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Research Article

**STUDIES ON THE SYNTHESIS OF SOME NEW 1,2,4-  
TRIAZOLES DERIVATIVES AND EVALUATION FOR  
THEIR ANTI-FUNGAL ACTIVITY PROFILES**Md Akram\*<sup>1</sup>, Abdul Sayeed<sup>1</sup>, Mohd Waseem Akram<sup>2</sup>, MD Ather Ali Soherwardi<sup>3</sup><sup>1</sup> Mesco College of pharmacy Hyderabad (T.S)<sup>2</sup> Luqman College of pharmacy, Gulbarga (KA)- 585102<sup>3</sup> Anwarul Uloom College of pharmacy Hyderabad (T.S)**Abstract:**

*The synthesis of new heterocyclic compounds has always drawn the attention of medicinal chemist over the years mainly because they possess diverse biological properties.*

*The literature survey on 1,2,4-triazoles revealed that they are endowed with wide variety of biological activities. During the present investigation a series of new 1,2,4-triazole derivatives N-(3-(2-(3-hydrazinyl-3-oxoalkanoyl)hydrazinyl)-5-(phenoxymethyl)-4H-1,2,4-triazol-4-yl)isonicotinamide (6a-6e) were synthesized by reacting with N-(5-mercapto-3-(phenoxymethyl)-4H-1,2,4-triazol-4-yl)isonicotinamide (5) and aliphatic dicarboxylic acid hydrazides (a-e). The structures of the newly All the compounds synthesised 6a to 6e were evaluated for antifungal activity against *Candida albicans* and *Aspergillus Niger* was carried out and MIC values were determined synthesized compounds were established by FT-IR, 1H-NMR and MASS spectral analysis.*

*The compound 6a (n=0) was found to be the most potent antifungal agent.*

**Keywords:** 1,2,4-triazole derivatives, antifungal, *Candida albicans*, *Aspergillus Niger*

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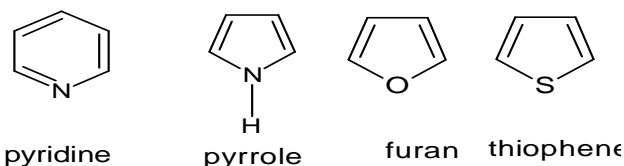
**INTRODUCTION:**

A strict definition of a heterocyclic compound is one which possesses a cyclic structure with at least two different kinds of atoms in ring. One of which is carbon, and can be aliphatic or aromatic. A heterocyclic compound usually possesses a stable ring structure which does not readily hydrolyze or depolymerize. It is obvious that the number and variety of heterocyclic compound with reported antimicrobial activity is extensive with respect to structure and application. Heterocyclic compounds exhibited remarkable pharmacological activities. The heterocyclic compounds containing pyrimidine, pyridine, and piperazine nucleus have wide range of therapeutic activity such as antitubercular, anticancer, antihelminthic, antioxidant and antimicrobial activities is also believed that the presence of N-C=S linkage is responsible for the amoebicidal, anticonvulsant, fungicidal and antiviral activities [1-4].

In this direction, the work is being pursued to investigate the antimicrobial activity of some heterocyclic compounds prepared in our laboratory. Various piperazine nucleus containing derivatives have been synthesized. All the synthesized derivatives shows better anti bacterial and anti fungal activity against various strains of bacteria and fungi viz. *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Aspergillus niger*, *Microsporum gypseum*. After the antimicrobial studies, it were found that heterocyclic Compound showed excellent activity against the bacterial strain of *Pseudomonas aeruginosa* and compound showed very good activity against the fungal strain of *Aspergillus niger*.

Incorporation of oxygen, nitrogen, sulphur, or an atom of a related element into an organic ring structure in place of a carbon atom gives rise to a heterocyclic compound. Since the heterocyclic atom must form more than one bond in order to be incorporated into a ring structure, halogens do not form heterocyclic compounds although they may be substituents on a heterocyclic ring structure. Heterocyclic compounds, like polycyclic ring compounds, are usually known by non-systematic names. They may be either simple aromatic rings or non-aromatic rings. Some

examples are pyridine, pyrrole, furan and thiophene.



Many heterocyclic compounds are biosynthesized by plants and animals and are biologically active. Some heterocycles are fundamental to life, such as haem derivatives in blood and the chlorophylls are essential for photosynthesis. Similarly, the paired bases found in RNA and DNA are heterocycles, as are the sugars that in combination with phosphates provide the backbones and determine the topology of these nucleic acids. The biological properties of heterocycles in general make them one of the prime interests of the pharmaceutical and biotechnology industries.

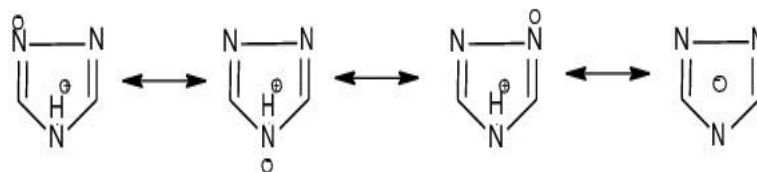
**TRIAZOLES**

In five-membered ring systems, the presence of three nitrogen atoms defines an interesting class of compounds, the triazoles. These may be of two structural types, the 1,2,3-triazoles or *v*-triazole (1) and 1,2,4-triazoles or *s*-triazoles (2).



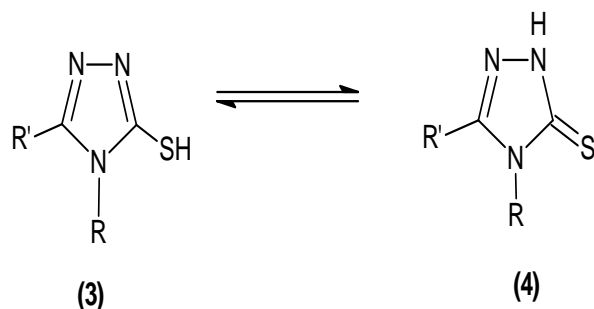
The name triazole was first given to the carbon nitrogen ring system  $C_2H_3N_3$  by Bladin, who described its derivatives in 1885. Later on because of various applications, triazoles took special attention particularly by the chemical industry. Different chemical reactions show that

1,2,4-triazole is an extremely stable nucleus, which can be regarded as aromatic in nature, Stabilized by resonance as shown below.

