SYTHESIS AND ANTITUBERCULAR ACTIVITY OF SOME TRIAZOLE DERIVATIVES OF PROPYL GALLATE

Sudeep K. Mandal[1], Dibyajyoti Saha[1], Vibhor K. Jain[1*], Bindu Jain[1]

1. School of Pharmacy, Chouksey Engineering College, Lalkhadan, Masturi Road, Bilaspur (C.G) 495004

ABSTRACT:

Triazole is a diunsaturated heterocyclic compound contains two nitrogen groups as hetero atoms. In present study an attempt was made to synthesis various derivatives of substituted 1,2,4-triazol-3-yl)benzene-1,2,3-triol and to assess their antitubercular efficiency. The synthesized compounds were subjected to physical characterization and spectral analysis by IR and NMR for structure elucidation. The compounds were than subjected to evaluation of antitubercular activity against bacterial strain M. Tuberculosis H_{37}Rv by MABA method using rifampicin as standard. The results of antitubercular activity shows that compounds S_3 and S_5 showed equivalent activity when compared with that of standard while rest of the compounds found to be less active than standard.

INTRODUCTION:

Tuberculosis (TB) is a bacterial infection caused by a germ called *Mycobacterium tuberculosis*. The bacteria usually attack the lungs, but they can also damage other parts of the body. TB spreads through the air when a person with TB of the lungs or throat coughs, sneezes or talks. If you have been exposed, you should go to your doctor for tests. You are more likely to get TB if you have a weak immune system. Symptoms of TB in the lungs may include:

- A bad cough that lasts 3 weeks or longer
- Weight loss
- Coughing up blood or mucus
- Weakness or fatigue
- Fever and chills
- Night sweats

If not treated properly, TB can be deadly. You can usually cure active TB by taking several medicines for a long period of time. People with latent TB can take medicine so that they do not develop active TB.

Infection and transmission

Tuberculosis (TB) is a contagious disease. Like the common cold, it spreads through the air. Only people who are sick with TB in their lungs are infectious. When infectious people cough, sneeze, talk or spit, they propel TB germs, known as bacilli, into the air. A person needs only to inhale a small number of these to be infected. Left untreated, each person with active TB disease will infect on average between 10 and 15 people every year. But people infected with TB bacilli will not necessarily become sick with the disease. The immune system "walls off" the TB bacilli which, protected by a thick waxy coat, can lie dormant for years. When someone's immune system is weakened, the chances of becoming sick are greater[11].

- Someone in the world is newly infected with TB bacilli every second.

- Overall, one-third of the world's population is currently infected with the TB bacillus.
- 5-10% of people who are infected with TB bacilli (but who are not infected with HIV) become sick or infectious at some time during their life. People with HIV and TB infection are much more likely to develop TB.

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. The simplest member of the Triazole family is triazole itself, white to pale yellow crystalline solids with a weak characteristic odor; soluble in water and alcohol, melts at 120^0C, boils at 260^0C[2-4].

The triazole antifungal drugs include fluconazole, isavuconazole, itraconazole, voriconazole, pramiconazole, and posaconazole.

The triazole plant protection fungicides include epoxiconazole, triadimenol, propiconazole, cyproconazole, tebuconazole, flusilazole and paclobutrazol, as well as the potent antiviral *N*-nucleoside ribavirin[5-7].

MATERIALS AND METHOD ;

All the chemicals were purchased from local market and purified according to established method. Melting points were recording using VEEGO digital 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 U.V light.

Perkin Elmer FT-IR spectrometer, Bruker advance II 400 MHz NMR spectrometer, LC-MSD- Tranp SL 2010 A SHIMADZU were used for structural elucidation of compounds.

