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Synthesis of Some Novel N-Substituted 1,2,4-Triazole derivatives and their Antimicrobial Evaluation.

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ABSTRACT:

A series of 1,2,4-triazole derivatives were synthesized by conventional method and characterized by IR, NMR, and mass spectral data. Pathogenic microorganisms are causative agents for different types of serious infectious diseases. Despite advancements in medication, bacterial infections continue to be a growing problem in health care. As more and more bacteria become resistant to antibiotics used in therapy there is considerable interest in the development of new compounds with antimicrobial activity. The compounds containing a heterocyclic ring play an important role among organic compounds with biological activity used as drugs in human, veterinary medicine or as insecticides and pesticides in agriculture. The compounds were evaluated for antimicrobial and activity. The pharmacological evaluation of 1,2,4-triazole derivatives revealed that, among all the compounds screened compound code 4 c showed leading antibacterial activity against the selected pathogenic strains of bacteria.

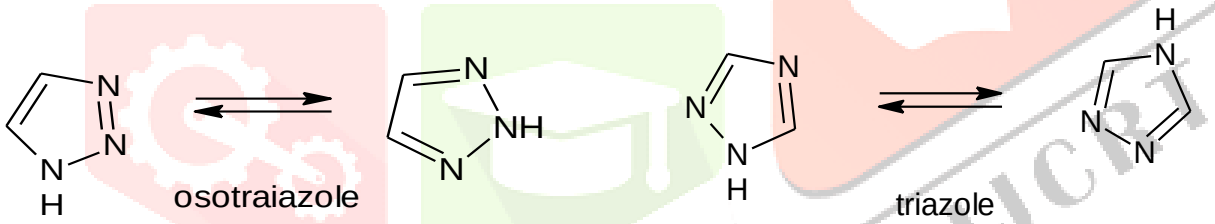
Keywords: *1,2,4-triazole, antimicrobial activity.*

1. INTRODUCTION:

Since last few decades, there is tremendous growth of research in the synthesis of nitrogen containing heterocyclic derivatives because of their utility in various applications, such as pharmaceuticals, propellants, explosives, pyrotechnics and especially in chemotherapy. A large number of ring systems containing 1,2,4-triazoles have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, CNS stimulants, sedatives, anti-anxiety, antimicrobial agents[1].

Nowadays research is concentrated towards the introduction of new and safe therapeutic agents of clinical importance. The heterocycles are enjoying their importance as being the centre of activity. The nitrogen containing heterocycles are found in abundance in most of the medicinal compounds. The success of the imidazole as an important moiety of number of medicinal agents led to the introduction of the triazoles. The triazoles are said to be the isosters of imidazoles in which the carbon atom of imidazole is isosterically replaced by nitrogen. Triazoles are 5 membered rings, which contain two carbon and three nitrogen atoms. According to the position of nitrogen atoms, the triazoles exist in isomeric forms[2].

Two structural isomeric triazoles are known , the 1,2,3-(1,2,5) and the 1,2,4-(1,3,4), the former being known as osotriazole, and the latter as triazole. Each exists in two disimilar tautomeric forms. The different isomers are characterized by the position of the nascent hydrogen. Thus, 1,2,4-triazoles exist in two isomeric forms i.e. 1H and 4H[2].



The antimicrobial agents available now have various drawbacks such as toxicity, drug resistance to microbes, and narrow spectrum of activity. Hence the design of new compounds to deal with the above problems has become one of the most challenging targets in antibacterial and antifungal research today[3].

Nitrogen containing heterocycles has fascinating applications in drug discovery and development. In particular, the synthesis of 1,2,4-triazoles has attracted considerable attention during the last years. Several potent pharmacological properties such as anti-bacterial, antimicrobial, anticancer, and antitubercular of 1,2,4-triazole derivatives have been reported[4].

Compounds containing triazole nucleus finds a unique place in medicinal chemistry and play a significant role as they are associated with immense biological activity. Triazole derivatives have gained considerable attention owing to their effective biological activity and extensive use. A survey of literature reveals that 1,2,4-triazole derivatives are known for their biological activities like antibacterial[5], antifungal[6], anti-inflammatory[7], analgesic[8], anticonvulsant[9], diuretic[10], antitb[11], anti tumor[12], etc.

The pharmacological importance of heterocycles derived from 1,2,4-triazole paved the way towards active

research in a triazole chemistry. As a result, variety of new compounds was being added to this field every year. A number of attempts were made to improve the activity of these compounds by varying the substituents on the triazole nucleus. Among these the mercapto and amino group substituted 1,2,4-triazole ring system have been reported for antimicrobial, anticancer, diuretic and hypoglycemic activities.

