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Review Article

ANTIMICROBIAL ASSAY AND 4, 6 - DIPHENYL- 3, 4-DIHYDROPYRIMIDINE-2(H)-ONE

Vishal Garg[1]*, Rambir Singh[2], Dinesh Jindal[1], Ajay Kumar Saini[1] and Kriti Gupta[1]

[1]Jaipur School of Pharmacy, MVGU, Jaipur, Rajasthan, India

[2]Department of Pharmacy, Maharishi Arvind University, Jaipur, Rajasthan, India

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ABSTRACT

Pyrimidine is a heterocyclic aromatic organic compound similar to benzene and pyridine, containing two nitrogen atoms at positions 1 and 3 of the six-member ring. It is isomeric with two other forms of diazine. The pyrimidine skeleton is of great importance to chemists as well as biologists as it is available in a large variety of naturally occurring compounds and in clinically useful molecule having diverse biological activities. Two general method are usually employed, the cylinder-plate (or cup-plate) method and the turbidimetric (or tube assay) method. The antifungal activity performed by either in *vitro* or in *vivo* methood.

Keywords: Antifungal activity, Antibacterial activity, Antimicrobial agents, Pyrimidine derivatives.

INTRODUCTION

Anything that destroys bacteria or suppresses their growth or their ability to reproduce is called antibacterial. Heat, chemicals such as chlorine, and antibiotic drugs all have antibacterial properties. Antimicrobials have transformed our ability to treat many infectious diseases that were killers only a few decades ago. The increasing use of antimicrobials in humans, animals, and agriculture has resulted in many pathogens developing resistance to these powerful drugs. All major groups of pathogens-viruses, fungi, parasites, and bacteria-can become resistant to antimicrobials. Many diseases are increasingly difficult to treat because of the emergence of drug-resistant organisms including: HIV and other viruses; bacteria such as Staphylococci, Enterococci and Escherichia coli; respiratory infections, such as Tuberculosis and Influenza; food-borne pathogens, such as Salmonella and Campylobacter; sexually transmitted organisms such as Neisseria gonorrhea; fungal infections, such as Candida; and parasites such as Plasmodium falciparum, the cause of malaria.[1]

Bacterial Infections[2]

Bacteria are living things that have only one cell. Under a microscope, they look like balls, rods or spirals. Some bacteria help to digest food, destroy disease-causing cells and give the body needed vitamins. Bacteria are also used in making healthy foods like yogurt and cheese. However, infectious bacteria can make us ill. They reproduce quickly in our body. Many give off chemicals called toxins, which can damage tissue and make us sick. Examples of bacteria that cause

infections include *Streptococcus*, *Staphylococcus*, and *E. coli*.

Bacteria are microorganisms that have circular double-stranded DNA and (except for Mycoplasma sp) cell walls. Only a small number are human pathogens.

Common Pathogenic Bacteria[2]

Bacteria may be cylindric (bacilli), spherical (cocci), or spiral (spirochetes). A few coccal, many bacillary and most spirochetal species are motile. Gram-positive bacteria retain crystal violet dye after iodine fixation and alcohol decolorization, whereas gram-negative bacteria do not. Gram-negative bacteria have an additional outer membrane containing lipopolysaccharide (endotoxin). Bacteria may be additionally enclosed in capsules, which may (eg, with Streptococcus pneumoniae and Haemophilus influenzae) impair their ingestion by phagocytes. Other factors may enhance bacterial pathogenicity. Aerobic bacteria grow in the presence of air. Anaerobic bacteria do not; Facultative bacteria can grow either aerobically or anaerobically. Some bacteria (eg, Salmonella typhi, Legionella sp, Mycobacteria sp, and Chlamydia and Chlamydophila spp) preferentially reside and replicate intracellularly. Most others do so extracellularly.

Antibacterial Drugs[3]

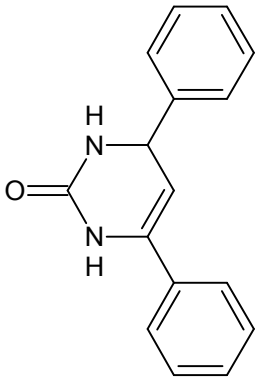
Antibacterial drugs are derived from bacteria or molds or from de novo synthesis. “Antibiotic,” which is often used synonymously with “antibacterial drug” technically refers only to antimicrobials derived from bacteria or molds. Antibacterials have many mechanisms of action, including inhibiting cell wall synthesis, activating enzymes that destroy the cell wall, increasing cell membrane permeability, and interfering with protein synthesis and nucleic acid metabolism. Antibacterials sometimes interact with other drugs, raising or lowering serum levels of other drugs by increasing or decreasing their metabolism or various other mechanisms. The most clinically important interactions involve drugs with a low therapeutic ratio. Many antibacterials are chemically related and are thus grouped into classes. Although drugs within each class share structural and functional similarities, they often have different pharmacology and spectra of activity.

