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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF SOME NEW HETEROCYCLES CONTAINING NITROGEN AND SULPHUR

Md. Akram *, Abdul Sayeed and Syed Yousuf Hussain

MESCO College of Pharmacy Hyderabad (T.S), Andhra Pradesh, India.

Keywords:

Chalcone Derivatives, Antibacterial, Antifungal, Antitubercular

Correspondence to Author:
Md akram

Assistant Professor
MESCO College of pharmacy
Hyderabad.

Email: mdakramid43@gmail.com

ABSTRACT: The literature survey on 1,2,4-Triazoles and chalcone derivatives revealed that they are endowed with wide variety of biological activities. During the present investigation newer series of chalcone derivatives S-4-(isonicotinamido)-5-(phenoxymethyl) - 4H-1,2,4 triazole-3-yl-3(-4-chlorophenyl) propanethioate **(7a-7f)** were synthesized by coupling suitably modified 1,2,4-Triazole nucleus with appropriate aryl aldehydes. The structures of the newly synthesized compounds were established by FTIR, ^{1}HNMR and Mass spectral analysis. All the compounds have been evaluated for *in vitro* antibacterial, antifungal and antitubercular activities. The compounds exhibited weak antibacterial activity only at higher concentrations. Whereas, the antifungal activity results showed that few of the compounds were able to show moderate antifungal potency. The fungi used in the anti-microbial study were found to resistant to the other compounds. The antitubercuar activity results were encouranging as the few compounds were able to possess growth inhibiting properties against the mycobacterium tested i.e the compounds 7b, 7e and 7f possessed the MIC as low as 6.25µg/ml. Even though the compounds failed to possess anti-bacterial and antifungal activity, it is quite interesting to note that most of the compounds were found to be promising biological agents as antitubercular.

INTRODUCTION: 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 hetrocyclic compound usually possesses a stable ring structure this does not readily hydrolyze or depolymerize. It is obvious that the number and variety of hetrocyclic compound with reported antimicrobial activity is extensive with respect to structure and application. Heterocyclic compounds exhibited remarkable pharmacological activities.

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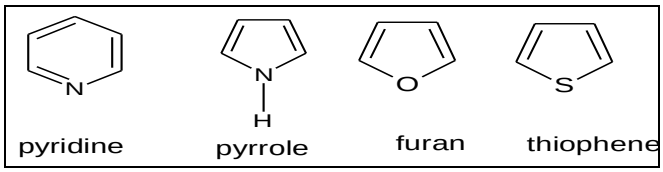
The hetrocyclic compounds containing pyrimidine, pyridine, and piperazine nucleus have wide range of therapeutic activity such as antitubercular, anticancer, antihelmintic, antioxidant and antimicrobial activities is also believed that the presence of N-C=S linkage is responsible for the amoebicidal, 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 show better anti bacterial and anti fungal activity against various strains of bacteria and fungai viz. *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonaie*., *Aspergillus niger*, *Microsporum gypseum*.

After the antimicrobial studies, it were found that hetrocyclic Compound showed excellent activity against the bacterial strain of *Pseudomonas aeruginosa* and compound showed very good activity against the fungal strain of *Aspergillus niger*.

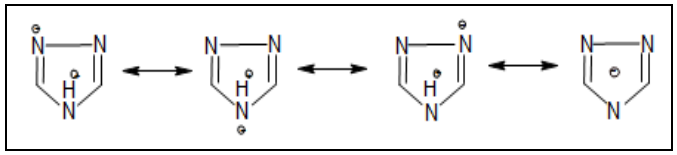
Incorporation of oxygen, nitrogen, sulphur, or an atom of a related element into an organic ring structure in place of a carbon atom gives rise to a heterocyclic compound. Since the heterocyclic atom must form more than one bond in order to be incorporated into a ring structure, halogens do not form 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.



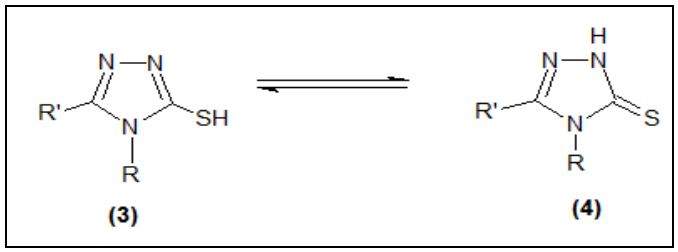
Many heterocyclic compounds are biosynthesized by plants and animals and are biologically active. Some heterocycles are fundamental to life, such as haem derivatives in blood and the chlorophylls are essential for photosynthesis. Similarly, the paired bases found in RNA and DNA are heterocycles, as are 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 a 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.

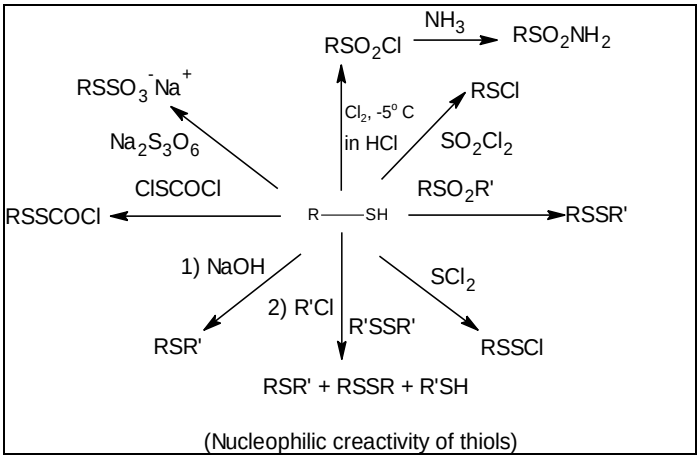


4,5-Disubstituted-1,2,4-triazole-3-thiones:
Triazoles having a mercapto group at C-3 **(3)** constitute a special class of compounds among the substituted 1,2,4-triazoles because of the tautomerism exhibited by them. The labile hydrogen atom may be attached to either nitrogen or sulfur atom. Previous studies of IR, NMR and UV spectra have provided strong evidence that mercaptotriazoles are present predominantly in the thione form **(4).**

