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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF SOME PYRIMIDINE DERIVATIVES

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Abstract:

The main objective of present work is to synthesis, characterization, and evaluation of antimicrobial activity of some pyrimidine derivatives containing O, N, and S in the ring. Pyrimidine derivatives were prepared in three steps. In the initial step, chalcones containing -NO_2 functional group were synthesized using Claisen Schmidt condensation of aromatic aldehydes with 2-acetyl pyridine/3-acetylpyridine in methanol in the presence of aqueous NaOH. In the next step, -NO_2 group was reduced to -NH_2 group. Resulting compounds containing NH_2 functional group were reacted with different dichlorothienopyrimidines and dichlorofuropyrimidines in the presence of N,N-diisopropylethylamine to obtain pyrimidine derivatives. Antibacterial and antifungal activity of pyrimidine derivatives were studied in vitro. Pyrimidine derivatives were synthesized and purified using column chromatography. Purity of synthesized pyrimidines was determined by HPLC. Pyrimidines were characterized by different analysis such as elemental analysis, infrared, nuclear magnetic resonance, and mass spectral analysis. Analytical data of synthesized pyrimidines indicates the proposed structures. Finally antibacterial and antifungal activity were observed in the synthesized pyrimidine derivatives.

Keywords: *Antibacterial, antifungal, chromatography, spectral, medicines*

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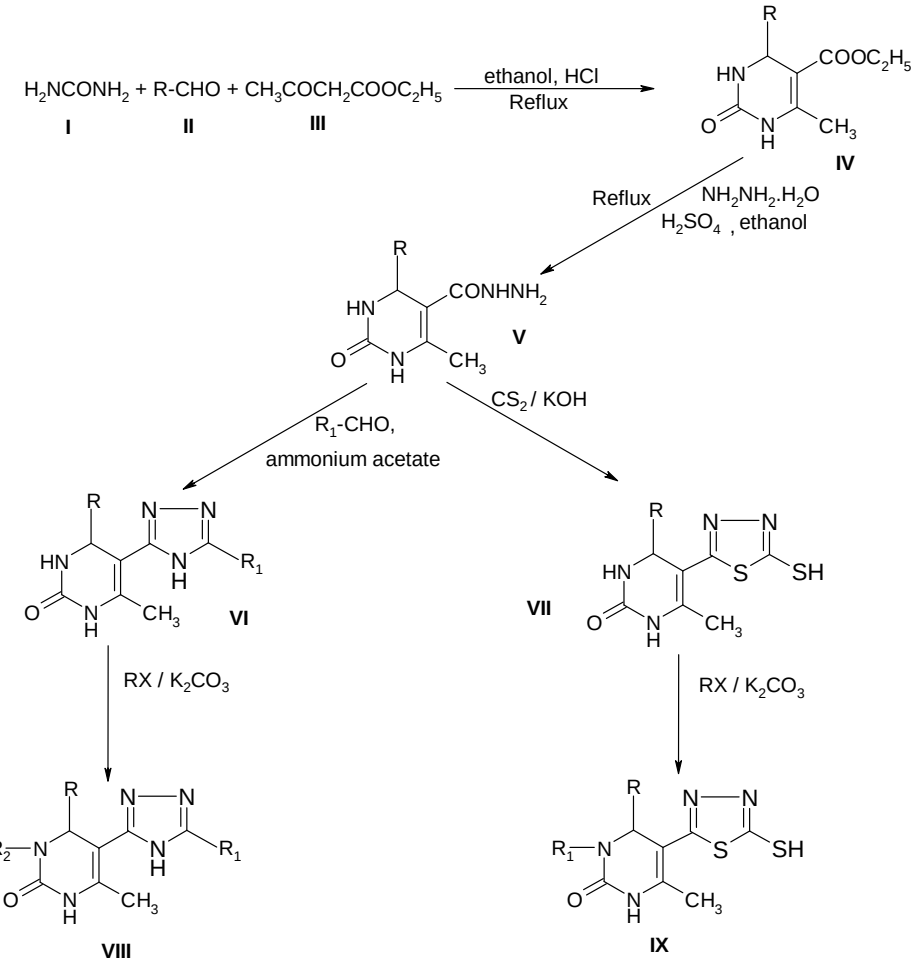
INTRODUCTION:

Man has been in the search of elixir of life from the age of the alchemists to the present age in which pharmaceutical giants are constantly meting out new drugs for the betterment of our lives. Each year millions of dollars are spent in R & D activities but due to the stringent FDA requirements the outcome is only a few chemical entities out of a several hundreds. It was always due to the ingenuity of the chemist that new chemical entities in pharmaceutical research were created. Today's medicine is based on traditional medicine. Traditional medicines exist in every continent of the globe and in every cultural area of the world. The most famous ones are traditional Chinese medicine in East Asia, Ayurvedic medicine in India, and formerly Galenic medicine in Europe, having same resemblance to each other. Each of these traditional medicines has their own origin and an individual basic philosophy. In India Ayurveda, Siddha and Unani systems of medicine provide health care for a large part of the population. Both traditional Chinese and Ayurvedic medicine developed further in terms of formulations. There is also the tendency to adopt the modern forms of clinical trials. But there has never been a change in paradigm as far as the basic philosophy is concerned. The most important achievements of modern Western medicine were made in several areas such as diagnosis, infectious diseases, endocrinology and medicinal chemistry. Virchow founded cellular pathology in the 19th century. The intensive use of the microscope in medicine with histological comparison of diseased and normal organs allowed the change from humoral to cellular pathology. Medicinal chemistry as an important science started less than 100 years ago. The active principles of plants, mostly alkaloids, were isolated and were the starting point for syntheses. Pharmacological research started in Europe in the second half of the 19th century when their founders, e.g., Rudolf Buchheim and Oswald Schmiedeberg, investigated the action of existing drugs in animal experiments. With the emergence of synthetic chemistry, the pharmacological evaluation of these products for therapeutic indications became necessary. Many new drugs were discovered by this classical approach during the 20th century. The classical way of pharmacological screening involves sequential testing of new chemical entities or extracts from biological material in isolated organs followed by tests in whole animals, mostly rats and mice but also higher animals if indicated. Most drugs in use nowadays in therapy have been found and evaluated with these methods. In the mid-1970s receptor binding assays were introduced as an approach for compound evaluation by the development of radioligand binding assays, based on evaluation procedures and mathematical calculations. The use of radioligand binding assays has facilitated the design of new chemical entities, especially as the information obtained has been used in deriving molecular models for the structure-activity relationship. The receptor technology provides a rapid means to evaluate small amounts of compound (5–10 mg) directly for their ability to interact with a receptor or enzyme, independent of its efficacy. But as new assays were developed, it also provides a means to profile the activity of compounds against a battery of binding sites, thereby yielding an in vitro radioligand binding profile. The designing and synthesis of a new molecule begins with the study of the properties of the existing molecules of a selected category, followed by the molecular modeling studies, the structure activity relationship studies and finally the synthesis of the designed compound.

METHODS

The steps adapted in the synthesis of the pyrimidine derivatives are depicted in the scheme below

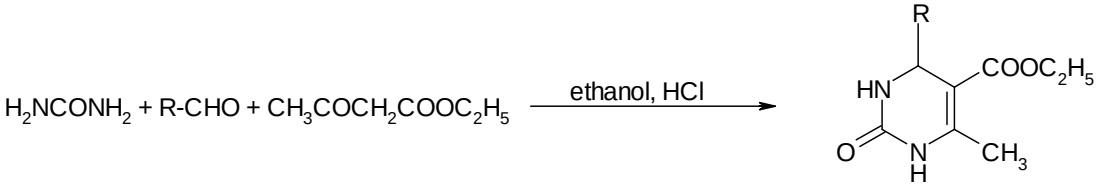


1. Synthesis of 4-phenyl-5-carboethoxy-6-methyl-3, 4-dihydropyrimidine-2- one.
2. Synthesis of 4- phenyl -5-carboxyhydrazide-6-methyl-3, 4-dihydropyrimidine-2-one.
3. Synthesis of 4- phenyl -5-(2'-substituted-1', 3', 4'-triazolo)-6-methyl-3, 4-dihydropyrimidine-2-one.
4. Synthesis of 4- phenyl -5-(1', 3', 4'-thiadiazolo)-6-methyl-3, 4- dihydropyrimidine-2-one.
5. Synthesis of N-substituted-4-phenyl-5-(2'-substituted-1', 3', 4'-triazolo)-6- methyl-3, 4-dihydropyrimidine-2-one.
6. Synthesis of N-substituted-4-phenyl-5-(1', 3', 4'-thiadiazolo)-6-methyl-3, 4- dihydropyrimidine-2-one.

