Original Article

Spectral Characterization And Biological Screening Of 1,2,4-Triazole Derivatives Of Isothiocyanates

Jyoti Sharma[1] , Nidhi Agarwal[*2]

[1]Principal, Govt. College Tijara, Alwar, sharma_ak002@yahoo.com

[2]Research Scholar, Raj Rishi Bhartrihari Matsya University, Alwar, nidhi.agarwal1210@gmail.com
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Abstract

Triazoles are the heterocyclic compounds having Nitrogen as heteroatoms with carbon. These are of 2 types on the basis of position of heteroatoms in five membered rings. Among which 1,2,4-triazole has been studied much in last decades due to its excessive antimicrobial activities. In this research work N-substituted derivatives of 1,2,4-triazole are prepared with the combination of substituted siothiocyanates and sodium hydroxide. The synthesized compounds were tested for their antimicrobial properties against bacteria E.coli, B.subtillis and fungus A.niger, P.chrysogenum. The compounds revealed moderate to high biological activities.

Key Words: Heterocyclic Compounds, Antimicrobial resistance, 1,2,4-trizole, Spectrocopic methods, Isothicyantes

I. INTRODUCTION:

Antibiotics are the powerful shield to fight with different kind of bacteria or fungi or any microorganism. Most of the antibiotics consists of heterocyclic moiety. Heterocyclic chemistry is an important arm of chemistry which deals with the two third part of organic chemistry. About 69 percent of drugs consist of heterocyclic compounds with different hetero atoms like nitrogen, oxygen and sulfur[(1-2)].

1,2,4-Triazole has the molecular fromula $C_2H_3N_3$, called triazoles, which have a five membered ring of two carbon atoms and three nitrogen atoms of azole ring readily able to bind with a variety of enzymes and receptors in biological systems via diverse non-covalent interactions and thus display versatile biological activities.[3] Triazole can be derived by replacement of oxygen of oxadiazole nucleus with nitrogen atom.[4] They are aromatic ring compounds similar to the azole, pyrazole and imidazole but with an additional nitrogen atom in the ring structure.[5]

There are two possible isomers of triazole: 1,2,4-triazole and 1,2,3-triazole.

1,2,3-triazole 1,2,4-triazole

Fig. 1: Types of Triazole compounds

A survey of the literature reveals that 1,2,4-triazoles and their fused heterocyclic derivatives exhibit a diverse range of biological activities. The 1,2,4-triazole core was being integrated into a wide range of clinically pertinent agents, including voriconazole (antifungal), posaconazole, ribavirin (antiviral), rizatriptan (antimigraine), alprazolam (anxiolytic), trazodone (antidepressant), letrozole, and anastrozole (antitumoral). In recent decades, scientists have focused on the synthesis of 1,2,4-triazole derivatives with various biological activities such as antifungal[6-7], antitubercular[8], antioxidant[9], anticancer[10,] anti-inflammatory[11], analgesic[12], antidiabetic [13], anticonvulsant[14], and anxiolytic[15]. Due to the lower toxicity and higher bioavailability of triazole compounds, as well as an increased specificity for fungal cytochrome p450 and a lower impact on human sterol synthesis, triazole-based pharmacophore has replaced the previously widely used imidazole pharmacophore in systemically active azoles[16].

According to the recent report of ICMR (Indian Council of Medical Research) uses of antibiotics are increasing indiscriminately in recent time. Due to this wide use of antibiotics pathogens are becoming resistant towards these drugs and number of these resistant microbes is increasing day by day. Therefore some infections can not be cured by these available drugs. This random use of drugs and resistance into pathogens could take the form of pandemic in future. So, however vast range of antibiotics are available commercially but still there is a huge demand for newer drugs due to the increasing resistance against microbes. Keeping this into mind researchers are continuously working in this field for the development of new antimicrobial drugs so that this resistance could be minimize.

In this scheme different 1,2,4-triazole derivatives were prepared. For this preparation we have used benzoic acid , ethanol as solvent, hydrazine hydrate, derivatives of aniline for the synthesis of aryl isothiocyanate, and Sodium hydroxide palletes. These derivatives were then tested for their capacity to prevent the development of fungus and bacteria.

II. EXPERIMENTAL SECTION:

The open capillary technique was utilized to determine the melting points, and the data obtained were not modified in any way. Iodine vapors were used to analyze the spots, while the reaction was observed silica gel coated glass plates for thin-layer chromatography. We generated ^{1}HNMR and ^{13}CNMR spectra by employing a Bruker Advance Neo 500MHz NMR spectrophotometer, by using tetramethyl silane internal standard, and DMSO solvent. To capture IR spectra, an “F. T. InfraRed Spectrophotometer Model RZX” was utilized (Perkin Elmer). An “LC-MS Spectrometer Model Q-ToF Micro Waters” was utilized to calculate the mass spectra. The triazole derivstives were synthesized according to the following scheme:

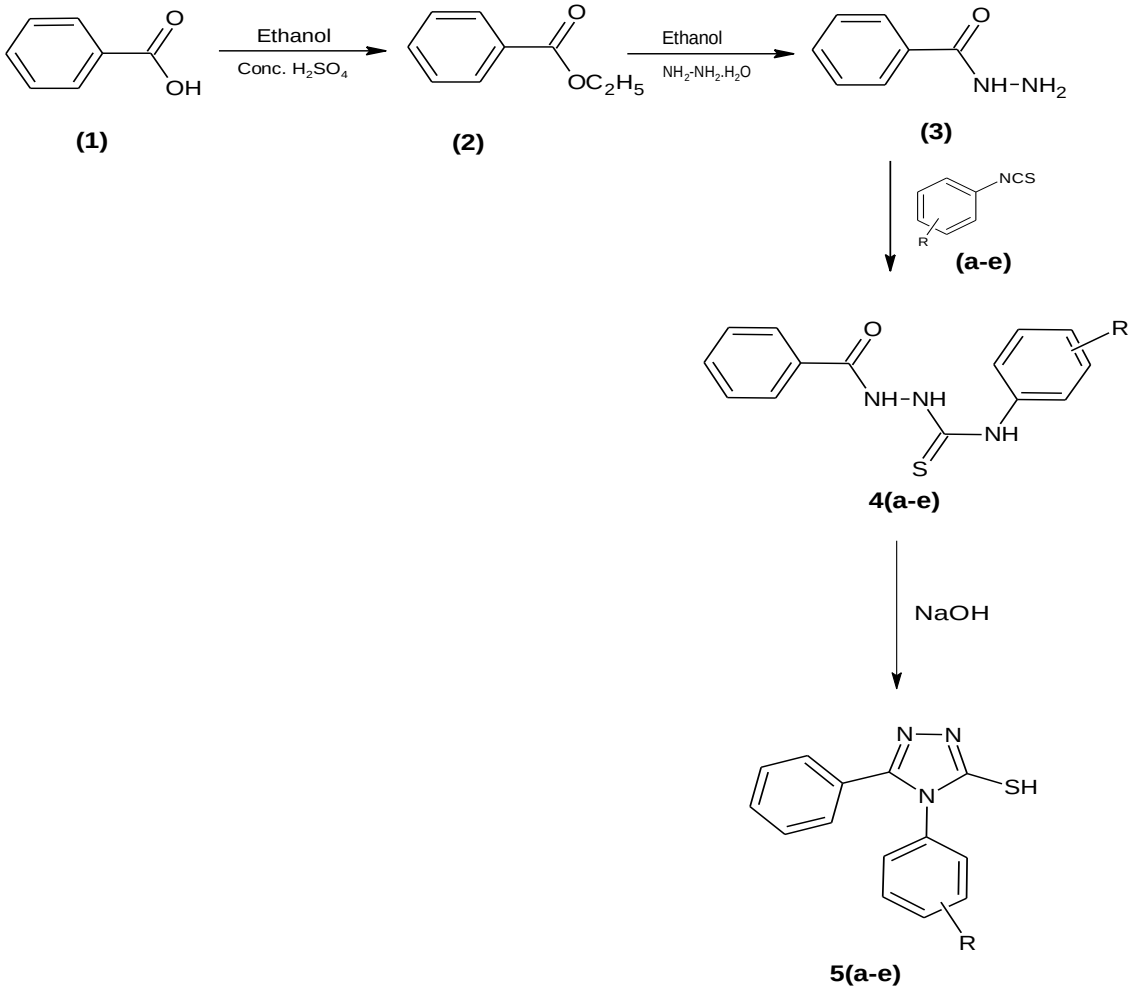


Fig. 2 : Synthetic scheme for 1,2,4-triazole derivatives

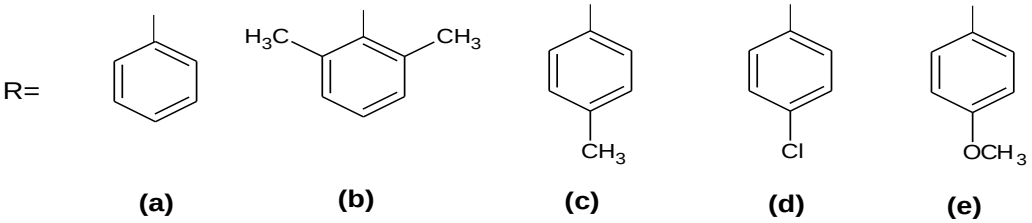


Fig. 3 : Substituents used in synthetic scheme for 1,2,4-triazole derivatives

General steps for the synthesis of precursor and 1,2,4-triazole derivatives:

Step-1: Ethyl benzoate synthesis (2):

A mixture of benzoic acid (0.0623 mol) and ethanol 12 ml was refluxed for 7 hours at 40-50 degree Celsius on water bath and 0.5 ml of concentrated sulfuric acid was mixed with this solution. After reflux reaction mixture was concentrated, cooled and recrystallized with ethanol.[17]

Yield: 70%; b.p. 211-213°C; Molecular formula: $C_9H_{10}O_2$; Molecular weight: 150.17, **^{1}H NMR**: δ 1.22 (3H, t, CH_3), 4.17 (2H, CH_2 of ethyl group, q,), 7.45 (2H, m-Ar-H), **IR(KBr, cm^{-1})**: 3183.1 (Ar-H stretching), 3040.9 (C-H stretching), 1233.8 (C-O stretching), 1700.7 (C=O stretching);

Step-2: Synthesis of benzohydrazide (3):

Compounds **3** was produced as a result of a reaction in which ethyl benzoate (**2)** (0.0523 mol) and hydrazine hydrate (6 ml) were refluxed together for nine hours at temperatures ranging from 30 to 40 degrees celsius. After filtering and washing the residue with water, ethanol was employed to recrystallize.

Yield: 79%; m.p. 112-114°C; Molecular formula: $C_7H_8N_2O$; Molecular weight: 136.15, **^{1}H NMR**: δ 7.47 (2H, m- Ar-H, dddd,), 7.58-7.83 (3H, Ar-H present on ortho and para position), **IR(KBr, cm^{-1})**: 3442.7 (N-H stretching), 3183.5 (Ar-H stretch), 1712.1 (Amide C=O stretching), 889.3 (NH-NH stretching);

Step-3: Synthesis of 2-benzoyl-N-(substitutedphenyl) hydrazine-1-carbothioamide 4(a-e) :

A combination of 24 ml ethanol and substituted phenyl isothiocynates[18] (a-e) was refluxed over water bath for 2 hours for the formation of substituted carbotioamides (4a-4e). This mixture was then cooled, concentrated and dried , filtered and crystallized with methanol.