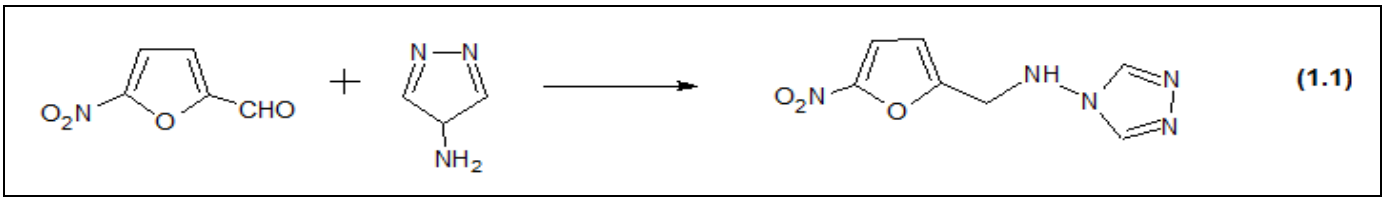


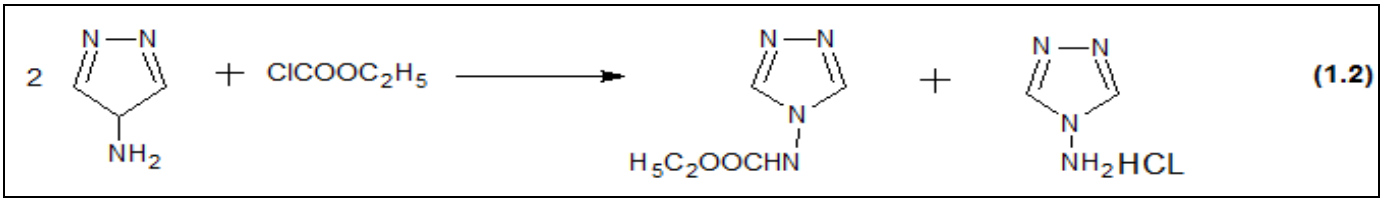
Due to lability of hydrogen, both the S-substitution and N-substitution can be carried out by changing the reaction to electron deficient alkenes and alkynes, ring opening of oxiranes and acylation.

At low temperatures, oxidative chlorination of mercaptotriazoes 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 methyliodide 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 aqueos 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-nitrofufural 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).

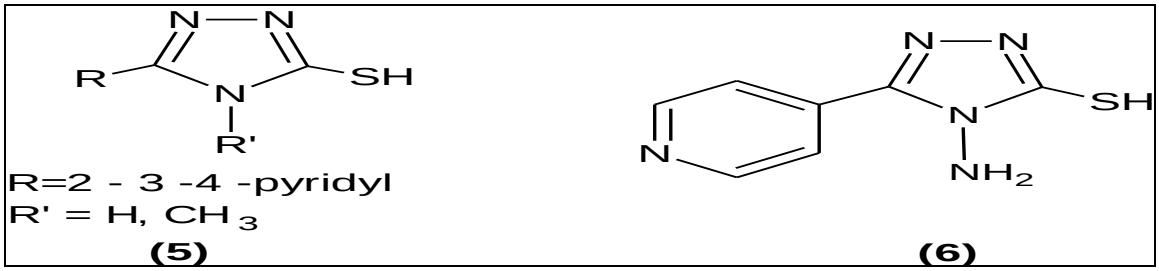


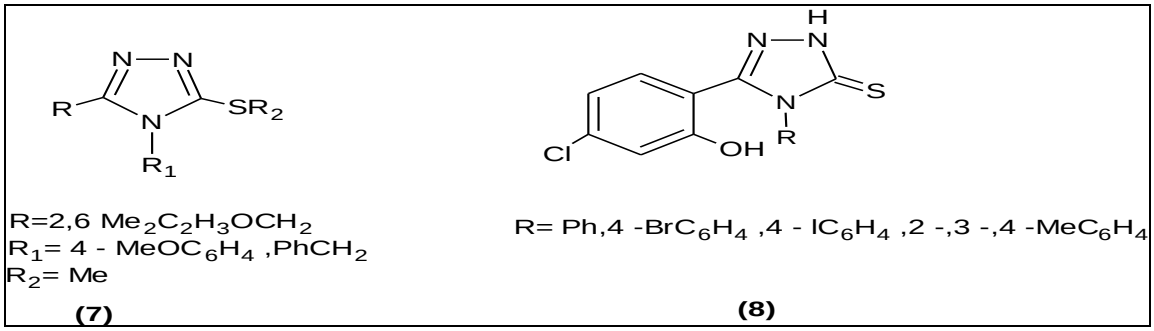


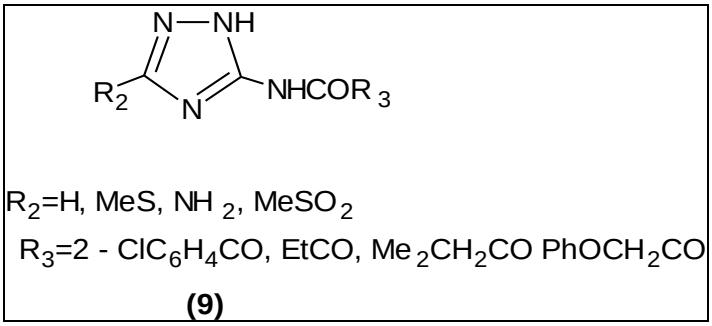
Biological Activities of 1,2,4-Triazoles: 4,5-disubstituted-2,4-dihydro-3H-1, 2, 4 – triazole - 3-thiones and their derivatives have gained a lot of interest in the last decade due to their biological, industrial and agricultural importance. A well known example is that of fluconazole, a broad spectrum 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-infalmmatory, anti-carcinogenic, hypnotic, sedatives, plant growth regulators and insecticidal. Some pyridyl and pyrimidyl substituted mercaptotriaoles **(5)** have been reported to exhibit antithyroid activity.

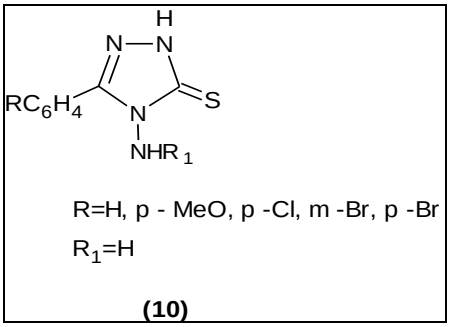
N-aminomercaptotriazoles **(6)** have been reported to show significant bactericidal and fungicidal activity. Some disubstituted mercaptotriazoles **(7)** and **(8)** have been tested *in vitro* against a number of fungi and they showed moderate to good activity.

