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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF SOME NOVEL NEW 1, 3, 4-TRIAZOLES

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Abstract

Five 1,3,4- Triazole derivative novel compounds have been synthesized combining pharmacological potential ofAcyl urea and hydrazine derivatives. A new series of substituted Benztriazoles derivative **TZ-1-TZ-5** were synthesized by cyclocondensation of different acyl ureas with hydrazines derivatives. The newly synthesized compounds were characterized by IR, MASS and 1H NMR spectral data. The synthesized compounds were evaluated for *in vitro* antibiotic activity by disc diffusion method. Compounds **TZ-04 AND TZ-05** are the most promising triazole, could serve as novel template for bacterial and fungal infection.

Keywords: Benztriazole, Hydrazine derivatives, Antimicrobial activity.

Introduction

Benztriazole is a heterocyclic aromatic organic compound which is an important pharmacophore and a privileged structure in medicinal chemistry. This compound is bicyclic in nature which consists of the fusion of benzene and triazole. The triazoles are said to be the isosters of imidazoles in which the carbon atom of imidazole is isosterically replaced by nitrogen. [1-2]Triazoles are 5-membered rings, which contain two carbon and three nitrogen Replacement of the imino hydrogen atom by an alkyl or aryl group prevents the tautomerism, and thereby gives rise to the possibility of two 1-substituted triazoles and two 1-substituted osotriazoles. Azoles form a versatile bunch of heterocycles and one of the most privileged scaffolds in modern medicinal chemistry, with an ample spectrum of biological activities such as anti microbial[3], anti viral[4], anticonvulsants[4], anti cancer[5], anti tubercular[6] and anti inflammatory[7] actions. The benztriazole scaffold is a useful structural modification for the development of molecules of pharmaceutical or biological interest. The optimization of benztriazole based structures has resulted in various drugs that are currently on the market, such as **Flucanazole, Tercanazole and Alprazolam**. Nowadays it is a moiety of choice which possess many pharmacological properties.

Many investigations have shown that the incorporation of substituted aromatic rings could have an important biological significance which prompted us to design some other azole derivatives in combination with substituted aromatic rings.[8] Such combination of triazoles is expected to give rise to a new range of highly bioactive molecules, particularly by designing substituents with optimum toxophoric requirements. The advent of sulfa drugs which has given rise to a vast progress

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in the field of sulfur containing heterocycles has also prompted us to synthesize some sulphur containing azoles and to study the effect of this substitution on the various biological activities. Thus, the aim of the current study was the synthesis of novel benztriazole derivatives in economical and to reduce environmental hazards.

Materials and method

All the chemicals were of synthetic grade and commercially procured from sigma Aldrich Chemicals. The melting points of all the synthesized compounds were determined using capillary tubes with Thermionic model C-LMP-1-Campbell melting point apparatus and are uncorrected. IR spectra were determined on JASCO FTIR 4100 using KBr pellets and wave number was reported in cm-1. 1H NMR spectra on AV-III-400 Fourier Transform NMR instrument using DMSO as solvent the chemical shifts (δ) were reported in ppm. Mass spectra were recorded on JOEL SX 102-GC MATE instrument employing electron impact ionization technique. The purity of the compounds by performing TLC over glass plates coated with silica gel with suitable (benzene: ethyl acetate, 1:4) mobile phase system and detected by iodine vapor. The reagent grade chemicals were purified by either distillation or recrystallization before use. The compounds were identified by IR,1H-NMR and MASS spectra. The physical data of compounds were presented in Table No.1

Chemistry

1st series: Synthesis of 3 - Amino, 5 -methyl, 4(H)-1, 2, 4-triazole

2.4 g(0.02moles) of acetyl urea was taken in a RB flask. To this Hydrazine hydrate (10-15ml), 20ml MeOH reagent was added and refluxed for 3hrs.The reaction mixture cooled and poured into an excess of ice-cold water (50-100ml).Yellow colour ppt was obtained, filtered and dried. Finally recrystalised from MeOH, yellow colour crystalline solid was obtained, showed single yellow spot on TLC (ethyl acetate: benzene,2:8).

2nd series : 4-carbamyl, 3 methyl /phenyl, 5-thio 1, 2, 4-triazole

2.4g (0.2moles) of Acyl urea was taken in a RB flask and to this Hydrazine hydrate (20ml), carbon disulphide (20ml) and KOH (15%, 20ml) were added. This reaction mixture was refluxed for 3hrs .The reaction mixture cooled and poured into an excess of cold water (50-100ml), yellow precipitate obtained and dried. Yellow colour ppt was obtained, filtered and dried. Finally recrystalised from MeOH, yellow colour crystalline solid was obtained, showed single yellow spot on TLC (ethyl acetate: benzene, 2:8).