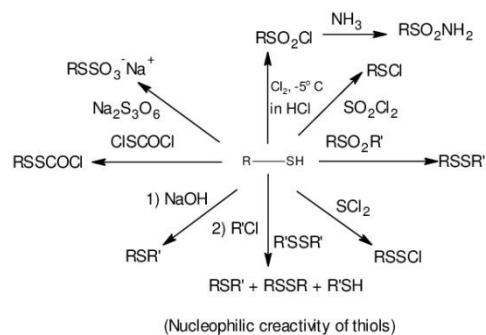
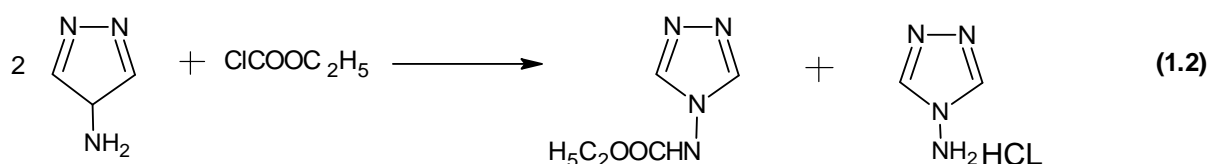
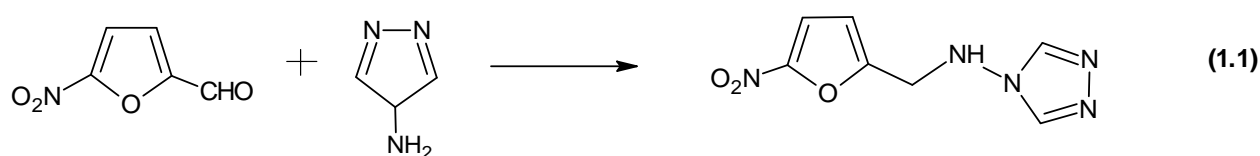


**4,5-Disubstituted-1,2,4-triazole-3-thiones:**

Triazoles having a mercapto group at C-3 (3) constitute a special class of compounds among the substituted 1,2,4-triazoles because of the tautomerism exhibited by them. The labile hydrogen atom may be attached to either nitrogen or sulfur atom. Previous studies of IR, NMR and UV spectra have provided strong evidence that mercaptotriazoles are present predominantly in the thione form (4).



Due to lability of hydrogen, both the S-substitution and N-substitution can be carried out by changing the reaction to electron deficient alkenes and alkynes, ring opening of oxiranes and acylation. At low temperatures, oxidative chlorination of mercaptotriazoles is an excellent method of obtaining the corresponding sulfonyl chloride which can be converted to sulfonamide. The mercapto group can be eliminated using reagents like Raney nickel, dilute nitric acid or hydrogen peroxide. Mercapto groups are easily converted into their methylethers with sodium hydroxide and methyl iodide and these ethers have little tendency to lose methanethiol on standing. Some important reactions of thiols are shown in below.

**5-(Substituted)-4-amino-1,2,4-triazole-3-thiones:**

Among the substituted triazoles, aminotriazoles constitute class because of their widespread biological activities eg. 3-aminotriazole, better known as amizol was the first triazole which was used as an important herbicide. Aminotriazoles may have a C-amino group or an N- amino group. C-aminotriazoles behave as normal aromatic amines and can be diazotized in aqueous mineral acid with nitrous acid, forming diazonium salts which couple with aromatic bases. The detection of 3-amino-1,2,4-triazole in plant extracts depends on this property. Both C- and N-aminotriazoles undergo other characteristic reactions of a primary amine. The amino group of 4-amino-1,2,4-(4H)-triazole reacts with aldehydes forming the corresponding Schiff base. The Schiff bases obtained from 5-nitrofurfural and 5-nitrofurylacylic aldehyde compare favorably with furacin (5-nitrofurfural semicarbazone) in bacteriostatic properties (Eq. 1.1). As expected, ethyl chloroformate readily forms ethyl N-(1,2,4-(4H)-triazol-4-yl) carbamate with 4-amino-(4H)-1,2,4-triazole (Eq. 1.2).

**Biological Activities of 1,2,4-Triazoles:**

4,5-disubstituted-2,4-dihydro-3H-1,2,4-triazole-3-thiones and their derivatives have gained a lot of interest in the last decade due to their biological, industrial and agricultural importance. A well known example is that of fluconazole, a broad spectrum antifungal agent for the treatment of superficial and systemic infections.

Recently it has been investigated that 1,2,4-triazoles are associated With A Variety Of Pharmacological Activities Such as antifungal, diuretic, antibacterial, hypoglycemic, antitubercular

Antidepressant, antiamoebic, antibiotic, anti-inflammatory, anti-carcinogenic, hypnotic, sedatives, Plant growth regulators and insecticidal.

Some pyridyl and pyrimidyl substituted mercaptotriazoles (5) have been reported to exhibit anti thyroid activity. N-amino mercaptotriazoles (6)

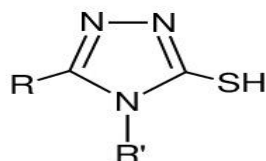
have been reported to show significant bactericidal and fungicidal activity. Some disubstituted mercaptotriazoles (7) and (8) have been tested in vitro against a number of fungi and they showed moderate to good activity.

The highest activity against all species was shown by (8) (R=4-IC<sub>6</sub>H<sub>4</sub>). Acylation of some aminotriazoles gives triazoleamides (9) which specifically inhibited rubella virus.

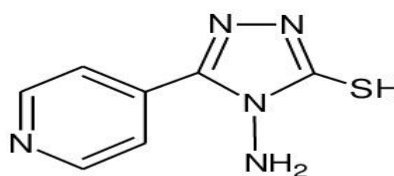
4-Amino-1,2,4-triazoles and their derivatives (10) and (11) have been prepared and found to possess bactericidal and/or fungicidal activity.

Certain 4-alkyl substituted triazoles (12) have been reported to inhibit reserpine-induced ptosis in mice with an ED<sub>50</sub> of 0.27 mg/kg.

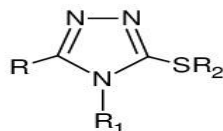
Certain substituted 5-(4-pyridyl)-3-mercapto-1,2,4-triazoles (13) have been used as additives.