Selection and Use of Antibacterial[4]

Antibacterial should be used only if clinical or laboratory evidence suggests bacterial infection. Use of antibacterial for viral illness or undifferentiated fever is inappropriate, subjects the patient to drug complications without any benefit, and contributes to bacterial resistance. Certain bacterial infections (eg, abscesses, infections with foreign bodies) require surgical intervention and do not respond to antibiotics alone. Cultures and antibiotic sensitivities are essential for selecting a drug for serious infections. However, treatment often must begin before culture results are available, necessitating selection according to the most likely infecting organisms (empiric antibiotic selection). Whether chosen according to culture results or empirically, drugs used should possess the narrowest spectrum of activity that will control the infection. For empiric treatment of serious infections that may involve any one of several pathogens (eg, fever in a neutropenic patient) or that may be due to multiple pathogens (eg, polymicrobial anaerobic infection), a broad spectrum of activity is desirable. The most likely organisms and the organisms' susceptibility to antibacterial vary according to geography (within cities or even within a hospital). Bactericidal drugs kill bacteria *in vitro*. Bacteriostatic drugs slow or stop *in vitro* bacterial growth but depend on body defenses to kill bacteria. Quantitative methods identify the minimum *in vitro* concentration at which an antibiotic can inhibit growth (minimum inhibitory concentration, or MIC) or kill (minimum bactericidal concentration, or MBC). However, in *vivo* antibacterial effectiveness involves other factors, including pharmacology (eg, absorption, distribution, concentration in fluids and tissues, protein binding, and rate of excretion or metabolism), the presence of drug interactions or inhibiting substances, and host defense

mechanisms. Usually, greater *in vitro* killing power is important only if local or systemic host defenses are weak (eg, in endocarditis, meningitis, serious infections in neutropenic or other immunocompromised patients). The predominant determinant of bacteriologic response to antibiotics is either the duration that blood levels of the antibiotic exceed the MIC (time-dependence) or the peak blood level relative to MIC (concentration-dependence). Complications of antibiotic therapy include superinfection by nonsusceptible bacteria or fungi and, commonly, cutaneous, renal, hematologic, and GI adverse effects. Adverse effects frequently require stopping the offending drug and substituting another antibiotic to which the pathogen is susceptible; sometimes, no alternatives exist.

The chemistry of pyrimidines and its derivatives have been studied since past century due to their close pharmacological association with diverse pharmacological properties. Pyrimidine was first isolated by Gabriel and Colman in 1899. Though pyrimidine itself does not exist in nature but substituted pyrimidines containing pyrimidine moiety are found as a part of more complex system and are widely distributed. Pyrimidines are considered to be important not only because they are an integral part of the genetic material viz. DNA and RNA as nucleotides and nucleosides but they also impart numerous biological activities such as bactericides, fungicides, viricides, insecticides. They have also found application in agricultural and industrial chemicals.[5] Many reviews on naturally occurring pyrimidines are available on general introduction including broad principles, detailed general procedure, synthetic procedures along with physical properties. But no collective information is available on role of pyrimidines as bioactive compound. This biodynamic property of the pyrimidine ring system prompted us to account for their pharmacological properties especially as anti-infective agents. 4-Aryldihydropyrimidinons and their derivatives are known to exhibit pharmacological activities as Calcium channel blocker, antihypertensive agents and wide range of biological activities such as antiviral, antitumor, antibacterial and anti-inflammatory. Several marine alkaloids containing the dihydropyrimidine unit have shown interesting biological properties.



4,6-diphenyl-3,4-dihydropyrimidin-2(1*H*)-one

So, there is a need of more research on this nucleus to increase its biological activity. In present work emphasis is given on antimicrobial activity. The activity can be increased by incorporation of side chain.