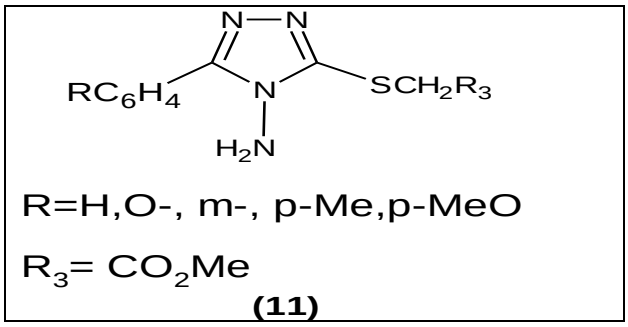
The highest activity against all species was shown by **(8)** (R=4-IC_6H_4). Acylation of some aminotriazoles gives triazoleamides **(9)** which specifically inhibited rubella virus 4- Amino-l,2,4-triazoles and their derivatives **(10)** and **(11)** have been prepared and found to possess bactericidal and/or fungicidal activity. Certain 4-alkylsubstituted triazoles **(12)** have been reported to inhibit reserpine-induced ptosis in mice with an ED50 of 0.27 mg/kg. Certain substituted 5-(4-pyridyl)-3-mercapto-l,2,4-triazoles **(13)** have been used as additives.

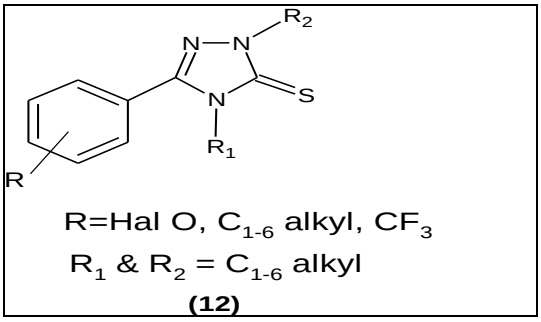


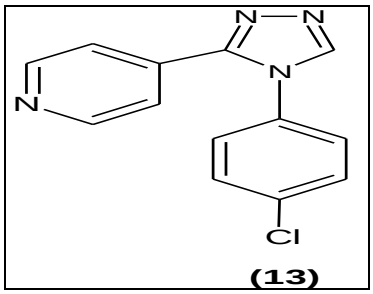




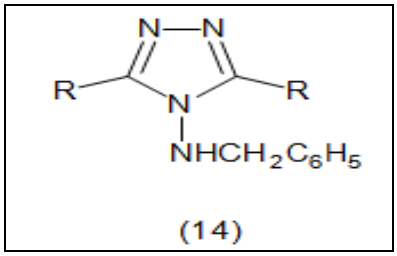








1,2,4-Triazolcs and condensed triazole systems have found considerable use in photographic industry. 1,2,4-triazole derivatives e.g. N,N'-bi(5-methyl-l,2,4-triazol-3- yl) formamidines are capable of inhibiting fog formation in silver halide emulsions by incorporating in the emulsions. The addition of 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: 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.



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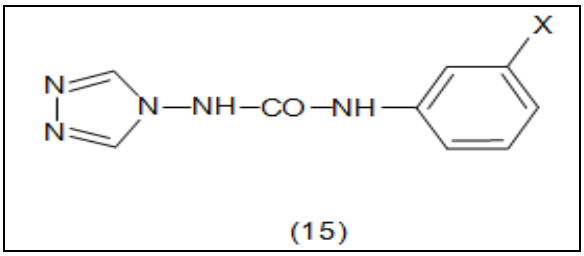
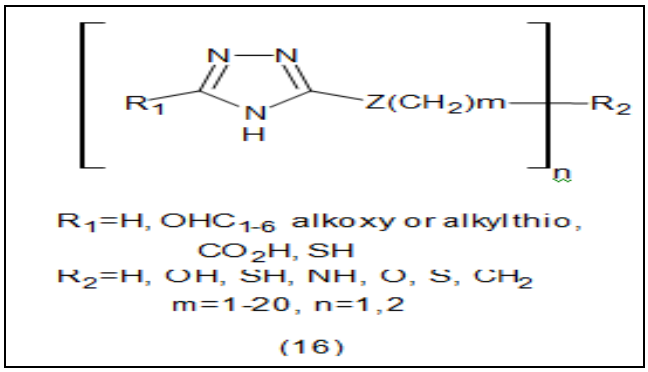


(15)

A new series of condensation polymers polyaminotriazoles have been prepared, which are similar to nylon. These are fibre forming and be melt 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-l,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.



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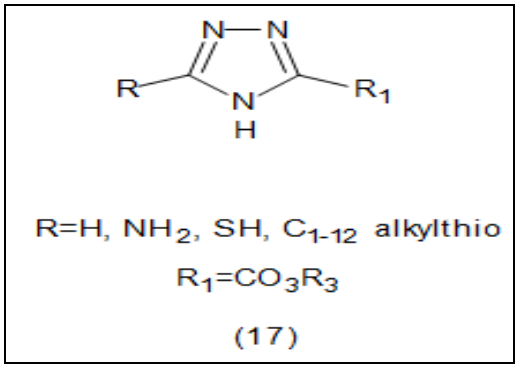


(17)

Objectives:

Need for the study: The chemistry of heterocyclic compounds is the field in the organic chemistry which is being explored continuously. The importance of 1,2,4-triazole derivatives lies in the field that, these derivatives represent one of the most biologically active classes of compounds, possessing a wide range of therapeutic properties [5-6]. Chalcones constitute an important family of substances belonging to flavonoids, a large group of natural and synthetic products with interesting physicochemical properties, biological activity and structural characteristics.

Chalcones are highly reactive substances of varied nature, and they experience chemical and physical transformations of great importance. These are characterized with diverse biological activities, among which anti- inflammatory, anti-malarial, anti-protozoal, anti bacterial, cytotoxic, anti- mitotic, anti-viral, anti-inflammatory, antileishmanial, and antitubercular activity.

