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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF SOME NOVEL TRIAZOLE DERIVATIVES

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Keywords:

Triazole, Antibacterial activity, Antifungal activity, Heterocyclic, Antimicrobial

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ABSTRACT: Triazole is a diunsaturated heterocyclic compound that contains two nitrogen groups as heteroatoms. In the present study, an attempt was made to synthesize various derivatives of substituted 1, 2, 4-triazol-3-yl) benzene-1-ol and assess their antimicrobial efficiency. The synthesized compounds were subjected to physical characterization and spectral analysis by IR and NMR for structure elucidation. The compounds were then subjected to evaluation of antimicrobial activity against bacterial strains *Escherichia coli (Gram -ve), Pseudomonas aeruginosa (Gram -ve), Bacillus Subtili (Gram +ve), Staphylococcus aureus (Gram +ve)* method using ciprofloxacin as standard and against fungal strain *Aspergillus niger* method using fluconazole as standard. The results of antibacterial activity show that compounds P1 and P2 and the results of antifungal activity show that compounds P3 and P4 showed equivalent activity when compared with standard while the rest of the compounds were found to be less active than standard.

INTRODUCTION: Triazole is one of a class of organic heterocyclic compounds containing a five-membered diunsaturated ring structure composed of three nitrogen atoms and two carbon atoms at nonadjacent positions [1]. Triazole nucleus containing drugs have been reported to possess diverse pharmacological activities such as fungicidal, insecticidal, bactericidal, herbicidal, antitumor, anti-inflammatory, CNS stimulant properties [2]. The chemistry of heterocyclic compounds continues to be an explore field in organic chemistry. The importance of triazole derivatives lies in the field that these have occupied a unique position in heterocyclic chemistry due to their antimicrobial activity [6].

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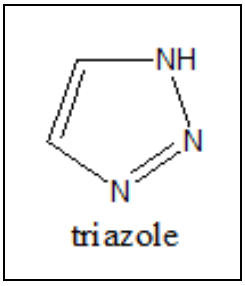


FIG. 1: TRIAZOLE RING

MATERIALS AND METHODS: Basing upon the developed Synthetic scheme raw materials, chemicals and apparatus of optimum quality were procured from renowned suppliers. Melting points were recording using electrically heating melting point apparatus. The homogeneity and purity of synthesized compound was ascertained by using TLC, performed on silica gel G coated plates using ethyl acetate and pet. Ether (1:1) an eluent, the developed plates were observed under an iodine chamber. SHIMADZU Model No.FTIR:8400S spectrophotometer, Bruker advance II 400 MHz NMR spectrophotometer, LC-MSD- Tranp SL

2010. A SHIMADZU were used for structural elucidation of compounds.

The compounds were prepared according to the established method shown in schematic diagram.

Scheme of Work:

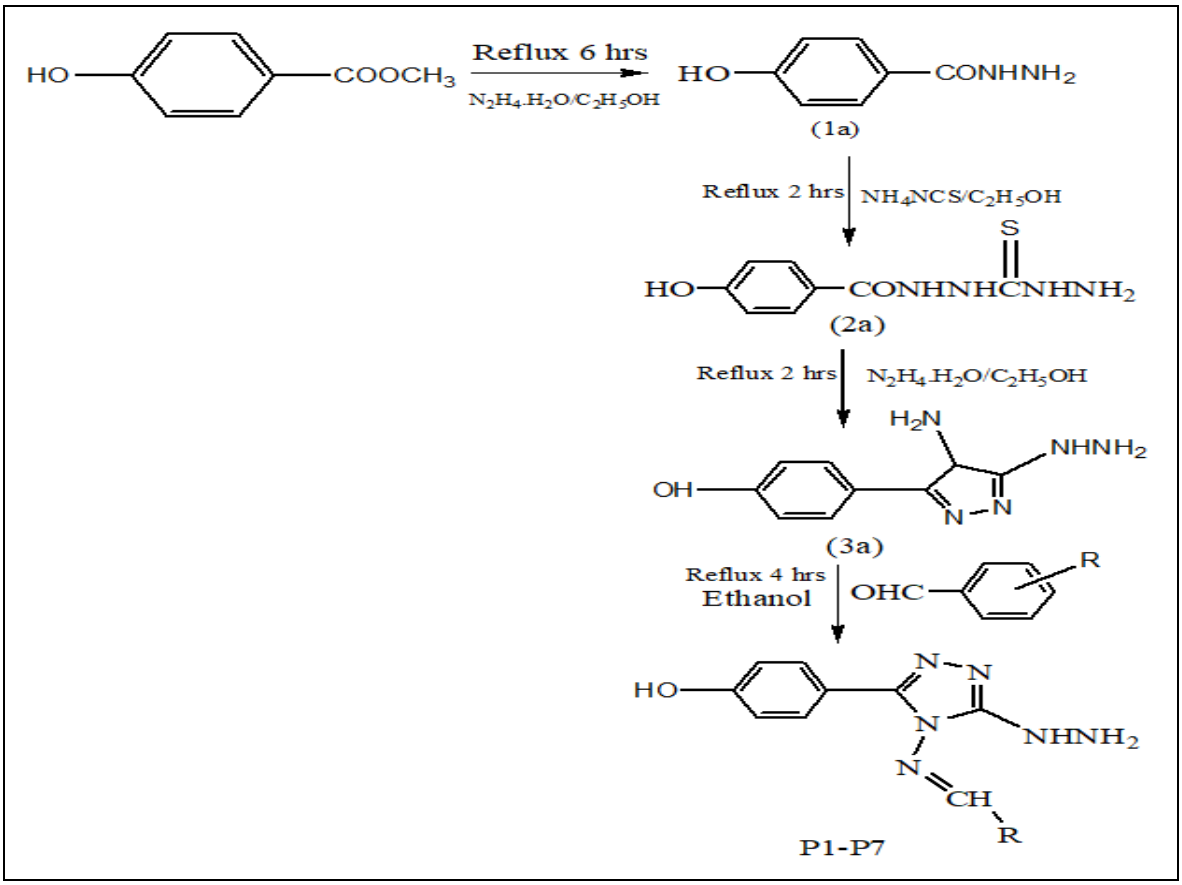
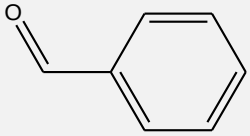
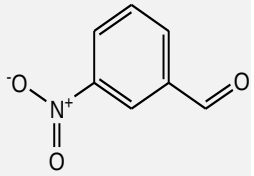
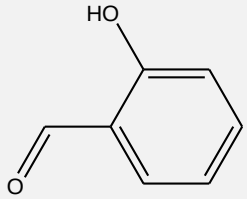
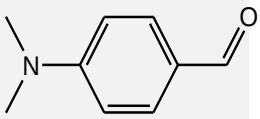
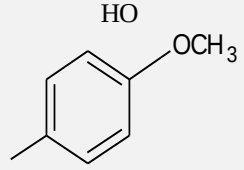
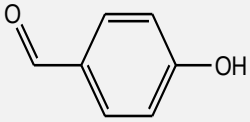


TABLE 1: LIST OF AROMATIC ALDEHYDE SUBSTITUENTS

Compound	Compound Name	R	Compound	Compound Name	R
P1	Benzaldehyde	 Benzaldehyde	P5	m-Nitro benzaldehyde	
P2	Salicylaldehyde	 salicaldehyde	P6	p-Dimethyl amino benzaldehyde	
P3	Anisaldehyde		P7	Formaldehyde	HCHO
P4	p-Hydroxy benzaldehyde				

General Synthetic Procedure:

Step 1: Synthesis of 4-hydroxybenzohydrazide: Methylparaben (0.01 moles) and hydrazine hydrate (0.01 moles) were mixed gently in ethanol and refluxed for 6 hrs. The mixture is then cooled and poured into ice-cold water. Filtered off the crystals and recrystallized from ethanol. Completing the reaction was monitored on TLC using silica gel-G coated plates using ethyl acetate and petroleum ether (1:1v/v) as the solvent system and observed in an iodine chamber.

Step 2: Synthesis of 2-[(4-hydroxybenzo-hydrazide) carbonyl]-N-hydrazine substituted-carbothioamide: A mixture of 4-hydroxybenzo-hydrazide (0.01 moles) and ammonium is thio-cyanate (0.001 moles) in ethanol (25.0 ml) was refluxed on a water bath for 2 hrs. The solvent was concentrated, and the precipitated product was filtered, dried, and recrystallized from methanol.

