ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 05 | May - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF [1,2,4 (H)] TRIAZOLE3-AMINE AND ITS DERIVATIVES.

Science

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ABSTRACT

In this research article we synthesized 1,2,4 (H) triazole 3-amine as a core molecule from aminoguinidine and formic acid in basic medium sodium carbonate heated at 120-123 ^{0}C for 12 h. It has excellent medicinal application. In synthetic modification especial emphasis on free –NH_2 as a pharmacophore which is synthetically active group. A series of 03 innovative molecules were synthesized by isocyanate in ethanol solvent at 0 ^{0}C to room temperature, 2 h. Urea as important moiety found in synthesized molecule. The purity of all compounds will be checked by TLC and wherever found necessary the compounds will be purified by column chromatography. The structure of all compounds will be established on the basis of microanalysis, FTIR, ^{1}HNMR, ^{13}CNMR, DEPT and mass spectral data. Antibacterial and antifungal evaluation of synthesized derivatives *in vitro* has done by Kirby Bauer Method. NCIM provides pure, non-pathogenic, viable and authentic cultures with their standard code number and testing symbols; Ciprofloxacin (10 lg) and fluconazole (5 lg) were used as standard antibacterial and antifungal drugs, respectively. Minimum zone of inhibition & % activity has been determined; from these values its predicted that antibacterial value of the derivatives are more prominent that core molecule against Gram positive bacterial strains *Staphylococcus aureus* & *Bacillus subtilis*. Antifungal activity is more prominent in TZA2 derivative where urease moiety connected with cyclohexyl group against *Aspergillus niger* & *Penicillium Chrysogenum*

KEYWORDS

1,2,4 (H) triazole 3-amine, FTIR, 1HNMR, 13CNMR, DEPT, Kirby Bauer Method etc.

INTRODUCTION:

1,2,4 (H) triazole 3-amine as a core molecule has numerous biological activities such as antitumor, anticancer, anti-inflammatory, anticonvulsant, antimalarial, antiviral, analgesics, antioxidant etc. By considering these we get attracted to synthesis its derivatives which may be enhance its medicinal value. 1,2,4 (H) Triazole & its isomeric form 1,2,3 (H) triazole exist in isomeric form; their derivatives showed massive importance in medicinal chemistry. A numerous heterocyclic compounds containing triazole moiety with different biological activities can be synthesized [1]. 1,2,4 (H)triazole are a class of heterocycles which have attracted significant interest in medicinal chemistry and they have a wide range of pharmaceutical and biological activities including antimicrobial, anti-fungal, anti-inflammatory, and antihypertensive [2–6]. These molecules are also utilized as pharmacophores due to their favorable metabolic profile and ability to engage in hydrogen bonding [7]. These drugs have been widely applied clinically. However, several factors have limited their practical applications. For example, *Aspergillus infections* have become so common in recent years that many experts believe that *Aspergillus* rather than *Candida* is the major problem [8] Unfortunately, this organism is intrinsically resistant to fluconazole. In addition, the number of fluconazole-resistant strains has increased significantly with the increased use of fluconazole [9-10] The use of another triazole antifungal compound, itraconazole, which demonstrates stronger activity against *Aspergillus* than does fluconazole, is hampered by its poor aqueous solubility and oral bioavailability [11].

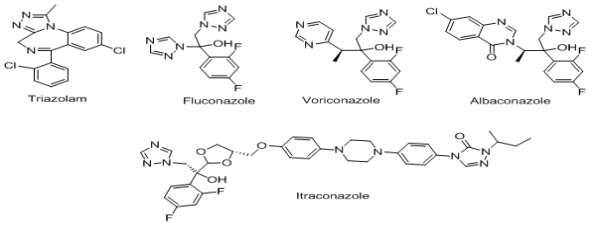
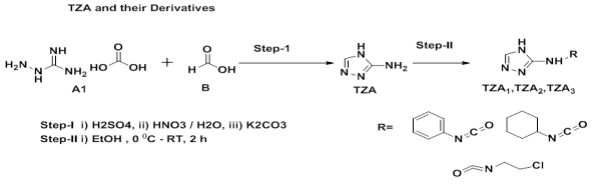


Figure 1. Structures of 1, 2,4triazole core containing drugs available in the market

EXPERIMENTAL METHODS:

General scheme: Synthesis of 4(H)-1, 2, 4-Triazole-3-amine (TZA) their Derivatives:



Step-1- for the synthesis of 4H-1, 2, 4-Triazole-3-amine (TZA)

To 1mole of finely powdered amino guanidine bicarbonate dissolved in aqueous H2SO4 and poured into a 500-ml round bottomed flask fitted with a thermometer. The reaction mixture was concentrated to obtained white residue. The obtained white colour residue is dissolved in (40 ml. 1.05 moles) of 98–100% formic acid. The foaming mixture is heated cautiously for 12h at temp, 120-130 ^{0}C gentle stirring of the flask to avoid local overheating, until gas evolution ceases and the whole mass is dissolved to form amino guanidine formate solution. Na_2CO_3 was added in small portion; the obtained suspension was evaporated to dryness in vacuo. 95% hot ethanol is added into the cooled solution; the solution is filtered while hot. The suspension was evaporated to dryness in water bath. The product obtained by evaporating the ethanol solution to dryness on the steam bath, and oven-drying at 100 °C, yield is about 90%. Recrystallization from ethyl acetate; melting point is about 152–156 °C.