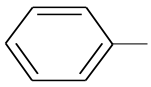
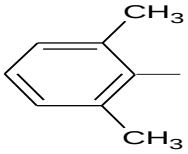
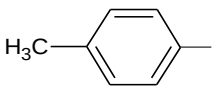
^{1}H NMR: δ 6.95 (1H, present on para position of phenyl ring, tt,), 7.18-7.40 (4H, Ar-H present on ortho and meta, 7.25), 7.51-7.65 (3H, Ar-H on para and meta of ring), 8.00 (2H, Ar-H on ortho position), **IR (KBr, cm^{-1})**: 3188.2 (Ar-H stretching), 1708.2 (Amide C=O stretching), 3407.7 (N-H stretching), 1316.6 (C=S stretching), 907.4 (N-N stretching).

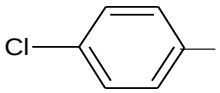
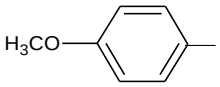
Step 4: Synthesis of 1,2,4-triazole derivatives 5(a-e) with N-substitutes:

Compounds **5a-5e** were produced as a result of technique given below, and they were subsequently identified as follows[19-20]:

A fusion of substituted carbothioamides (4a-4e) and ethanol 30 ml was mixed with 4N NaOH solution (2.5 ml) for the preparation of clear solution and then refluxed in round bottom flask for 1 hour on water bath. After reflux filtered, cooled and pH was maintained by adding dilute glacial acetic acid.

Table 1: Physiochemical data of the synthesized compound 5(a-e) Scheme 3

Compound	R	Molecular Formula	Mw.	% Yield	m.p. (°C)	Rf$^{solvent\ system}$
5a		$C_{14}H_{11}N_3S$	253.31	70	367-369	0.87[a]
5b		$C_{16}H_{15}N_3S$	281.37	83	188-190	0.73[a]
5c		$C_{15}H_{13}N_3S$	267.34	78	201-203	0.54[a]

5d		$C_{14}H_{10}ClN_3S$	287.76	69	207-209	0.98[a]
5e		$C_{15}H_{13}N_3OS$	283.34	59	211-213	0.72[a]

[a]Ethyl benzoate: Petroleum ether (4:6 v/v)

4,5-diphenyl-4H-1,2,4-triazole-3-thiol (5a):
^{1}H NMR: δ 7.35-7.73 (8H, Ar-H at para, meta & ortho position of phenyl ring), 8.02 (2H, Ar-H at ortho position, dtd, J = 7.8, 1.5, 0.4 Hz). **IR(KBr, cm^{-1})**: 3194.1 (Ar-H stretching), 2345.3 (S-H stretching), 1381.4 (ter. Amine stretching), 1480.8 (=C-N stretching), 1399.6 (C-N-C stretching), 1221.8 (N-N stretching).**MS m/z**: 253.06(M^+). **Anal. Calculated For: $C_{14}H_{11}N_3S$**: C-66.37, N-16.59, H-4.38, S-12.66; Found: C-65.45%, N-16.39%, H-3.88%, S-11.88%.

4-(2,6-dimethylphenyl)-5-phenyl-4H-1,2,4-triazole-3-thiol (5b):
^{1}H NMR: δ 2.14 (6H, s, CH_3), 6.93-7.12 (3H, Ar-H at meta, para of amine 6.99 (t, J = 7.8 Hz), 7.34-7.57 (3H, Ar-H at meta, para of acid group), 7.93 (2H, Ar-H at ortho of acid group, dtd, J = 7.8, 1.5, 0.4 Hz). **IR(KBr, cm^{-1})**: 3211.1 (Ar-H stretching), 3045.1 (C-H stretching), 2329.2 (S-H stretching), 1461.6 (ter. Amine stretching), 1533.2 (=C-N stretching), 1397.6 (C-N-C stretching), 1210.0 (N-N stretching).**MS m/z**: 281.09 (M^+). **Anal. Calculated For: $C_{16}H_{15}N_3S$**: C-68.30, N-14.93, H-5.37, S-11.40; Found: C-67.95%, N-14.90%, H-5.98%, S-10.08%.

4-(4-methylphenyl)-5-phenyl-4H-1,2,4-triazole-3-thiol (5c):
^{1}H NMR: δ 2.16 (3H, s, CH_3), 7.36 (2H, Ar-H at meta of amine, ddd, J = 8.0, 1.3, 0.5 Hz), 7.43-7.57 (3H, Ar-H at meta, para of acid group), 7.75 (2H, Ar-H at ortho of amine, ddd, J = 8.0, 1.3, 0.5 Hz), 8.01 (2H, Ar-H at ortho of acid group, dtd, J = 7.8, 1.5, 0.4 Hz). **IR(KBr, cm^{-1})**: 3202.4 (Ar-H stretching), 3040.5 (C-H stretching), 2342.2 (S-H stretching), 1155.9 (ter. Amine stretching), 1491.1 (=C-N stretching), 1376.1 (C-N-C stretching), 1209.6 (N-N stretching).**MS m/z**: 267.08 (M^+). **Anal. Calculated For $C_{15}H_{13}N_3S$**: C-67.39, N-15.72, H-4.90, S-11.99; Found: C-66.83%, N-14.02%, H-4.77%, S-10.48%.

4-(4-chlorophenyl)-5-phenyl-4H-1,2,4-triazole-3-thiol (5d):
^{1}H NMR: δ 7.44-7.57 (3H, Ar-H at meta, para of acid group), 7.65-7.89 (4H, Ar-H at ortho, meta of amine) 8.02 (2H, Ar-H at ortho of acid, dtd, J = 7.8, 1.5, 0.4 Hz). **IR(KBr, cm^{-1})**: 3211.8 (Ar-H stretching), 2351.2 (S-H stretching), 1348.5 (ter. Amine stretching), 1532.7 (=C-N stretching), 1405.2 (C-N-C stretching), 1222.7 (N-N stretching), 1044.1 (C-Cl stretching).**MS m/z**: 287.02 (M^+). **Anal. Calculated For $C_{14}H_{10}ClN_3S$**: C-58.43, N-14.60, H-3.50, S-11.14, Cl-12.32; Found: C-57.25%, N-15.02%, H-3.99%, S-10.32%, Cl-11.54%.