Characteristics of 4, 6 - Diphenyl-3,4-dihydropyrimidine-2(H)-one

Molecular formula	$C_{16}H_{14}N_2O$
Molecular weight	322
Physical state	Solid
Colour	White
Odour	Odourless
M.P.	233-235^oC
solubility	Soluble in ethanol

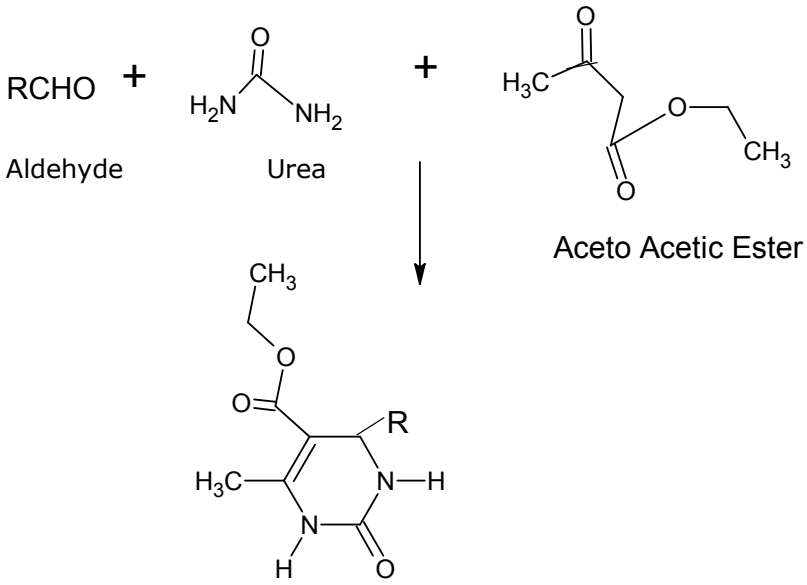
SYNTHESIS OF 3, 4-DIHYDRO-PYRIMIDINE –2-(1H)-ONES[5,6]

3,4-dihydropyrimidine-2-(1H)-ones nucleus can be conveniently derived from number of substrates and methods.

From calcium chloride catalyzed three component, one pot condensation reaction[5]

Biginelli reported this reaction shown in **figure 2.** A variety of Lewis acid catalysts are employed eg. LiBr, $ZrCl_4$, $CeCl_3.7H_2O$, $LaCl_3.7H_2O$, $SnCl_2.2H_2O$ etc. These conditions were applied to enolisable ketones, urea and benzaldehyde to

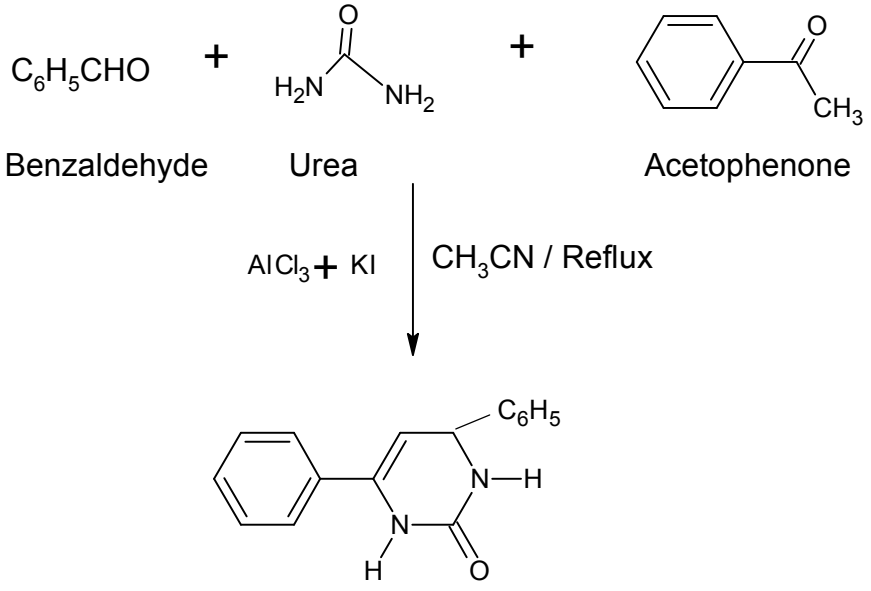
obtain 3, 4- dihydropyrimidine –2- (1H)-one in 82-90% yield.