Step-II- For the synthesis of 4H-1, 2, 4-Triazole-3-amine and their derivatives: (TZA1, TZB2, TZB3) - In a round bottom flask aryl/alkyl substituted isocyanate (0.714mL, 6.54mmol) was taken in Ethanol (10mL) at 0 °C. To this cooled solution 4H-1, 2, 4-triazol-3-amine (0.5g, 5.95mmol) was added and the reaction was then stirred at room temperature for 2h. The progress of reaction was monitored by TLC analysis. Formation of new single spot and complete consumption of comp. 1 was observed; reaction mixture was concentrated on rotary vacuo and the residue was purified by silica gel column chromatography. The new spot was isolated and concentrated to dryness. White solid product obtained was analyzed by ^{1}H NMR, ^{13}C NMR, MS and IR analysis.

Table No. 1.1 Reaction Conditions used for synthesis of 4(H)-1, 2, 4-Triazole-3-amine (TZA) and their Derivatives (TZA1, TZA2, TZA3) are depicted below;

Table No. 1.1 **Conditions used for the synthesis of 4H-1, 2, 4-Triazole-3-amine Sr.No.**	**Starting Material D1 and D2**	**Substituted R part**	**Solvent**	**Temp**	**Time**	**Desired Product**
1			C_2H_5OH	0°C-RT	2h	
2			C_2H_5OH	0 °C-RT	2h	
3			C_2H_5OH	0 °C-RT	2h	

RESULTS AND DISCUSSION:

Table no.1.2 Physical and analytical data of synthesized Derivatives of 4(H)-1, 2, 4-Triazole-3-amine (TZA) are depicted below;

Entry	R	R'	Mol. Formula	Mol. Wt.	Appearance	% Yield	M.P °C
TZA	-H	-H	$C_2H_4N_4$	84	White solid	55	185
TZA1	-H	Isocyanato-benzene	$C_9H_8N_5O$	203.2	White solid	17	210
TZA2	-H	Isocyanato Cyclohexane	$C_9H_{15}N_5O$	209.2	White solid	68	212
TZA3	-H	1-chloro-cyanoethane	$C_5H_8N_5OCl$	189.6	White solid	71	215

In ^{1}HNMR spectrum of **4-(H)-1, 2, 4-Triazole-3-Amine (TZA)**, NH_2 group appears at 5.81δ, -NH proton appears at downfield region at δ value 12.11 more deshielded, =C-H proton appear at aromatic region of 4-(H)-1, 2, 4-Triazole ring at 7.45 δ. [12]

From 4-(H)-1, 2, 4-Triazole-3-Amine synthesized derivatives such as **TZA1**, **TZA2**, **TZA3** spectral analysis spectrums and chemical shifts values. **^{1}HNMR** spectrum of **phenyl-3-(4H-1, 2, 4-triazol-3-yl) urea (TZA1)** NH proton of 4H-1, 2, 4-triazol-3-yl appear at around δ 10.13 ppm in singlet as per references N-H peaks of the triazole rings were seen at 11.70–12.30 ppm as singlet which correspond to a triazole NH; methionine proton (=C-H) appears at 7.7 δ as a singlet in a downfield region due to triazole moiety. 4-(H)-1, 2, 4-Triazole-3-Amine as a core compound synthesized into urea derivative as a pharmacophore. It is flanked in between 4-H-1, 2, 4-triazole core and phenyl group. N-H peak of urea appeared at 8.22–9.33 δ as singlet as per reported data. In the synthesized derivative N-H peak appear at 7.66 δ in slightly up field region compare to reported peak value of urea compound. Phenyl group is substituted at another side of urea moiety in that aromatic proton appears from o & m-side for 4-H at 7.39-7.31δ as a multiplate and at 7.17 to 7.09δ multiplate is appeared for single proton of phenyl ring present at para position.

