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Synthesis, Characterization and Evaluation of 3, 5-Disubstituted-1, 2, 4-Triazole Derivatives Using Propylamine

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ABSTRACT

Heterocyclic compounds have great applications in pharmaceutics because they have characteristic chemical reactivity. The large number of synthetic heterocyclic compounds has found broad use, for example as an anticancer agent, antitubercular, analeptics, analgesic, hypnotics and as pesticides, insecticides and weed killers. Antibiotic resistance is a fact of life that we must accept and confront. Nowadays immunity of human beings is decreasing day by day. Because of strong antibiotics taken for long time there is an alteration in the balance of microorganisms. Increasing problem of antimicrobial resistance has made highly urgent to design and develop novel types of antimicrobial agents consisting different chemical structures from the traditional drugs. Taking in a view of the applicability of heterocyclic compounds, we have been synthesizing 3-Amino-5-Substituted phenyl-1,2,4-Triazole analogues. The structures were confirmed with help of IR, NMR, and Mass spectroscopy. The compounds were tested for antibacterial and antifungal activity and have shown poor to moderate activity.

1. INTRODUCTION

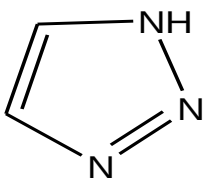
The discovery and development of a new drug entity (NDE) to become a commercial drug is a costly, complex and time-consuming process. A NDE is expected to meet an unmet medical need or to improve therapy where existing drugs have proved ineffective. The need of compound is satisfied by searching leads, isolating and purifying active principles of animals, plants, by synthetic routes and by microorganisms and their fermentation. Most of the drugs fit in the class of heterocyclic compounds. Heterocyclic compounds are an integral part of chemical, life sciences and play a vital role in metabolism of all living organisms and have broad spectrum of pharmacological activities.[1]

Synthesis of compounds including five membered heterocyclic rings has drawn interest over the past decade because of their use in various applications. In the field of medicinal chemistry, azoles are widely used and studied class of antimicrobial agents due to their high therapeutic index and safety profile. Ribavirin, Rizatriptan, Alprazolam, Vorozole, Letrozole, and Anastrozole are the best examples of drugs containing 1,2,4-triazole moiety.[2][3]

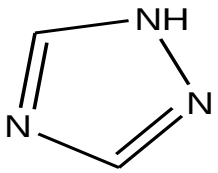
1.1 Rationale for Synthesis of 1,2,4- Triazole Derivatives:

Triazole heterocycles occupy a central position in modern heterocyclic chemistry, principally because this heterocyclic ring is an important recognition element in biologically active molecules.[4]

Triazole refers to either one of a pair of isomeric chemical compounds with molecular formula $C_2H_3N_3$, having a five membered ring of two carbon atoms and three nitrogen atoms. The two isomers are: 1,2,3-triazole and 1,2,4-triazole. Each of these has two tautomers that differ by which nitrogen has a hydrogen bonded to it.[5]



1,2,3-Triazole



1,2,4-Triazole

1,2,4-Triazole and its derivatives represent one of the most biologically active class of compounds and are associated with diverse pharmacological activities such as antibacterial, antifungal, hypoglycemic, antihypertensive and analgesic properties. The substituted 1,2,4-

triazole nucleus is particularly common and can be found in marketed drugs such as Fluconazole, Terconazole, Rizatriptan, Alprazolam and Triazolam. Increasing problem of antimicrobial resistance has made highly urgent to design and develop novel types of antimicrobial agents consisting different chemical structures from the traditional drugs. Many pieces of research have focussed their attention on triazole ring by as antimicrobial agents. It is rational to investigate triazole compounds as novel antimicrobial agents since triazole has shown a broad range of pharmacological activity.[6][7]

Recent literature survey demonstrates that the 1,2,4-Triazoles are becoming of great practical significance. 1,2,4-Triazole derivatives possess Analgesic, Antipyretic and Antiphlogistic properties. A group of 1,2,4-Triazole-5-ones and their Mannich bases have shown antitubercular activity and have been investigated with regard to their mode of action.[8][9]

In view of this, a series of 3-Amino-5-Substituted Phenyl-1,2,4-Triazole analogues have been synthesized and their antibacterial and antifungal activities were evaluated.

2. MATERIALS AND METHODS

2.1 Materials

All the chemicals required for the work were purchased partly from Loba Chemicals and Pallav Chemicals.

2.2 Instrumentation

Throughout this work solvent were used after distillation. Melting points of synthesized organic compounds were determined on “Veego” VMP-I apparatus and are uncorrected. Monitoring the reactions and checking the purity of the final compounds was performed by the Thin Layer Chromatography (TLC) using silica G 60 F254 plates and visualized under ultraviolet (UV). The IR spectra were recorded in the 4000-400 cm-1 range in SHIMADZU IR INFINITY by placing sample directly on probe. 1H NMR was recorded on BRUKER AVANCE II (400MHz) spectrometer in DMSO as solvent using Trimethylsilane (TMS) as internal reference standard and values were expressed in **δ** ppm. LCMS were done using WATER MICROMASS Q- Tof MICRO. Spectral analysis was recorded at Sophisticated Analytical Instrumentation Facility, Panjab University, and Chandigarh. Biological activities were done at Maratha Mandal’s Nathajirao G. Halgekar Institute of Dental College & Research Centre, Belgaum, Karnataka.