5-(ethoxycarbonyl)-4-alkyl-6-methyl-3, 4-dihydropyrimidin-2(1H)-one

From aluminium, chloride mediated three-component cyclocondensation[6]

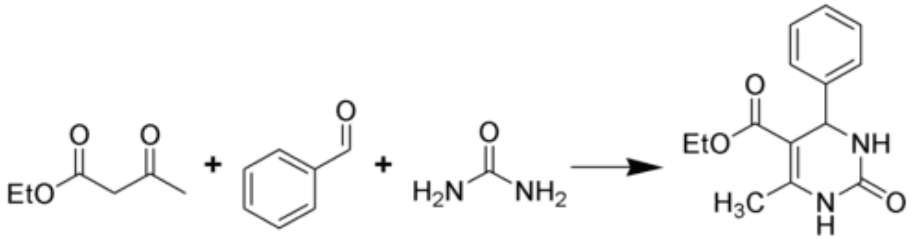
Michael reported the mechanistic pathway that involve the $AlCl_3$ and KI stem which activates the carbonyl function, thereby making the methyl group readily enolisable, which in turns react with aldehyde and urea derived imines in the Michael way to produce product. In recent years, the development of more economical and environmental friendly conversion processes is gaining interest. Herein we report an efficient, practical, environmentally benign and high yielding method for the one pot synthesis of dihydropyrimidinones using $CaCl_2$ as catalyst. $CaCl_2$ is inexpensive, commercially available reagent. In a typical experimental procedure a solution of ketoester, aldehyde and urea in ethanol was heated under reflux in the presence of dihydropyrimidinones. The reaction mixture was then poured into crushed ice and the solid product separated was filtered. The crude product obtained was of high purity.



4, 6-diphenyl-3, 4-dihydropyrimidine-2(1H)-one

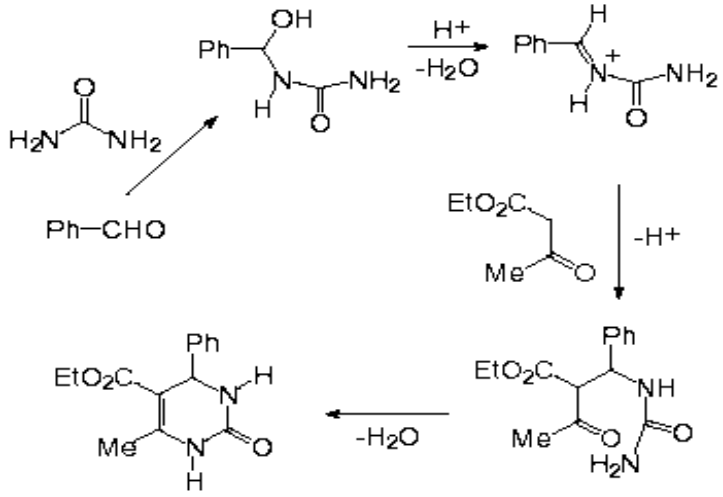
Biginelli Reaction[7]

This acid-catalyzed, three-component reaction between an aldehyde, a ß-ketoester and urea constitutes a rapid and facile synthesis of tetrahydropyrimidones, which are interesting compounds with a potential for pharmaceutical application.



Mechanism

The first step in the mechanism is believed to be the condensation between the aldehyde and urea, with some similarities to the Mannich Condensation. The iminium intermediate generated acts as an electrophile for the nucleophilic addition of the ketoester enol, and the ketone carbonyl of the resulting adduct undergoes condensation with the urea NH_2 to give the cyclized product.



Mechanism of Biginelli Reaction

Antimicrobial Activity[8]

Antibacterial activity

The inhibition of growth under standardized conditions may be utilized for demonstrating the therapeutic efficacy of antibacterial agents. Any subtle change in the antibacterial molecule, which may not be detected by chemical methods will be revealed by a change in the antimicrobial activity and hence microbiological assays are very useful for resolving doubts regarding possible change in potency of antibiotics and their preparations.

The microbiological assay is based upon a comparison of the inhibition of growth of microorganisms by measured concentration of the antibacterial to be examined with that produced by known concentrations of a standard preparation of the antibiotic having a known activity. Two general method are usually employed, the cylinder-plate (or cup-plate) method and the turbidimetric (or tube assay) method.

The Cylinder-Plate Method (Method A)

This method depends upon diffusion of the antibiotic from a vertical cylinder through a solidified agar layer in a petri dish or plate to an extent such that growth of the added microorganism is prevented entirely in a zone around the cylinder containing a solution of the antibiotic.

The Turbidimetric Method (Method B)

This depends upon the inhibition of growth of a microbial culture in a uniform solution of the antibiotic in a fluid medium that is favorable to its rapid growth in the absence of the antibiotic

Standard drug for antibacterial activity

Norfloxacin

Norfloxacin is a broad-spectrum chemotherapeutic agent, primarily used for urinary and genital tract infections.

Molecular formula : $C_{16}H_{18}FN_3O_3$

Molecular weight **:** 319.34

Melting point **:** 228°C

Solubility **:** Soluble in glacial acetic acid, mineral acid and bases, and slightly soluble in water, ethanol and chloroform.