R=2 - 3 -4 -pyridyl  
R' = H, CH<sub>3</sub>  
(5)



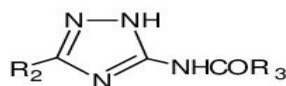
(6)



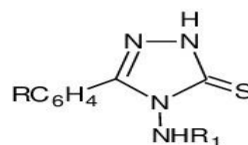
R=2,6 Me<sub>2</sub>C<sub>2</sub>H<sub>3</sub>OCH<sub>2</sub>  
R<sub>1</sub>= 4 - MeOC<sub>6</sub>H<sub>4</sub>, PhCH<sub>2</sub>  
R<sub>2</sub>= Me  
(7)



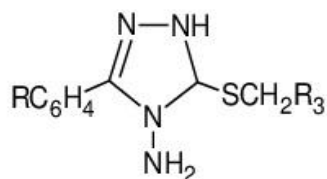
R= Ph, 4 -BrC<sub>6</sub>H<sub>4</sub>, 4 - IC<sub>6</sub>H<sub>4</sub>, 2 -, 3 -, 4 -MeC<sub>6</sub>H<sub>4</sub>  
(8)



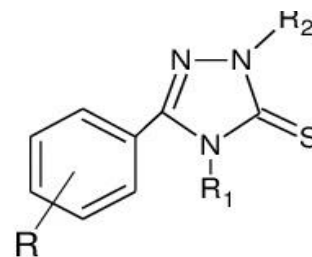
R<sub>2</sub>=H, MeS, NH<sub>2</sub>, MeSO<sub>2</sub>  
R<sub>3</sub>=2 - ClC<sub>6</sub>H<sub>4</sub>CO, EtCO, Me<sub>2</sub>CH<sub>2</sub>CO PhOCH<sub>2</sub>CO  
(9)



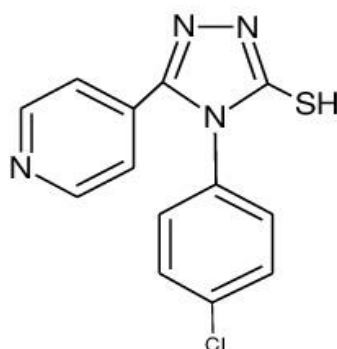
R=H, p - MeO, p -Cl, m -Br, p -Br  
R<sub>1</sub>=H  
(10)



R=H, o-, m-, p-Me, p-MeO  
 $R_3 = \text{CO}_2\text{Me}$   
**(11)**



R=Halo,  $\text{C}_{1-6}$  alkyl,  $\text{CF}_3$   
 $R_1$  &  $R_2 = \text{C}_{1-6}$  alkyl  
**(12)**

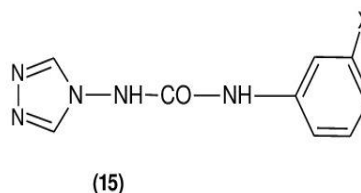
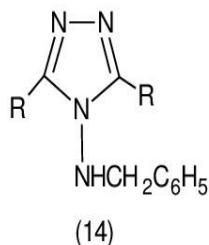


**(13)**

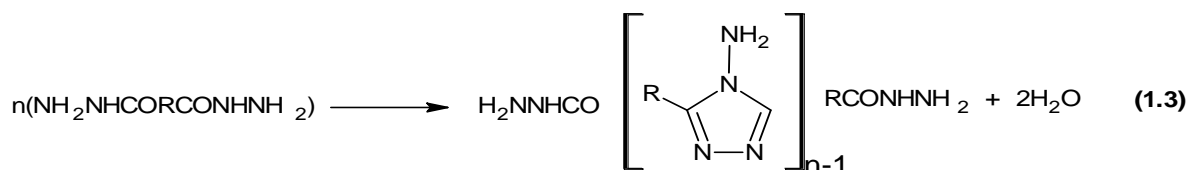
#### Uses of 1,2,4-Triazoles:

1,2,4-Triazoles and condensed triazole systems have found considerable use in photographic industry. 1,2,4-triazole derivatives e.g. N,N'-bi(5-methyl-1,2,4-triazol-3-yl) formamidines are capable of inhibiting fog formation in silver halide emulsions by incorporating in the emulsions. The addition of 5-mercaptotriazole or their soluble salts to the emulsion after development produces a greater maximum image density and prevents bronzing effect. The sodium salt of a sulfonated

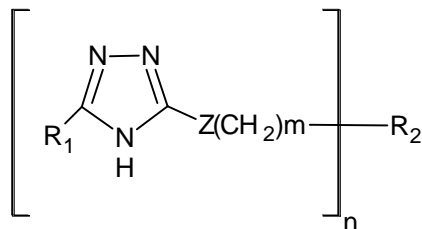
derivative possesses good detergent action e.g. N-benzylated aminotriazole (14) have useful properties in inhibiting the acid fading of dyestuff. Some 4-(phenylureido)-1,2,4-triazole derivatives have been used as defoliants e.g. (15; 50.0 wt. parts) when formulated with sodium-dodecylbenzenesulfonate 3.0, sodium-ligninsulfonate 2.0, SiO<sub>2</sub>: 15.0 and clay 30.0 wt. parts gives 50% wettable powder and it causes 50% defoliation of cotton at 0.5 kg/ha, Vs 20% with (BuS)PO.



A new series of condensation polymers polyaminotriazoles have been prepared, which are similar to nylon. These are fiber forming and can be melting spun to give filaments which after drawing possess high strength and good affinity for dyestuffs.



These polyaminotriazoles are condensation polymers which are produced by heating dihydrazides in presence of small amount of hydrazine. The name refers to the rearranging linkage, the 4-amino-1,2,4-triazole ring (Eq, 1.3). Some 1,2,4-triazole derivatives (16) and (17) have been used as stabilizers for chlorine-containing thermoplastic polymers.

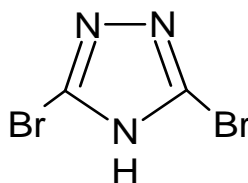


$R_1 = \text{H, OHC}_{1-6} \text{ alkoxy or alkylthio CO}_2\text{H, SH}$

$R_2 = \text{H, OH, SH, NH, O, S, CH}_2$

$m = 1-20, n = 1, 2$

(16)



$R = \text{H, NH}_2, \text{SH, C}_{1-12} \text{ alkylthio}$

$R_1 = \text{CO}_3\text{R}_3$

(17)

## OBJECTIVES

### PURPOSE OF THE WORK

The treatment of infectious disease still remains an important and challenging problems because of emergence of new infectious disease and the increasing number of multidrug resistant microbial organisms. In spite of a large number of chemotherapeutic agents existing for medicinal use,

at the same time the emergence of development of resistance to most of these in the last decades reveals a substantial need for the new classes of antimicrobial agents. There is really perceived need for the discovery of new compounds endowed with antimicrobial activity, possibly acting through mechanisms of action, which are distinct from those of well known antimicrobial agents, for which the

pathogens are now resistant[5].

Tuberculosis is believed to be present in about 1/3<sup>rd</sup> of the world's population[6]. Active disease following new infection, as well as reactivation of latent tuberculosis, is particularly prevalent in individuals with compromised immune systems such as those HIV positive. In addition, the emergence of drug resistance strains of *Mycobacterium tuberculosis* has led to the increased pressure on current chemotherapy regimens[7]. Hence there exists, urgent need for the discovery of newer molecules, which may have potential to curb the above mentioned infectious diseases.