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

SCHEME[2-6]

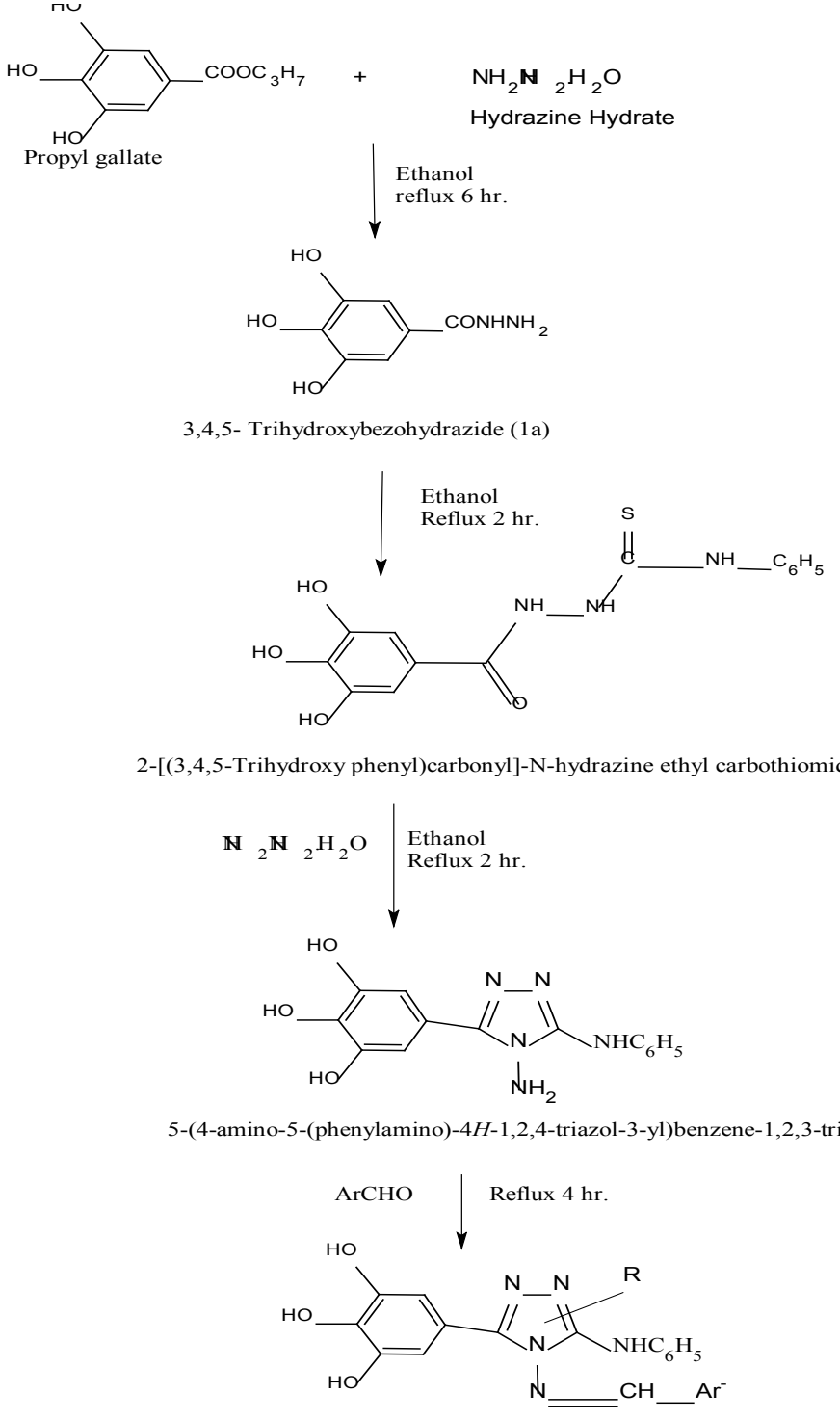
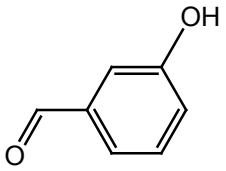
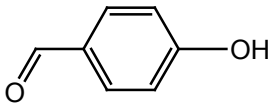
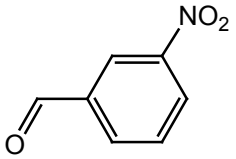
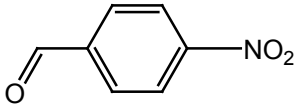
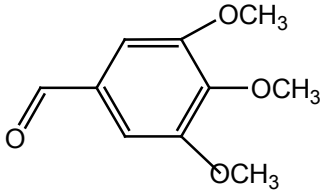


Fig 1. Synthetic scheme

Table 1: List of aromatic aldehyde substituents

S. NO.	COMPOUNDS	STRUCTURE
1.	m- hydroxy benzaldehyde	
2.	p- hydroxy benzaldehyde	
3.	m- nitro benzaldehyde	
4.	p-nitro benzaldehyde	
5.	3,4,5- trimethoxy benzaldehyde	