4-(4-methoxyphenyl)-5-phenyl-4H-1,2,4-triazole-3-thiol (5e):
^{1}H NMR: δ 3.69 (3H, s, CH_3), 7.16 (2H, Ar-H at meta of amine, ddd, J = 8.6, 1.4, 0.5 Hz), 7.40-7.70 (5H, Ar-H at para, meta of acid group & ortho of amine), 7.93 (2H, Ar-H at ortho of acid, dtd, J = 7.7, 1.5, 0.4 Hz) **IR(KBr, cm^{-1})**: 3195.8 (Ar-H stretching), 3015.3 (C-H stretching), 2330.3 (S-H stretching), 1386.7 (ter. Amine stretching), 1533.5 (=C-N stretching), 1403.4 (C-N-C stretching), 1223.7 (N-N stretching), 1190.8 (=C-O stretching).**MS m/z**: 283.07(M^+). **Anal. Calculated For $C_{15}H_{13}N_3OS$**: C-63.58, O-5.65, N-14.83, H-4.62, S-11.32; Found: C-63.00%, O-5.02%, N-14.44%, H-3.84%, S-10.38%.

III. BIOLOGICAL SCREENING:

The Agar well diffusion technique[21] was employed to assess the newly synthesized compounds **5(a-e)** for their potential to prevent bacterial growth in vitro. In addition, these tests were carried out to determine the capabilities of the substances. Depending on the specific type of pathogen used, 30, 60 or 90 µg/ml was

the concentration used for the pathogens. This collection of pathogens comprised both Gram-positive and negative strains of bacterium B. subtillis and E. coli. When the common antibiotic ciprofloxacin was used, it revealed an inhibitory zone of 31millimetres for E. coli and 13millimetres for B. subtillis at 100 milligrams per milliliter concentration. The findings are outlined in **Table-5.49**, which may be seen further down on this page.

In a manner analogous to this, the Modified Agar well diffusion technique[22] was utilized to evaluate the newly synthesized compounds 5(a-e) for their ability to inhibit fungal growth. Aspergillus niger and Penicillium chrysogenum, separate fungus species, were tested at levels of fifty and one hundred µg/ml, respectively. In the experiment, which used the medication ketoconazole as a point of comparison, the zone of inhibition for A. niger measured 31 millimetres, while the zone for P. chrysogenum measured 23 millimetres. The findings are summarized in **Table-5.50**, which may be seen below.

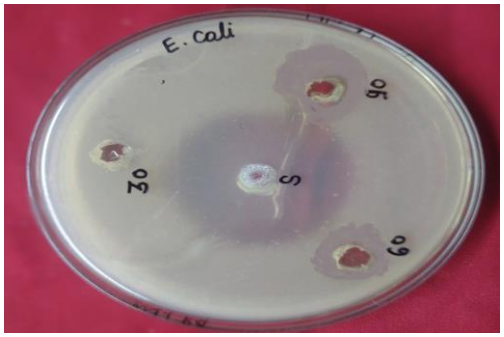


Fig.4 Compound 5c inhibits growth of Bacteria

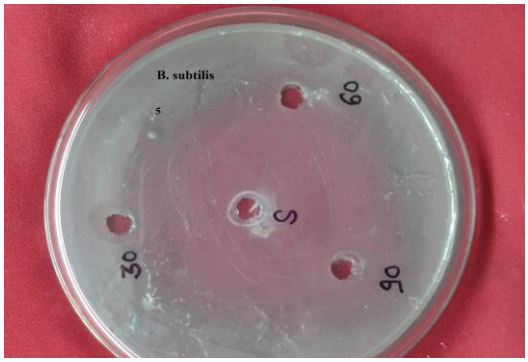


Fig.5 Compound 5d inhibits growth of Bacteria B.subtiliis

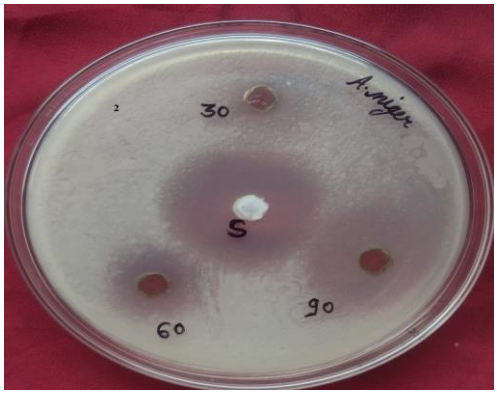


Fig.6 Compound 5b inhibits growth of fungi A,niger

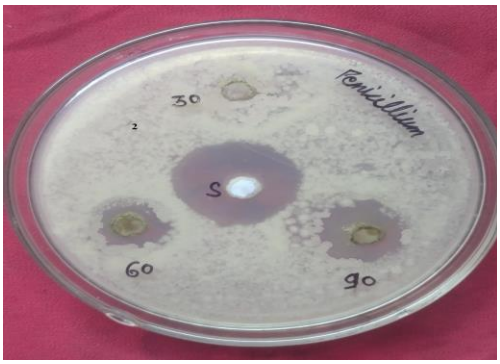


Fig.7 Compound 5d inhibits growth of fungi Penicillium

Table 2: Antibacterial activity of synthesized compounds 5(a-e)

Compound	**Inhibition Zone (mm)**					
	Gram –ve (E. coli)			**Gram +ve (B. subtillis)**		
Conc. µg/ml	30 µg	60 µg	90 µg	30 µg	60 µg	90 µg
5a	**11**	**18**	**25**	00	05	09
5b	10	17	20	01	04	09
5c	**09**	**20**	**27**	00	05	08
5d	00	10	18	**04**	**08**	**11**

5e	00	09	17	00	00	04
Ciprofloxacin	30	31	31	11	12	13

Table 3: Antifungal activity of synthesized compounds 5(a-e)

Compound	**Inhibition Zone (mm)**					
	A. niger			**P. chrysogenum**		
Conc. µg/ml	30 µg	60 µg	90 µg	30 µg	60 µg	90 µg
5a	**09**	**14**	**25**	09	15	17
5b	**13**	**18**	**27**	00	04	15
5c	00	09	15	00	06	13
5d	06	10	13	**10**	**16**	**21**
5e	00	13	18	**04**	**14**	**19**
Ketoconazole	30	31	31	21	22	23

IV. RESULTS & DISCUSSION:

Spectral Characterization:

Compounds were prepared in high yield (ranging from 60-90%) and identified with the help of spectroscopic methods.