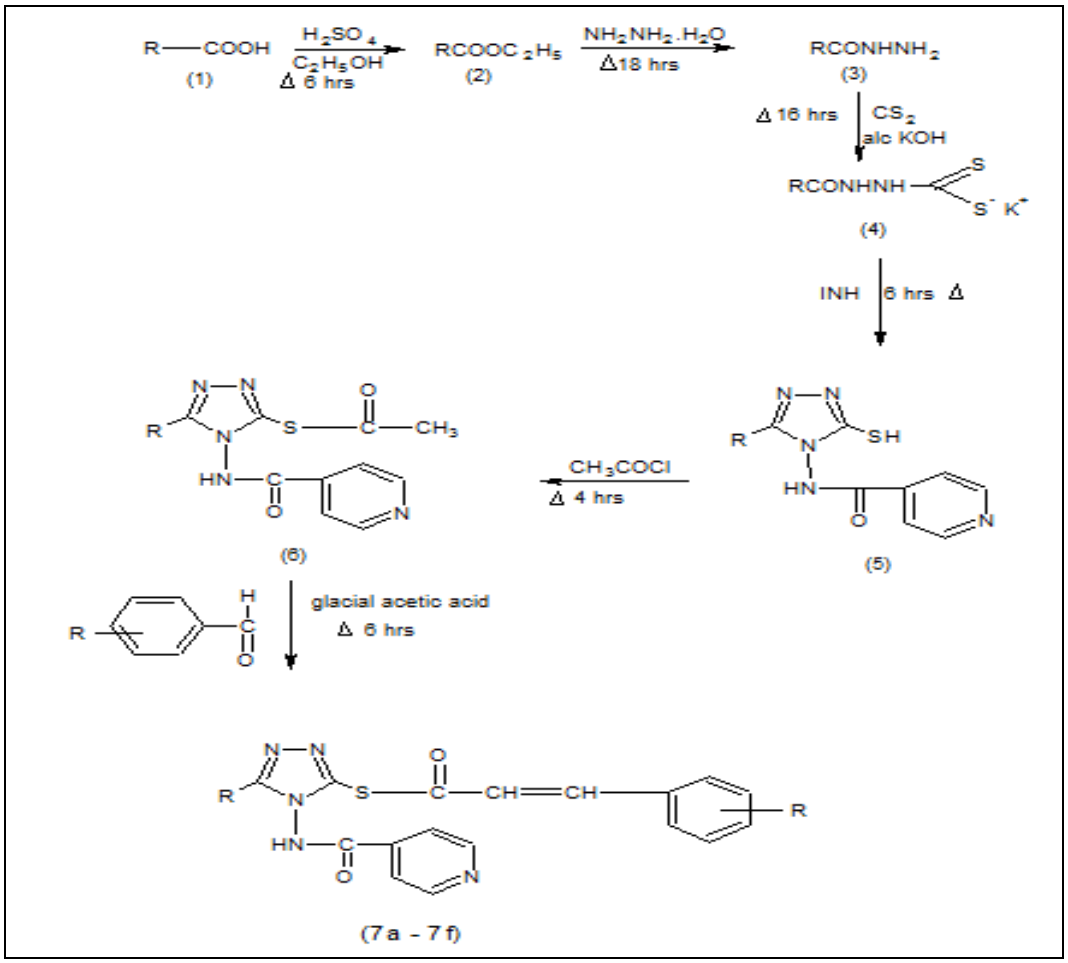
The above mentioned reports encouraged us to modify 1,2,4-triazole scaffold into various bioactive structures. Hence, in the present study we have synthesised some new derivatives of 1,2,4-triazole and evaluate them for antimicrobial and antitubercular activities.

Chalcones have been studied extensively because of their ready accessibility, diverse chemical reactivity and broad spectrum of biological activities.

The present investigation includes the following;

1. Preparation of 3-phenoxmethyl-4-N-(pyridyl carboxamido)- 5-mercapto 1,2,4-triazole by following known method.
2. Conversion of the above compound to form S-4 (isonicotinamido)-5- (phenoxymethyl) - 4H -1, 2, 4-triazol-3-yl ethanethioate.
3. The reaction of the above compound with substituted benzaldehydes in presence of 2% NaOH to yield the title derivatives.
4. Chemical characterization of the newly synthesised compounds by IR, ^{1}HNMR and
5. Mass spectral data.
6. To screen the synthesised compounds for the anti-microbial and antitubercular activity.

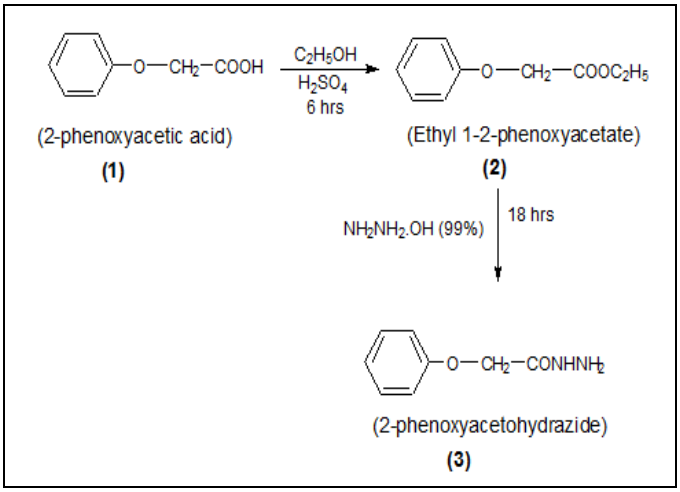
MATERIAL AND METHODS: The entire chemicals used were procured from Qualingens, Himedia and Loba- chemicals. Purity of starting materials used for reaction was confirmed by checking their melting point or boiling point and by thin layer chromatography.



SCHEME: 1

(B). Synthetic Studies:

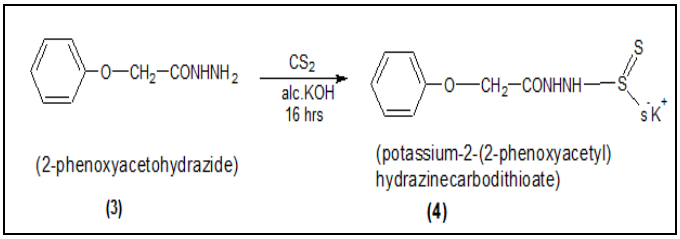
Step 1: Preparation of 2-Phenoxyacetohydrazide (3):-



The acid **(1)** (0.1 mole) and ethanol (50 ml) were taken with a few drops conc. H_2SO_4 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.