Step-I: Synthesis of 3, 4, 5-trihydroxybenzohydrazide(Galloyl hydrazide)

Propyl Gallate (0.01 moles) and hydrazine hydrate (0.01 moles) are mixed gently and refluxed for 6 hrs. and the mixture is then cooled and pour into ice cold water. Filtered off the crystals and recrystallized from ethanol. Completion of reaction was monitored on TLC using silica gel-G coated plates by using ethyl acetate and petroleum ether as the eluent and observed in U.V. light.

1 (a) Yield 74%

Melting point 134^0C

Step II: Synthesis of 2-[(3,4,5-trihydoxyphenyl)carbonyl]-*N*-hydrazine phenyl carbothioamide.

A mixture of 3, 4, 5-trihydroxybenzohydrazide (0.01 mol) and phenyl isothiocyanate (0.001 mol) 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. Completion of reaction was monitored on TLC using silica gel-G coated plates by using ethyl acetate and petroleum ether(1:1) as the eluent and observed in U.V. light.

2 (a) Yield 69%

Melting point 154^0C

Step III: Synthesis of 5-(4-amino-5-(phenylamino)-4*H*-1,2,4-triazol-3-yl)benzene-1,2,3-triol

Compound 2-[(3,4,5-trihydoxyphenyl) carbonyl–N-hydrazine phenyl carbothioamide (0.002 mol) and hydrazine hydrate (0.025 mol) was refluxed in methanol for 2 hrs. at a temperature between 50-60 ^{0}C, reaction mixture was cooled and poured over crushed ice. Solid was filtered and recyrastallized from methanol. The completion of reaction was monitored on TLC using silica gel-G coated plates by using ethyl acetate and petroleum ether(1:1) as the eluent and observed in U.V. light.

Yield 71% ; Melting point 167^{0}C

Step IV: Synthesis of 5-(4-(substituted benzylideneamino)-5-(phenylamino)-4H-1,2,4-triazol-3-yl)benzene-1,2,3-triol

To a solution of 5-(4-amino-5-(phenyl amino)-4H-1,2,4-triazol-3-yl)benzene-1,2,3-triol (0.01 mol) in absolute ethanol (30 ml), the appropriate aromatic aldehydes ().012 mol) was added. The reaction mixture was refluxed for 4 hrs. The formed solid after cooling was filtered off and recrystallized to give the title compounds respectively.

IDENTIFICATION AND CHARACTERIZATION[9,10]:

The compounds synthesis were identified and characterized by following methods such as:

A. Melting point determination.

B. Thin layer chromatography.

C. Infra red spectroscopy.

D. Nuclear magnetic resonance spectroscopy.

A. Melting Point determination (M.P.) :

The melting point of organic compound was determined by Thiel's melting point tube (capillary tube method). The determination of melting point is the most important and easy way of differentiating this physical constant of one compound from other.

B. Thin Layer Chromatography (TLC) :

TLC is an important method for synthetic chemistry which helps to characterize the different properties of the compound based on the R_f values since different compound will have different R_f values. It also helps in confirming the progress of the reaction.

The Ethylacetate:petroleum ether was used as solvent system. Iodine chamber and U.V. lamps were used for visualization of spots.

C. Infra Red Spectroscopy (IR) [10,11] :

IR is one of the most important tools for determining the various functional groups and the possible chemical structure. The important advantage of IR over the other techniques is that it gives fingerprints (1300-650 cm) information about the structure (functional group, bonding with each other) of molecules easily. No two compounds have identical fingerprint region.

This technique is based upon the molecular vibration of the compound such that each and every bond will vibrate at the different frequency and this vibration frequency corresponds to the IR frequency. Thus IR spectra of each and every bond will be formed. The infra red spectra of compounds were recorded in JASCO FTIR Spectrometer.