- Compound's purity was examined by TLC on silica Gel plates with solvent system ethyl benzoate: Petroleum ether (4:6 v/v) and exposure of spots was done with the help of iodine vapours.
- IR spectra of compounds shows N-N stretching vibration at 1209.6 -1223.7 cm^{-1} , C-N-C vibrations at 1376.1-1403.4 cm^{-1} and C=N stretching vibrations at 1480.8-1533.5 cm^{-1} which reveals the presence of triazole ring in the compound. Here one peak of S-H stretching is found at 2329.2-2351.2 cm^{-1} which denotes the presence of thiol moiety. In this spectra peak at 1155.9-1461.6 cm^{-1} denotes the presence of tertiary amine in the molecule.
- NMR spectra of synthesized compounds shows multiplate at δ 7.35-7.73 which indicates the presence of hydrogen at para, meta position of the N-substituted phenyl ring. Multiplate at δ 7.67-8.02 indicates the presence of 4 aromatic protons at ortho position of triazole in phenyl ring. NMR spectra of synthesized compound **5b & 5c** shows singlet at δ 2.14 which indicates the presence of $–CH_3$ group. NMR spectra of synthesized compound **5e** shows singlet at δ 3.69 which indicates the presence of $–OCH_3$ group.
- Mass spectra of compounds **5a-5e** shows molecular ion peak at m/z 253.06, 281.09, 267.08, 287.03 & 283.07 respectively.

^{1}HNMR and IR spectra of some biologically active compounds are as follows:

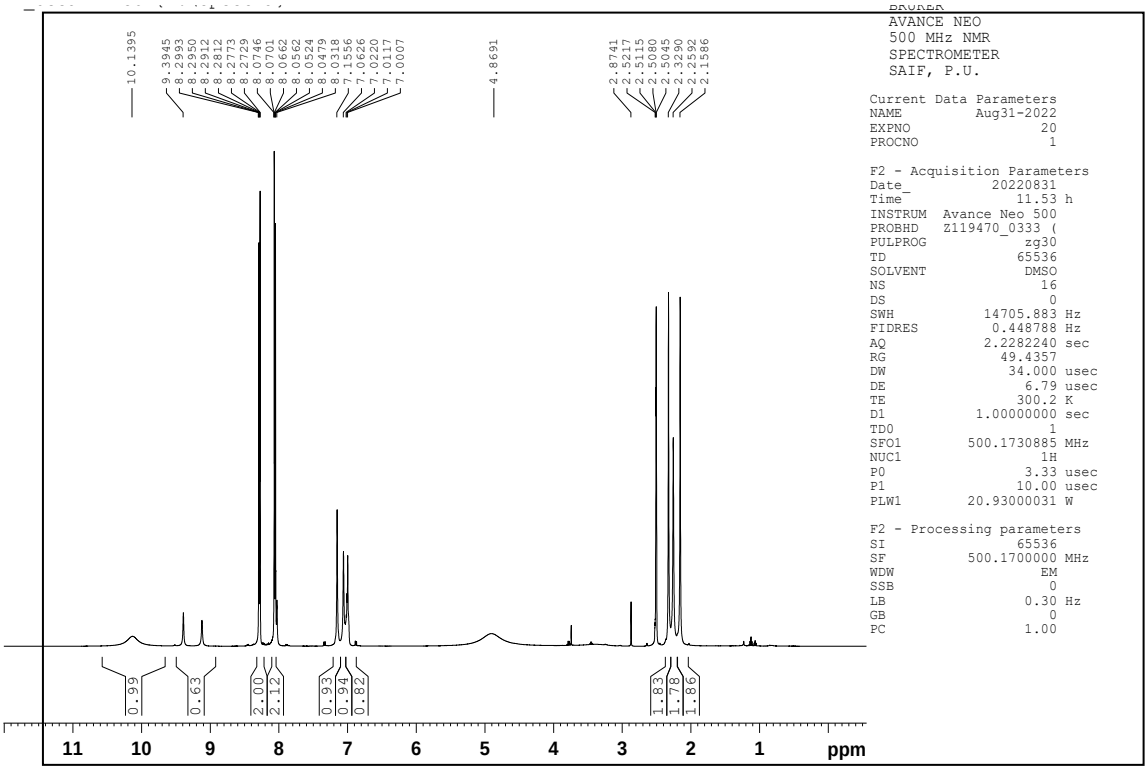


Fig. 8: ^{1}HNMR spectra of 4-(4-methylphenyl)-5-phenyl-4H-1,2,4-triazole-3-thiol (5c)