Completing the reaction was monitored on TLC using silica gel-G coated plates using ethyl acetate and petroleum ether (1:1v/v) as the solvent system and observed in the iodine chamber.

Step 3: Synthesis of 4-(4-amino-5-(substituted amino)-4H-1, 2, 4-triazol-3-yl) benzene-1-ol: Compound 2-[(4-hydroxyphenyl) carbonyl]-N-hydrazine substituted carbothioamide (0.002 mol) and hydrazine hydrate (0.025 mol) was refluxed in methanol for 2 hrs, at a temperature between 50-60°C, reaction mixture was cooled and poured over crushed ice. Solid was filtered and recrystallized from methanol. The completion of the reaction was monitored on TLC using silica gel-G coated plates by using ethyl acetate and petroleum ether (1:1v/v) as the solvent system and observed in an iodine chamber.

Step 4: Synthesis of 4-(4-(substituted benzy-lidene amino)-5-(substituted amino)-4H-1, 2, 4-triazol-3-yl) benzene-1-ol (P1-P7): To a solution of 5-(4-amino-5-(substituted amino)-4H-1, 2, 4-triazol-3-yl) benzene-2-ol (0.01 Moles) in absolute ethanol (30 ml), the appropriate aromatic aldehydes (0.012 moles) was added.

The reaction mixture was refluxed for 4 h. After cooling, the formed solid was filtered off and recrystallized to give the title compounds respectively (P1-P7).

The completion of the reaction was monitored on TLC using silica gel-G coated plates by using ethyl acetate and petroleum ether (1:1v/v) as the solvent system and observed in an iodine chamber.

Characterization of Synthesized Compounds: Yield 80.52% as a solid; m.p °C; UV (Et OH) λ_{max} (log ε) 272.5; IR (KBr) νmax3215 (O-H str.), 3043(=C-H str.), 2640 (C=N str.), 1269 (C-O str.), 1618 (C=C str.), 1276 **(**C-N str.) cm-1; ^{1}HNMR (400 MHz, DMSO): δ (ppm): 4.0 (s,1H,NH); 6.94-7.87 (m, 8H, Ar-H); 8.88 (s, 1H, CH); 2.56(s, 2H, NH_2); 6.91(s, 2H, OH); 13CNMR ($CDCl_3$, 300 MHz): δ=154.3, 124.9, 162.9, 122.6, 130.1, 102.8, 152.6, 138.2, 151.8, 102.2, 56.1, 55.3, 56.8.

4-(4-(2-hydroxy benzylidene amino)-5-(substituted amino)-4H-1, 2, 4- triazol-3-yl) benzene-1-ol: Yield 74.2 % as a solid; m.p 168-170 °C; UV (Et OH) λ_{max} (log ε) 262.5; IR (KBr) νmax 3365 (O-H str.), 3116(=C-H str.), 2362(C=N str.), 1016 (C-O str.), 1613(C=C str.), 1044 (C-N str.) cm-1; ^{1}HNMR (400 MHz, DMSO): δ (ppm): 3.88 (s, 1H, NH); 6.84-8.38 (m, 8H, Ar-H); 8.60 (s, 1H, CH); 2.56(s, 2H, NH_2); 6.82 (s, 1H, OH), 3.82(s, 3H, CH_3); 13CNMR (CDCl3, 300 MHz): δ=184.3, 134.9, 182.9, 132.6, 140.1, 102.8, 152.6, 138.2, 141.8, 112.2, 58.1, 54.3, 56.8; Anal. Cacld for $C_{16}H_{15}N_6O$ S2: C, 56.81; H, 2.80; F, 5.29; N, 3.90; O, 13.36; S, 17.84.

4- (4- (2- Methoxy benzylidene amino)- 5-(substituted amino)- 4H- 1, 2, 4- triazol-3-yl) benzene-1-ol: Yield 71.0% as a solid; m.p 180-182°C; UV (Et OH) λ_{max} (log ε) 235; IR (KBr) νmax 3514(O-H str.), 3216(=C-H str.), 2399 (C=N str.), 1171(C-O str.), 1603(C=C str.), 1240 (C-N str.) cm^{-1}; ^{1}HNMR (400 MHz, DMSO): δ (ppm): 3.49 (s, 1H, NH); 6.86-7.97 (m, 8H, Ar-H); 8.33 (s, 1H, CH); 2.55(s, 2H, NH_2); 6.79(s, 2H, OH).; 13CNMR (CDCl3, 300 MHz): δ=164.3, 144.9, 172.9, 135.6, 148.1, 142.8, 182.6, 148.2, 149.8, 152.2, 57.1.