2.3 Experimental Section [10][11]

Step 1: Synthesis of Semicarbazones:

0.01 mole of Semicarbazide hydrochloride and 0.02 mole of Sodium acetate was mixed in 10ml water. To this substituted 0.01 mole of Aromatic aldehyde was added slowly with continuous stirring. Turbid solution was formed. To form clear solution Methanol was added till clear solution is formed. It was stirred on magnetic stirrer and reaction was monitored using TLC. On completion of reaction, it was dumped in ice cold water. Final product was filtered off. Hexane: Ethyl acetate (2:1).

Step 2: Synthesis of 3,5-Disubstituted-1,3,4-Oxadiazole:

Mixture of 0.01 mole of Semicarbazone and 0.02 mole of Sodium acetate was taken in round bottom flask. To this Glacial acetic acid was added till all dissolves. It was stirred on magnetic stirrer for 2 minutes, then to this liquid Bromine in Glacial acetic acid was added (0.7ml in 5ml). It was stirred on magnetic stirrer and reaction was monitored using TLC. On completion of reaction, it was dumped in ice cold water. Final product was filtered off. Hexane: Ethyl acetate (2:1).

Step 3: Synthesis of 3,5-Disubstituted 1,2,4-Triazole:

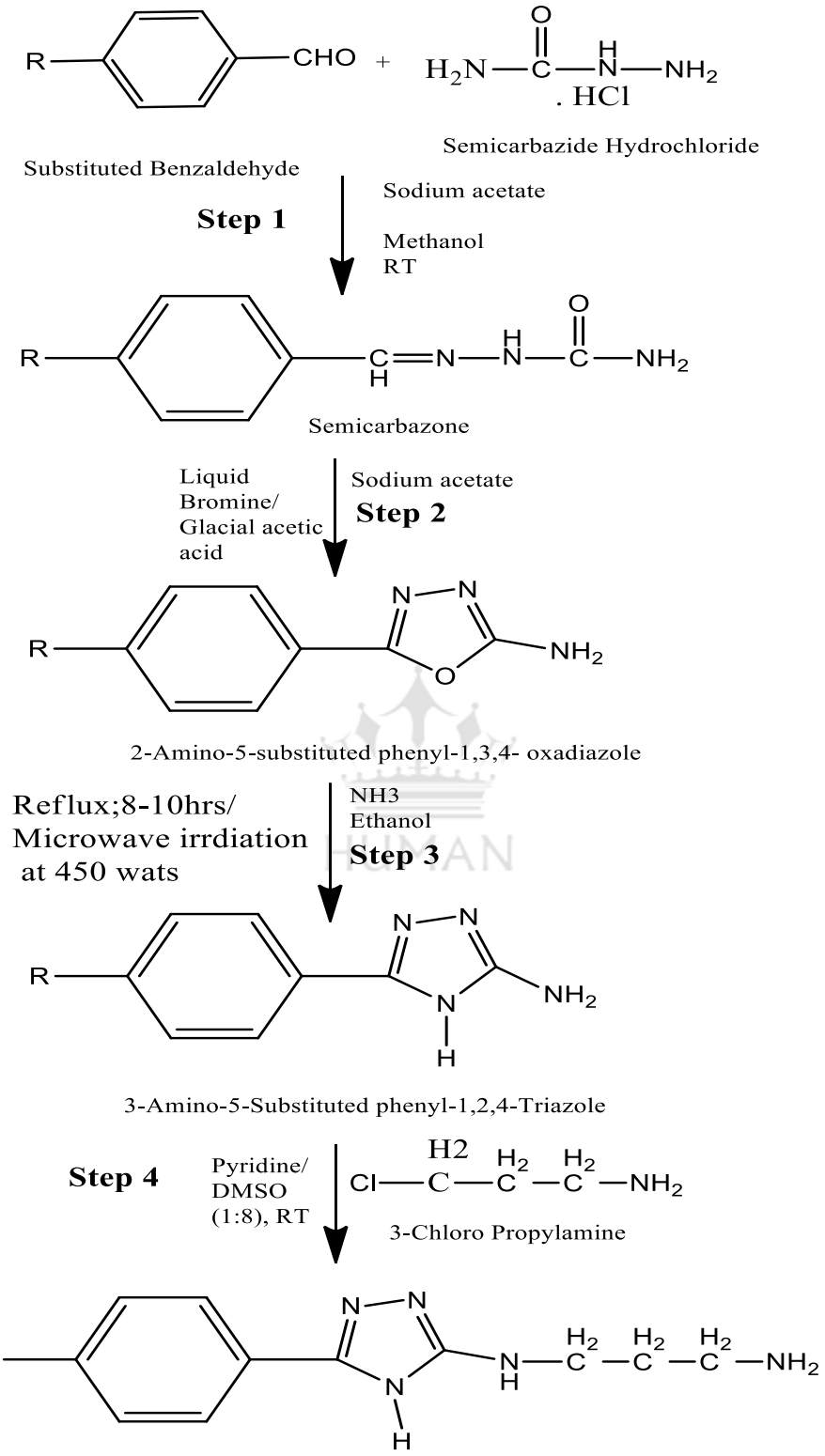
0.01 mole of 3,5-Disubstituted-1,3,4-Oxadiazole was refluxed in Ethanol with 25% Ammonia in microwave for 20 mins at 450 watts/ Heating mantle for 8 hrs. and reaction was monitored using TLC. On completion of reaction, it was dumped in ice cold water. Final product was filtered off. Hexane: Ethyl acetate (2:1).