There is significant and continuous concern in the chemistry of five-membered N-heterocyclic compounds, mainly tetrazole, triazoles, and their substituted derivatives. Five-membered N-heterocyclic compounds are important structural fragments and considered as biologically active compounds. In 1885, Bladin was the first scientist who gave the name triazole to the carbon nitrogen ring system[14].

2. MATERIALS AND METHODS:

2.1. CHEMISTRY:

All the synthetic work was done by procuring available laboratory grade reagents and analytical grade solvents. The solvents and reagents were purified and dried according to the procedure given in Vogel's text book of practical organic chemistry. TLC was preformed to monitor the reactions and to determine the purity of the products. Further the compounds were purified by recrystallisation using suitable solvents. The melting points of the synthesized compounds were determined in open capillaries using a Veego VMP-1 apparatus and expressed in ^{o}c and are uncorrected. The IR spectrum of compounds was recorded on a Shimadzu FT-IR spectrometer using the KBr pellet technique and is expressed in cm-1. 1H-NMR spectra were recorded on a Bruker DRX-300 (300 MHz FT-NMR) using CDCl3 as solvent and TMS as Internal standard. Mass spectra were obtained using Shimadzu LC-MS 2010A mass spectrometer.

2.2 SYNTHESIS:

2.2.1 Synthesis of methyl benzoate from benzoic acid (1)

Substituted carboxylic acid (1.5gm) was refluxed with ethanol (10ml) for 4 hrs. After heating few drops of concentrated H2SO4 was added.

2.2.2 Synthesis of benzoic acid hydrazide from methyl benzoate (2)

Methyl benzoate (1) (1.5gm) was mixed with Hydrazine Hydrate (5.8ml) in round bottom flask and refluxed for 1 hour. And then 25 ml of ethanol was added and refluxed for more 3 hrs. Total volume of solution got reduced to half. Then solution was cooled with ice water causing production of white crystals recrystallized from ethanol.

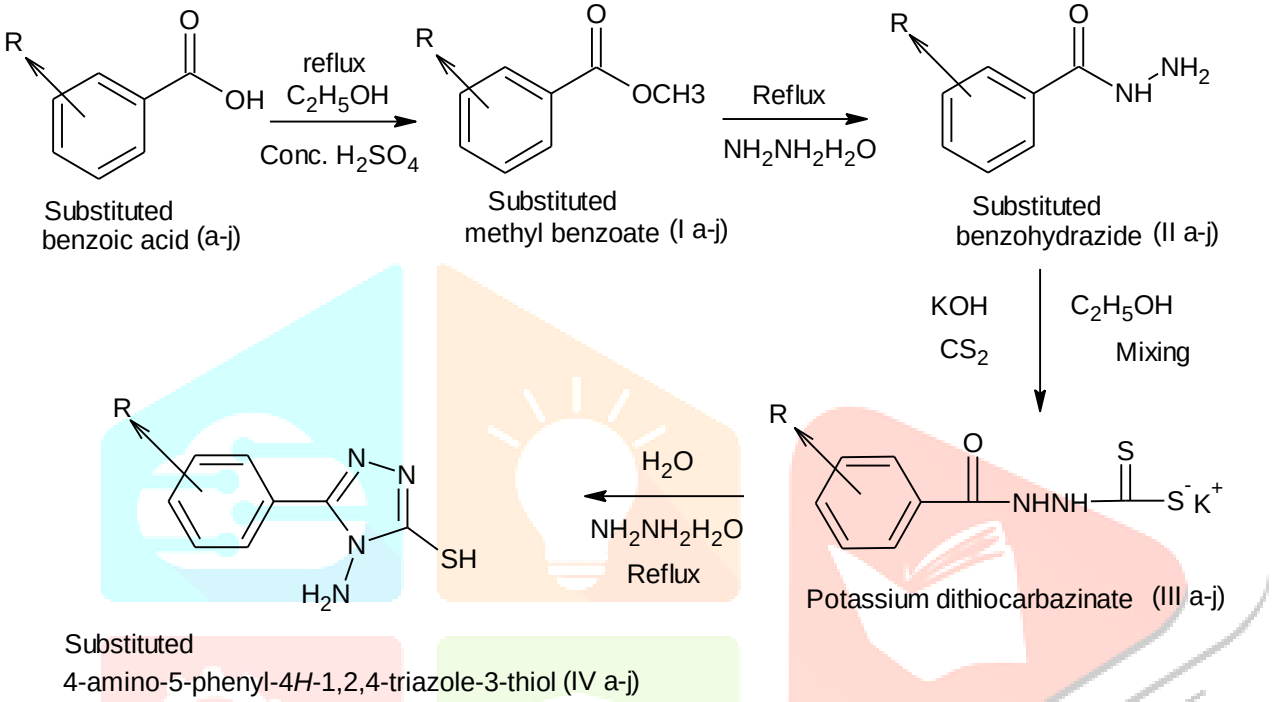
2.2.3. Synthesis of Potassium Dithiocarbazinate From Benzoic Acid Hydrazide (3)