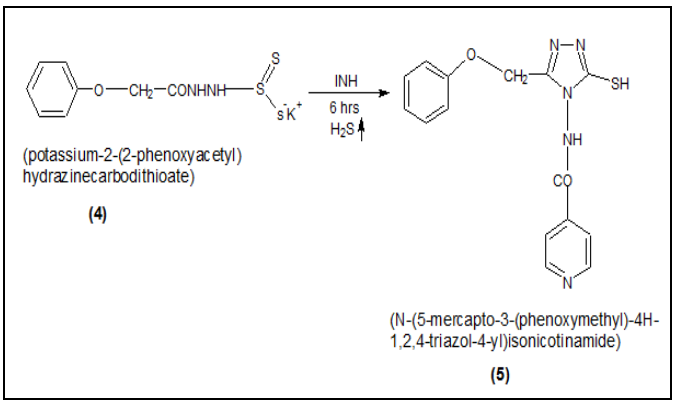
The ester **(2)** (0.1 mole) was dissolved in appropriate quantity of ethanol and to this hydrazine hydrate (0.1 mole) was added. The reaction mixture was refluxed for a period of 12-18 hours. Excess of ethanol was distilled off under reduced pressure. It was then poured into ice cold water and the solid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

Step 2: Preparation of potassium-2-(2-phenoxyacetyl) hydrazinecarbodithioate (4):-



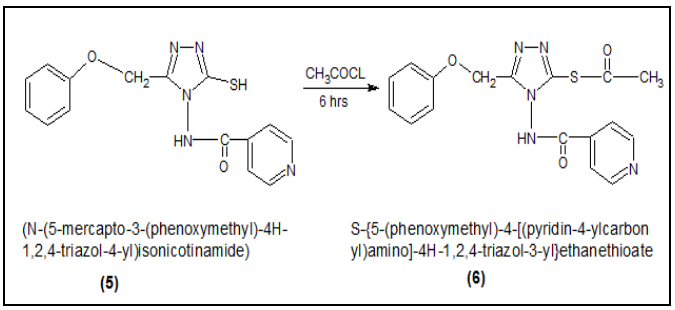
To a solution of potassium hydroxide (KOH) (0.15 mole) in absolute ethanol (125 ml), 2-phenoxyacetohydrazide **(3)** (0.1 mole) and carbon disulphide (CS_2) (0.15 mole) were added and the mixture was agitated for 16 hours. To the resulting solution, anhydrous ether (250 ml) was added and the precipitated product **(4)** was collected by filtration, washed with ether and dried under vacuum at 65ºC. This potassium salt **(4)** was used in the next step without further purification.

Step 3: Preparation of N-(5-mercapto-3-(phenoxymethyl) - 4H-1, 2, 4-triazol-4- yl) iso nicotinamide (5) :-



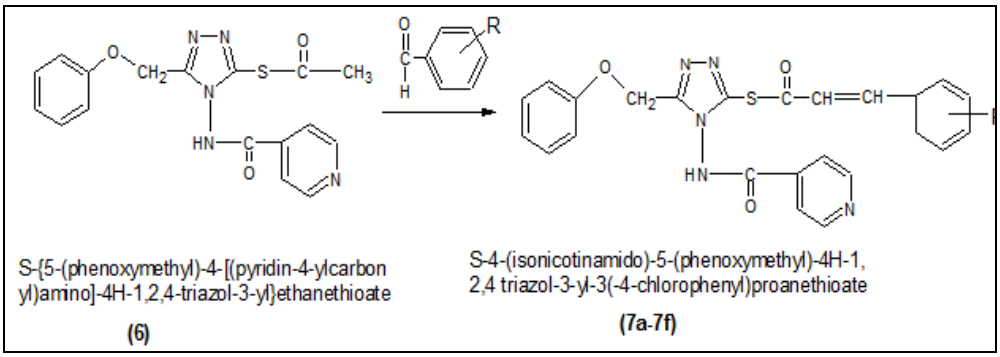
A suspension of the potassium salt (4) (0.1 mole), Isonicotinic acid hydrazide (INH) (0.1 mole) and water (5 ml) were heated under reflux for 6 hours and hydrogen sulphide (H_2S) gas was evolved and clear solution was resulted. The dilution of reaction mixture with cold water (50 ml) and subsequent acidification with dilute hydrochloric acid (HCl) gives the triazole (5), which was filtered, washed with water and recrystallized from aqueous ethanol.

Step 4: Preparation S-{5-(phenoxymethyl)-4-[(pyridin-4-ylcarbonyl) amino]-4H-1,2,4-triazol-3-yl} ethanethioate (6) :-



The mixture of triazole (5) (0.01 mole) and (0.01mole) of potassium carbonate is taken in a RBF to this add 50ml of actone and stir the mixture on magnetic stirrer for 10min. Then add 0.01mole acetyl chloride by dropwise using funnel. After complete addition reflux the reaction mixture for about 4hours cool the reaction mixture and add 100ml of water filter and wash with water.

Step 5: Preparation of S-4-(isonicotinamido)-5-(phenoxymethyl 4H-1, 2, 4 triazole- 3-yl-3 (substituted phenyl) propanethioate (7a-7f) :-



To a mixture of equimolar quantities of triazole and aromatic aldehyde (0.005 mole) in ethanol(25ml) was added and stirred for 10hours at room temperature. Then it was refluxed for 6hrs on water bath. The excess of solvent was removed under reduced pressure it was poured into ice-cold water. The solid thus separated was filtered dried and recrystallized from ethanol. The physical data of compounds (7a-7f) are summarized in **Table 1**.