4- (4- (4- Hydroxy benzylidene amino)- 5-(substituted amino)-4H- 1, 2, 4- triazol- 3-yl) benzene-1-ol: Yield 72.6 % as a solid; m.p 235-237°C; UV (Et OH) λmax (log ε) 245; IR (KBr) νmax 3352(O-H str.), 3165(=C-H str.), 1263 (C-O str.), 1645 (C=C str.), 1350 (C-N str.), 1508 (C-NO_2 str.) cm^{-1}; ^{1}HNMR (400 MHz, DMSO): δ

(ppm): 3.79 (s, 1H, NH); 6.81-8.87 (m, 8H, Ar-H); 8.54 (s, 1H, CH); 2.53(s, S 2H, NH_2); 5.82(s, 1H, OH)..; 13CNMR ($CDCl_3$, 300 MHz): δ=174.3, 144.9, 162.9, 125.6, 158.1, 142.8, 172.6, 138.2, 149.8, 132.2, 58.1. Anal. Cacld for $C_{16}H_{15}N_6O$ S2: C, 5481; H, 1.80; F, 6.29; N, 4.90; O, 3.36; S, 7.84.

4-(4-(3-Nitro benzylidene amino)-5-(substituted amino)-4H-1, 2, 4- triazol-3-yl) benzene-1-ol: Yield 71 % as a solid; m.p 199-201°C; UV (Et OH) λ_{max} (log ε) 245; IR (KBr) ν_{max} 3235 (O-H str.), 3150(=C-H str.), 1234 (C-O str.), 1605 (C=C str.), 1369(C-N str.) cm^{-1}; ^{1}HNMR (400 MHz, DMSO): δ (ppm): 3.55 (s, 1H, NH); 6.86-8.31 (m, 8H, Ar-H); 9.97 (s,1H, CH); 2.95(s, 2H, NH_2); 6.66 (s, 1H, OH), 3.01(s, 6H, CH_3); 13CNMR (CDCl3, 300 MHz): δ=154.3, 154.9, 162.9, 135.6, 148.1, 132.8, 142.6, 128.2, 139.8, 135.2, 54.1. Anal. Cacld for $C_{18}H_{20}N_7O_2$ S2: C, 581; H, 3.80; F, 5.39; N, 6.90; O, 7.36; S, 4.84.

4-(4-(4-dimethyl amino benzylidene amino)-5-(substituted amino)-4H-1, 2, 4- triazol-3-yl) benzene-1-ol: Yield 73.2 % as a solid; m.p 188-190°C; UV (Et OH) λ_{max} (log ε) 235; IR (KBr) ν_{max} 3235 (O-H str.), 3150(=C-H str.), 1234 (C-O str.), 1605 (C=C str.), 1369(C-N str.) cm^{-1}; ^{1}HNMR (400 MHz, DMSO): δ (ppm): 3.55 (s, 1H, NH); 6.86-8.31 (m, 8H, Ar-H); 9.97 (s,1H, CH); 2.95(s, 2H, NH_2); 6.66 (s, 1H, OH), 3.01(s, 6H, CH_3); 13CNMR ($CDCl_3$, 300 MHz): δ=154.3, 154.9, 162.9, 135.6, 148.1, 132.8, 142.6, 128.2, 139.8, 135.2, 54.1. Anal. Cacld for $C_{18}H_{20}N_7O_2$ S2: C, 581; H, 3.80; F, 5.39; N, 6.90; O, 7.36; S, 4.84.

Biological Evaluation: Bacterial strain *A. Escherichia coli (Gram -ve) B. Pseudomonas aeruginosa (Gram -ve) C. Bacillus Subtili (Gram +ve) D. Staphylococcus aureus (Gram +ve)* in present study the cup-plate method was used to evaluate the antimicrobial activity in vitro of the synthesized compounds. This method was used for determining the selective effectiveness of the anti-bacterial activity.