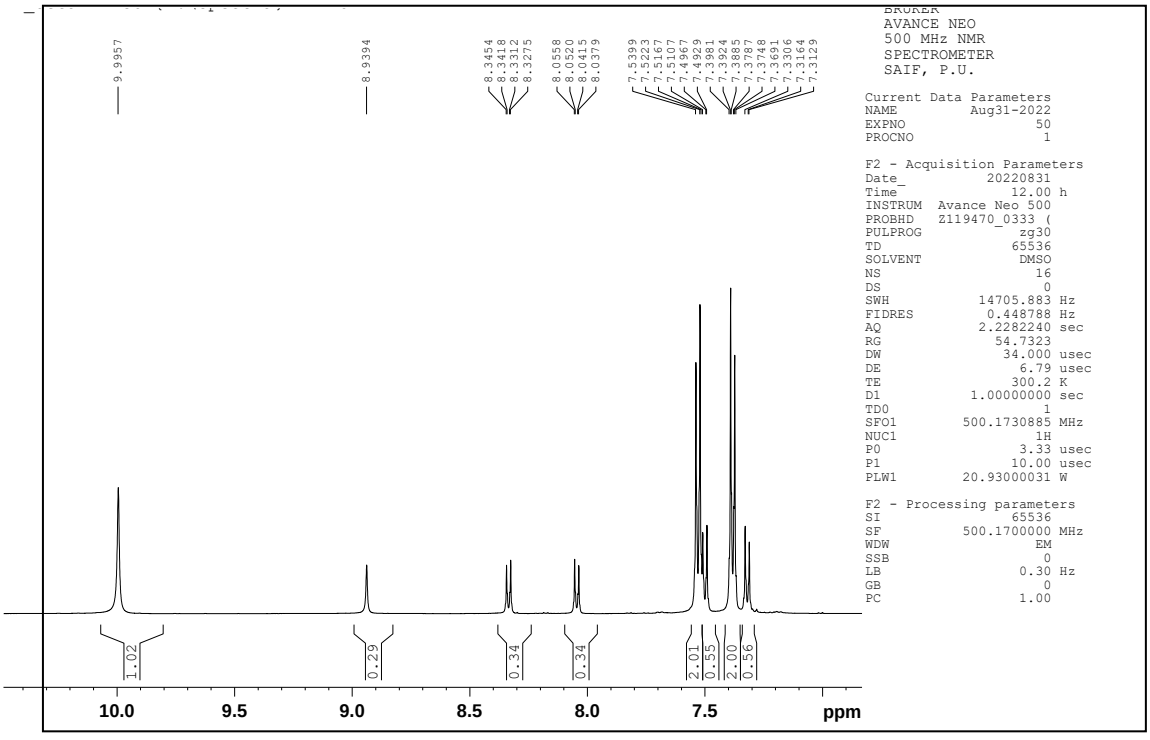


Fig. 9: ^{1}HNMR spectra of 4-(4-chlorophenyl)-5-phenyl-4H-1,2,4-triazole-3-thiol (5d)

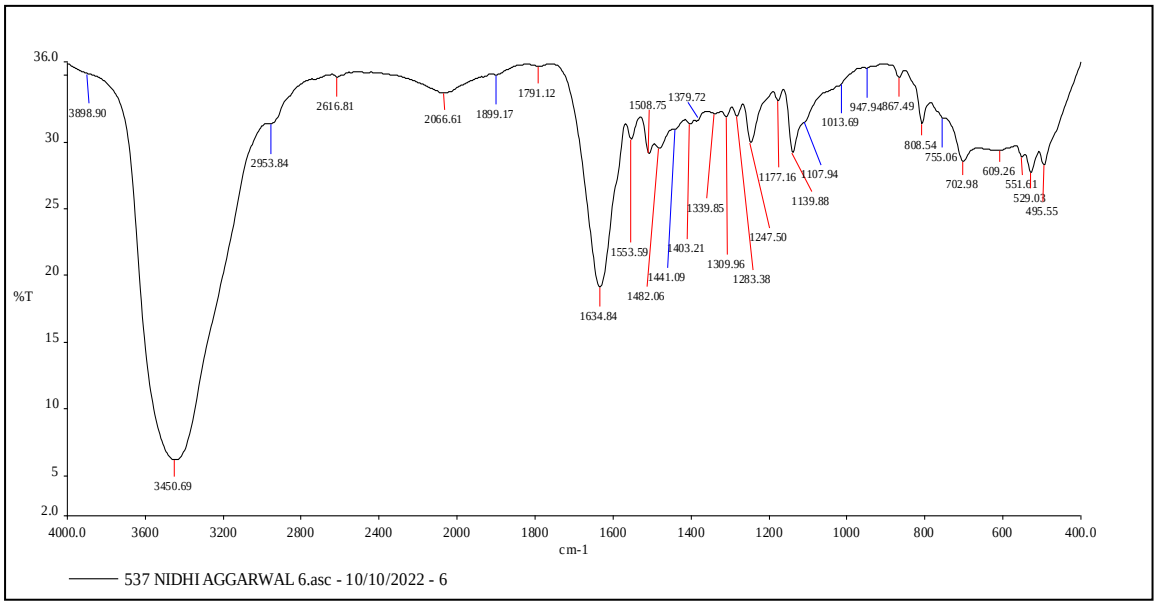


Fig. 10: FT-IR spectra of 4-(4-methylphenyl)-5-phenyl-4H-1,2,4-triazole-3-thiol (5c)

Anti-Microbial Activity:

The synthesized compounds were screened for antibacterial and antifungal activity by Agar well diffusion method and Modified agar well diffusion method respectively. Outcomes of this analysis shows that 1,2,4-triazole derivative **5a** without any substituent on the phenyl ring and **5c** having $–CH_3$ at para position of N-substituted phenyl ring revealed significant antibacterial activity against Gram negative bacteria E. coli. Other derivatives show good to moderate antibacterial activity against E.coli whereas compound **5d** containing –Cl group at the para position of N-substituted phenyl ring showed significant antibacterial activity against Gram positive bacteria B. subtillis.

According to the antifungal assay of 1,2,4-triazole derivatives **5a** without any substituent and **5b** having 2,6-dimethyl groups at N-substituted phenyl ring revealed very good antifungal activity against A. niger whereas compounds **5d** with –Cl substituent at 4^{th} position of N-substituted phenyl ring and **5e** having methoxy at 4^{th} position of the N-substituted phenyl ring revealed significant antifungal activity against P. chrysogenum strain. Other compounds showed low to moderate antifungal activity. The results are represented in the **Fig:11, Fig:12, Fig:13** and **Fig:14** for antibacterial and antifungal activity respectively.