TABLE 1: PHYSICAL DATA OF (E)-S-4-(ISONICOTINAMIDO)-5-(PHENOXYMETHYL)-4H 1,2,4-TRIAZOL-3-YL 3-(SUBSTITUTED PHENYL)PROP-2- ENETHIOATE(7A-7F)

S.no	Compound Code	Substituent R_2	Molecular Formula	Molecular Weight	R_f value	Melting Point (ºC)	% Yield
1	7a	m-Nitro	$C_{24}H_{18}O_5N_6S$	502.01	0.56	152	68.52%
2	7b	p-Cl	$C_{24}H_{18}O_3N_5Cl$	492.43	0.68	165	70.20%
3	7c	o-Cl	$C_{24}H_{18}O_3N_5Cl$	492.43	0.65	166	59.02%
4	7d	p-floro	$C_{24}H_{18}O_3N_5SF$	475.98	0.41	163	65.55%
5	7e	p-Nitro	$C_{24}H_{18}O_5N_6S$	502.01	0.48	166	63.23%
6	7f	3,5 diNitro	$C_{24}H_{17}O_7N_7S$	547.08	0.38	169	69.18%

All melting points were uncorrected Mobile phase: Acetone: Benzene (1:1)

Biological activities:

Anti-Tubercular Activity: All the synthesised 1,2,4- triazole derivatives have been evaluated for Anti- tubercular activity against *Mycobacterium tuberculosis* H37 Rv using Microplate alamar blue dye assay(MABA) .The minimum inhibitory concentration(MIC) was determined for each of the sample. The first line antitubercular drug INH was used as a refrence standard. The resulte are tabulated in table no-4 and graphically depicted in **Fig. 1**.

Anti tubercular activity using Alamar Blue Dye Method: The anti-mycobacterial activity of the title compounds were assessed against *Mycobacterium tuberculosis* H37 Rv using Microplate Alamar Blue Assay (MABA) method. This method is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for seven days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth and pink color was scored as growth. The MIC is defined as lowest drug concentration which prevented the color change from blue to pink.

Antimicrobial activity: [7-8] In general, any compound or drug which inhibits the growth or causes the death of micro-organisms is known as antimicrobial agent. Any drug which inhibits

the growth of bacteria or fungi, it is said to possess bacteriostatic and fungi static activity respectively.

If it kills the bacteria or fungi, it is said to possess bactericidal and fungicidal activity. *In-vitro* tests are used as screening procedure for new agents and for testing the susceptibility of individual isolates from infections to determine which of the available drug might be useful therapeutically important factors for the antimicrobial activity and size of the inoculums, metabolic state of organisms, pH, temperature, duration of interaction, concentration of the inhibitors and presence of interfering substance. Sensitivity testing is done to determine the range of microorganisms that are susceptible to the compound under specified conditions. It can be done by cup-plate method. This method is suitable for the organisms that grow well overnight such as most of the common aerobes and facultative anaerobes and rapidly growing fungi. Several forms of disk diffusion methods have been advocated.

Biological evaluation involves testing of microbial susceptibility to chemotherapeutic agents. Determination of antimicrobial effectiveness against pathogens is essential for therapy. Testing can show the efficiency of antimicrobial against a pathogen and give an estimate of proper therapeutic dose. The idea of the effectiveness of a chemotherapeutic agent against a specific pathogen can be obtained from the minimum inhibitory concentration (MIC). The MIC is the lowest concentration of the drug that can prevent the growth of the pathogen.

The important factors to be considered in the testing of the antimicrobial activity are as follows:

1. Type of test organism.
2. Temperature and time of incubation.
3. Composition and pH of culture.
4. Inoculums concentration.

1, 2, 4, triazole derivatives described in the literature are known for their antimicrobial activity. Hence, in the present study, substituted triazole derivatives synthesized were screened for their antibacterial as well as antifungal activity using various bacterial strains as well as fungal strains.

Evaluation of Antibacterial Activity: Antibacterial activity was determined based on the *in vitro* activity in pure cultures. *In vitro* susceptibility test were done by the cup-plate method. The antibacterial activity of formazan derivatives was evaluated by cup-plate method against the strains of common pathogens; gram negative organisms *Escherichia coli, Pseudomonas aeruginosa* and Gram positive organisms *Staphylococcus aureus Bacillus subtilis.* Ciprofloxacin is used as a standard drug.

MATERIAL AND METHODS:

Test organisms (bacteria)

Bacillus subtilis- Gram positive bacteria

Staphylococcus aureus- Gram positive bacteria

Escherichia coli- Gram negative bacteria

Pseudomonas aeruginosa- Gram negative bacteria

All the synthesized compounds were screened for antibacterial activity against the above mentioned strains by cup-plate method. The following materials were used for the testing.

1. Nutrient agar.
2. Sterilized petridish, pipettes and beakers.
3. Sterilized tuberculin syringes.
4. 18-24 hr old growth culture in nutrient broth.
5. Sterilized test tubes containing solution of test compounds in desired concentration

Preparation of Nutrient agar media

Nutrient agar (40g), bacteriological peptone (1g), beef extract (5g) and sodium chloride (5g) were dissolved in distilled water (1000 ml). The pH of the solution was adjusted to 7 to 7.4 by using sodium hydroxide solution (40%, approximately 0.25 ml for 100 ml of nutrient broth) and then sterilized for 30 min. at 15 lbs pressure in an autoclave.

Preparation of sub culture: One day prior to test the microorganisms were inoculated into the sterilized nutrient broth and incubated at 37ºC for 24 hr on the day of testing the organisms were sub-cultured into sterile nutrient broth. After incubating for 3hr, the growth thus obtained was used as inoculums for the test.

Sterilization of media and glass wares: The media used in the present study, nutrient agar and nutrient broth were sterilized in a conical flask of suitable capacity by autoclaving the same at 15 lbs pressure for 20 min. The petridishes, test tubes and pipettes were sterilized by employing hot air oven at 160ºC for 1 hr.

Preparation of solution of test compound: The test compound (10 mg each) was dissolved in freshly distilled DMF (10 ml) in serially labeled sterile test tubes, thus giving a final concentration of 50µg/0.1ml,100µg/0.1ml.

Preparation of standard solution: The standard compound ciprofloxacin (10 mg) was dissolved in freshly distilled DMF (10 ml) in serially labeled sterile test tubes, thus giving a final concentration of 50µgm/0.1ml, 100µgm/0.1ml.