3,5-disubstituted 1,2,4-triazole and its derivatives have been reported to possess wide spectrum of activities ranging from anti-bacterials[8], anti-inflammatory[9], anti-convulsant[10], anti-neoplastic[11], antimalarial[12], anti-viral[13], anti-cancer[14], anti-TB[15] and anti-proliferative[16].

Pyridine, a heterocyclic nucleus played a vital role in the development of different medicinal agents and in the field of agrochemicals.

This nucleus is present in many products such as drugs, vitamins, food, flavorings, plant dyes, adhesives and herbicides[17].

It is seen from the current literature that pyridine congeners are associated with different biological properties like pesticidal[18,19], insecticides[20] and fungicidal[21] activity.

In view of the above mentioned facts and in continuation of our interest in the synthesis of nitrogen heterocycles to identify new candidate, that may be value in designing new, potent, selective and less toxic chemotherapeutic agent, we propose herein for the synthesis of some novel derivatives incorporating suitably substituted 1,2,4-triazole moiety with long aliphatic  $\alpha,\omega$ -dicarboxylic acid of variable lengths.

This combination suggested is an attempt to investigate the influence of such hybridization and structure variation on the anticipated biological activities, hoping the possibility that, the target derivatives might be more efficacious as antimicrobial agents.

#### Objectives:

##### The present investigation includes:

1. Preparation of N-(3-phenoxyethyl-5-mercapto-4H-1,2,4-triazol-4-yl) isonicotinamide nucleus by following literature method.
2. Preparation of dihydrazides using various  $\alpha,\omega$ -aliphatic dicarboxylic acids namely oxalic acid, malonic acid, succinic acid, glutaric acid, adipic acid by the reaction with hydrazine hydrate(99%) through their corresponding ethyl ester formation.
3. Condensing the above prepared acid dihydrazides (a-e) with 3-phenoxyethyl-5-mercapto-1,2,4-triazole to form various 5-

substituted N-(3-phenoxyethyl-5-mercapto-4H-1,2,4-triazol-4-yl)isonicotinamides (6a-6e).

4. Determination of homogeneity of all the synthesized compounds by TLC technique and confirmation of the structures by determining their IR, <sup>1</sup>HNMR, MASS spectra.

5. Evaluation of all the synthesized compounds for their antifungal profiles.

#### METHODOLOGY

##### INTRODUCTION

The importance of 1,2,4-Triazole moiety has been discussed in the previous chapter. Among the many methods available for the synthesis of 1,2,4-Triazole derivatives, in the present chapter a convenient and versatile methodology has been adopted for the synthesis of 1,2,4-Triazole derivatives.

##### MATERIAL AND METHODS:

a) The entire chemicals used were procured from Qualingens, Himedia and Loba-chemicals.

Purity of starting materials used for reaction was confirmed by checking their melting point or boiling point and by thin layer chromatography.

b) Purity of compounds was checked on "Silica Gel G" coated on laboratory micro slides prepared by dipping method or pre-coated plates, eluent was the mixture of different polar and non-polar solvents in varying proportions and detection was done either by observing in UV (ultra-violet) light or exposure to iodine vapours as required. The absence of TLC spots for starting materials and appearance of new TLC spot at different R<sub>f</sub> value ensured the completion of reaction.

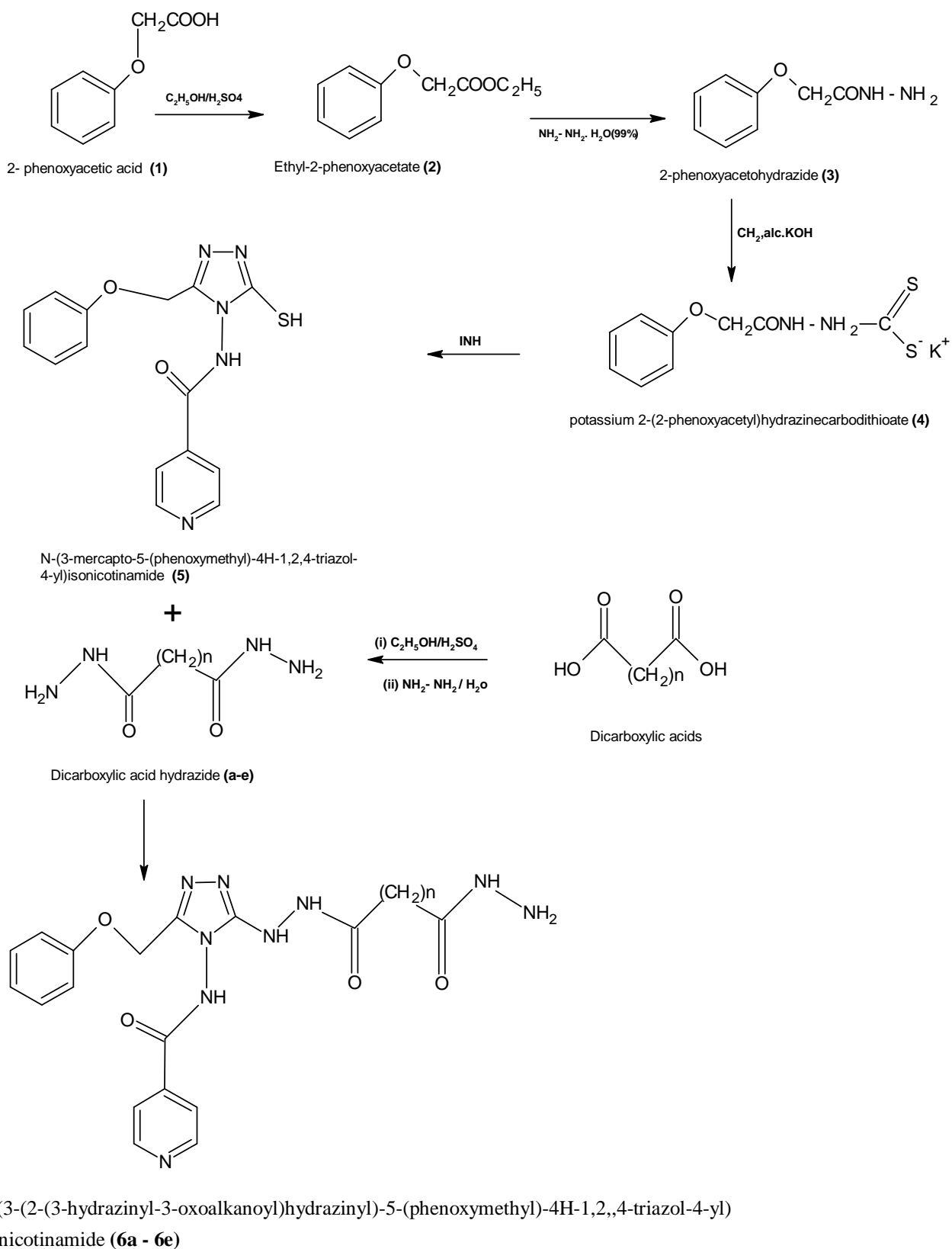
c) Melting points were determined in open capillary tube using precision melting point apparatus and uncorrected.