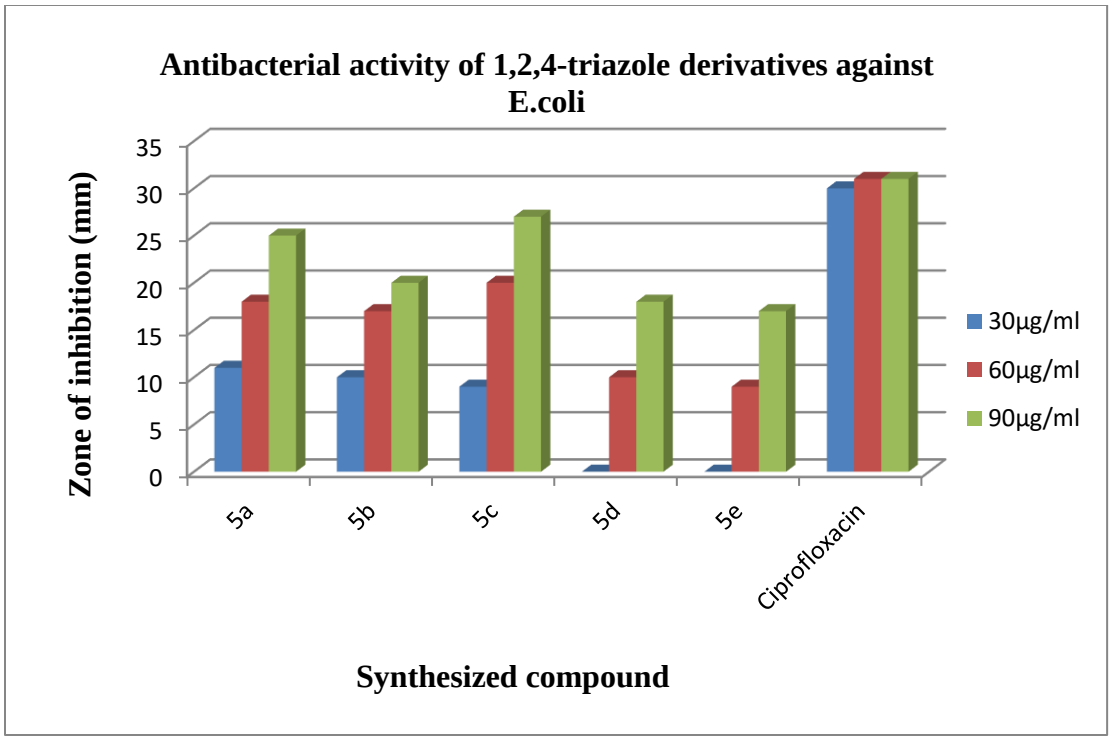


Fig. 11: Antibacterial activity of 1,2,4-triazole derivatives 5(a-e) against E.coli

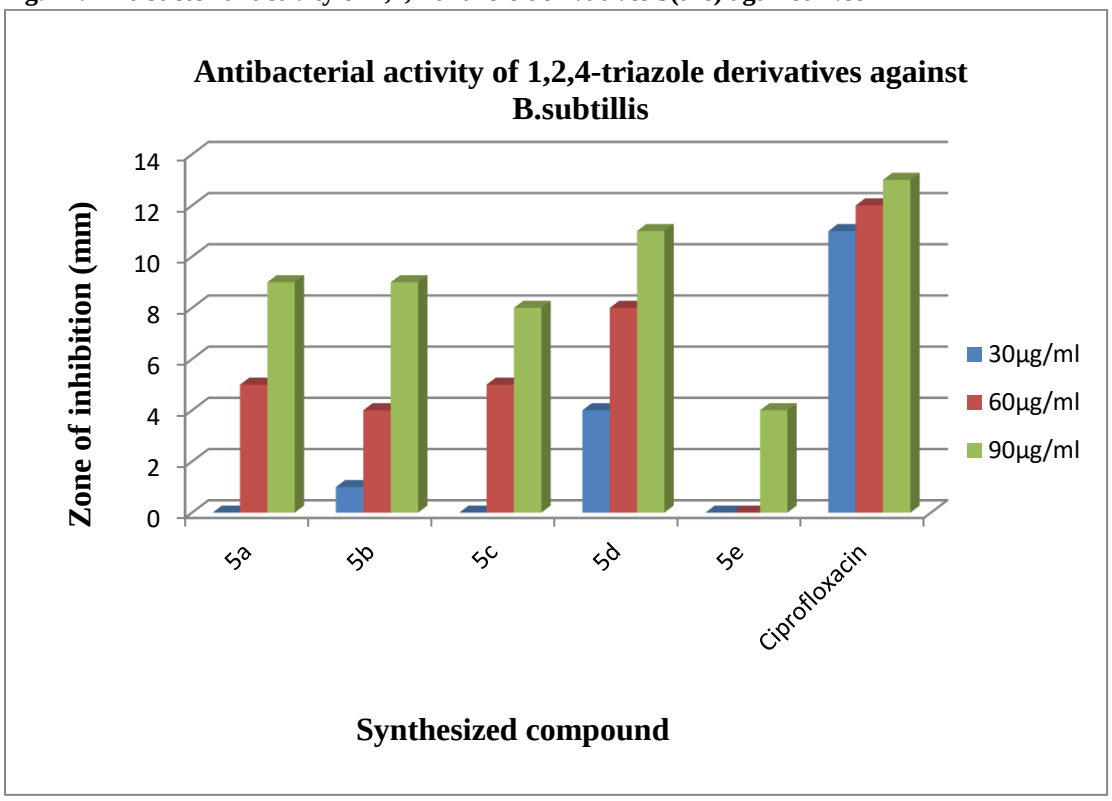


Fig. 12: Antibacterial activity of 1,2,4-triazole derivatives 5(a-e) against B. subtillis