3rd series: 1 Amino,2 anilino,4 carbamyl,3 methyl/phenyl, 5 thio ,1,2,4-triazole

2.4g (0.02moles) of Acyl urea was taken in a RB flask and to this phenyl hydrazine(20ml) , carbon disulphide(20ml) and KOH(20ml) were added. This reaction mixture was refluxed for 3hrs .The reaction mixture cooled and poured into an excess of ice cold water (50-100ml).Green colour powder was obtained ,filtered and dried . Finally recrystalised with methanol green colour crystals are obtained, shows single yellow spot on TLC.

Identification and Characterization

IR (KBr):Cm^{-1}=3324.44(N-H str triazole) , 2956.07 (C-H str methyl) , 1655.32 (N-H bending amine) , 1454 (N-N srt ,triazole)1279.94(C-N str,aryl) , 1038(C-N str,aliphatic).

^{1}HNMR: δppm =1.64(s,Methyl group), 2.50(s, triazole –NH-), 4.11(S,NH_2).

MS *m/z* 98.11 [M]+.

4-carbamyl, 3 methyl, 5-thio 1, 2, 4-triazole

Identification and characterization

IR (KBr):Cm^{-1}=3431.80,s(N-Hstr,amide), 2954.19,m(C-H str methyl), 1642.84-1554(amide N-H bending,Amide-II), 1413 (N-N str,triazole), 1297.14(C-N str),1202(C=S str), 1063.42 (C-N str,aliphatic).

^{1}HNMR: δppm =1.64(s,methyl), 2.50(s,$CONH_2$), 3.410 (s,N-H triazole). - **MS *m/z*** 158.18 [M]+.

4-carbamyl,3 phenyl,5-thio 1,2,4-triazole

Identification and characterization

IR (KBr):Cm^{-1}=3390.04-3283,m(N-H str-amide), 3056.17(Ar C-H str **),** 1660.61-1596(amide N-H bending **),**1414.95,m(Ar C=C str), 1230.86-996,M,(C=S str), 757.68(C-C bending ,aryl mono substituted).

^{1}HNMR: δppm =1.64(s,methyl), 2.50(s,$CONH_2$), 4.317 (s,N-H triazole) ,7.346(phenyl).

MS *m/z 220.25*[M]+.

1 Amino,2 anilino,4 carbamyl,3 methyl, 5 thio ,1,2,4-triazole

Identification and characterization

IR (KBr):Cm^{-1}=3433.67 (N-Hstr,amide), 3263.75 (N-H str,2^0 amine), 3114.86(Ar C-H str), 2926.04(Ali C-H str), 1609.21(C=O str .amide), 1468.78(C-H def), 1270.51(C-N str,aryl), 1127.66-920(C=S str), 746.22(C-Cbending,aryl mono substituted.)

^{1}HNMR: δppm =1.08(s,-CH of triazole), 2.50(s,NH_2), 6.99(s, $CONH_2$).

MS *m/z*266.32 [M]+.

1 amino,2 anilino ,4 carbamyl,3 phenyl, 5 thio-,1,2,4 triazole

Identification and characterization

IR (KBr):Cm^{-1}=3390.04 (N-H str,2^0amine), 3092.87 (Ar C-H str), 1680.96 -1645(N-H str bending amide), 1490.97 (Ar C=C)str, 1293.85(C-N str.aryl), 1025(C-N str ,aliphatic)743.90 &683 (C-C bending,,mono substituted aryl).

^{1}HNMR: δppm =1.74(s,-CH of triazole), 2.50(s,NH_2), 3.327 (s, $CONH_2$), 7.22-7.54(d,aryl).