d) The FT-IR spectra of the synthesized compounds have been obtained from **BLDE College of Pharmacy, Bijapur**. The IR spectra were recorded on SHIMADZU PERKIN EKMER 8201 PC IR SPECTROMETER using a thin film on potassium bromide pellets.

e) The <sup>1</sup>HNMR spectra of the selected compounds have been obtained from Astra **Zeneca Pharma India Ltd, Bangalore**. The <sup>1</sup>HNMR spectra were recorded on **BRUKER AVANCE II 300 NMR SPECTROMETER** in a mixture of DMSO. Chemical shift ( $\delta$ ) values are reported as values in ppm relative to TMS ( $\delta=0$ ) as internal standard.

f) The Mass spectra of the selected synthesized compounds have been obtained from **Oxygen Healthcare Research P. Ltd, Ahmedabad**. The FAB mass spectra were recorded on **JEOL SX-102/DA-6000 Mass Spectrometer**.

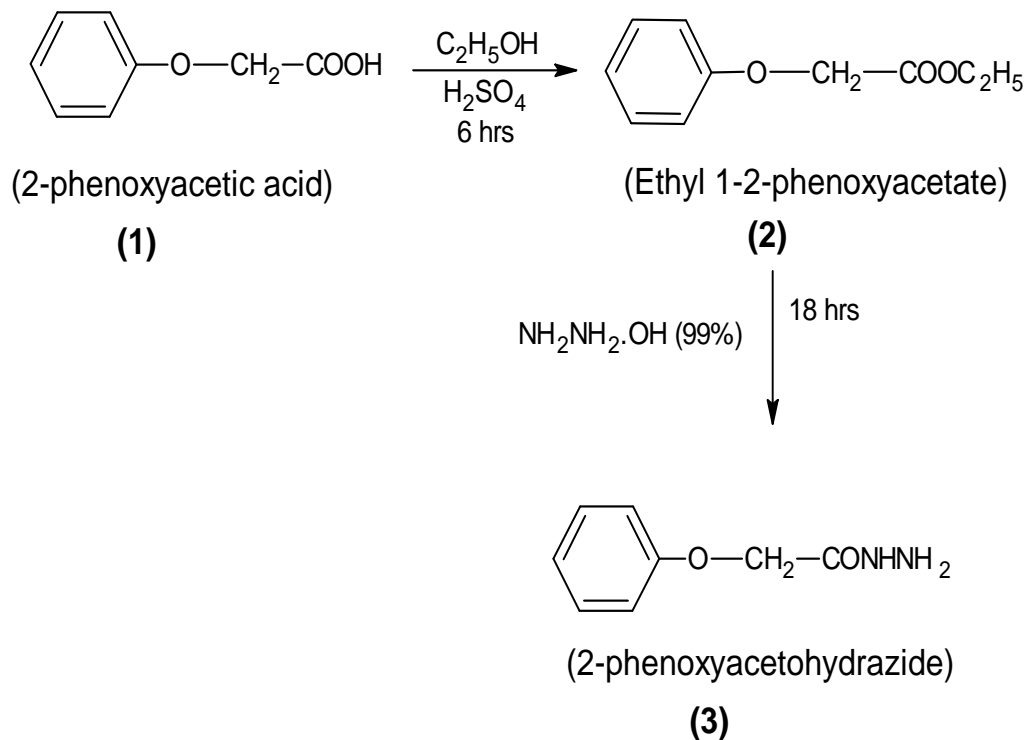
## SCHEME:





## SYNTHETIC STUDIES:

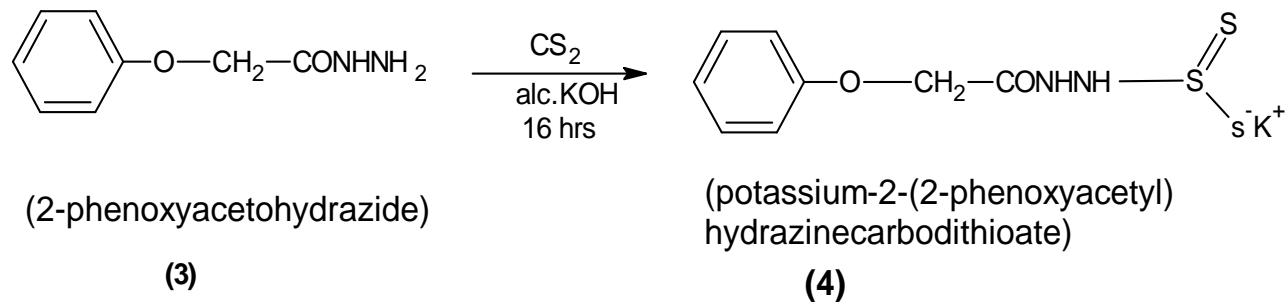
## STEP 1: Preparation of 2-phenoxyacetohydrazide (3):-



The acid (1) (0.1 mole) and ethanol (50 ml) were taken with a few drops conc.H<sub>2</sub>SO<sub>4</sub> and was refluxed for 6 hours. The reaction mixture was concentrated by distilling off the excess of ethanol under reduced pressure. The ester (2) obtained was used for the preparation of hydrazide directly.

The ester (2) (0.1 mole) was dissolved in appropriate quantity of ethanol and to this hydrazine hydrate (0.1 mole) was added. The reaction mixture was refluxed for a period of 12-18 hours. Excess of ethanol was distilled off under reduced pressure. It was then poured in to ice cold water and the solid obtained was filtered. It was recrystallized from ethanol.