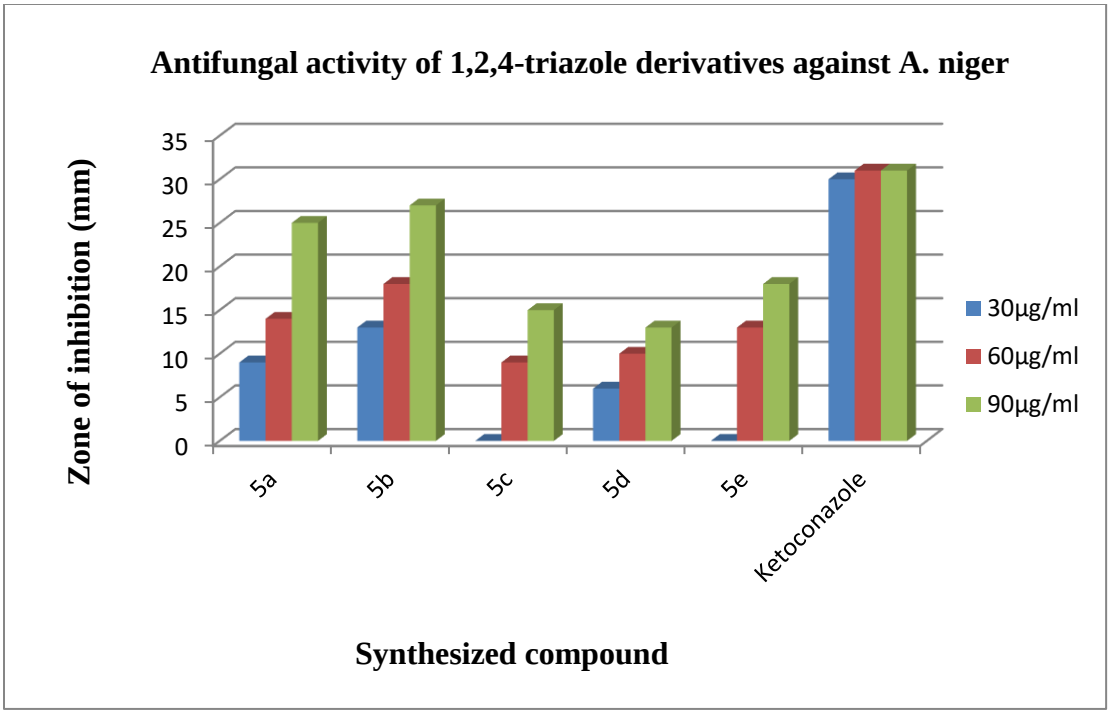


Fig. 13: Antifungal activity of 1,2,4-triazole derivatives 5(a-e) against A. niger

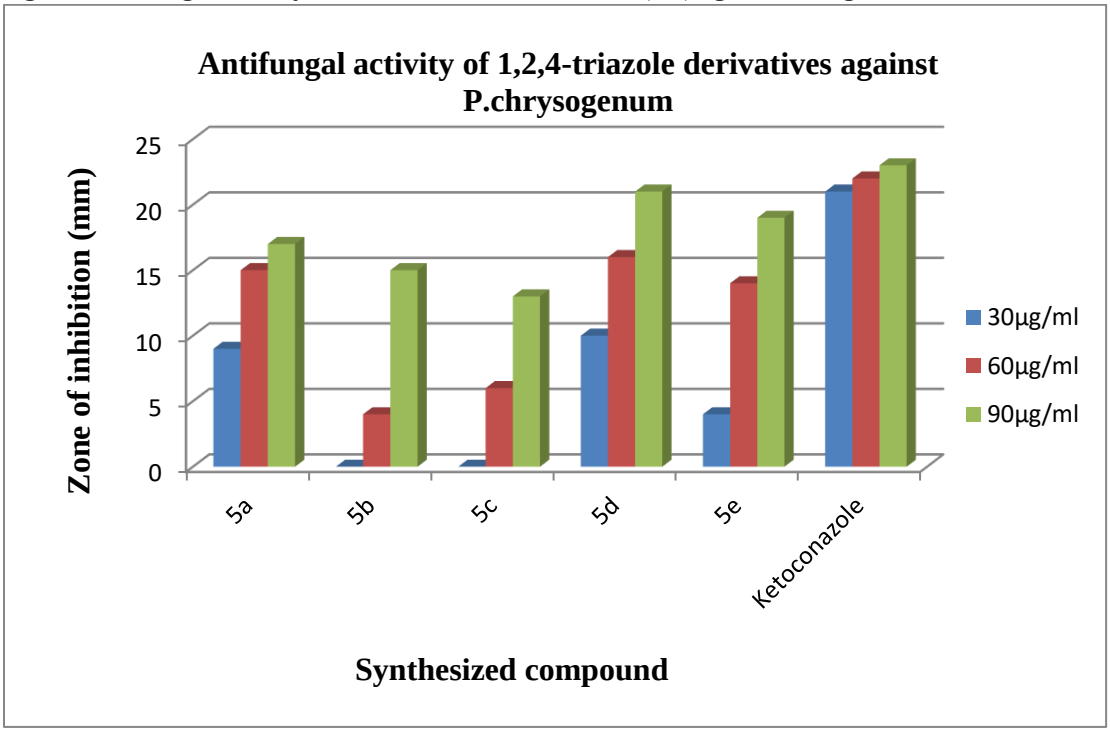


Fig. 14: Antifungal activity of 1,2,4-triazole derivatives 5(a-e) against P. chrysogenum

V. CONCLUSION:

In present research work different N-substituted 1,2,4-triazole derivatives were prepared. Synthesis was being started with benzoic acid and it was converted into benzohydrazide with hydrazine hydrate. Then carbothioamides derivatives were synthesized with the reaction of benzohydrazide and aryl isothiocyantes.

When these carbothioamides reacted with NaOH solution substituted-1,2,4-triazole-3-thiol derivatives **(5a-5e)** were crystallized. Among these compounds **4,5-diphenyl-4H-1,2,4-triazole-3-thiol** (**5a)**, **4-(4-methylphenyl)-5-phenyl-4H-1,2,4-triazole-3-thiol** (**5c)** and **4-(4-chlorophenyl)-5-phenyl-4H-1,2,4-triazole-3-thiol** (**5d)** showed significant antibacterial as well as antifungal activity. These findings make the produced 1,2,4-triazole derivatives an intriguing lead chemical for further synthetic and biological testing.

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