SYNTHESIS OF PYRIMIDINE DERIVATIVES

Step 1: Synthesis of 4-phenyl-5-carboethoxy-6-methyl-3, 4-dihydropyrimidine-2- one.

The synthesis of 3, 4-dihydropyrimidine was performed by using benzaldehyde, urea and ethylacetoacetate as depicted in the section below by Biginelli condensation.

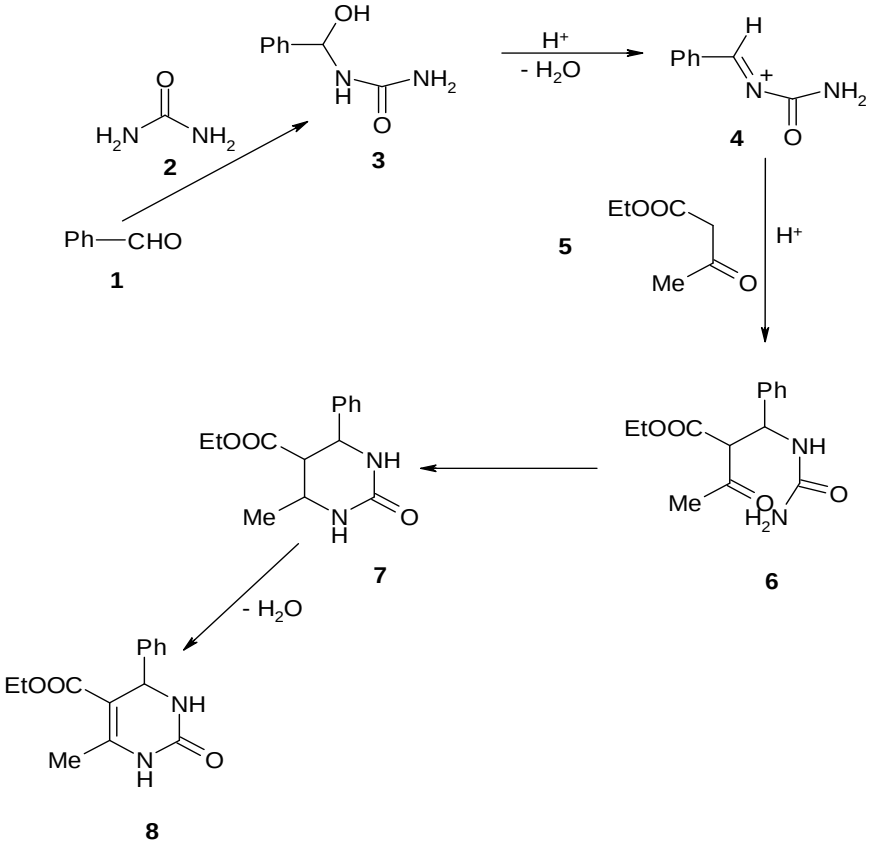


BIGINELLI CONDENSATION

The Italian chemist Pietro Biginelli first reported the one pot method for the synthesis of 3,4-dihydropyrimidine in 1893. This method is an acid catalyzed multi-component reaction of an aldehyde, a β-keto ester and urea in presence of ethanol with a catalytic amount of concentrated hydrochloric acid at reflux temperature. The mechanism of Biginelli condensation was investigated in 1997 using ^{1}H / ^{13}C NMR spectroscopy and trapping experiments.

Mechanism of Biginelli condensation.

The mechanism of the Biginelli condensation is depicted in the following reaction pathway.

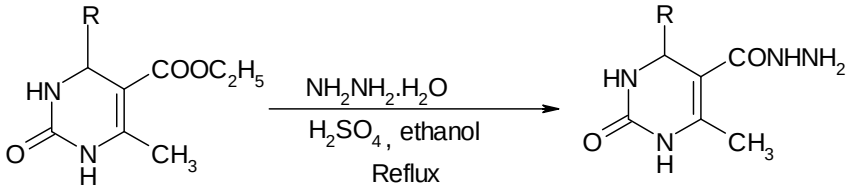


PROCEDURE

0.5 moles of urea (**I**), 0.75 moles of ethylacetoacetate (**III**) and 0.5 moles of benzaldehyde (**II**) were mixed in 25 mL of ethanol.