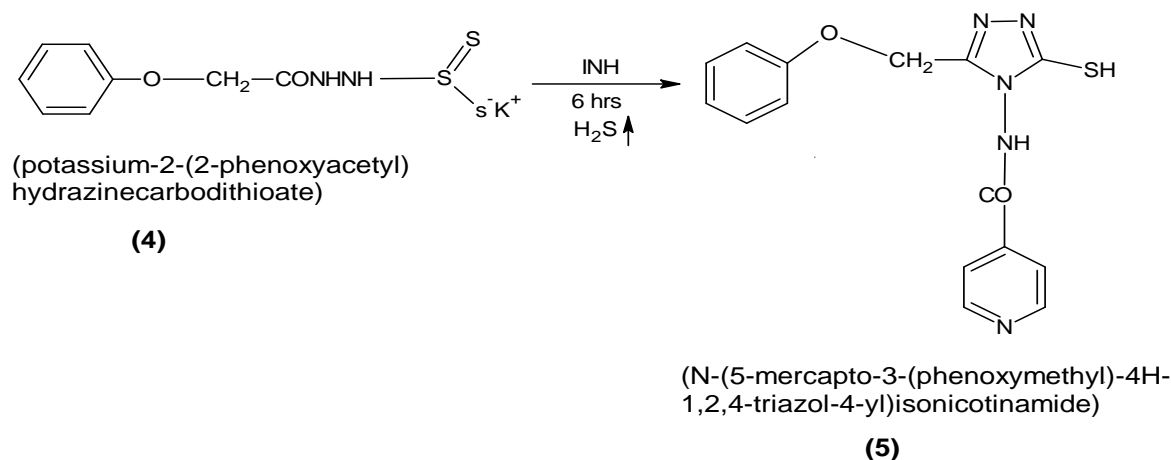
## STEP 2 : Preparation of potassium-2-(2-phenoxyacetyl)hydrazinecarbodithioate (4) :-



To a solution of potassium hydroxide (KOH) (0.15 mole) in absolute ethanol (125 ml), 2-phenoxyacetylhydrazide (3) (0.1 mole) and carbon disulphide (CS<sub>2</sub>) (0.15 mole) were added and the mixture was agitated for 16 hours. To the resulting solution, anhydrous ether (250 ml) was

added and the precipitated product (4) was collected by filtration, washed with ether and dried under vacuum at 65<sup>0</sup>C. This potassium salt (4) was used in the next step without further purification.

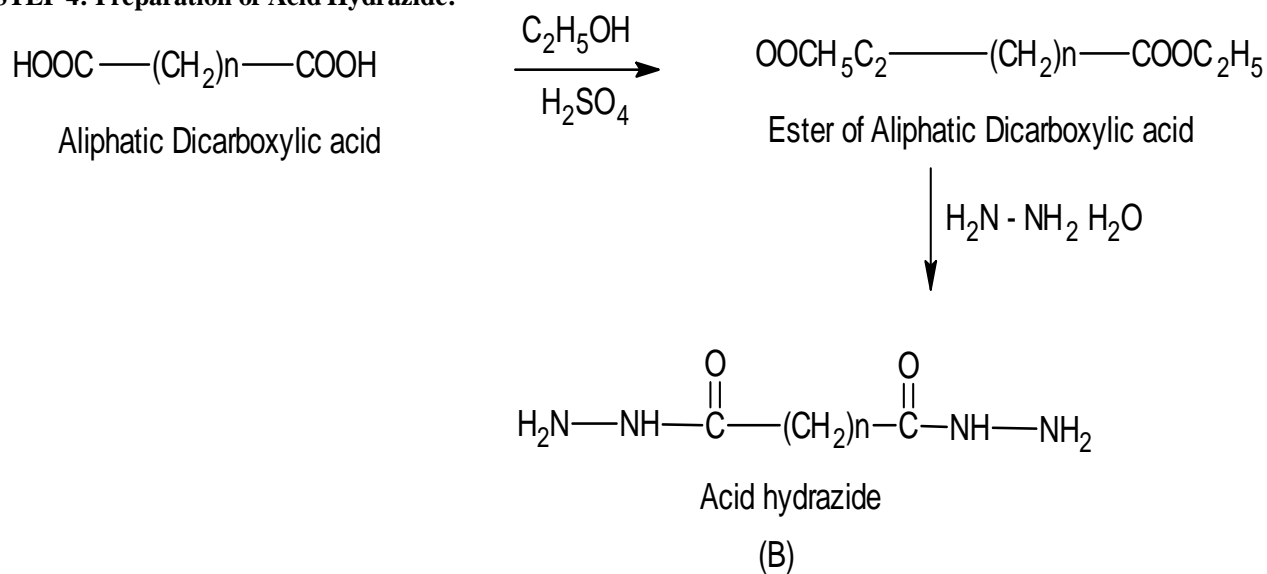
**STEP 3: Preparation of N-(5-mercapto-3-(phenoxyethyl)-4H-1,2,4-triazol-4-yl) isonicotinamide (5) :-**



A suspension of the potassium salt (4) (0.1 mole), Isonicotinic acid hydrazide (INH) (0.1mole) and water (5 ml) were heated under reflux for 6 hours and hydrogen sulphide (H<sub>2</sub>S) gas was evolved and clear solution was resulted. The dilution of reaction

mixture with cold water (50 ml) and subsequent acidification with dilute hydrochloric acid (HCl) gives the triazole (5), which was filtered, washed with water and recrystallized from aqueous ethanol.

**STEP 4: Preparation of Acid Hydrazide:**

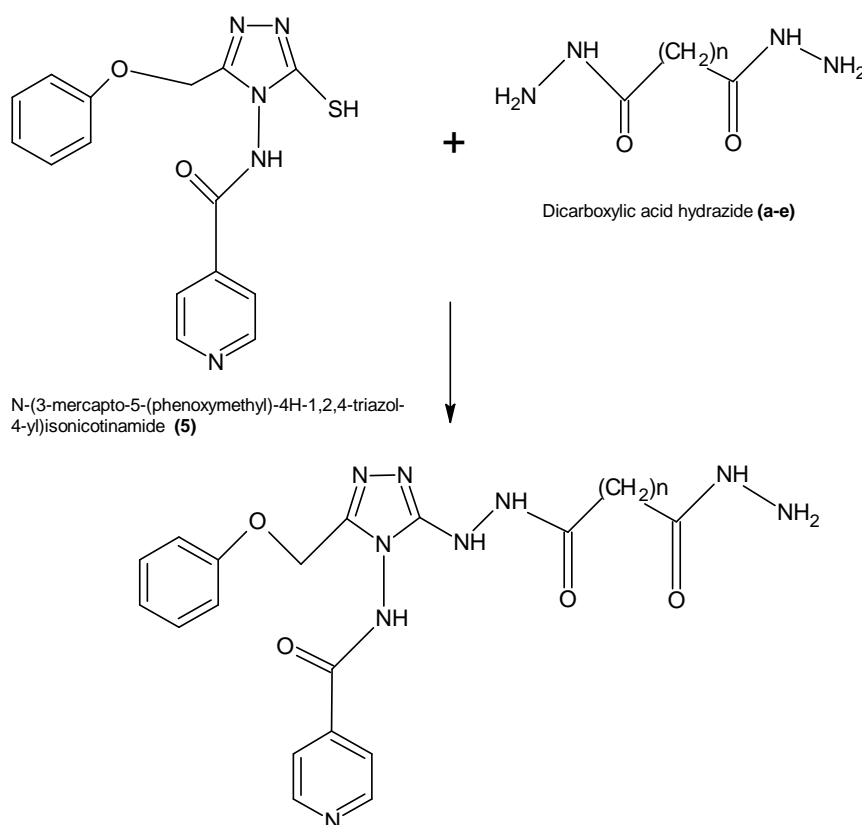


The acid (0.1 moles) and absolute ethanol (50 ml) were taken with a few drops conc. H<sub>2</sub>SO<sub>4</sub> and was refluxed for 6 hours. The reaction mixture was concentrated by distilling off the Excess of ethanol under reduced pressure. The ester obtained was used for the preparation of hydrazide directly.