MS *m/z*328.39 [M]+

Biological screening

Antimicrobial activity

Antimicrobial activity of all synthesized compounds was determined by disc diffusion method[9]. All human pathogenic bacteria viz (*BacillusSubtitis* NCIM 2063) , (*Pseudomonas aeruginosa*NCIM2206) and fungi viz (*Pencillium notatum* MTCC 3100,(*Aspergillus niger* MTCC 277), were procured from National Collection of Industrial Microorganisms (NCIM), Pune and Microbial Type Culture Collection (MTCC). The agar medium was purchased from Hi-media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. Discs measuring 6 mm in diameter were punched from Whatman No.1 filter paper. Stock solutions of synthesized compounds diluted in dimethylsulphoxide (1% DMSO) to give final concentration of 250µg/ml and 500 µg/ml. A reference standard for both gram positive and gram negative bacteria and fungi was made by ***gatifloxacin*** (0.5 µg/µl) and *Miconazole Nitrate* (1µg/ml) separately. The incubation was carried out at 37ºC for 24h. All the experiments were carried out in triplicate. Simultaneously, controls were maintained by employing 0.1 mL of dimethyl sulfoxide which did not reveal any inhibition. Zones of inhibition produced by each compound were measured in mm. The results of antibacterial studies are given in Table-2.

Results and discussion

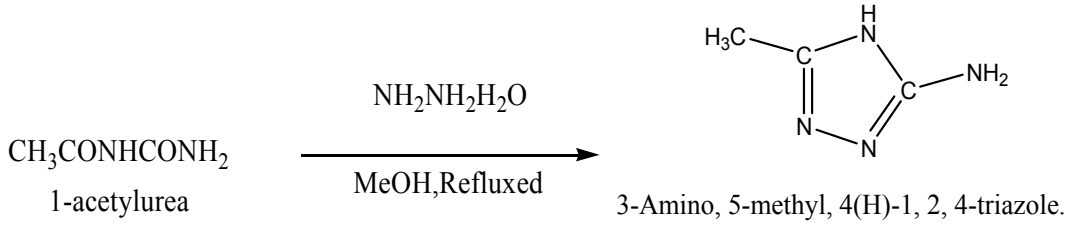
The structures of synthesized compounds were confirmed by IR,1HNMR and mass spectral analysis. The titled compounds were confirmed by IR spectral data showing sharp bands in the range between3324-3433 cm^{-1} for associated NH of Triazoles, 2956-3114 cm^{-1} for Aryl C-H stretching of methyl, 1297-1270 cm^{-1} for C-N stretching and 1230-1127 cm^{-1} for C=S stretching. Compounds (tz01-05) were also confirmed by 1HNMR spectral analysis. Inspection of the 1HNMR spectra suggested that the 1,2,4-triazoles were geometrically pure. Compounds(tz01-05) was also verified their molecular ion peaks (M+).The results revealed that majority of the synthesized compounds showed varying degrees of inhibition against Gram positive bacteria shown in Table. **TZ-05** showed significant antibacterial activity against Gram negative organisms ***P.aeruginosa NCIM 2206***.

Table No. 01: Physical and analytical data of synthesized compounds

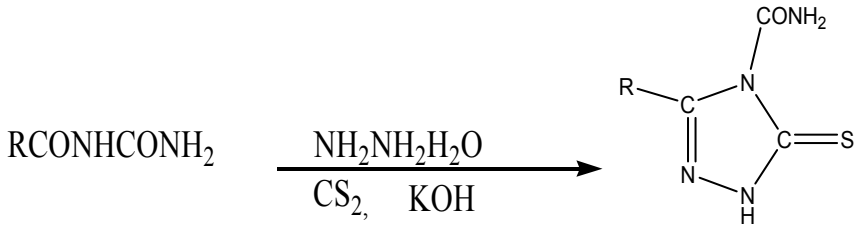
Code	IUPAC Name	Molecular Formula	Molecular weight	% yield	Melting point	Rf	solubility	Colour
TZ-01	3-Amino 5-methyl, 4(H)-1, 2, 4-triazole.	$C_3H_6N_4$	98.11	65	110	0.76	Methanol	yellow
TZ-02	4-carbamyl 3 methyl, 5-thio 1, 2, 4-triazole	$C_4H_6N_4OS$	158.18	74	121	0.74	Methanol DMSO	White
TZ-03	4-carbamyl 3 phenyl 5-thio- 1,2,4-triazole	$C_9H_8N_4OS$	220.25	76	145	0.69	Methanol	Green
TZ-04	1 amino 2 anilino 4 cabamyl 3 methyl 5 thio -1,2,4 triazole.	$C_{10}H_{14}N_6OS$	266.32	75	132	0.69	Methanol	Green
TZ-05	1 amino 2 anilino 4 carbamyl 3 phenyl 5 thio 1,2,4 triazole	$C_{15}H_9N_7OS$	328.39	79	130	0.69	Methanol Chloroform	Grey