Step 2: Synthesis of 4- phenyl -5-carboxyhydrazide-6-methyl-3, 4-dihydropyrimidine-2-one.

The hydrazide derivative of the dihydropyrimidine-2-one was synthesized by the treatment of the product of step 1 by hydrazine hydrate in presence of ethanol with catalytic amount of concentrated sulfuric acid.



Mechanism

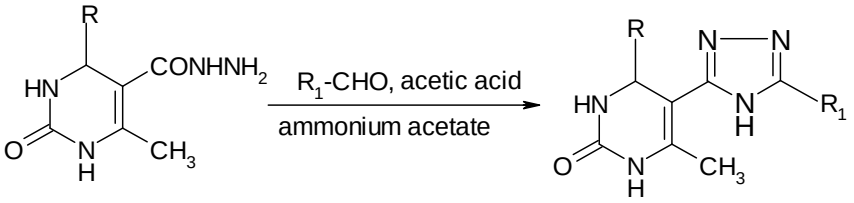
The reaction proceeds by the way of ammonolysis of esters. The mechanism involves nucleophilic attack on the electron deficient carbon atom of the ethoxy group, $-OC_2H_5$, by the base, hydrazine hydrate. The alkoxy group gets replaced by $–NHNH_2$ to yield the product, hydrazide derivative.

PROCEDURE

To 0.1 mole of the product **IV** in 20 mL ethanol, 0.1 mole of hydrazine hydrate was added.

Step3(a): Synthesis of 4- phenyl -5-(2'-substituted-1', 3', 4'-triazolo)-6-methyl-3, 4-dihydropyrimidine-2-one.

The triazolo derivatives were synthesized by the condensation of the hydrazide derivative, ammonium acetate and an aldehyde in the presence of acetic acid.

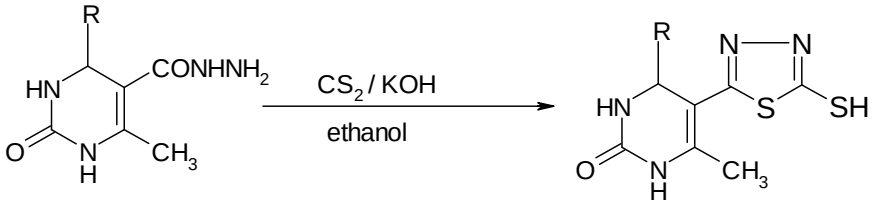


PROCEDURE

To 0.1 mole of product **V** in 20 mL acetic acid, a pinch of ammonium acetate was added, followed by the addition of 0.1 mole of Benzaldehyde / formaldehyde solution.

Step 3(b):Synthesis of 4- phenyl -5-(1', 3', 4'-thiadiazolo)-6-methyl-3, 4- dihydropyrimidine-2-one.

The product precipitated when the mixture was neutralized with concentrated hydrochloric acid.

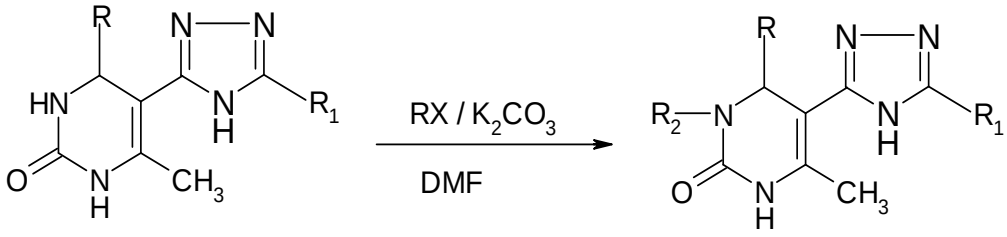


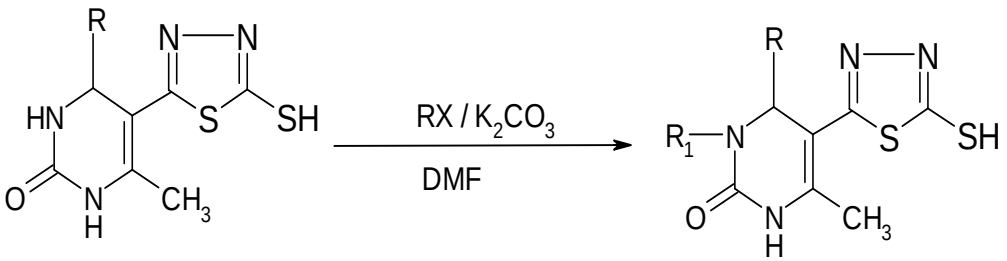
PROCEDURE

To a solution of 0.15 moles potassium hydroxide in ethanol and 0.15 mole of the compound **V** was added 0.15 mole of carbon disulfide.