Mode of action **:** It is bacterial DNA gyrase (Topoisomerase) inhibitor.

Use **:** Norfloxacin is indicated for the treatment of urinary tract infections, caused by *E. coli,*and *S. aureus* etc.

Antifungal activity

Fungal infections of the skin are caused by dermatophytes, such as *Trichophyton rubrum, Trichophyton tonsurans, Trichophyton mentagrophytes, Microsporum canis, Epidermophyton floccosum, Microsporum gypseum*, and *Trichophyton verrucosum.* Most fungal skin infections, such as tinea pedis and tinea cruris, respond to topical therapy, although widespread or chronic infections that do not respond to local measures may require systemic treatment. The evaluation of the antifungal activity can be performed *in vitro* or *in vivo*.

In vitro inhibitory activity

In vitro tests are performed to investigate whether the test compound in comparison to standards covers the most relevant pathogens of dermal mycoses. The test conditions are rendered more difficult by addition of protein since the main infection site for fungi is the horny layer of the epidermis, which has high protein content. The studies are performed by means of conventional serial dilution procedures in test medium without and with addition of 4% bovine albumin. The test medium is Sabouraud dextrose broth containing 1% Neopeptone Difco and 2% glucose. The basic medium is sterilized in an autoclave at 121 °C for 15 min. The pH is adjusted to 6.5 with 1 N NaOH. The medium-containing albumin is sterilized by filtration through a membrane filter. For preparation of the test series, the inhibiting substances are dissolved in methanol and then rapidly diluted with slightly warmed test medium, so that series with a continuous dilution factor of 2 are obtained: 125–0.03 µg/ml in medium without protein, 500–1 µg/ml in medium with protein. The test organisms are various strains of dermatophytes (*Trichophytum mentagrophytes*, *T. rubrum*, *T. verrucosum*, *T. equinum*, *T. gallinum*, *Microsporum canis*, *Microsporum gypseum*) and yeasts (*Candida albicans*, *Ca. tropicalis*, *Ca. pseudotropicalis*, *Ca. krusei*, *Ca. parapsilosis*, *Ca. lipolytica*, *Ca. brumpti*, *Ca. utilis*, *Torulopsis glabrata*). The organisms are pre-cultured on a modified Grutz agar at 28 °C for periods of 1–4 weeks. The suspensions are adjusted by photometry that about 105 micro conidia of dermatophytes and 104 yeast cells per ml are obtained in each inoculated test tube. The minimal inhibitory concentrations are measured after 14 days incubation at 28 °C.

The percentage of strains of *Trichophytum mentagrophytes*, *Trichophytum rubrum*, and C*andida albicans* is plotted against dilution steps for each test compound with and without albumin and IC_{50} values are calculated.

In vivo activity in the guinea pig trichophytosis model

To evaluate antimycotic compounds, several authors have used the guinea pig trichophytosis model. Male albino guinea pigs (Pirbright White), bred mycosis-free, weighing 450–550 g are fed Altromin® pellets and tap water ad libitum. On both sides of the back, areas of 5 x12 cm are shorn to a fur length of 1 mm. Three areas with a diameter of 3 mm are inoculated with a pipette on either side. Per injection site, 104 spores of *Trichophyton mentagrophytes* 2114 in 0.05 ml suspension in physiological saline solution are inoculated. Three days after inoculation, infections with redness and scale formations are observed. From days 3–7 after the infection, 1 ml of the test preparation or standard is applied onto the right animal sides and rubbed in once daily. The diameters (mm) of all alopecias are measured with a ruler 3.5 weeks after the infection. The values of alopecias, separated according to the treated group and animal side, are determined and statistically evaluated using Duncan's new multiple range test.

Standard drug for antifungal activity:

Ketoconazole: Ketoconzole is a broad-spectrum chemotherapeutic agent, primarily used for blasto mycosis and candidiasis.

Molecular formula: $C_{26}H_{28}Cl_2N_4O_4$

Molecular weight: 531.44

Solubility: Soluble in glacial acetic acid, mineral acid and bases and slightly soluble in water, ethanol and chloroform.

Mode of action: It inhibit mycolic acid synthesis essential for fungal cell wall synthesis

Uses: Blastomycosis, Histoplasmosis, Ringworm infection.

CONCLUSION:

In this article we included different methods for the synthesis of 3,4-dihydropyrimidine-2-(1H)-ones and methods of antimicrobial assay for the exploring Information regarding pyridine derivatives.

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