Step 4: Synthesis of 3,5-Disubstituted 1,2,4-Triazole derivative:

0.01 mole of 3,5-Disubstituted-1,2,4-Triazole was dissolved in DMSO. To this 0.08 mole of Pyridine was added and was stirred on magnetic stirrer for 30 minutes at 4°C. To this 0.01 mole of 3-Chloropropylamine was added slowly over a period of 2hrs. It was stirred on magnetic stirrer and reaction was monitored using TLC. On completion of reaction crude product was obtained from ice cold water.

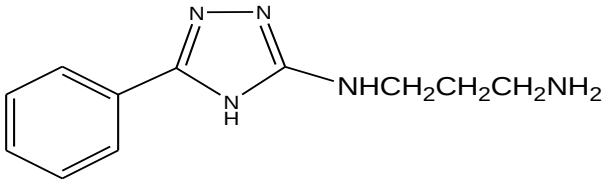
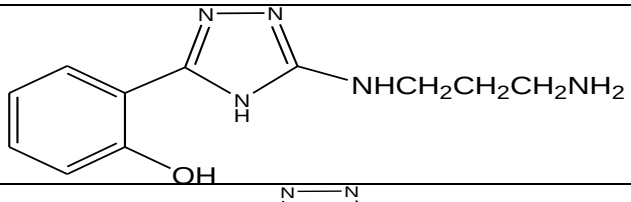
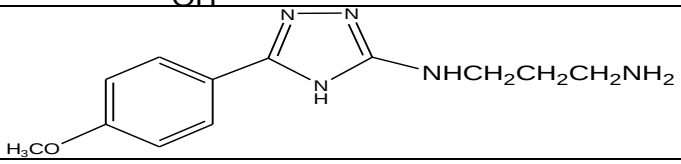
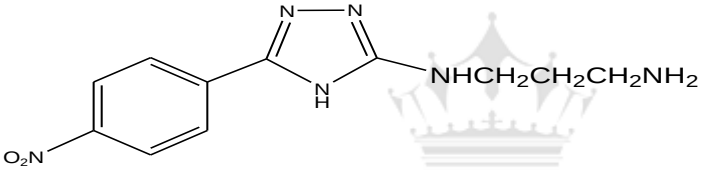
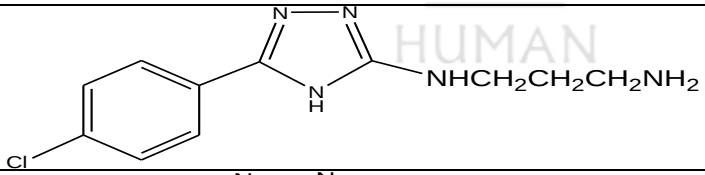
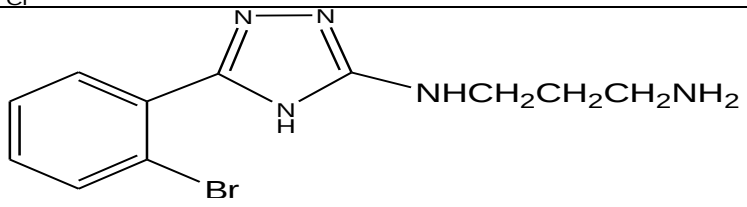
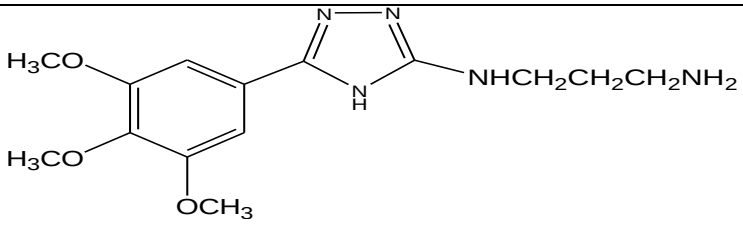
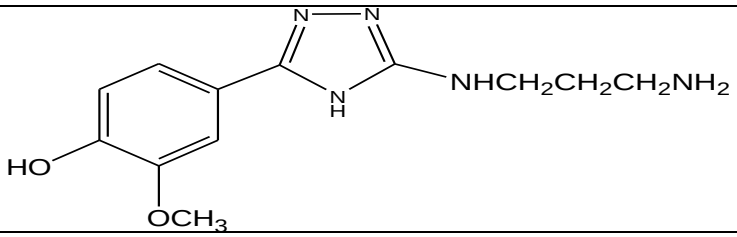
2.3.1 Scheme of Synthesis:

A



The structure, Rf values, Melting point are given in **Table No.1.**

Table No. 1: List of Final Compounds Synthesized

Compound Code	Structure	Rf Value	M.P. (ºC)
F1		0.83	230-233
F2		0.67	239-242
F3		239-242	208-211
F4		0.49	198-202
F5		0.55	229-231
F6		0.64	212-215
F7		0.56	218-221
F8		0.51	187-189

2.3.2 Characterization:

Spectral Characteristics of the Synthesized Compounds is as follows:

1. N-(3-aminopropyl)-5-phenyl-4H-1,2,4-triazol-3-amine

IR (cm^{-1}): NH stretch (3269.34), NH_2 stretch (3101.54), KC=N Imine stretch (1656.85), C-N Vibration (1402.25).

^{1}HNMR (DMSO-d_6, δ, ppm): 12.12 (1H, s, N-H), 7.83 (1H, s, N-H), 7.70-7.33 (3H, m, aromatic phenyl), 6.48 (2H, s, NH_2), 2.50-2.49 (6HK, m, CH_2)

MS: M/z = **217.36** $(M-1)^+$

2. 2-(5-(3-aminopropylamino)-4H-1,2,4-triazol-3-yl) phenol

IR (cm^{-1}): O-H stretch (3147), C=N Imine stretch (1687.71), N-H stretch (3493.09), C-H aromatic stretch (3053.32) & 2985.81 cm^{-1}, C=C aromatic stretch (1489), C-H bending vibration (756.10).

^{1}HNMR (DMSO-d_6, δ, ppm): 12.1369 (1H, s, N-H), 7.6285-7.6476 (1H, s, N-H), 7.1326-7.1752 (1H, m, C-H aromatic), 6.7907-6.8616 (2H, m, C-H aromatic), 6.2967 (2H, s, N-H), 5.1789 (1H, m, O-H), 2.5201-2.5334 (4H, m, CH_2).

MS: M/z = **233.65** $(M-1)^+$

3. N-(3-aminopropyl)-5-(4-methoxyphenyl)-4H-1,2,4-triazol-3-amine

IR (cm-1): O-H stretch (1114), OCH_3 stretch (2985.81), C=C aromatic (1506), C=N Imine= (1579), C-N vibration (1409.96), N-H stretch (3159.40), NH_2 = (3412).

^{1}HNMR (DMSO-d_6, δ, ppm): 12.35 (1H, s, N-H), 7.80 (1H, s, N-H), 7.60-7.62 (2H, m, C-H aromatic), 6.89-6.92 (2H, m, C-H aromatic), 6.35 (2H, s, NH_2), 3.36 (3H, s, CH_3), 2.52-2.53 (6H, m,CH_2).

MS: M/z = **247.56** $(M-1)^+$

4. N-(3-aminopropyl)-5-(4-nitrophenyl)-4H-1,2,4-triazol-3-amine

IR (cm^{-1}): NO_2 stretch (1334.74), C=C aromatic (1506), C=N Imine= (1579), C-N vibration (1409.96), N-H stretch (3159.40), NH_2 (3412).

^{1}HNMR (DMSO-d_6, δ, ppm): 12.3261 (1H, s, N-H), 8.1825-8.2043 (2H, m, C-H aromatic),

7.9433-7.9738 (2H, m, C-H aromatic), 7.4354 (1H, s, NH), 6.5809 (2H, s, N-H), 2.5321-2.5746 (4H, m, CH_2), 1.2419 (2H, m, CH_2).

MS: M/z = **262.76** $(M-1)^+$

5. N-(3-aminopropyl)-5-(4-chlorophenyl)-4H-1,2,4-triazol-3-amine

IR (cm-1): C-Cl stretch (775.38), C-N vibration= (1402.25), C=N Imine stretch (1593.20), N-H stretch (3101.64), C=C aromatic stretch (1485.19), NH_2 stretch (3269.34).

^{1}HNMR (DMSO-d_6, δ, ppm): 12.03 (1H, s, N-H), 7.62-7.64 (1H, s, N-H), 7.13-7.17 (1H, m, C-Haromatic), 6.79-6.86 (2H, m, C-H aromatic), 6.29 (2H, s, N-H), 2.52-2.53 (4H, m, CH_2), 1.40 (1H, m, CH_2).

MS: M/z = 253.64 $(M-1)^+$

6. N-(3-aminopropyl)-5-(2-bromophenyl)-4H-1,2,4-triazol-3-amine

IR (cm^{-1}): C-Br stretch (752.24), NH_2 stretch (3456.44), N-H stretch (3061.03, C-vibration (1408.04), C=N imine stretch (1643.35), C=C aromatic stretch (1512.19), CH_2 bend (1352.10). **MS:** M/z = **296.19**$(M-1)^+$

7. N-(3-aminopropyl)-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-3-amine

IR (cm^{-1}): OCH_3 stretch (2985.81), NH_2 stretch (3510.45), N-H stretch (33381.2) C-N stretch (1357.89), C=N stretch (1670.35), C=C stretch aromatic (1610.56, 1411.89), CH_2 bending (1448.54).