In the solution of potassium hydroxide (8.5gm) and ethanol (15ml), benzoic acid hydrazide (2) (1.5gm) from previous step and carbon disulphide (14ml) was added and mixture was stirred for 16 hrs. To that resulting solution anhydrous ether (20ml) was added and precipitated potassium dithiocarbazinate was collected by filtration, washed with ether and dried.

2.2.4. Synthesis of 4-Amino-5-Phenyl-4H-1, 2, 4-Triazole -3-Thiol from potassium dithiocarbazinate (4)

A suspension of potassium salt (3) (5gm), hydrazine hydrate (2ml) and water (40ml) was refluxed for 3 hrs. color of reaction mixture changed from yellow to green. And hydrogen sulphide was evolved and homogenous solution resulted. The reaction mixture was cooled to room temperature and diluted with (30ml) of cold water. On acidification with HCl white powder was precipitated out, which was recrystallized from ethanol.

2.3. REACTION SCHEME:



2.4 BIOLOGICAL EVALUATION:

- ❖ **Antimicrobial screening:**
- ➢ **Materials and method:**
- ➢ **Chemicals:**

All chemicals and solvents were procured from commercial sources, purified and sterilized using standard procedures from literature whenever required.

- Macconkey agar medium (Research lab, Mumbai)
- Nutrient agar medium (Research lab, Mumbai)

❖ **Dilution of the compounds:**

All the synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) so as to get concentration of 200µg/ml and standard drugs Ciprofloxacine in DMSO as a concentration of 10mg/ml.

❖ **Sterilization of equipments and the chemicals :**

MacConkeyagar [M081], nutrient agar medium [NO11] and normal saline solution were sterilized in autoclave at 15 lbs pressure [121ºC] for 15mins. Petri plates, Whatman filter paper disc and cotton swabs were sterilized in oven at 160ºC for 2hrs.

❖ **Preparation of Slants:**

- **Preparation of MacConkay agar slant:**

MacConkey agar 206 mg was dissolved in 4 ml of distilled water, boiled and poured in test tube then plugged with cotton and sterilized in autoclave at 15l lbs pressure (121ºC) for 15 min. after sterilization the tubes containing the Macconkey agar were kept in inclined position for 30 min. Then on the surface of slants pure culture staphylococcus aureus were streaked in aseptic condition and incubated at37ºC for 24 hrs.

- **Preparation of nutrient agar medium slant:**

Nutrient agar medium 112 mg and agar powder 100 mg was dissolved in 4 ml distilled water, boiled and then poured in the test tube then plugged with cotton and sterilized in autoclave at 15lbs pressure (121ºC) for 15 min. after sterilization the tubes containing the nutrient agar medium were kept in inclined position for 30min.then on the surface of slants pure culture of bacillus Substilis, Escherichia coli were streaked in aseptic condition and incubated at 37ºC for 24 hrs.

- **Preparation of Saboraud's agar slant:**

250 mg of Saboraud's agar was dissolved in 4 ml of distilled water, boiled and then poured it in the test tube and the test tube was plugged with cotton and then sterilized in autoclave at 15 lbs pressure (121ºC) for 15 min. After the sterilization the tubes containing the Saboraud's agar were kept in inclined position for ½ hrs. Then on the solid surface of these slants the pure culture of the test fungi i.e. Candida Albicans were streaked in aseptic condition and then incubated at 37ºC for 24 hrs.

- **Prepration of suspension of test bacteria and test fungi (standardized inoculums):**

By using the 24hrs old growth of the bacteria, fungi from the slants, suspension of the bacteria/fungi were separately in sterile normal saline solution (0.85%NaCl in distilled water) in aseptic condition, to get moderate turbidity of the solution resulting by mixing0.5ml of 1.175% of barium chloride and 99.5ml of 0.36N H2SO4 acid.

