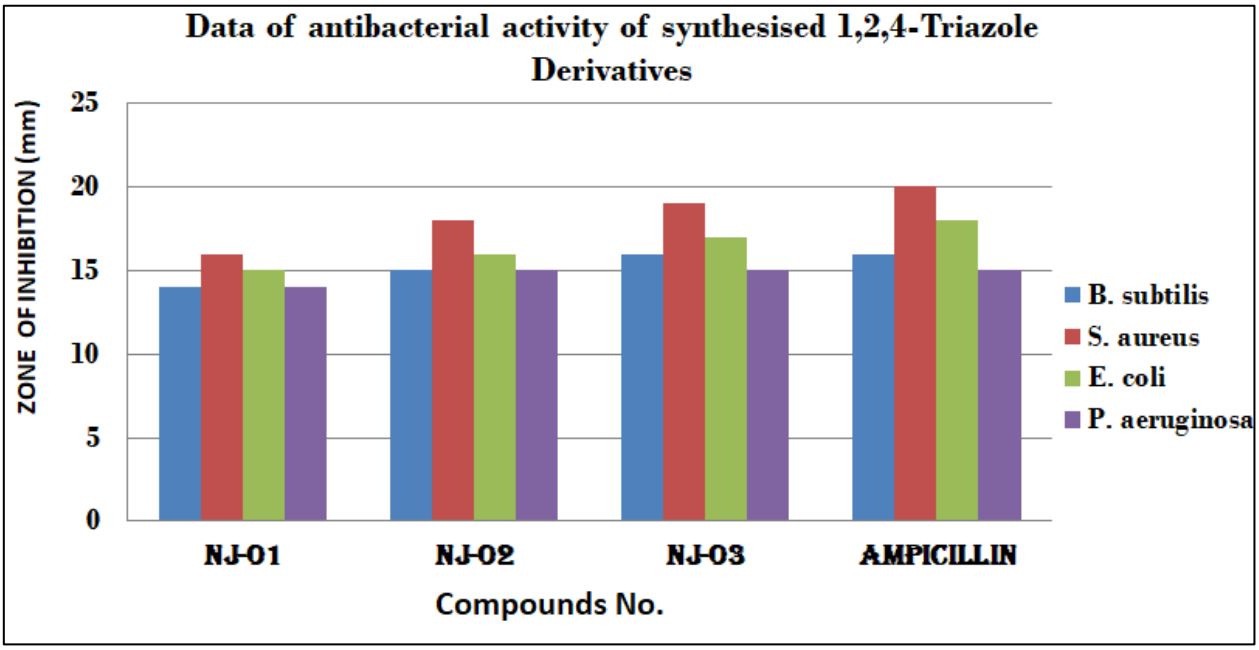


Fig 3 The Bacterial Screening Indicated that Among the Compound No. the Compounds NJ-02 and NJ-03 Moderately Activity Against All Tested Bacterial Stain *staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa.*

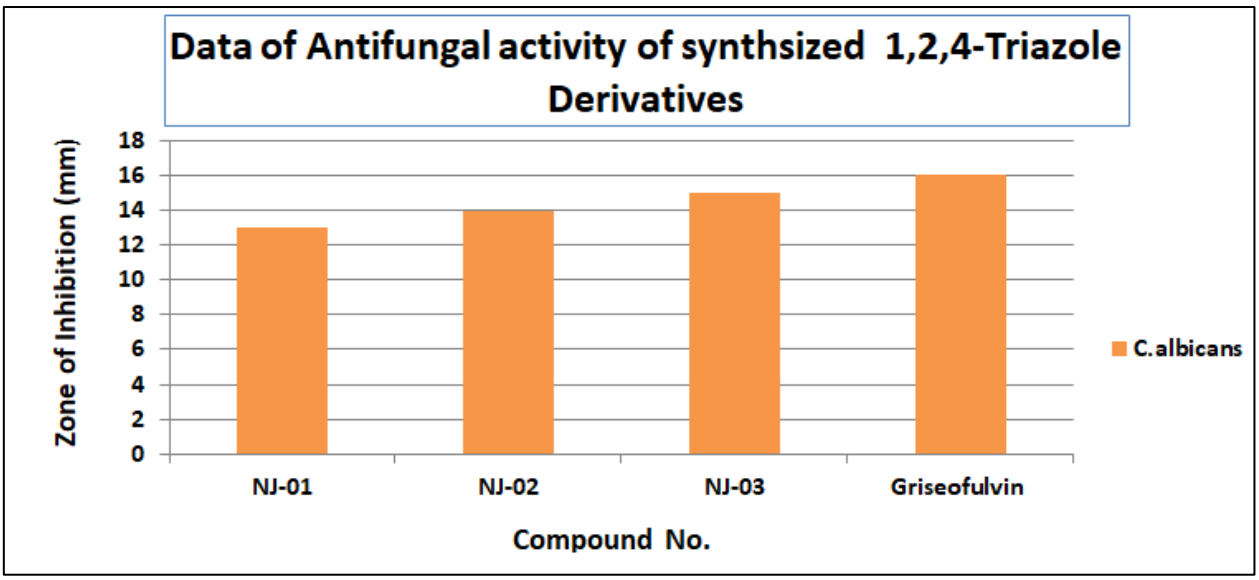


Fig 4 Antifungal Screening Indicated that Among the Compound NJ-03 Reveled that the Test Compounds Showed Moderate Activity Against *Candida albicans.*