^{1}HNMR (DMSO-d_6, δ, ppm): 12.5463 (1H, s, N-H), 7.4354 (1H, m, N-H), 6.8984-6.9203 (2H, m, CH aromatic), 6.5809 (2H, s, NH_2), 3.7263 (3H, s, OCH_3), 2.5321-2.5746 (5H, m, CH_2), 1.2419 (1H, m, CH_2)

8. 4-(5-(3-aminopropylamino)-4H-1,2,4-triazol-3-yl)-2-methoxyphenol

IR (cm^{-1}): O-H stretch (3334.92), OCH_3 stretch (3263), C=C aromatic (1409), C=N Imine (1566.20), C-N vibration (1641.42), N-H stretch (3159.40), NH_2 (3385.07).

^{1}HNMR (DMSO-d_6, δ, ppm): 12.6123 (1H, s, N-H), 7.8672 (1H, s, N-H), 6.9264-6.9474 (2H, m, CH_2 aromatic), 6.4398 (1H, s, NH_2), 5.0010 (1H, s, OH), 3.8007(3H, s, OCH_3), 2.5253-2.5721 (4H, m, CH_2), 1.0769-1.2433 (2H, m, CH_2)

2.3.3 Biological Activity [12][13][14]

All derivatives of 3,5-Disubstituted 1,2,4-Triazole were screened for antibacterial, antitubercular and antifungal activities.

Antibacterial Activity:

1. The bacterial strains were procured from ATCC (ATCC, Manassas, VA, USA) and Mueller– Hinton broth II (MHB II) (Becton Dickinson) was used to propagate the bacteria.

2. The compounds were serially diluted utilizing 2-fold dilutions and bacteria were subsequently added to a final count of 104-105cfu/ml.

3. The 96-well plates were incubated at 37°C for 18–24 h and the antimicrobial activity was determined by visual inspection.

4. The MIC of the active compounds was determined and was defined as the lowest concentration of the compound that inhibited visible growth after 24h.

Antifungal Activity:

1. Materials - The media Brain Heart Infusion (BHI) broth used was provided by HIMEDIA.

2. Experimental Procedure - Determination of MIC - 9 dilutions of each drug are done with BHI for MIC.

3. In initial tube, 20 microliter of drug was added into the 380 microliter of BHI broth.

4. For dilutions 200 microliter of BHI broth was added into the next 9 tubes separately.

5. Then from the initial tube 200 microliter was transferred to the first tube containing 200 microliter of BHI broth.

6. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube 200 microliter was transferred to the second tube to make 10^{-2} dilution.

7. This serial dilution was repeated up to10^{-9} dilution for each drug. From the maintained stock cultures of required organisms, 5 microliter was taken and added into 2mL of BHI broth.

8. In each serially diluted tube, 200 microliter of above culture suspension was added.

9. The tubes were incubated for 24 hrs and observed for turbidity.

3. RESULTS AND DISCUSSION:

3.1 Biological Evaluation

3.1.1 Antibacterial Activity - For antibacterial activity Gram positive strain of *Staphylococcus aureus* and Gram-negative strain of *Pseudomonas aeruginosa* were used and standard used was Ciprofloxacin. MIC of all the targeted compounds are shown in **Table No.2.**

Table No. 2: MIC values of final compounds (Antibacterial Activity)

MIC (µg/ml)		
Compound Code	SA (***Staphylococcus aureus)*** - gram positive)	PA (***Pseudomonas aeruginosa*** - gram negative)
F1	>250	>500
F2	>250	>500
F3	>250	>500
F4	>250	>500
F5	>250	>500
F6	>250	>500
F7	>250	>500
F8	>250	>500
Ciprofloxacin	**<2**	**<4**

Ciprofloxacin was used as the standard and has MIC values of **<2 µg/ml** against *Staphylococcus aureus,* and **<4 µg/ml** against *Pseudomonas aeruginosa* as shown in **Table No. 2**. The Above results indicated that all synthesized compounds were found to be less active as compared to the standard against Gram –ve bacteria & Gram +ve bacteria. The synthesized compounds showed MIC values **>250 µg/ml for Gram +ve bacteria and >500 µg/ml for Gram –ve bacteria**

3.1.2 Antifungal Activity

For antifungal activity *Candida albicans* were used and standard used was Fluconazole. MIC of all the targeted compounds are shown in **Table No. 3.**

Table No. 3 g- MIC values of final compounds (Antifungal activity)

Compound Code	*Candida albicans* MIC (µg/ml)
F1	>125
F2	>125
F3	>125
F4	>125
F5	>125
F6	>125
F7	>125
F8	>125
Fluconazole	**16µg/ml**

Fluconazole was used as a standard and has a MIC value of **16 µg/ml** against ***Candida albicans*** as shown in **Table No. 3.** The above results indicated that all synthesized compounds were found to be less active as compared to the standard against ***Candida albicans*** with MIC value**>125µg/ml.**