❖ **Antimicrobial Drug sensitivity Test:**

Antimicrobial sensitivity test have been carried out by using disc-diffusion method, performed in nutrient agar for bacterial and saboraud's agar for fungi.

- **Preparation of culture media for antibacterial sensitivity test**

Soybean casein digest agar was prepared by weighing 3 gm of soybean casein digest medium and 2.5 gms of agar powder in 100 ml of distilled water. Then it was sterilized in autoclave at 15 lbs pressure (121ºC) for 15mins. After sterilization the media was cooled up to 45ºC and then it was poured in sterile Petri plates in

aseptic condition. Approximately 20-25 ml of media was poured in each plate. Then the media from the plate was allowed to get solidified.

- **Inoculation of suspension of bacteria & fungi on culture media:**

Sterile, non-toxic cotton swab were dipped in to the standardized inoculums (turbidity as adjusted as to obtained confluent growth on the Petri plate) and then the entire agar surface of the plate was streaked with the swab three times, turning the plate at 60o angle between streaking. Then the streaked inoculum was allowed to dry for 5-15mins with lid in place. Sterile paper disc made by punching whatman paper were dipped separately in to the solutions containing synthesized drug (300µg/ml of DMSO) and standard drug ciprofloxacin (10mg/ml of DMSO.) & Fluconazole (10mg/ml of DMSO) in aseptic condition with help of sterile forceps and were then placed on the surface of inoculated culture media after which the plates were kept in refrigeration for 30 min for the diffusion of the drug from the paper disc in to the culture media. After 30min the plates were incubated at 37ºC.

❖ **Preparation of nutrient:**

Formula for preparation of nutrient agar:

Sr.no	Ingredients	Quantity
1	Agar	20 gm
2	Peptone	10 gm
3	Beef extract	10 gm
4	NaCl	5 gm
5	Distilled water	Up to 1000ml

❖ **Preparation of MacConkeyagar**

Formula for preparation of mac-conkey agar:

Sr.no	Ingredients	Quantity
1	Lactone	20 gm
2	Peptone	10 gm
3	NaCl	5 gm
4	Bile Salt	5 gm
5	Neutral red solution	10 gm
6	Agar	20 gm
7	Distilled water	Up to 1000 ml

3. RESULTS AND DISCUSSION:

3.1. Chemistry:

In first step mixture of substituted benzhydrazide and carbon disulphide was irradiated for 15 min at 340 watt under microwave. The reaction was monitored by TLC using chloroform: methanol (9:1) as mobile phase. A product of 2-(2-substituted) hydrazine carbodithioic acid was added in hydrazine hydrate and methanol and mixture was irradiated for 20 min at 340 watt under microwave. The reaction was monitored by TLC using butane: chloroform: water (7:2:1) as mobile phase. The solid product was washed with water and recrystallized with methanol.

Microwave assisted synthesis is faster, better and safer green chemistry approach for the traditional reactions. The time taken for the synthesis of 1,2,4-triazole is drastically reduced by the microwave assisted synthesis. This technique offers clean, simple, efficient, fast and economic for the synthesis of a number of organic molecules such reaction has new tool in the organic synthesis and highly accelerated rate of the reaction time with an improvement in yield and quality of product. The IR, NMR and mass spectra are fully consistent with the structure.

3.2. Antimicrobial activity:

Antibacterial activity of the newly synthesized compounds (4a-c) was evaluated by the disc diffusion method against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus strains of bacteria. Compound code 4c was found to be highly active against all the tested strains of bacteria showing the broadest spectrum of antibacterial activity when compared with standard drug ciprofloxacin.

Antibacterial screening results of synthesized compounds measuring the zone of inhibition in millimeters:

Comp. No.	Zone of inhibition in mm							
	E. coli		B. substilis		S. aureus		Protease	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
4a	15	12	10	7	18	18	11	10
4b	14	13	12	9	17	18	13	9
4c	20	16	14	11	22	19	17	14
Standard (Ciproflo xacin)	22	19	20	17	27	23	19	18

4. CONCLUSION:

A series of 4-amino-5-(substituted phenyl)-4H-1,2,4-triazole-3-thiol were synthesized by microwave method and characterized by IR, NMR and mass spectra. All newly synthesized were screened for antibacterial activity. Among them compound code 4c showed excellent antibacterial activity.

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