The standard antibiotic selected for the study of the antibacterial activity was ciprofloxacin Baselayer was obtained by pouring about 10-15ml of the base layer medium into each sterilized Petri dish and allowed to attain room temperature. The overnight grown sub-culture was taken into definite volume of inoculated, then with the help of cotton swab the organisms were streaked the entire agar surface horizontally, vertically, and around the outer edge of the plate to ensure a heavy growth over the entire surface. Allow all culture plates to dry for about 5 minutes. Scooping out nutrient agar with sterilized cork borer made the cups. The solution of the test compounds (0.1ml) was added into the cups by using micropipettes, and these plates were subsequently incubated all the plate cultures in an inverted position for 24 hats 37°C and observed for antimicrobial activity. Ciprofloxacin (10μg/ml) was used as a standard drug, and the solvent control (DMSO) was kept separately. After 24 hrs, the diameters of zone of inhibition were measured for the plates in which the zones of inhibition and minimum inhibitory concentration (MICs) were measured in mm for each organism. Zone of inhibition was determined for all the ten compounds the results in the form of percent inhibition were summarized.

Fungal Strain was used *Aspergillus niger:* In the present study, the cup-plate method was used to evaluate the antimicrobial activity in vitro of the synthesized compounds.

This method was used for determining the selective effects of the anti-fungal activity. The standard antibiotic selected for the study of the antibacterial activity was fluconazole. Sabouraud's dextrose agar (SDA) was used for the growth of the fungal culture.

The same procedure as that for assaying the antibacterial activity was adopted, and fungal cultures were kept for 48 hr to determine the diameter of the zone of inhibition. Fluconazole (1 mg/ml) was used as standard.

The Microbiological testing has been performed for bacterial and fungal species and the following values were determined.

- MIC value.
- Zone of Inhibition.

Antimicrobial Activity:

Antimicrobial Activity of Compounds P1-P7 using Cup-Plate Method: Antibacterial activity

Growth Medium-Nutrient Agar.

TABLE 2: MICS (MINIMUM INHIBITORY CONCENTRATIONS) (µg/ml) ZONE OF INHIBITION OF THE SYNTHETIC COMPOUNDS

	Zone diameter in mm			
	Gram-positive bacteria		Gram-negative bacteria	
Compounds	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
P1	9.31 (6.25)	9.11(6.25)	9.45(6.25)	9.69(6.25)
P2	9.23 (6.25)	9.32 (6.25)	9.61(6.25)	9.51(6.25)
P3	9.24 (6.25)	8.87 (6.25)	9.17 (6.25)	9.53(6.25)
P4	9.13 (6.25)	8.99 (6.25)	9.23(6.25)	9.33(6.25)
P5	9.21 (6.25)	9.12 (6.25)	9.17(6.25)	9.59(6.25)
P6	–	–	–	–
P7	–	–	–	–
Ciprofloxacin as standard	25.21 (6.25)	23.68 (6.25)	22.41(6.25)	18.85 (6.25)

Antifungal Activity:

Growth Medium: Subouraud dextrose agar.

TABLE 3: *IN-VITRO* ANTIFUNGAL ACTIVITY OF SYNTHESIZED COMPOUNDS

S. no.	Sample ID	*Aspergillus niger*
1	P1	9.07 (12.5)
2	P2	9.24 (12.5)
3	P3	9.08 (6.25)
4	P4	9.22 (6.25)
5	P5	9.35 (12.5)
6	P6	–
7	P7	–
Standard	Fluconazole	9.02 (6.25)

CONCLUSION: Antibacterial activity of synthesized compounds revealed that compounds P1 and P2 possess potent antibacterial activities over rest of the compounds. P1 and P2 are more potent against *Pseudomonas aeruginosa* and *E. coli* with respect to ciprofloxacin with MIC value 6.25 µg/ml & 12.5 µg/ml, respectively. Compound P3, P4 and P5 exhibited moderate activity. Whereas comparable MIC against *Bacillus subtilis* and *Staphylococcus aureus*. And antifungal activity of the synthesized compounds revealed that compounds P3 and P4 possess potent antifungal activities over rest of the compounds. P3 and P4 are more potent against *Aspergillus niger* with respect to fluconazole with MIC values 6.25 µg/ml and 12.5 µg/ml, respectively. And rest of the compound has moderate antifungal activity. The preliminary SAR revealed that different aromatic aldehyde favors antibacterial and anti-fungal activity of 1, 2, 4 triazoles. Anti-fungal activity data indicated that they have more potency over reference.

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CONFLICTS OF INTEREST: Nil

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