D. Nuclear Magnetic Resonance Spectroscopy (NMR) [10,11] :

The interaction between matter and electromagnetic forces can be observed by subjecting a substance simultaneously to 2 magnetic forces, one stationary and other varying at some radio frequency. At a particular combination of fields, energy is absorbed by the sample and absorption can be observed as a change in signal developed by a radio frequency detector and amplifier. This energy of absorption can be related to a magnetic dipolar nature of the spinning nuclei. This technique is known as Nuclear Magnetic Resonance. This technique is useful in assuming the structure of the molecule. The proton magnetic resonance spectra (^{1}HNMR) were recorded on Bruker Avance II 400 NMR spectrometer in DMSO using Tetramethyl Silane [$(CH_3)_4Si$] as internal standard.

Pharmacological evalution[1]:

Bacterial strain M. Tuberculosis $H_{37}Rv$ ATCC (American Type Culture Collection), $H_{37}Rv$ inoculated was grown on 100 ml of Middlebrook 7H9 broth (Difco, Detroit Mich.) supplemented with 0.2% (v/v) glycerol (Sigma Chemical Co., Stain Louis, Mo.), 10% (v/v) OADC (Oleic acid, albumin, dextrose, catalase, Difco) and 0.5% (v/v) Tween 80 (Sigma). The complete medium referred to as 7H9GC-T80.

Rifampicin (RMP) was positive control (Sigma). MIC was determined after incubated for seven days at 37^0C.

Microplate Alamar Blue Assay (MABA) an anti-TB susceptibility testing was performed in black, clear bottomed, 96-well microplates (Black view plates; Packard Instrument Company, Meriden, Conn.) in order to minimize background fluorescence. Initial drug dilution was prepared in dimethyl sulfoxide and subsequent two fold dilutions were performed in 0.1ml of 7H12 media in the microplates.

The H37 Rv was diluted in 7H9 media to reach approximately $2X10^5$ cfu/ml and 0.1 ml was added to wells. Wells containing compounds only were used to detect autofluorescence of the compounds. Plates were incubated at 37^0C. At day 7 of incubation, 20µl of Alma Blue solution (Trek Diagnostic System, Clevenland, Ohio) and 12.5 ml of 20% Tween 80 were added to all the wells and the plates were reincubated at 37^0C for 24 hr. Fluorescence was measured in a victor II multilabel fluorometer (Perkin Elmer Life Science Inc., Boston, MA) at 530nm and 590 nm.

Percent inhibition was defined as 1-(test well FU/mean FU of triplicate B wells)X100

The lowest drug concentration effecting an inhibition ≥90% was considered the MIC.

RESULTS AND DISCUSSION:

Physical characterization:

Table2 :Physical parameters of synthesized compounds

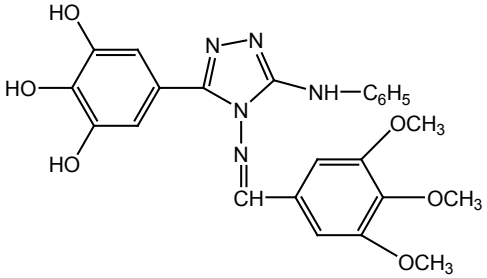
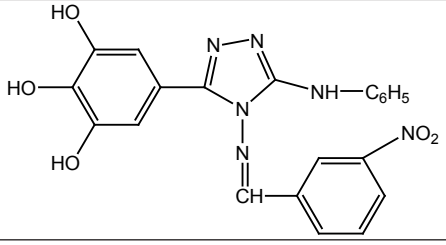
S. No.	Code of compound	R_f value	Melting point (ºC)	Appearance	% of yield	Molecular formula	Molecular weight (gm)
1.	S_1	0.64	159^0C	Brown	80	$C_{24}H_{23}N_5O_6$	477.47
2.	S_2	0.68	169^0C	Yellow	69	$C_{21}H_{16}N_6O_5$	432.39
3.	S_3	0.83	179^0C	Orange	63	$C_{21}H_{16}N_6O_5$	432.39
4.	S_4	0.69	187^0C	Brown	77	$C_{21}H_{17}N_5O_4$	403.39
5.	S_5	0.75	197^0C	Yellow	79	$C_{21}H_{17}N_5O_4$	403.39