***MOBILE PHASE**: Ethyl Acetate:Benzene (2:8) **DETECTING AGENT**:Iodine vapors

Scheme- 1st series



Scheme- 2nd series



R=CH_3 TZ-02, R=C_6H_5 TZ-03

Scheme- 3rd series

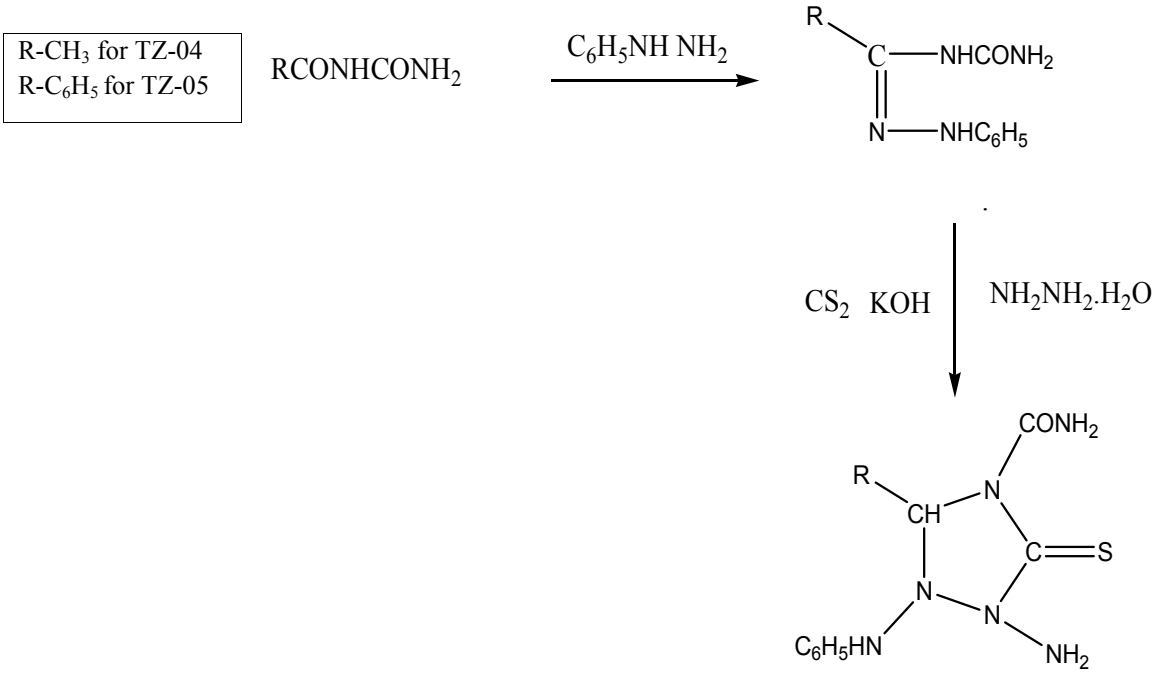


Table No. 02: Antibacterial screening

S.No.	Compound code	*Bacillus Subtilis*		*Pseudomonas aeruginosa*	
		250µg/ ml	500µg /ml	250µg /ml	500µg/ ml
1.	TZ-01	10	12	6	8
2.	TZ-02	12.5	14	8	12
3.	TZ-03	9	12	6	8
4.	TZ-04	8	10	4	6
5.	TZ-05	13.5	15	11	12
6.	STANDARD	16	18	16	17

Table No. 03: Antifungal screening

S.No.	Compound code	*Aspergillus niger*		*Pencillium notatum*	
		250µg/ml	500µg/ml	250µg/ml	500µg/ml
1.	TZ-01	8	10	-	4
2.	TZ-02	8	12	8	12
3.	TZ-03	11	12	6	8
4.	TZ-04	14	15	4	6
5.	TZ-05	10	10	6	10
6.	STANDARD	16	18	16	18

Conclusion

Five novel derivatives of benztriazole were synthesized, characterized by FTIR and 1HNMR, MASS and evaluated for in *vitro* antimicrobial activities by disc diffusion method. Compound **(TZ-05)and (TZ-04)** exhibited significant antibacterial and antifungal activity was observed against (***B.Subtitis,P.Auriginosa***) and (***A.Niger,P.Notatum)***respectively. The result obtained, encourages me to make further research for synthesis different derivatives and *in vivo* trials in experimental animals to broaden their Pharmacological assessment, may provide a new analogue that can overcome drug resistance, prolonged treatment, complex drug regimen and side effects involved.

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