3.2 IR Spectra of Compound F1

The IR spectrum of Final Compound F1 (***N-(3-aminopropyl)-5-phenyl-4H-1,2,4-triazol-3-amine)*** is as shown in **Fig. No. 1.**

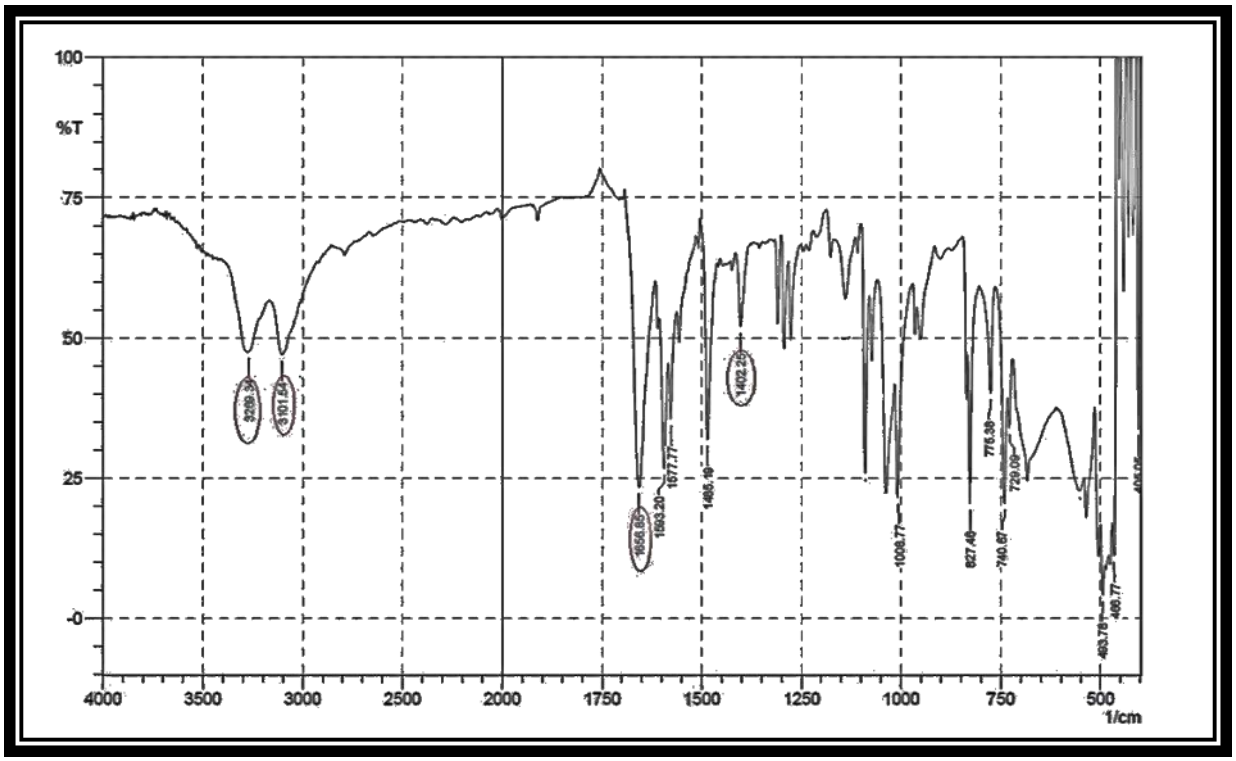


Fig. No. 1: IR Spectra of Compound F1 (cm-1): NH stretch (3269.34), NH2 stretch (3101.54), C=N Imine stretch (1656.85), C-N Vibration (1402.25)

3.3 [1]HNMRof Compound F1

[1]HNMR assignments of Final Compound F1 (***N-(3-aminopropyl)-5-phenyl-4H-1,2,4-triazol-3-amine)*** is as shown in **Fig. No. 2.**