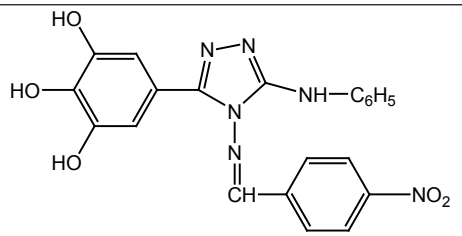
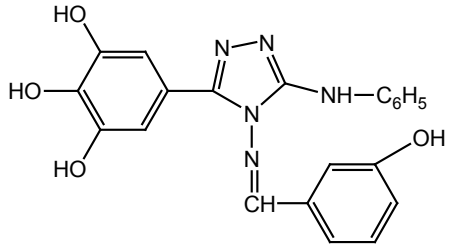
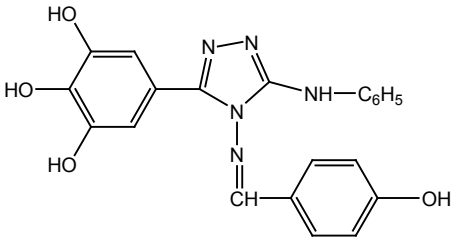
Spectral analysis:

Table3:Spectral data of synthesized compounds

S . No.	Compounds Code	NMR Data	IR Data
1.	S_1	3.65 (s, 9H, CH_3),4.20(s, 1H, NH),5.10 (s, 3H, OH), 6.2-6.75 (m, 9H, Ar-H),8.2(s, 1H, CH)	O-H stretching 3415,=C-H stretching 3158, -C-H stretching 2981,C-O stretching1036, C=C stretching 1658,C=N stretching 2353 C-N stretching 1093
2.	S_2	4.15 (s, 1H, NH) 5.2 (s, 3H, OH) 6.2-6.8 (m, 11H, Ar-H) 8.2 (s, 1H, CH)	O-H stretching 3458, =C-H stretching 3192, C=N stretching 2333, C-O stretching 1104 , =C stretching 1660, C-N stretching1153, C-NO_2 stretching 1383
3.	S_3	4.15 (s, 1H, NH) 5.20 (s, 3H, OH) 6.1-6.9 (m, 11H, Ar-H), 8.1 (s, 1H, CH)	O-H stretching 3295,=C-H stretching 3142, C=N stretching 2347,C-O , stretching 1016, C=C stretching 1613, C-N stretching 1044,C-NO_2 stretching 1323
4.	S_4	4.1 (s, 1H, NH), 5.2 (s, 4H, OH) 6.2-6.85 (m, 11H, Ar-H), 8.10 (s, 1H, CH)	O-H stretching 3458, =C-H stretching 3153, C=N stretching 2342, C-O stretching 1104, C=C stretching 1660, C-N stretching 1153
5.	S_5	4.20 (s, 1H, NH), 5.10 (s, 4H, OH) 6.2-6.75 (m, 11H, Ar-H) 8.2 (s, 1H, CH)	O-H stretching 3365, =C-H stretching 3116, C=N stretching 2362, C-O stretching 1016, C=C stretching 1613, C-N stretching 1044

Table 4 :Structure of synthesized compounds

S.No.	Compounds	Structure of the Compounds
1.	S_1	
2.	S_2	

3.	S_3	
4.	S_4	
5.	S_5	

Anti tubercular activity:

Table 3: Anti tubercular activity of compounds S_1-S_5 using MABA Method

Compound code	% inhibition at (100 µl/ml)
S_1	98
S_2	95
S_3	100
S_4	91
S_5	100
Standard	100