Method of Testing:

Cup-plate Method: This method depends on the diffusion of an antibacterial agent from a cavity through the solidified agar layer in a petridish to an extent such that growth of the added microorganisms is prevented entirely in a circular area or zone around the cavity containing a solution of test compounds. About 15-20 ml of molten nutrient agar was poured into each of the sterile petridishes. The cups were made by scooping out nutrient agar with a sterile cork borer. The agar plates so prepared were divided into different set and each set of the plates were inoculated with the suspension of particular organism by spread plate technique.

The cups of inoculated plates were then filled with 0.1 ml of the test solution; the plates were then incubated at 37ºC for 24 hours. The zone of inhibition (diameter in mm) developed, if any, was then measured for the particular compound with each organism. The solvent DMF was used as negative-control to know the activity of the solvent.

The results of antibacterial testing are summarized in the following **Table 3.** The antibacterial activity of the tested compounds are then compared with that of standard drug used i.e. Ciprofloxacin.

Antifungal activity: The antifungal activity of formazan derivatives was carried out by Cup-plate method in comparison with that of standard antifungal drug clotrimazole. The fungi cultures used were *Candida albicans* and *Aspergillus niger*.

MATERIAL AND METHODS:

Cup-plate method: Antifungal activity of the test compounds was assessed against the above strains of fungi by disc-diffusion method. The following materials were used:

1. Sabourauds agar and tuberculin syringes with needles.
2. Sterilized petri-dishes and pipettes of 0.1 ml and 0.2 ml.
3. 16-18 hr old cultures grown in Sabourauds broth.
4. Sterilized test tubes for preparation of solution of the test compounds in desired concentration.

Preparation of media:

Sabourauds agar: Bacteriological peptone **(1 g)** and glucose (4 g) were dissolved in distilled water (100 ml) and filtered. Agar powder (2 g) was added and sterilized for 30 min at 15 lbs pressure.

Preparation of sub cultures: One day prior to the test, inoculation of the microorganisms (*Candida albicans* and *Aspergillus niger*) was made in sabourauds broth and incubated at 37ºC for 18 hr.
Sterilization of media and glass wares.

The media used in the present study was sterilized in conical flask of suitable capacity by autoclaving at 15 lbs pressure for about 20 min. The petridish, test tubes and pipettes were sterilized in hot air oven at 160ºC for one hour.

Preparation of solution:

1. Compounds: 10 mg of each test compounds was dissolved in 10 ml of DMF in serially and suitably labeled in sterile test tubes thus giving a final concentration of 50µg/0.1ml, 100µg/0.1ml.

2. Clotrimazole: 10 mg of the clotrimazole was dissolved in 10 ml of DMF (dimethyl formamide) to get a concentration of 50µg/0.1ml, 100µg /0.1ml.

Method of Testing:

Cup-plate Method: This method depends on the diffusion of an antibacterial from a cavity through the solidified agar layer in a petridish to an extent such that growth of the added microorganisms is prevented entirely in a circular area or zone around the cavity containing a solution of test compounds. About 15-20 ml of molten nutrient agar was poured into each of the sterile petridishes. The cups were made by scooping out nutrient agar with a sterile cork borer. The agar plates so prepared were divided into different set and each set of the plates were inoculated with the suspension of particular organism by spread plate technique.

The cups of inoculated plates were then filled with 0.1 ml of the test solution; the plates were then incubated at 37ºC for 24 hours. The zone of inhibition (diameter in mm) developed, if any, was then measured for the particular compound with each organism. The solvent DMF was used as negative-control to know the activity of the solvent. The results of antifungal testing are summarized in the following **Table 4.** The anti-fungal activity of the tested compounds are then compared with that of standard drug used i.e. Clotrimazole.

RESULTS AND DISCUSSION: During the present investigation, the title compounds 1-(4-(isonicotinamido)-5- (phenoxymethyl)-4H-1,2,4-triazol - 3 –y l) - 3, 5 - diphenylformazan were synthesized as per the scheme described.

By analysis of spectral data of the representative compounds reveals the successful information of the synthesized substituted formazan derivatives possessing 1,2,4-Triazole scaffold. All the synthesized derivatives remitted in the products with good yield. Purity of all the synthesized compounds were checked by their melting point as well as TLC. The structure of synthesized compounds has been established and confirmed by spectral data obtained viz, FT-IR, ^{1}HNMR and Mass. All the synthesized compounds were evaluated for *in vitro* antimicrobial and some of the selected compounds for anti- cancer activities. Few of the selected compounds were also screened for their *in vivo* analgesic and anti-inflammatory activities.

Biological Activities:

Antimicrobial Evaluation: 1,2,4 triazole derivatives described in the literature are known for their antimicrobial activity; hence, in the present study also substituted triazole derivatives synthesized were screened for their antibacterial as well as antifungal activity using various bacterial strains as well as fungal strains. *In vitro* antimicrobial study was carried out by Cup-plate method. All the compounds were screened for antimicrobial activity at different concentration levels against the bacterial strains *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis.* Antifungal activity was tested on Sabouraud dextrose agar (Himedia) plates (26ºC, 48-72h) by Cup-plate method against *Candida albicans* and *Aspergillus niger* at different concentration levels. Ciprofloxacin and Clotrimazole were used as reference standards for comparison of antibacterial and antifungal activity respectively. The results are tabulated in **Table 2** and **Table 3**.

TABLE 2: ANTIBACTERIAL ACTIVITY OF (E)-S-4-(ISONICOTINAMIDO)-5-(PHENOXYMETHYL)- 4H-1, 2, 4-TRIAZOL-3-YL 3-(SUBSTITUTEDPHENYL)PROP-2- ENETHIOATE. (7A - 7F);