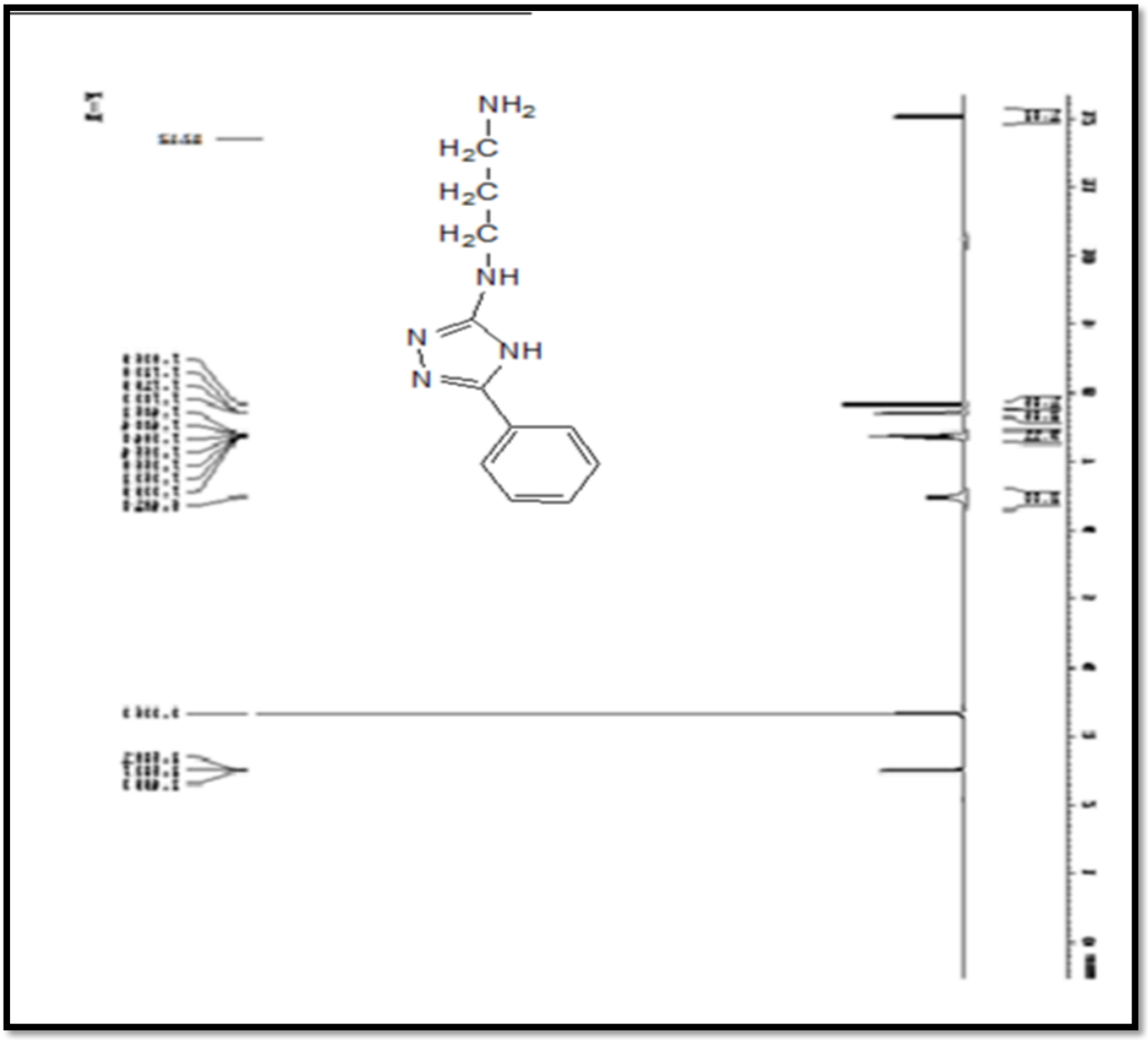


Fig. No. 2: ^{1}HNMR of Compound F1 (DMSO, ppm): 12.12 (1H, s, N-H), 7.83 (1H, s, N-H), 7.70-7.33 (3H, m, aromatic phenyl), 6.48 (2H, s, NH2), 2.50-2.49 (6H, m, CH2)