*** Rifampicine (RMP) 10 µl/ml used as standard drug**

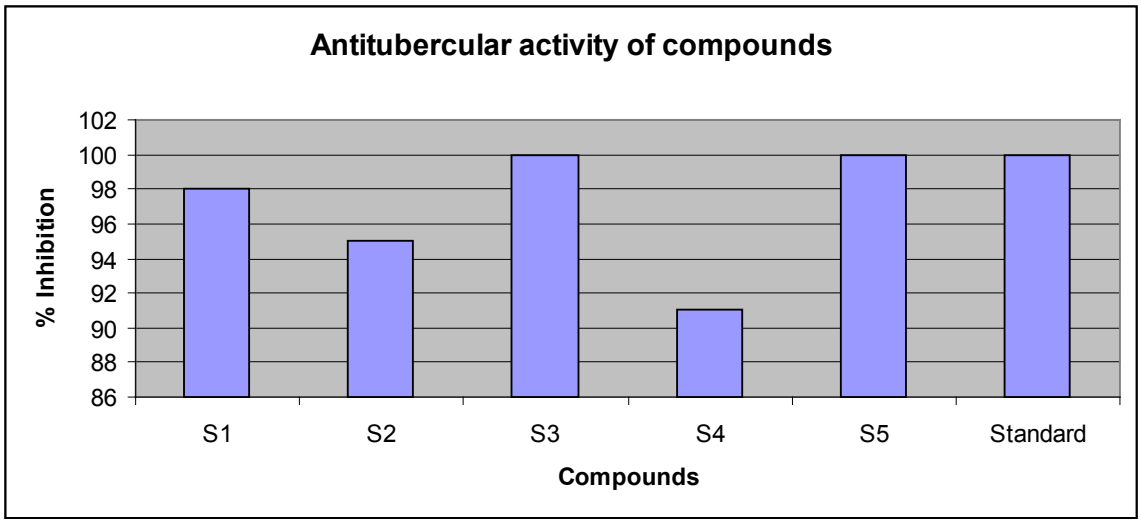


Fig 2: % inhibition of compounds

Conclusion:

The antitubercular activity reveals that S_3 and S_5 shows comparable activity with that of standard against M. tuberculosis $H_{37}Rv$. The remaining compounds found to be less active than standard. Rifampicin was used as the standard in this assay.

REFERENCES:

[1] Collins L A, Franzblau S G, Micro plate alamar blue assay versus BACTEC 460 system for high throughput screening of compounds against Mycobactrium avium, Antimicrob agents Chemother, 1997, 14, pp 1004-1009

[2] Wilson and Gisvold's Text book of organic medicinal and pharmaceutical chemistry, Eleventh edition, pp.1-3.

[3] Thomas L. Lemke, David A. Williams, Victoria F. Roche, S. Williams Zito, Foye's principle of medicinal chemistry, 6th edition, pp.1-2.

[4] Harkishan Singh and V.K. Kapoor, Medicinal and pharmaceutical chemistry edition 2005, pp. 1-2.

[5] Ashutosh Kar,Text book of Medicinal chemistry, revised 3rd edition, pp. 1-3.

[6] Udupi RH, P Purushottamachar. Synthesis and biological activity of 3-pyridyl-4-[*N*-substituted phenyl carboxamido]-5-mercapto-1, 2, 4-triazoles. Indian J. Heterocyc Chem, March 2000;9:189.

[7] Kalluraya Balakrishnan, Chimbslkar Ramesh M, Hegde Jyoti. Anticonvulsant activity of Nicotinyl / Isonicotinyl substituted 1, 2, 4-triazole-5-thione Mannich bases. Indian J. of Heterocyc Chem, July 2005;16:15-18.

[8] Pathak US, Rathod IS, Jain KS, Laddha NS, Kolhe KS. Synthesis of novel 1, 2-disubstituted -6, 7-dimethyl-1*H*, 5*H*-thieno[2, 3-*d*]-[1, 2, 4]triazolo[1,5-*a*]pyrimidin-5-ones. Indian J. Chem, Jul 1997;36B:566-571.

[9] Connors, K.A (2004), 'A Textbook of Pharmaceutical Analysis', John Wiley and Sons, Inc., IIIth edition.

[10]Kalsi, P.S. (2004), 'Spectroscopy of Organic Compounds', New Age International (P) Ltd., Vth edition.

[11]Kemp, W. (2002), 'Organic Spectroscopy', Palgrave, IIIth edition

[12]Ziyi Zhang, Pengfei Xu, Xinping Yang and Shaozu Wu. Synthesis of 1,2,4-trizole, 1,3,4-thiadiazole and 1,3,4-oxadiazolederivatives of 6-nitrobenzimidadazole. Indian J. Chem, feb 1998;37B:127-131.

[13]Mohan Jag, kumar Vineet. Bridgehead nitrogen heterocyclic systems: Synthesis and antimicrobial activity of *s*-triazolo[3,4-b] [1,3,4]thiadiazoles, s-triazolo[3,4-b][1,3,4] thiadiazines and s-triazolo[3',4':2,3]-thiadiazino[5,6-b]quinaxaline. Indian j. Chem, Feb 1998;37B:183-186.