^{13}CNMR spectrum of phenyl-3-(4H-1, 2, 4-triazol-3-yl) urea (**TZA1**) showed sp^2 hybridized methionine carbon peak appears at 157 ppm match with the value of triazole ring carbon; carbon adjacent to urea moiety appears at 150 ppm; carbonyl peak appears at 149.05ppm in expected region of reported base value. Aromatic carbon adjacent to -NH appears at 137.2 as single peak; ortho position carbons appear at 121.14ppm; meta position carbons at 128.68ppm and para position carbons at 128.81ppm. All values are obtained as per recorded base value.

Infrared spectrum of synthesized derivative**(TZA1)** exhibited strong absorption band at 3431.71cm^{-1} (NH str.), medium absorption bands were observed at 1520, 1425, cm^{-1} (aromatic ring, C=C-H str.) 1718.26cm^{-1} strong band observed for (-C=O) amide functional group; 1379,1361,1213 cm^{-1}observed for (C=N str) of triazole ring; 839.38 cm^{-1} (-C-H str) of triazole ring; strong absorption bands appear at 743,618,608,501 cm^{-1} for aromatic ring, (C-H str out of plane bend)

In LCMS of synthesized derivative (**TZA1**) showed Total Ion Current is observed as intense peak at retention time 1.069 min. and mass observed is 204.1 confirmed the structure of **TZA1**.

^{1}HNMR spectrum of **1-cyclohexyl-3-(4H-1, 2, 4-triazol-3-yl) urea (TZA2)** NH proton of 4H-1, 2, 4-triazol-3-yl appear at around 7.19δ as singlet very close to=C-H methionine proton which appears at 7.19 δ as a singlet in a slightly up field region compare to **TZA1** of triazole moiety. N-H peak of urea appeared at 7.53 δ as singlet and another N-H peak appear at 7.90-7.94δ as doublet, coupling constant (J) is 7.9 Hz, doublet appears due to adjacent proton of cyclohexane ring; 3.53–3.58 δ is the downfield region of single proton in multiplate due to electron withdrawing force of amide functional group; remaining 10-Hs are appeared in the region 1.04-1.78δ value as a multiplate.

^{13}CNMR spectrum of (**TZA2**) showed sp^2 hybridized methionine carbon peak appears at 156.60 ppm as per base value of triazole ring carbon; carbon adjacent to urea moiety appears at 150.13 ppm; carbonyl peak appears at 149.58ppm as base value. Aliphatic carbon appears at shielded position which observed in cyclohexyl carbon atoms. -CH adjacent to -NH appears at 49.07ppm; ortho position carbon appears at 121.14ppm; meta position carbon at 128.68ppm and para position carbon at 128.81ppm. All values are obtained as per recorded base value.

DEPT-135 spectrum of (**TZA2**) Triazole ring carbon (N=C-H) gives positive signal appears at 149.54 ppm and -CH of cyclohexyl appear at 49.02 gives positive signal; methylene carbons of cyclohexyl ring at ortho, meta and para position gives negative signal appears at 24.82 ppm,24.91ppm and 31.93ppm respectively.

Infrared spectrum of synthesized derivative (**TZA2**) exhibited strong absorption band at 3390.24 cm^{-1} (NH str.), medium absorption bands were observed at 1493, 1405,1358 cm^{-1} (cyclohexane ring, C-C-H str.)1686.26cm^{-1} strong band observed for (-C=O) amide functional group; 1629 cm^{-1}(N-H bend) 1207, 1134, cm^{-1}observed for (C=N str) of triazole ring; 936-855cm^{-1} (=C-H str) of triazole ring; strong absorption bands appear at 634,582,497,431 cm^{-1} for (C-H str out of plane bend)

In LCMS of synthesized derivative (**TZA2**) showed Total Ion Current is observed as intense peak at retention time 1.601 min. and mass observed is 210.1 confirmed the structure of **TZA2**.

^{1}HNMR spectrum of **1-(2-chloroethyl)-3-(4H-1, 2, 4-triazol-3-yl) urea (TZA3)** NH proton of 4H-1, 2, 4-triazol-3-yl appear at around 7.23δ as singlet similar to =C-H methionine proton which also appears at 7.23 δ as a singlet in a slightly up field region compare to **TZA2** of triazole moiety. N-H peak of urea appeared at 7.57 δ as singlet and another N-H peak appear at 8.41δ as triplet, coupling constant (J) is 7.9 Hz, triplet appears due to adjacent -CH2; 3.51–3.57 δ is the -CH2 region in multipate similarly 3.76–3.70δ for 2H in a triplicate slightly downfield due to electron withdrawing chlorine atom.

^{13}CNMR spectrum of (**TZA3**) showed sp^2 hybridized methionine carbon peak appears at 156.60 ppm as per base value of triazole ring carbon another carbon adjacent to urea moiety appears at 151.08 ppm; carbonyl peak appears at 149.92ppm as base value. Aliphatic carbon appears at shielded position which observed in ethyl chloride. -CH2 adjacent to -NH appears at 41.31ppm; CH2 position carbon attached to electron withdrawing chlorine atom appears at 42.58 ppm. All values are obtained as per recorded base value.