S. no.	Comp. code	Diameter of zone inhibition (mm)																			
		Escherichia coli					*Pseudomonas*					*Stephylococcus aureus*					*Bacillus subtilis*				
	-	5 µg/	10 µg/	25 µg/	50 µg/	75 µg/	5 µg/	10 µg/	25 µg/	50 µg	75 µg/	5 µg/	10 µg/	25 µg/	50 µg/	75 µg/	5 µg/	10 µg/	25 µg	50 µg/	75 µg/
1	7a	R	R	10	20	25	R	R	9	19	26	10	11	14	18	21	R	9	11	13	20
2	7b	R	R	R	R	10	R	R	R	9	11	R	R	10	13	14	R	R	9	11	14
3	7c	R	R	R	R	R	R	R	R	R	9	R	10	13	15	18	R	11	14	16	19
4	7d	R	R	R	9	11	R	R	R	10	12	R	R	R	R	10	R	R	R	R	9
5	7e	R	R	R	R	11	R	R	R	R	9	R	R	R	9	12	R	R	R	10	13
6	7f	R	R	R	8	10	R	R	R	9	11	R	R	R	R	10	R	R	R	R	8
7	Ciprofloxacin	23	26	30	35	37	23	30	34	36	38	23	26	29	33	35	14	23	28	30	34
8	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 3: ANTIFUNGAL ACTIVITY OF (E)-S-4-(ISONICOTINAMIDO)-5-(PHENOXYMETHYL)-4H-1,2,4-TRIAZOL-3-YL 3-(SUBSTITUTEDPHENYL)PROP-2- ENETHIOATE. (7A-7F)

SI No.	Comp. Code	Diameter of zone inhibition (mm)									
		Candida albicans					*Aspergillus niger*				
	-	5	10	25	50	75	5	10	25	50	75
1	7a	12	14	15	17	25	10	13	16	20	27
2	7b	10	13	16	18	22	R	15	20	23	26
3	7c	10	15	19	20	23	R	R	20	21	26
4	7d	10	12	13	16	18	12	13	15	18	22
5	7e	8	10	15	16	10	13	15	18	20	24
6	7f	8	9	13	15	17	R	12	15	16	23
11	clotrimazole	24	26	30	35	37	23	26	29	33	35
12	Control	-	-	-	-	-	-	-	-	-	-

Antitubercular Activity Studies: The literature survey has revealed that, the moieties containing 1,2,4- triazole nucleus have shown to posses antitubercular activity. Hence, in the present investigation all the synthesised 1,2,4-triazole derivatives (7a-7f) have been evaluated for antitubercular activity against *mycobacerium tuberculosis H37Rv* following microplate alamar blue assay method. MIC was determined for each of the compound and first line antitubercular drug INH was used as the refrence standard. The results of the study are tabulated in **Table 4** and graphically depicted in **Fig. 1.**

TABLE 4: ANTITUBERCULAR ACTIVITY OF AGAINST MYCOBACERIUM TUBERCULOSIS H37RV

Sl.no.	Samples	100	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
1	7a	S	S	S	S	R	R	R	R	R	R
2	7b	S	S	S	S	S	R	R	R	R	R
3	7c	S	S	R	R	R	R	R	R	R	R
4	7d	S	S	S	R	R	R	R	R	R	R
5	7e	S	S	S	S	S	R	R	R	R	R
6	7f	S	S	S	S	S	R	R	R	R	R
7	INH	S	S	S	S	S	S	S	S	S	S

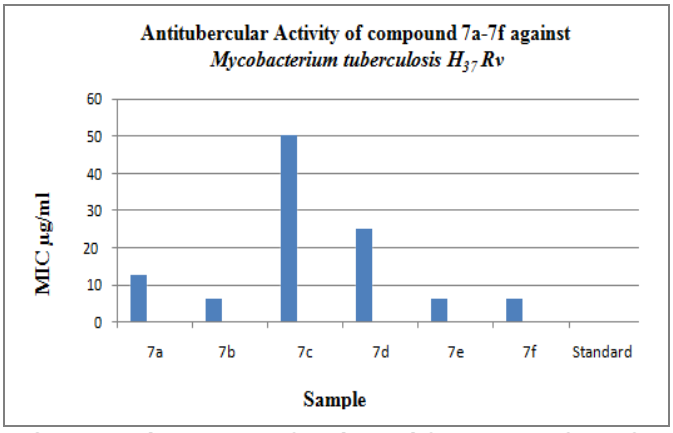


FIG. 1: ANTITUBERCULAR ACTIVITY OF S-4-(ISONICOTINAMIDO)-5-(PHENOXYMETHYL)- 4 H- 1,2,4 TRIAZOLE - 3 - YL- 3 (SUBSTITUTED PHENYL) PRO PANETHIOATE (7A-7F) AGAINST MYCOBACERIUM TUBERCULOSIS H37RV

Antibacterial and Antifungal Studies: All the synthesized compounds were evaluated for antibacterial activity among which only the compound 7a has shown weak activity.

Whereas the other compounds failed to show growth inhibiting properties even at higher concentration levels tested.

The antifungal studies revealed that the compounds 7a-7d were able to show moderate activity when compared to that of standard clotrimazole.

Rest of the compounds have failed to show the potency as antifungal.

Antitubercular Activity Studies: The results of antitubercular activity are encouranging because

the compounds 7b,7e and 7f have shown to possess the lowest MIC of 6.25µg/ml.

The other compounds 7a, 7d and 7c evaluated for antitubercular activity have also exhibited significant potency by possessing MIC of 12.5,25 and 50µg/ml respectively.

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 and chalcone derivatives revealed that they possess wide range of biological properties.

It has been observed that combination of biologically active moieties in to one molecule and synthesizing newer moieties have been the methods of research. Based on these observations,

Melting points were determined in open capillary method and were uncorrected. The structure of newly synthesised compounds was established by IR, ^{1}HNMR and Mass spectral studies.

The *in vitro* antimicrobial activity results showed that all the compounds failed to possess antibacterial properties. However, most of the compounds have shown to exhibit properties in inhibiting the growth of the fungal organisms.

The results of antitubercular activity studies were encouranging as most of the compounds have exhibited excellent growth inhibiting properties against the mycobacterium tested.

All the above results only indicate that, the compounds of the above type has emerged as promising antitubecular agents. Further study on the above type of moiety is quite reasonable and desirable Slight modification in the above type of moieties may lead to the discovery of new useful during the present investigation newer chalcone derivatives were synthesized by coupling suitably modified 1,2,4-Triazoles nucleus and evaluated them for *in vitro* antimicrobial and antitubercular activities.

Synthesis of all the chalcone derivatives by the procedure described in methodology, resulted in products with good yields**.** All the reactions were carried out under prescribed laboratory conditions. The products were purified by recrystallization.

therapeutic agents.

Given more attention on the above type of chalcone derivatives, which can be rich source for further exploitation, can still give lead compounds.

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