Step 4: Synthesis of the N-substituted compound.

Alkylation of the pyrimidine derivatives obtained in the steps 3a and 3b above was accomplished by the treatment with alkyl / aryl halide and K_2CO_3 in presence of DMF.





PROCEDURE

A mixture of 2.17 mmoles of **VI** or **VII**, 4.35 mmoles of K_2CO_3 and 4.48 mmoles of the alkyl or aryl halide in 6 mL of DMF was stirred for 4 hours at room temperature.

CHARACTERIZATION OF THE SYNTHESIZED COMPOUNDS

Chemical Characterization

The synthesized pyrimidine derivatives are pale yellow to off white crystals, with no characteristic odour. These derivatives are soluble in chloroform, ethanol, slightly soluble in methanol and practically insoluble in benzene.

The characterization of the physicochemical properties of these compounds was done as follows:

(1) The **melting points** were determined by open capillary method and are uncorrected.

(2) The purity and homogeneity of the compounds was determined by **thin layer chromatography**[45], using silica gel G as the stationary phase on glass plates. Iodine vapors were used for development of the chromatogram. The solvent system used for running the compounds was chloroform: methanol in the ratio 9:1.

(3) **Infrared spectroscopy**: The I.R. spectra of the synthesized compounds were obtained using FT-IR spectrophotometer, Jasco FT/IR-470 plus.

(4) **Mass spectroscopy and NMR spectroscopy**: Samples are sent to CDRI, Lucknow for NMR and Mass spectroscopy. The results are still awaited.

Pharmacological Characterization

The anti microbial activity of the synthesized pyrimidine derivatives was performed on different gram positive and gram-negative bacteria and also on fungi.

The different methods available for the evaluation of the antimicrobial activity are:

1. Serial dilution method
2. Agar streak dilution method
3. Agar diffusion method.

a. Cup-plate method
b. Cylinder method
c. Paper-disc method

4. Turbidimetric method
5. Miscellaneous methods.

a. Phenol-coefficient method
b. Nephelometric method
c. Counting method
d. Determination of MIC.

The Diffusion Methods: In these methods the substance to be assayed is allowed to diffuse through solid inoculated culture medium

Cylinder method: The cylinders used can be made of Pyrex glass, glazed porcelain, aluminum or stainless steel.

Cup and Plate method: In this method, cups are cut in the agar plate. The cuts can be made in either of two ways. A sterile rubber stopper, test tube or marble is placed in the molten agar. When the agar solidifies, the object is removed leaving a depression. The cuts must be made vertically into the agar and should be completely filled with the substance to be assayed. The thickness of the agar should be 5 to 6 mm.

Drop plate method: In this method two techniques can be employed for the assay of the substance. A loop, a pipette or a syringe may be used to apply the drop.

Paper disc method: In this method, the disc, instead of the cup or the cylinder, is used as the reservoir for the substance being assayed. The solution to be assayed is placed on the disc either by the use of a pipette or a loop, or the disc may be dipped into the solution.

Zone of Inhibition: Various degrees of inhibition may occur and may affect the accurate reading of the zones obtained. The range is from the ideal zone, with well-defined edges that are distinct and easily read, to zones that are blurred, diffuse and with or without halos and concentric rings.

Measurement of the zone diameter: The diameter of the zones of inhibition may be read using any convenient measuring device. Millimeter rulers, calipers and specially designed equipment have been utilized.

Accuracy of Results: The limits of error have been ranged from ±15 percent to ±20 percent. The precision has been improved in the recent years by the development of sophisticated instruments.

Among the several methods reported for the determination of the antimicrobial activity, the serial dilution method was selected for the determination of the minimum inhibitory concentration (MIC) of the synthesized compounds.