DEPT-135 spectrum of (**TZA3**) Triazole ring carbon (N=C-H) gives positive signal appears at 149.88ppm; methylene carbons of ethyl chloride gives negative signal appears at 41.26 ppm and -CH2 gives negative signal at 42.81ppm.

Infrared spectrum of synthesized derivative(**TZA3**) exhibited strong absorption band at 3396 cm^{-1} (NH str.), strong band seen at 3096 cm^{-1}(C-H str) strong band observed at 1692$cm^{-1}$for (NH-C=O) amide functional group;1634 cm^{-1}(N-H bend) 1507,1405,1306,1186 cm^{-1}observed for (C=N str) of triazole ring; 839.38 cm^{-1} (=C-H str) of triazole ring; strong absorption bands appear at 743-642 cm^{-1} for alkyl chloride, (C-Cl str out of plane bend)

In LCMS of synthesized derivative (**TZA3**) showed Total Ion Current is observed as intense peak at retention time 1.343 min. and mass observed is 190.0 confirmed the structure of **TZA2**.

Antimicrobial Evaluation of synthesized Derivatives *in vitro* - Kirby Bauer Method:

Test microorganisms were procured from National Collection of Industrial Microorganisms-CSIR-National Chemical Laboratory (NCIM-NCL), Pune.NCIM provides pure, non-pathogenic, viable and authentic cultures with their standard code number and testing symbols are mentioned below [13-17]; Gram positive bacterial strains *Staphylococcus aureus* (Sa- 2178) *Bacillus subtilis* (Bs-2239) Gram negative bacterial strains *Eschereschia coli* (Ec-25744) *Klibesiella aerogenus* (Ka-2239) Antifungal strains are *Aspergillus niger* (An-504) and *Penicillium Chrysogenum*(Pc-709)

Table no. 1.3 Antibacterial screening of the synthesized derivatives are depicted below;

Sr. No	Comp code	Gram positive				Gram negative			
		S.aureus		*B.subtilis*		*E. coli*		*K. aurogenus*	
		MZI (mm)	% activi	MZI (mm	% activit	MZI (mm	% activit	MZI (mm	% activit
1	TZA	10.2	40.8	...	...	...	...	7.5	30
2	TZA1	9.0	36	10.4	41.6	...	...	..	..
3	TZA2	10.4	41.6	7.2	28.8	10	40	7.0	28
4	TZA3	11.25	45	7.5	30	6.8	27.2	...	...

Table no.1.4 Antifungal screening of the synthesized derivatives are depicted below;

Sr. No	comp. code	*Aspergillus niger*		*Penicillium chrysogenum*	
		MZI (mm)	% activity	MZI (mm)	% activity
1	TZA	11	39.28	6.6	33.57
2	TZA1	...	...	...	...
3	TZA2	9.8	35	12	42.85
4	TZA3	...	...	7.2	25.71

TZA and their derivatives such as $\mathbf{TZA_1}$, $\mathbf{TZA_2}$, $\mathbf{TZA_3}$ showed activity against *S. aureus* and *K.aureogenus* but TZB is **inactive** against *S. aureus, B.subtilis and E.coli* but showed **activity** against *K.aureogenus*. $\mathbf{TZA_1}$ is **inactive** against gram negative bacteria *E.coli* and *K.aureogenus*. $\mathbf{TZB_2}$, $\mathbf{TZB_3}$ is **inactive** against *B.subtilis*. Overall 1,2,4 (H)-triazole derivatives and methyl thio- substituted 1,2,4 (H) triazole derivatives are antibacterial actives. These increases in activity of synthesized derivatives compare to lead molecule due to QSAR and structural effect. TZA_3 and TZB_3 have showed 11.25 % activity due to chloro ethyl substituent it lower down in cyclohexyl substituent and more than that than phenyl ring. **TZA** has been completely inactive against fungal strain. It may be due to the presence of free NH_2 exert solvation effect and basicity of the molecule similarly $\mathbf{TZA_1}$ due to phenyl ring hydrophobic interaction. $\mathbf{TZA_2}$, $\mathbf{TZA_3}$, $\mathbf{TZB_2}$ and $\mathbf{TZB_2}$ have been active against *P. Chrysogenum.*

CONCLUSION:

All of the compounds showed antibacterial activity that was more potent than both reference drugs, ciprofloxacin and fluconazole against all bacteria tested. Antifungal activity against *P. Chrysogenum* was active for cyclohexyl and ethyl chloride connected to urea moiety and 1,2,4 (H) triazole scaffold. In future based on in *silico* / virtual screening of these molecules it will be tested for anticancer activity.

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