The ester (0.1mole) was dissolved in appropriate

quantity of ethanol and to this hydrazine hydrate (0.2 mole) was added. The reaction mixture was refluxed for a period of 12-18 hours. Excess of ethanol was distilled off under reduced pressure. It was then poured into ice cold water and the solid obtained was filtered and dried. It was recrystallized from aqueous ethanol.

**STEP 5: Preparation Of *N*-(3-(2-(3-hydrazinyl-3-oxoalkanoyl) hydrazinyl)-5- (phenoxy methyl)-4*H*-1,2,4-triazol-4-yl)isonicotinamide (6a-6e) :**



N-(3-(2-(3-hydrazinyl-3-oxoalkanoyl)hydrazinyl)-5-(phenoxy methyl)-4*H*-1,2,4-triazol-4-yl) isonicotinamide (**6a - 6e**)

0.1 mol of *N*-(5-mercapto-3-(phenoxy methyl)-4*H*-1,2,4-triazol-4-yl) isonicotinamide (**5**) and 0.1 mol of acid hydrazide (**a-e**) in the presence of glacial acetic acid taken in 50 ml of ethyl alcohol and refluxed in an anhydrous condition for 8hr. The reaction mixture was cooled to room temperature and filtered the product and separated. It was dried and recrystallised from absolute ethanol.

**BIOLOGICAL ACTIVITIES:****Anti-Fungal activity:**

The antifungal activity of 1,2,4- triazoles work carried out against the fungi *Candida albicans* and *Aspergillus niger*. MIC for each of the compound was determined. Clotrimazole was used as a reference standard for the study. The results of antifungal activity against *Candida albicans* are tabulated in Table no-1 and that against *Aspergillus niger* are tabulated in Table no-2.

**Determination of minimum inhibitory concentrations (MICs) of antimicrobial agents by agar dilution method[22]:**

**INTRODUCTION:**

Dilution methods are used to determine the minimum inhibitory concentrations (MICs) of antimicrobial agents and are the reference methods for antimicrobial susceptibility testing against which other methods, such as disc diffusion are calibrated. MIC methods are widely used in the comparative testing of new agents. In clinical laboratories, they are used to establish the susceptibility of organisms that give equivocal results in disc tests, for tests on organisms where disc tests may be unreliable and when a more accurate result is required for clinical management.

In dilution tests, microorganisms are tested for their ability to produce visible growth on a series of agar plates (agar dilution) or in microplate wells of broth (broth micro dilution) containing dilutions of the antimicrobial agent. The lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism is known as the MIC.

**MEDIUM**

Although several susceptibility testing media are available in Europe, a clear choice for a reference medium remains to be determined. Mueller-Hinton (MH) agar shows no performance advantages over some other media but is probably the most widely used medium internationally, and there is a USA National Committee for Clinical Laboratory Standards (NCCLS) document which describes procedures for evaluating MH agar. MH agar which meets the requirements of the NCCLS standard is considered the reference medium.

**Preparation of stock solutions**

Use an analytical balance when weighing agents. Allowance for the potency of the powder can be made by use of the following formula:

Weight of powder (mg) = Volume of solvent (mL) X Concentration (mg/L) Potency of powder (mg/g) /  
Alternatively, given a weighed amount of

antimicrobial powder, the volume of diluent needed may be calculated from the formula:

Weight of solvent (mL) = Weight of powder (mg) X Potency of powder (mg/g) Concentration (mg/L) /  
Concentrations of stock solutions should be 1000 mg/ L or greater, although the solubility of some agents will be limiting.

The actual concentrations of stock solutions will depend on the method of preparing working solutions. Sterilization of solutions is not usually necessary. If required, sterilization should be by membrane filtration, and samples before and after sterilization must be compared by assay to ensure that adsorption to the membrane has not occurred. Unless otherwise instructed by the manufacturer, store stock solutions frozen in aliquots at -20<sup>0</sup>C or below. Most agents will keep at -60<sup>0</sup>C for at least 6 months. Stock solutions must be frozen as soon as possible after preparation, used promptly on defrosting and not re-frozen.

**Preparation of working solutions**

The range of concentrations tested will depend on the organisms and antimicrobial agent being tested, but a two-fold dilution series based on 1mg/L is conventionally used. Twenty- milliliter volumes of agar are commonly used in 9 cm Petri dishes for agar dilution MICs. Two alternative dilution schemes are there which involve adding 19 ml volumes of molten agar to 1ml volumes of antimicrobial solution. The more conventional method is based on diluting a 10 240 mg/ L stock solution, always measuring 1mL volumes of antimicrobial solution. The other method is based on diluting a 10000 mg/ L stock solution and involves measuring various volumes of antimicrobial solution by the use of high precision variable volume micropipettes, which are now widely available. An alternative to the method is to omit the distilled water added to make antimicrobial volumes up to 1 mL and instead to add a variable volume of molten agar to make the total volume 20 mL.

**PREPARATION OF PLATES**

Prepare agar as recommended by the manufacturer. Allow the sterilized agar to cool to 50<sup>0</sup> C in a water-bath. Prepare a dilution series of antimicrobial agents, in 25-30 mL containers. Include a drug-free control. Add 19 mL of molten agar to each container, mix thoroughly, and pour the agar into relabeled sterile petri dishes on a level surface. Allow the plates to set at room temperature and dry the plates so that no drops of moisture remain on the surface of the agar. Do not over dry plates.

Plates should not be stored unless the agents have

been shown to be stable on storage. Clavulanic acid and carbapenams are particularly unstable.

#### PREPARATION OF INOCULUM

Standardize the density of inoculums to give  $10^4$  colony-forming units (CFU) per spot on the agar. Use four or five colonies of a pure culture to avoid selecting an atypical variant. The inoculums may be prepared by emulsifying overnight colonies from an agar medium or by diluting a broth culture. The broth used must not be antagonistic to the agent tested. A 0.5 McFarland standard may be used for visual comparison to adjust the suspension to a density equivalent to approximately  $10^8$  CFU/mL. Alternatively, inoculate can be adjusted photo metrically. Dilute the suspensions of organisms in 0.85% saline or broth to give  $10^7$  CFU/mL. Plates must be inoculated within 30 min of standardizing the inoculums, to avoid changes in inoculum density.