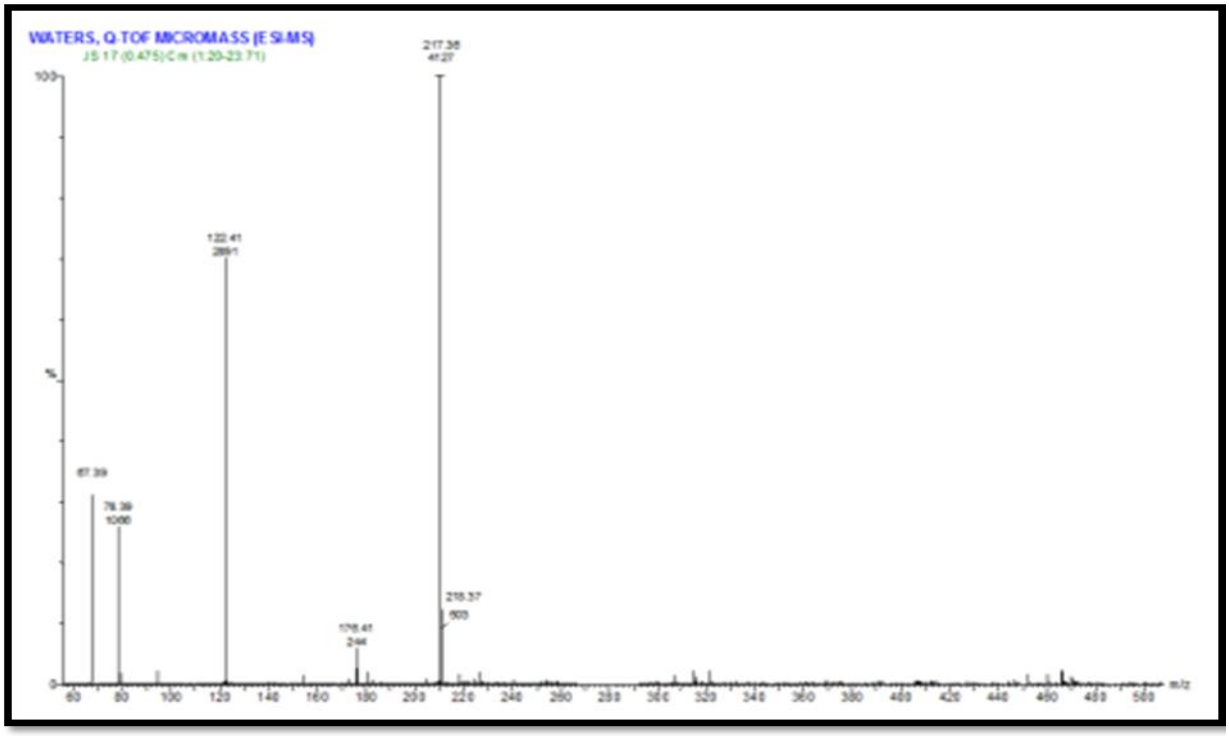


Fig. No. 3: Mass Spectrum of Compound F1 Molecular weight: **217;** M/z = **217.36**$(M-1)^+$

3.4 Mass spectrum of Compound F1

The Mass Spectrum of Final Compound F1 is as Shown in **Fig. No. 3.**

4. CONCLUSION

Derivatives of 1, 2, 4-Triazole were characterized on the basis of IR, ^{1}H -NMR and Mass spectral data. Spectroscopic studies confirmed proposed structures of these compounds. All compounds were screened for their *in vitro* antibacterial and antifungal activity. However, the results indicated that the synthesized compounds possess poor to moderate activity with reference to their respective standards.

5. ACKNOWLEDGEMENT

We would like to acknowledge CDRI, Lucknow for anti-bacterial and anti-fungal activity. We are grateful to Sophisticated Analytical Instrument Facility (SAIF), Punjab for ^{1}H NMR and Mass spectral data.

6. REFERENCES

[1] Surati Madhvi, Jauhari Smita, Desai K. R., A brief review on Microwave assisted organic reactions Arch. Appl. Sci. Res.2012;4 (1):645-661.
[2] Ravichandran S, Karthikeyan E. Microwave synthesis-a potential tool for green chemistry. International Journal of ChemTech Research. 2011 Mar; 3(1):466-70.
[3] Rakesh S, Shashikant P, Sandeep N, Prabhakar S, Vilasrao K. Microwave assisted synthesis and antimicrobial activity of some Schiff's bases. International Journal of ChemTech Research. 2010; 2(2):860-4.
[4] Dolzhenko A. V, Pastorin G, Dolzhenko A. V, Chui W. K. An aqueous medium synthesis and tautomerism study of 3 (5)-amino-1, 2, 4-triazoles. Tetrahedron Letters. 2009 May 6;50(18):2124-8.
[5] Daszak P, Cunningham A. A, Hyatt A. D. Emerging infectious diseases of wildlife--threats to biodiversity and human health science. 2000 Jan 21; 287(5452):443-9.
[6] Soni N. Synthesis and *In Vitro* anthelmintic activity of novel substituted oxadiazole bearing benzimidazole derivatives. Der Pharma Chemica. 2016; 8(7):181–4.
[7] Gao Q, Liu S, Wu X, Zhang J, Wu A. Direct annulation of hydrazides to 1, 3, 4-oxadiazoles via oxidative C (CO)–C (methyl) bond cleavage of methyl ketones. Organic Letters. 2015 Jun 19; 17(12):2960-3.
[8] Yin P, Ma WB, Chen Y, Huang WC, Deng Y, He L. Highly efficient cyanoimidation of aldehydes. Organic Letters. 2009 Dec 3; 11(23):5482-5.
[9] Gangrade D, Lad S. D, Mehta A. L. Overview on microwave synthesis-Important tool for green Chemistry. International Journal of Research in Pharmacy & Science. 2015 Apr 1(2).
[10] Ashok M, Holla B. S, Poojary B. Convenient one pot synthesis and antimicrobial evaluation of some new Mannich bases carrying 4-methylthiobenzyl moiety. European journal of medicinal chemistry. 2007 Aug 1; 42(8):1095-101.
[11] Shneine, J.; Alaraji, Y. Chemistry of 1, 2, 4-Triazole: A Review Article, *International Journal of Science and Research (IJSR)* 2016, *5*, 1411-1423.
[12] Guillon R, Giraud F, Logé C, Le Borgne M, Picot C, Pagniez F, Le Pape P. Design of new antifungal agents: synthesis and evaluation of 1-[(1H-indol-5-ylmethyl) amino]-2-phenyl-3-(1H-1, 2, 4-triazol-1-yl) propan-2-ols. Bioorganic & medicinal chemistry letters. 2009 Oct 15;19 (20):5833-6.
[13] Gupta A. K, Prachand S, Patel A, Jain S. Synthesis of some 4-amino-5-(substituted-phenyl)-4H-[1, 2, 4] triazole-3-thiol derivatives and antifungal activity. Int. J. Pharm. Life Sci. 2012 Jul;3(7):1848-57.
[14] Ye D. J, Feng X. L, Zhang Z. H., Synthesis and Crystal Structure of 1, 2, 4-Triazol-5 (4H)-one Derivative. Asian Journal of Chemistry. 2012 Nov 1; 24(11).

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