Determination of the Minimum Inhibitory Concentration (MIC)

The MIC of an antimicrobial agent, for a particular organism, is the lowest concentration that prevents growth of that organism. There are two types of serial dilution methods.

- Broth dilution method
- Agar dilution method

Broth dilution method: In this method, the graded concentration of the test compounds are added to the broth and accurate volume of a suspension of the

microorganism is added to each. After proper shaking, these dilutions are incubated for specified period of time at the optimum temperature (37ºC) and then examined for growth.

Agar dilution method: In this method, dilutions of the compounds to be evaluated are mixed agar before pouring or applied to the surface of the medium after it has just set.

ANTIMICROBIAL EVALUATION: In the experimental part of antimicrobial evaluation of the synthesized compounds, the evaluation of antibacterial activity was performed.

Evaluation of the antimicrobial activity.

Bacteria are very small, unicellular, prokaryotic organisms with rigid cell wall. Christian Gram in 1884 devised a differential staining procedure, which classified the bacteria broadly into two categories: Gram negative and Gram positive bacteria and Gram positive bacteria

Proteus mirabilis: Proteus species is gram negative bacteria, actively motile, uncapsulated pleophormic, coliform bacillus.

Bacillus subtilis: B. subtils is gram-positive, rod-shaped bacteria, capable of producing endospores.

Pseudomonas aeruginosa: P. aeruginosa is a gram negative, encapsulated, does not produce spores and is usually motive by virtue of a single polar flagellum.

Staphylococcus aureus: S. aureus is gram positive bacteria occurring on the skin and mucous membrane of the warm-blooded animals.

In the present dissertation work, the antimicrobial evaluation of the synthesized pyrimidine derivatives involved the following steps:

Preparation of the solution of the standard drug: A stock solution of the standard drug (Norfloxacin)[50,51] of the concentration 1000µg/mL was prepared in N, N-dimethyl formamide (DMF). The stock solution was diluted further with DMF to give a concentration of 100µg/mL.

Preparation of the solutions of the synthesized compounds: A stock solution of each of the synthesized pyrimidine derivative of the concentration 1000µg/mL was prepared in DMF.

Sterilization: The sterilization of the nutrient broth, culture tubes, pipettes and other glasswares, was done by autoclaving at 15 lb/sq inch pressure for 30 minutes.

Incubation: The incubation was carried out in an electrically heated oven at 37± 1ºC for 48 hours.

Determination of the minimum inhibitory concentration (MIC) of Norfloxacin : A set of 10 sterilized test tubes were taken and numbered serially.

Determination of the minimum inhibitory concentration (MIC) of the synthesized compounds: A set of 18 sterilized test tubes were taken and numbered serially. Required solutions of synthesized compounds were transferred to each of the test tubes.

SUMMARY AND CONCLUSION:

The yield of all the synthesized compounds is found to be significant. The structural confirmation of the compounds is done by IR spectra and the percentage nitrogen content found in the synthesized compounds. All the synthesized compounds show peaks in the IR spectrum at wave number (cm^{-1}) 3500, 3120, 2980, 1690, 756, 1370, and 1456. These peaks are characteristics of N—H, C—H (Aromatic), C—H, C=O stretching and C—H bending. Compounds PYMD-1 and PYMD-2 show peaks at 2525, which is the characteristic stretching of S—H group. All the synthesized pyrimidine derivatives show anti-bacterial activity against both gram positive and gram-negative bacteria. The compounds are found to be active against the gram-positive bacteria in comparatively lower dose than that required for the activity against the gram-negative bacteria in most of the cases. The triazolo-2'-phenyl substituted pyrimidine derivatives are found to be the most potent compounds of all the synthesized compounds. The thiadiazolo substituted pyrimidine derivatives are found to be almost equipotent to the triazolo-2'- phenyl substituted pyrimidine. The results indicate that the 5-position of the pyrimidine does play an important role in the anti microbial activity of the compounds. In addition to the type of heterocyclic substitution on the 5- position of the pyrimidine, in case of the triazolo substituted pyrimidine, the 2'-positon of the triazole nucleus is also vital for the activity. The presence of a cyclic substitutent at this position enhances the activity of the compound against the bacteria. The result also indicates that the substitution of an aryl derivative at the nitrogen of the pyrimidine ring leads to a more

potent anti microbial agent as compared to the nitrogen substituted with an alkyl group.

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