#### INOCULATION OF PLATES

Mark the plates so that the orientation is obvious. Transfer diluted bacterial suspensions to the wells of an inoculums replicating apparatus. Use the apparatus to transfer the inocula to the series of agar plates, including a control plate without antimicrobial agent. Replicator pins 2.5 mm in diameter will transfer about 1  $\mu$ L, i.e. an inoculum of  $10^4$  CFU/spot. Alternatively, a micropipette or standard loop may be used to inoculate plates. Allow the Inoculum spots to dry at room temperature before

inverting the plates for incubation.

#### INCUBATION OF PLATES

Incubate plates at 35-37<sup>0</sup>C in air for 18 h. In order to avoid uneven heating, do not stack plates more than five high. If the incubation period is extended for slow-growing organisms, the stability of the agent over the incubation period must be assessed by the inclusion of Control strains with known MICs. Avoid incubation in an atmosphere containing 5% CO<sub>2</sub> unless necessary for growth of the organisms (e.g. *Neisseria* spp.).

Incubate methicillin/oxacillin susceptibility tests on staphylococci at 30<sup>0</sup>C.

#### READING RESULTS

The MIC is the lowest concentration of the agent that completely inhibits visible growth as judged by the naked eye, disregarding a single colony or a thin haze within the area of the inoculated spot. A trailing endpoint with a small number of colonies growing on concentrations several dilutions above that which inhibits most organisms should be investigated by subculture and retesting. Such trailing endpoints may indicate contamination, resistant variants,  $\beta$ -lactamase producing organisms, or, if incubation is prolonged, regrowth of susceptible organisms following deterioration of the agent. With sulfonamides and trimethoprim, endpoints may be seen as a reduction in growth, and a haze of growth may be seen at several dilutions above the actual MIC.

triazole nucleus in them. The anti fungal activity for all the synthesised compound was evaluated against the fungi *Candida albicans* and *Aspergillus niger*. The MIC was determined for each of the compound and clotrimazole was used as a reference standard. The results are tabulated in Table no-1 and 2.

### RESULTS AND DISCUSSION:

#### Biological Activities

##### Antifungal Activity Studies

The literature survey prompted us to evaluate the synthesised compounds for their antifungal activity as many of the established antifungal agents contained

**Table-1: Antifungal activity of N-(3-(2-(3-hydrazinyl-3-oxoalkanoyl) hydrazinyl)-5-(phenoxyethyl)-4H-1,2,4-triazol-4-yl) isonicotinamide (6a-6e) against *Candida albicans***

Sl No	Samples	100	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
1	6a	S	S	R	R	R	R	R	R	R	R
2	6b	R	R	R	R	R	R	R	R	R	R
3	6c	R	R	R	R	R	R	R	R	R	R
4	6d	R	R	R	R	R	R	R	R	R	R
5	6e	R	R	R	R	R	R	R	R	R	R
6	Clotrimazole	S	S	S	S	S	S	S	S	S	S

**Table –2: Antifungal activity of *N*-(3-(2-(3-hydrazinyl-3-oxoalkanoyl) hydrazinyl)-5-(phenoxyethyl)-4*H*-1,2,4-triazol-4-yl)isonicotinamide (6a-6e) against *Aspergillus niger***

No	Samples	100	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
1	<b>6a</b>	S	S	S	S	R	R	R	R	R	R
2	<b>6b</b>	S	R	R	R	R	R	R	R	R	R
3	<b>6c</b>	S	S	R	R	R	R	R	R	R	R
4	<b>6d</b>	R	R	R	R	R	R	R	R	R	R
5	<b>6e</b>	R	R	R	R	R	R	R	R	R	R
6	<b>Clotrimazole</b>	S	S	S	S	S	S	S	S	S	S

### DISCUSSION:

The results of antifungal activity has revealed that , the fungus *Candida albicans* was found to be resistant to most of the compounds at all the tested dose levels except the compound **6a** for which the MIC was found to be 50 µg/ml. The results of antifungal activity against *Aspergillus niger* were of significance as the compound **6a** possesses MIC value of 12.5 µg/ml. The compound **6e** shown to have MIC of 50µg/ml and that for **6b** was found to be 100µg/ml. The fungal strain *Aspergillus niger* was found to be resistant to the compounds **6d** and **6e** at all the tested dose levels i.e. 0.2 µg/ml to 100 µg/ml.

### SUMMARY AND CONCLUSION:

The development of new antimicrobial therapeutic agent with enhanced potency, high selectivity and reduced toxicity is a constant process in medicinal chemistry. Exhaustive literature survey on 1,2,4-Triazoles derivatives revealed that they possess wide range of biological properties.

The purpose of the present study was to examine whether molecular modification might results in the discovery of new potential biological agents.As per the given scheme **N-(5-mercapto-3-(phenoxyethyl)-4*H*-1,2,4-triazol-4-yl) isonicotinamide (5)** was synthesised following the procedure described in methodology. The various aliphatic dicarboxylic acids differing in their length of alkyl chains were converted into their respective hydrazides and were reacted with **N-(5-mercapto-3-(phenoxyethyl)-4*H*-1,2,4-triazol-4-yl) isonicotinamide (5)**

**1,2,4-triazol-4-yl)isonicotinamide (6a-6e)**, with good yields.

This combination suggested is an attempt to investigate the influence of such hybridization and structure variation on the anticipated biological activities hoping the possibility that the target derivatives might be more efficacious as Antifungal agents.

The homogeneity of all the derivatives was established by TLC technique. The structures of the compounds were successfully established by means of IR, Proton NMR and Mass spectral studies.All the title compounds were evaluated for antifungal activity against *Candida albicans* and *Aspergillus niger* by following MIC determination method. During this study, it is found that the fungal strain *Candida albicans* was found to be resistant to all the compounds that all the tested dose levels except for the compound **6a (n=0)** that has MIC of 50 µg/ml.

The fungus *Aspergillus niger* was found to be susceptible for the compounds **6a, 6b** and **6c** and was resistant to the compounds **6d** and **6e**.

The compounds **6a,6b** and **6c** were shown to possess MIC values of 12.5, 100 and 50 µg/ml.

Among the compounds evaluated for antifungal activity study the compound **6a** was found to be the most potent antifungal agent with the MIC values of 50 µg/ml for *Candida albicans* and 12.5 µg/ml for *Aspergillus niger*.

Eventhough, the structural activity relationship could not be arrived at, the present study is of significance in that few of the synthesised compounds shown to possess good antifungal properties. All these results only indicate that the above type of 1,2,4-triazole moiety needs more attention and that further study the compounds **N-(3-(2-(3-hydrazinyl-3-oxoalkanoyl) hydrazinyl)-5-(phenoxyethyl)-4*H*-1,2,4-triazol-4-yl)isonicotinamide (6a-6e)** suitably exploited can still give better lead compounds.

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